

AN EVALUATION OF NURSERY TECHNIQUES AND FEED MANAGEMENT DURING
CULTURE OF MARINE SHRIMP *Litopenaeus vannamei*

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AN EVALUATION OF NURSERY TECHNIQUES AND FEED MANAGEMENT DURING
CULTURE OF MARINE SHRIMP *Litopenaeus vannamei*

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Oscar Zelaya, son of Captain Jose Dario Zelaya and Nolvía (Montes) de Zelaya was born in February 28, 1975, in Tegucigalpa, Honduras. He graduated from Elvel High School in 1992. He attended Zamorano University, Honduras, from 1993-1995, supported by a full scholarship granted from the Deutsche für Internationale Entwicklung DSE (German Foundation for the International Development), and graduated as an agronomist. He worked for over a year with Grupo Granjas Marinas S.A. in Choluteca, Honduras, and returned to Zamorano in May 1997 for the agronomic engineering program, supported by a full scholarship from DSE, and graduated in May 1998. Upon graduation, He worked with Grupo Granjas Marinas S.A. through March 1999. He began graduate studies in the Department of Fisheries and Allied Aquaculture, Auburn University, on April 23, 1999 supported by a full scholarship granted from the Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP) and received a Master of Science degree in Aquaculture in 2001. In May 2001, he started graduate studies under a Mississippi-Alabama Sea Grant research project to pursue a Doctorate degree from the Department of Fisheries and Allied Aquaculture, Auburn University. Following the successful defense of his dissertation on July 22, 2004, he began the MBA program at Auburn University in August 2004. With the completion of this program his studies officially culminated on December 2005.

DISSERTATION ABSTRACT

AN EVALUATION OF NURSERY TECHNIQUES AND FEED MANAGEMENT
DURING CULTURE OF MARINE SHRIMP *Litopenaeus vannamei*

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The use of indoor shrimp nurseries is often encouraged, especially prior to intensive and super-intensive shrimp culture. Potential benefits in subsequent grow out include; better control of stock inventory, better management of feed inputs, reduced predation, and the ability to extend the growing season. More recently, nurseries have been recommended for biosecurity reasons. There has been limited research to identify suitable nursery protocols that optimize shrimp performance in indoor nurseries. There is even less information on shrimp performance in the growout phase following various nursery practices. Additionally, feeding is one of the most important activities when

considering pond dynamics, management and production economics for pond growout. Despite the importance of feed management there are few studies that have evaluated feeding strategies or feeding programs.

These studies were conducted to evaluate various management practices for shrimp nurseries and possible effects on growout following nursing. Objectives of the nursery studies were to evaluate the influence of: 1) nursery stocking rates 2) nursery duration and 3) the use of dried feed, algae and newly hatched artemia; on survival, growth and feed conversion during the nursery phase and on survival, individual shrimp size and total production during the following grow out phase.

The evaluation of feed management strategies was conducted with the objective of incorporating general aquaculture considerations into a management and feeding program and evaluating the effects and economic implications of three feeding schedules: 1) Early aggressive feeding schedule with high feed inputs early in the cycle to maximize early growth of the shrimp but then to minimize feed inputs during the end of the production when water quality is most unstable, 2) Late aggressive feeding schedule which minimized early feed inputs when natural productivity is high and maximize feed inputs late in the cycle when natural productivity is more likely to be limiting, and 3) Intermediate feeding (IF) which is intermediate to EAF and LAF.

The experiments were conducted at the Claude Peteet Mariculture Center, Gulf Shores, Alabama. The studies included nursery phases and a growout phases. Six fiberglass tanks (3.0 x 1.5 x 0.9 m) located inside a greenhouse and sixteen round plastic tanks of 1 m³, located under a plastic cover, were used for the nursery phase of the

experiments. Twelve 0.1-ha plastic lined production ponds were used for the grow out phase.

Findings of the studies suggest that nursery densities in the range of 25 to 65 PL/L have no influence on subsequent growth and survival during grow out. However at a higher density (65pl/L), improved feed and culture systems were required. Postlarvae in the best performing nursery treatments also had higher yields and better size distributions during the growout phase. When comparing a nursery period of 14 and 21 days, it was found that a longer nursery period enhanced larger juveniles and improved nursery biomass loading, however under grow out conditions nursed juveniles did not differ significantly in production criteria from direct stocked shrimp. When evaluating type and combination of diets, in terms of final average weight and biomass loading results suggest that there is a clear advantage in supplementing dried feeds with artemia for 3 days. The use of algal paste did not produce better results than algae that grew naturally in nursery tanks.

No significant differences were found among treatments in early and late aggressive feeding experiments, but significantly better FCR and lower feed costs were found in the early aggressive feeding strategy. Gross income and returns above selected variable costs were not different among treatments.

The targeted FCR method under the implemented feeding program was useful in reducing feed by an average of 17% when compared to a previous study that was based on typical feed tables.

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I. INTRODUCTION

Commercial shrimp landings have reached maximum sustainable yield (FAO 1998), yet a demand for shrimp has increased in the last decade (Keefe and Jolly 2002). These shortages have resulted in increased value which has stimulated development of shrimp aquaculture in certain regions of the world. Between 1984 and 1997, the contribution from shrimp aquaculture to the total world supply has increased at 14%/year, and in 1997 around 941,000 tons of shrimp and prawns valued at 6.1 billion dollars were cultured (Barg *et al.* 1999).

In the US, domestic production from the wild is at maximum yield, while domestic consumption, per capita consumption, and imports all have continued to increase (Hopkins 1992, Keefe and Jolly 2002). The U.S. market outlook indicates that consumption for shrimp will continue to grow (United States Department of Commerce 1988).

As a result of these factors, expansion of shrimp culture is taking place with shrimp farms built in the southeastern and western United States. From this expansion, economically successful farms have been characterized by Hopkins (1990, 1992) as those having good technology, business management, financial structure and marketing and selling to high value niche markets. Fast (1991) considered that the main constraint to their economic viability is in the costs of rearing shrimp to market size after they leave the hatchery.

A natural resource that might aid U.S. shrimp culture is that about two thirds of the continental United States is underlain with saline water. Mariculture was suggested as means of utilizing these salt-affected lands (Feth 1970). This has encouraged the development of an inland shrimp culture industry in addition to the development of a coastal industry. The ability of shrimp to tolerate changes in salinity appears to be influenced in a large extent by age (Aquacop 1991, Samocha et al. 1998, McGraw et al. 2002). A nursing period to obtain older post larvae (PL) may be required to allow shrimp to be acclimated to lower salinities often found in underground water. McGraw et al. (2002) suggests that PL younger than 15 days old should not be acclimated to salinities below 4 ppt, whereas older PL can be acclimated to 1 ppt with good survival.

However these regions are limited in their culture period to the warm season because no areas of the continental U.S. (between 25° and 45°N latitude) are adequate for year-round shrimp production. Even at best, only six to eight months per year are warm enough for shrimp culture in most regions (Sandifer et al. 1988, Hopkins 1992). With direct stocking the growing season is too short for two crops to be economical (Griffin et al. 1981, Clifford 1985, Sadeh et al. 1986).

The inclusion of an early indoor nursery phase has been considered as a possible way to extend the growing season (Sturmer and Lawrence 1987, Lawrence et al. 1985, Sandifer et al. 1988). This could add one or two months for the first crop and might increase the potential for production of two crops per year.

A nursery phase in the production cycle is a significant management strategy that has been implemented by many shrimp farmers in other regions in the world (Lawrence

1985). In the early 1980's about 6% of the shrimp farmers in Ecuador produced 20% of the national production using two phase systems (Hirono 1983). In temperate regions such as the United States, the two phase growout is becoming a common technology for semi-intensive and intensive shrimp farming (Samocha 1983, Lawrence and Huner 1987, Williams *et al.* 1996).

There are several potential benefits associated with nurseries and multi-stage production systems that have been identified for crustacean aquaculture. The technology was first used for freshwater shrimp culture (Sandifer and Smith 1977) and eventually used in greenhouse raceways for intensive culture of penaeid shrimp (Mahler *et al.* 1974). Among the documented benefits are increased control, efficiency, predictability and profits in both phases of production (Hirono 1983, Wang and Leiman 2000). The use of a nursery system allows control of stock inventory, reduce exposure to predation, and can be used to extend the effective growing season (Fast 1991, Sandifer *et al.* 1991, Sturmer *et al.* 1991, Samocha and Lawrence 1992, Stern and Letellier 1992).

Disadvantages of the use of nurseries also have been noted. Increased mortality may occurred if the PL are stressed during transfer (Stern and Letellier 1992). Additionally, the use of a nursery system or multiple crops does not necessarily mean a more cost effective operation (Juan *et al.* 1988). Implementing an indoor nursery phase also involves greater initial investment, greater operational costs and higher skilled labor. These additional costs can be justified if the nursery system increases yields or greater market value of the final product (Samocha and Lawrence 1992).

Currently, indoor nursery practices normally involve the use of green house structures and tanks or raceways. Post larvae stocking densities vary between 2 and 70/L

with survivals ranging from 41-100%, depending on age, size, and culture period (Stern and Letellier 1992, Samocha and Lawrence 1992, Stumer et al. 1992). The quantity and nutritional quality of the diet offered to shrimp post larvae is a determinant factor in its growth and survival. Proper feeding is a key to producing healthy juveniles that will do well once transferred to the growout system. Therefore, common feeding practices include newly hatched *Artemia nauplii* for the initial phase, eventually replaced with high protein (45-50%) prepared diets (Fegan 1992). Specific algal blooms (preferably diatoms) are enhanced by fertilization and inoculation to increase natural productivity for optimal growth and water quality stabilization (Krom et al. 1985, Sturmer et al. 1992).

In shrimp culture growout phase, feeding is one of the most important activities when considering pond dynamics and management (Boyd and Tucker 1998) and production economics (Jolly and Clonts 1993). It has been estimated that feed accounts for 55 to 60% in intensive systems and around 40% of the operating costs in an semi-intensive systems (Chanratchakool *et al.* 1994, Lovell 1998). According to Wyban *et al.* (1989) and based on financial analyses, survival and growth have the greatest impact on the economic performance of shrimp production, and adequate feeding is essential in attaining both of them. Therefore, adequate management should aim to optimized feed inputs, and feed conversion ratio (FCR) along with minimizing the potential impact of effluents (Jory *et al.* 2001). However, over the last decade, the tendency toward greater intensity in shrimp production, implying greater stocking densities and greater feed inputs commonly resulted in better FCR (Peterson 1999, Peterson and Walker 2002). Most production failures, have been blamed on PL quality, feed, water quality and disease, although in most cases the origin of the problem is poor feed management (Cruz 1991,

Piedad-Pascual 1993). The need for improved feed management efficiency stems not only from economic reasons, but also from environmental issues. Shrimp pond water represents a potential environmental impact because only a portion of the nutrients in feed are consumed, assimilated and utilized for shrimp growth. When the environmental condition of water sources areas are deteriorated, the effect is not isolated, but promotes the outbreak of various diseases and water quality related problems, directly affecting production (Goddard 1996, Boyd and Tucker 1998, Jory *et al.* 2001, Samocha *et al.* 2001).

In spite of the recognized importance of nurseries, there is need for more information concerning the production of juvenile shrimp in nursery systems. Research has been conducted to identify suitable nursery protocols that optimize shrimp performance in indoor nurseries (Emmerson and Andrew 1981, Sturmer *et al.* 1991, Samocha *et al.* 1993, Stern and Lettelier 1992). However, information linking various nursery conditions and practices (densities and duration) with shrimp performance in the growout phase in production ponds that follows nursery is limited. Also, although several references support the individual usage of diet components, there is limited research related to shrimp growth response under different combinations and offering rates of those that are commercially available. And despite the importance of growout phase feed management there are few studies that have evaluated feeding strategies or feeding programs. The majority of the information available includes the basic nutrient requirements of various shrimp species during their different stages. Few references are available that incorporate effective pond management practices to help develop feeding

programs. Most feed management protocols are based mainly on the use of feeding tables.

Therefore, these series of studies were conducted to evaluate nursery management procedures, nursery effects on subsequent pond production and growout pond feed management.

II. INFLUENCE OF INDOOR NURSERY DENSITIES ON FINAL POND PRODUCTION OF THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*.

Abstract

The objective of this study was to evaluate the influence of post larval stocking density during the indoor nursery period on production characteristics of the Pacific White shrimp *Litopenaeus vannamei* during the growout phase that followed. The hypothesis was that different nursery densities produce post larvae (PL) of various qualities (size and health) which influence shrimp performance during the growout phase. The PL were purchased from a commercial hatchery as 7-8 day old PL which weighed an average of 1.31 mg/PL with a coefficient of variation of 67.3%. A 21-day nursery was conducted at three densities; high density 65 PL/L (HD), medium density 38 PL/L (MD) and low density 25 PL/L (LD). Densities were obtained by reducing water depths in nursery tanks from 85 cm to 65 and 47.5 cm thus decreasing the tanks volumes. After the nursing period, mean shrimp survivals were 61%, 58% and 49% for HD, MD and LD respectively. Final weights of the shrimp were 21.4, 18.5 and 19.1 mg and final individual coefficients of variation for weights were 66%, 156% and 106%, for the HD, MD and LD, respectively. Significantly greater biomass loading (1.3 kg/m³) and lower FCR was found in the HD treatment tanks. No significant differences in shrimp survival

or harvest weights were found between treatments, however high survival (61%) and a lower coefficient of variation for weight was observed in the HD treatment. Nursed juveniles from the two replicates of each of the three treatments were pooled and stocked into four replicated 0.1-ha production ponds at a density of approximately 35 shrimp/m² and managed under standard conditions. After 12 weeks in production ponds, shrimp mean average weights were 12.5, 13.6 and 10.7 g, survivals were 89%, 68% and 83%, FCR were 1.6, 2.1 and 2.1, and average yields were 4,091, 3,193 and 2,914 kg/ha, for shrimp originating from HD, MD and LD nursery treatments, respectively. No significant difference in final average weight or FCR was found. Significantly greater yields and lower observed FCR were obtained in production ponds stocked with shrimp from the HD nursery tanks. Findings of this experiment suggest that nursery densities in the range of 25 to 38 PL/L have minimal influence on subsequent growth and survival during growout. However, shrimp in the high density tanks had the best performance in several areas such as improved feed and culture system utilization and that shrimp from the best performing nursery treatment resulted in the greatest yields in production ponds. This study suggests that high density nursery may be best, provided adequate feed and water quality are maintained. The study also suggests that the quality of shrimp produced in the nursery will have an effect in the performance in production ponds.

Introduction

Commercial shrimp landings have reached maximum sustainable yield (FAO 1998), yet a demand for shrimp has increased in the last decade (Keefe 2002). These shortages have resulted in increased value which has stimulated development of shrimp aquaculture in certain regions of the world. Between 1984 and 1997, the contribution from shrimp aquaculture to the total world supply has increased at 14%/year, and in 1997 around 941,000 tons of shrimp and prawns value at 6.1 billion dollars were cultured (Barg *et al.* 1999).

Within the activities associated with shrimp aquaculture, there has been a shift from extensive culture systems to more intensive systems during the last 10 to 20 years. Besides market forces, factors such as environmental regulations, influencing coastal land and water use, also have played a major role in this shift. Regulations have been introduced mainly in response to the potential discharge of waste products into the estuarine environment (Boyd and Musig 1992).

As culture system intensity increases, two-phase culture is becoming more common. A first or nursery phase, can be run in indoor facilities or outdoors, such as greenhouses and intensive ponds. In indoors nurseries, post larvae (PL) from hatcheries are stocked into tanks for one to four weeks before being transferred to final production ponds. Indoor nursery systems with water conditions similar to the hatchery, eliminate predators, provides greater control over water quality, and increases feeding efficiency during critical initial life stages (Pretto 1983). Stocking juvenile shrimp, as opposed to

direct stocking of PL, makes it possible for managers to more accurately predict survival, standing crop, adjust feeding rates and production levels in growout ponds. Post larvae straight out of the hatchery are most fragile, so handling and transportation can cause mortality which may be difficult to predict. Some studies have predicted mortality due to transportation of PL to be around 10-20% (Smith and Ribelin 1983).

The inclusion of a nursery phase in the production cycle is a significant management strategy that has been implemented by many shrimp farmers in the world (Lawrence 1985). In the early 1980's about 6% of the shrimp farmers in Ecuador produce 20% of the national production using two phase systems (Hirono 1983). In temperate regions such as the United States, the two phase growout is a common technology for semi-intensive and intensive shrimp farming (Samocha 1983, Lawrence and Huner 1987, Williams *et al.* 1996).

There are several potential benefits associated with nurseries and multi-stage production systems that have been identified for crustacean aquaculture. The technology was first used for freshwater shrimp culture (Sandifer and Smith 1977) and eventually used in greenhouse raceways for intensive culture of penaeid shrimp (Mahler *et al.* 1974). Among the documented benefits are: increased control, efficiency, predictability and profits in both phases of production (Hirono 1983, Wang and Leiman 2000). The use of nursery systems allow control of stock inventory, reduce exposure to predation, and can be used to extend the effective growing season (Fast 1991, Sandifer *et al.* 1991, Sturmer *et al.* 1991, Samocha and Lawrence 1992, Stern and Letellier 1992).

Disadvantages of the use of nurseries also have been noted. Increased mortality may occurred if the PL are stressed during transfer (Stern and Letellier 1992).

Additionally, the use of a nursery system or multiple crops does not necessarily mean a more cost effective operation (Juan *et al.* 1988).

One of the key aspects in the management of a nursery is the stocking density. Shrimp population density usually influences growth and mortality rates with their relationship expected to be inversely proportional (Emmerson and Andrews 1981, Sandifer *et al.* 1988, Ray and Chien 1992, Wyban and Sweeney 1993). Stocking density plays a primary role because a system can support a population of a given size depending on rearing conditions such as tank size, water management practices like water flow, circulation rate and filtration capacity as well as waste removal capacity. Beyond this biomass limit or critical standing crop (CSC), water quality deteriorates as the biomass increases (Martin *et al.* 1998). Deteriorating water quality is often followed by increased incidence of disease and growth rate reduction (Wang and Leiman 2000). For a nursery phase, the initial stocking density should be set in accordance with critical standing crop. As the shrimp biomass increases and shrimp become stronger and more adaptable, they should be transferred to the next phase prior to the system reaching critical standing crop. In order to operate efficiently, the stocking density that maximizes shrimp growth and survival and that is within the site specific CSC should be determined.

In spite of the recognized importance of nurseries, there is need for more information concerning the production of juvenile shrimp in nursery systems. Research has been conducted to identify suitable nursery protocols that optimize shrimp performance in indoor nurseries (Emmerson and Andrew 1981, Sturmer *et al.* 1991, Samocha *et al.* 1993, Stern and Lettelier 1992). However, information linking various

nursery conditions and practices with shrimp performance in the growout phase in production ponds that follows nursery is limited.

The hypothesis of this study was that different nursery densities produce PL of various qualities (size and health) and that this effect influences the shrimp performance in the growout phase. Therefore, the objectives of this study were to evaluate the influence of stocking rates during nursery on production characteristics during the nursery and determine if there is an influence on performance during the growout phase that followed.

Materials and Methods

Nursery

The study was conducted at the Claude Peteet Mariculture Center, in Gulf Shores, Alabama (30 16.981 N, 087 39.914 W). Post larvae with initial mean weights \pm standard deviation of 0.42 ± 0.026 (based on samples selected randomly) were received from Harlingen shrimp farm, Harlingen, Texas on July 6, 2001.

The shrimp (550,000 PL shipped at an approximate density of 1,500 PL/L) were received in 30 Styrofoam boxes, each holding two double plastics bags with about 6 liters of 25.4 ppt salt water at 22 C, pH of 6.8, 13.1 mg/L dissolved oxygen and negligible concentration of total ammonia-nitrogen. A 940 L acclimation tank was filled with sea water, and adjusted to the salinity of the shipping water. The PL were released after the water in the bags and the acclimation tank were within 0.5 C of each other. Once the PL

were pooled together in the acclimation tank, their condition in terms of size, coloration, and activity was evaluated. During acclimation, decapsulated and newly hatched *Artemia nauplii* (INVE Americas, Inc., Salt Lake City, UT, USA) were offered at a rate of 100 artemia/PL (Treece and Yates 1990). Once the PL warmed up and their activity increased (approximately 1 h), the PL were concentrated and quantified volumetrically.

A volumetric quantification (Hardin *et al.* 1985, Juarez *et al.* 1996) was done to verify the quantity supplied and to allow an accurate distribution of PL among nursery tanks. Post larvae were concentrated into a 57-L tank, vigorously mixed by hand and subsampled with a 60-ml beaker to obtain the density for quantification purposes. Means, standard deviation and coefficients of variation were recorded (Appendix 1). The coefficients of variation of all counts were in the range of 4% to 14% and considered acceptable population estimates for management decisions (Juarez *et al.* 1996). Indoor nursery treatments included high density at 65 PL/L (HD), medium density at 38 PL/L (MD) and low density at 25 PL/L (LD), that were nursed for 21 days (Appendix 2).

The six, fiberglass tanks (3.0 x 1.5 x 0.9 m) used for the nursery phase of the experiment were located inside a clear polyethylene plastic enclosed quonset-style greenhouse. Depending on the need for cooling, airflow within the greenhouse was created with a propeller-type fan. The nursery system was designed as a semi-closed recirculating system containing culture tanks, common biological filter, a rapid-rate sand filter Model TR100 (AREA, Homestead, FL, USA) and a circulation 1hp high head pump pump (AREA, Homestead, FL, USA).

Supplemental aeration was provided by a common 1hp regenerative blower, (Sweetwater, Lapwai, ID, USA) through six air-lifts installed evenly along the sides of

each tank. The airlifts helped aerate and circulate the water throughout the water columns as well as to help distribute the feed and the PL evenly throughout the tank. In each nursery tank, incoming water was distributed along the bottom centerline through perforated openings spaced at regular intervals (2.5 cm) in a 2.5 cm pipeline, which also helped suspend feed particles and settled solids. Water volumes were maintained by an internal 5 cm diameter stand pipe, 80 cm long and nested within a larger pipe fitted with an external screened pipe (250 μ m mesh). Water was drained from the opposite end of the tank relative to the incoming water. The biofilter was composed of six partitions of filter media (1.3 x 0.7 m) placed perpendicular in the tank. Tanks were filled with full strength seawater, filtered through a sand filter for 24 hours to remove debris and organisms. The culture system water was then disinfected by chlorination using sodium hypochlorite at approximately 10 ppm of Cl. The system was then run for four days, tested for chlorine residues, then seeded with algal paste, *Chaetoceros sp.* (Instant Algae, Reed Mariculture Inc, San Jose, CA, USA) and then PL were stocked. A week after inoculation, algal counts were around 60,000 cells/ml.

Water quality was maintained by an initial water circulation through the biofilter for three hours per day between days 4 and 11. Continuous circulation was initiated at day 12 and circulation through the sand filter was initiated at day 16. Freshwater was added to the system to acclimate the juveniles to lower salinities during the last three days of the nursery phase. Intermittently, the tank bottoms were visually inspected and the accumulated solids removed by siphoning on an as need basis.

Four feedings were scheduled each day. At each feeding, pre-weighed dry feed was mixed with tank water to form a slurry and then distributed evenly around the tank.

During the first three days, PL were offered a 50% protein diet, (PL Redi-Reserve, Zeigler, Gardners, PA, USA) and brine shrimp at a rate of 100 Artemia /PL/ day (INVE Americas, Inc., Salt Lake City, UT, USA). Thereafter, PL were fed with a combination of two 45% protein commercial shrimp starter feeds (Table 1), Zeigler 50% protein and Rangen #0 and #1, (Rangen Inc., Buhl, Idaho, USA). Types of feed offered varied in size from an initial 400 micron size to a final size of 1,800 micron. Feeding inputs were adjusted every three days based on biomass determinations and feeding rates. The feed rate was initially set at 50% body weight and was reduced to 15 %. The biomass on each tank was estimated based on the mean weight of PL and an assumed 100% survival. Random PL-samples were taken from each tank every three days. Samples included at least 30 PL, which were towel dried and weighed to determine an average weight. At the conclusion of the nursery period, the water level was reduced to approximately 20 cm and the PL were captured by hand nets and quantified gravimetrically. For each tank juvenile shrimp final average, Coefficient of variation (CV) of individual weights ($CV = \text{standard deviation} / \text{mean} * 100$), survival, feed conversion ratio (FCR = feed input / yield), and total biomass were determined.

Growout phase

At the conclusion of the 21-day nursery period, juveniles from tanks were pooled by treatment, then stocked into four replicate production ponds at a density of approximately 35 shrimp/m². Ponds used for the growout phase were approximately 0.1 ha in surface area, rectangular (46 x 20 m), with a 1.0 m average depth. Each pond was equipped with a 20-cm diameter standpipe, a concrete catch basin, and lined with

Table 1. Feeding rates as percentage biomass and feed type utilized throughout the 21 days nursery period for *Litopenaeus vannamei* post larvae. Feed inputs were based on an assumed 100% survival and sampled shrimp weights.

Days	% Biomass	Feed Type
1 to 3	50	PL Ready ^a & 100 Artemia ^b / PL
4 to 6	25	PL ready
7 to 9	25	PL ready & Crumble ^c # 0
10 to 12	25	Crumble # 0
13 to 15	20	Crumble # 0
16 to 18	20	Crumble # 0 & # 3
19 to 21	15	Crumble # 0 & # 3

^a PL Ready 50% Protein, Zeigler Bross, Inc., Gardners, PA, USA.

^b INVE Americas, Inc., Salt Lake City, UT, USA

^c Rangen 45% protein, Rangen Inc., Buhl, Idaho, USA

1.52 mm thick high-density polyethylene sheeting (Grundle Lining System, Inc., Houston Texas, USA). The sloped pond bottoms were covered with a 25-cm deep layer of sandy-loam soil which was dried and tilled to a depth of 10-15 cm prior to filling. Ponds were filled with water from the Intracostal Canal between Mobile and Perdido Bay three weeks prior to stocking. Fill water was filtered through a nylon filter sock (Domestic Lace Mfg., Inc.) to prevent the introduction of large predators, and minimize the introduction of larval fish and crabs while allowing the introduction of small planktonic organisms. Two weeks before stocking, all ponds were fertilized with an application of liquid, inorganic fertilizers, at a ratio of 1:2 (N:P₂O₅), and a rate of 4 kg/ha N (Boyd and Tucker 1998). A mixture of 1.68 L of 10-34-0 and 402 ml 32-0-0 and pond water was prepared in a 208 L container, then slowly dripped into the ponds while operating a paddlewheel aerator. Fertilization was used to maintain a minimum Secchi disk reading within the range of 25-40 cm. Depending on the response of the algae individual pond response to fertilization, a second application at half the initial rate was added two weeks after first application. Twenty four hours before stocking, a 1:15 motor oil and diesel fuel mixture at a rate of around 9 L/ha, was applied evenly over each pond surface to reduce the number of air breathing insects.

Each pond had a 1-hp (0.75kW/ha) spiral paddle wheel aerator (Little John aerator, Southern Machine Welding Inc. Quinton AL) representing aeration capacity of 10 hp/ha. In emergency situations an additional 1 hp propeller aspirator aerator (Aire-O₂, Aeration Industries International, Inc. Minneapolis, Minnesota) was added to the ponds to maintain dissolved oxygen levels.

Water was added to the ponds only to replace evaporation losses. Aerators were operated during the night (8 hrs.) to maintain oxygen concentrations above a set limit of 3 mg/L. Dissolved oxygen (DO) concentrations, were monitored with a YSI 85 DO meter, (Yellow Spring Instrument Co., Yellow Springs, OH, USA) twice a day, at sunrise (0500) and after dark (1900). Weekly water samples were taken in all ponds with an 80-cm water column sampler (Boyd and Tucker 1992) early in the morning and analyzed for total ammonia-nitrogen measured with a spectrophotometer (Spectronic Instrument Inc. Rochester, NY, USA) and the Nesslerization method (APHA 1989), pH and salinity with a YSI 30 salinity meter (Yellow Spring Instrument Co. Yellow Springs, OH, USA). Secchii disk visibility readings were taken once a week. Results of water quality determinations were averaged over time for each treatment.

Shrimp were fed twice a day with a sinking 35% protein pelleted feed (Appendix 3) (Burriss Mill & Feed, Inc., Franklinton, L.A, USA). Feeding took place in the morning and late in the evening. Feed inputs were adjusted weekly based on the estimated shrimp biomass. Shrimp were sampled by seine for the third and fourth weeks and by cast net (monofilament net, 1.22 m radius and 0.95 cm opening) during the remainder of the culture period. Weekly assessments included a visual observation of appearance and average weight. Sampling took place in the early morning hours to reduce stress. Samplings did not allow for reliable estimates of shrimp survival, so an assumed 25% mortality for the culture period was utilized. Feed calculations also incorporated Feed Conversion Rates (FCR < 2:1). Feed consumption was monitored with feeding trays. For the first two weeks of the growout phase, ponds were fed at a rate of 8 kg/ha. Beginning the third week, feeding rates were based on 15% of the estimated biomass and then

Table 2. Feeding schedule for *Litopenaeus vannamei* reared in 0.1ha ponds over a 12 weeks culture period. Biomass estimates were based on weekly sampling for weight and an assumed survival of 83%.

Culture week	Assumed Survival	Calculation based on	Daily Feed input (kg/ha)
1	99	8 kg/ha	8
2	97	8 kg/ha	8
3	96	15% biomass	14-15
4	94	14% "	48-52
5	93	10% "	98-100
6	92	10% "	110-150
7	90	7.5% "	140-180
8	89	7.5% "	140-180
9	87	2.5 g/ shrimp/ wk	110-115
10	86	2.5 g/ shrimp/ wk	100-115
11	85	2.5 g/ shrimp/ wk	100-110
12	83	2.5 g/ shrimp/ wk	90-100

¹ Shrimp Feed 35% protein, Buris Mill & Feed Inc. Franklinton, LA

gradually reduced each week as the shrimp biomass increased, following guidelines from a feeding table (Table 2). Maximum feeding rates were set at 180 kg/ha. As the temperatures dropped during the last four weeks feeding rates were standardized at 2.5g/shrimp/wk. Feeding ceased two days prior to harvest. Harvest took place during the 11th and 12th week of the culture period (Oct 12 to 18). Harvesting was accomplished by draining two thirds of the water from each pond during the night before harvest. Aeration through the night was accomplished as needed, using only paddlewheel aerators to minimize erosion on pond bottoms. On the day of harvest, the rest of the water in the pond was pumped out through a hydraulic fish pump with a 25 cm suction (Aqualife-Life pump, Magic Valley Heli-arc and Mfg, Twin Falls, Idaho, USA). The pump was placed in the catch basin and shrimp were pumped and dewatered as they were moved to the harvest truck. Shrimp were then taken to a wet lab to be washed and weighed. During weighing, a random sample of 100 shrimp was collected for individual weights. Individual weights were used to calculate mean weights, survivals and size distributions.

Data Analyses

The collected data was analyzed by a one-way Analysis of Variance using SAS program version 8.2 (SAS Institute Inc., Cary, NC). The Student-Newman-Keuls multiple comparison test was utilized to determine significant differences among treatment means. Due to high variability of pond research, significant differences were determined at a probability level of $P \leq 0.10$.

Table 3. Production characteristics of *Litopenaeus vannamei* nursed for 21 days at three densities, 25, 38 and 65 PL/L and stocked at 35 shrimp/m² in grow out ponds for a 12 week culture period¹.

Nursery Phase	Density (PL/L)			P
	25	38	65	
Final average weight (mg)	19.2 ^a	18.5 ^a	21.5 ^a	0.7072
Survival (%)	49 ^a	58 ^b	61 ^b	0.0182
FCR ²	1.4 ^a	1.6 ^a	1.1 ^b	0.0362
Biomass loading (kg/m ³)	0.4 ^b	0.5 ^b	1.4 ^a	0.0002
CV (%) ³	106.3 ^a	156.0 ^a	66.6 ^a	0.2000
n	2	2	2	
Grow out phase				
Final average weight (g)	10.7 ^a	13.6 ^b	12.5 ^{ab}	0.0790
Survival (%)	83 ^a	68 ^b	89 ^a	0.0350
FCR	2.1 ^a	2.1 ^a	1.6 ^a	0.2430
Yields (kg/ha)	2,914 ^a	3,193 ^a	4,091 ^b	0.0700
CV (%)	23.56 ^a	13.91 ^b	14.08 ^b	0.0100
n	4	4	3	

¹Means not sharing a common superscript within a row are significantly different ($P \leq 0.10$) based on Student-Newman-Keuls test.

²FCR = Total weight of feed given / Biomass increase

³CV= Standard deviation / mean * 100

Results

Nursery phase

At the conclusion of the 21 day nursery period, mean weights of juvenile shrimp were 19.2, 18.5 and 21.5 mg, FCR were 1.4, 1.6 and 1.1, biomass loading were 0.4, 0.5 and 1.4 kg/m³, for LD, MD and HD, respectively (Table 3). No significant difference in final weights were observed. Rapid growth became noticeable around the 10th day of the culture period when growth began to double each week (Fig.1). The three treatments followed similar growth patterns, however the HD treatment experienced the best growth. No treatment showed a growth rate decline during the 21-day nursery period. Survivals were 49%, 58% and 61% for shrimp stocked at LD, MD, HD respectively. A better FCR and higher biomass loadings were found for shrimp reared for shrimp reared in the HD treatment as compared to the other treatments. Upon reception of PL, individual weight coefficient of variation was 67.3%, and after the nursery, were 66%, 156% and 106% for HD, MD and LD, respectively.

Water quality analyses results for the nursery trial are summarized in Table 4 and 5. Water temperatures were maintained within a range of 27- 29 C. Dissolved oxygen readings in all tanks were consistently in the range of 6 to 6.9 mg/L. However, maximum recorded readings of dissolved oxygen (10 mg/L) were observed related to photosynthetic activity of the algae which developed in tanks.

Salinity throughout the experimental period ranged from 23 to 25 ppt. During the last three days salinity was gradually reduced to 17 ppt to match that of the growout ponds. The pH generally fluctuated between 7 and 8. Total ammonia nitrogen (TAN) in

Figure 1. Mean weights (n=2) of post larvae at various ages of *Litopenaeus vannamei*, reared in an indoor nursery stocked at densities of 65 PL/L(HD), 38 PL/L (MD) and 25 PL/L (LD).

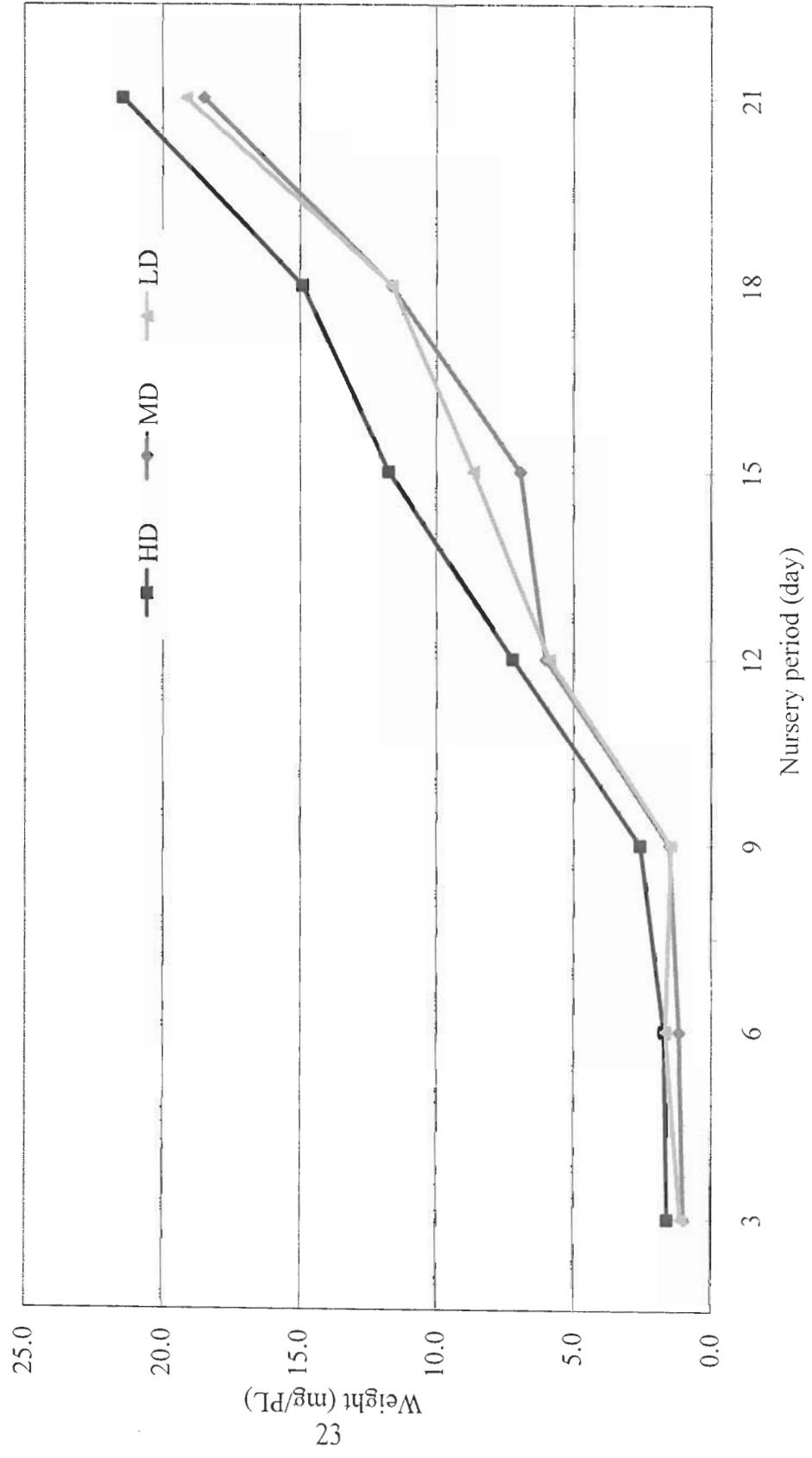


Table 4. Mean values for water temperature (C) and dissolved oxygen (mg/L) averaged by treatment over the 21 day nursery period for *Litopenaeus vannamei* stocked at three densities 25 (LD), 38 (MD) and 65 (HD) PL/L. Values are displayed as mean \pm standard deviation with maximum/ minimum values below in parentheses.

Treatments	Temperatures C			
	6am	11am	3pm	9pm
HD	27.7 \pm 0.9 (29.1, 25.8)	28.4 \pm 1.0 (29.7, 26.2)	29.6 \pm 0.7 (30.8, 28.3)	29.1 \pm 0.7 (30.1,27.8)
MD	28.1 \pm 0.8 (29.2, 26.8)	28.8 \pm 0.8 (28.7, 27.5)	28.9 \pm 3.4 (31.1,15.6)	29.3 \pm 0.7 (30.4, 27.8)
LD	28.2 \pm 0.7 (29.2, 27.2)	28.7 \pm 0.7 (29.6, 27.5)	29.5 \pm 0.8 (31.1, 28.0)	29.2 \pm 0.7 (30.4, 27.8)
Dissolved oxygen (mg/L)				
HD	6.4 \pm 1.1 (8.0, 4.2)	6.9 \pm 1.5 (10.7, 5.1)	6.7 \pm 1.4 (10.1, 4.9)	6.5 \pm 1.6 (10.2, 4.8)
MD	6.5 \pm 1.2 (8.8, 4.1)	6.9 \pm 1.6 (10.7, 5.1)	6.6 \pm 1.4 (10.3, 5.2)	6.5 \pm 1.5 (10.3, 5.0)
LD	6.4 \pm 1.2 (8.2, 4.3)	6.9 \pm 1.6 (10.9, 4.9)	6.6 \pm 1.5 (10.5,4.6)	6.5 \pm 1.6 (10.5,4.8)

Table 5. Mean values for pH, salinity and total ammonia nitrogen (TAN) averaged by treatment over the 21 days nursery period for *Litopenaeus vannamei* stocked at three densities 25 (LD), 38 (MD) and 65 (HD) PL/L . Values are displayed as mean \pm standard deviation with maximum/minimum values in parentheses.

Treatments	pH	Salinity ppt	TAN mg/L
HD	7.4 \pm 0.3 (7.8, 6.9)	24 \pm 0.5 (25.1, 23.7)	0.42 \pm 0.73 (1.77, 0)
MD	7.5 \pm 0.3 (7.9, 7.0)	24 \pm 0.4 (25.1, 24.0)	0.28 \pm 0.61 (1.66, 0)
LD	7.5 \pm 0.3 (8.0, 7.0)	24.0 \pm 0.5 (25.1, 23.8)	0.28 \pm 0.59 (1.61, 0)

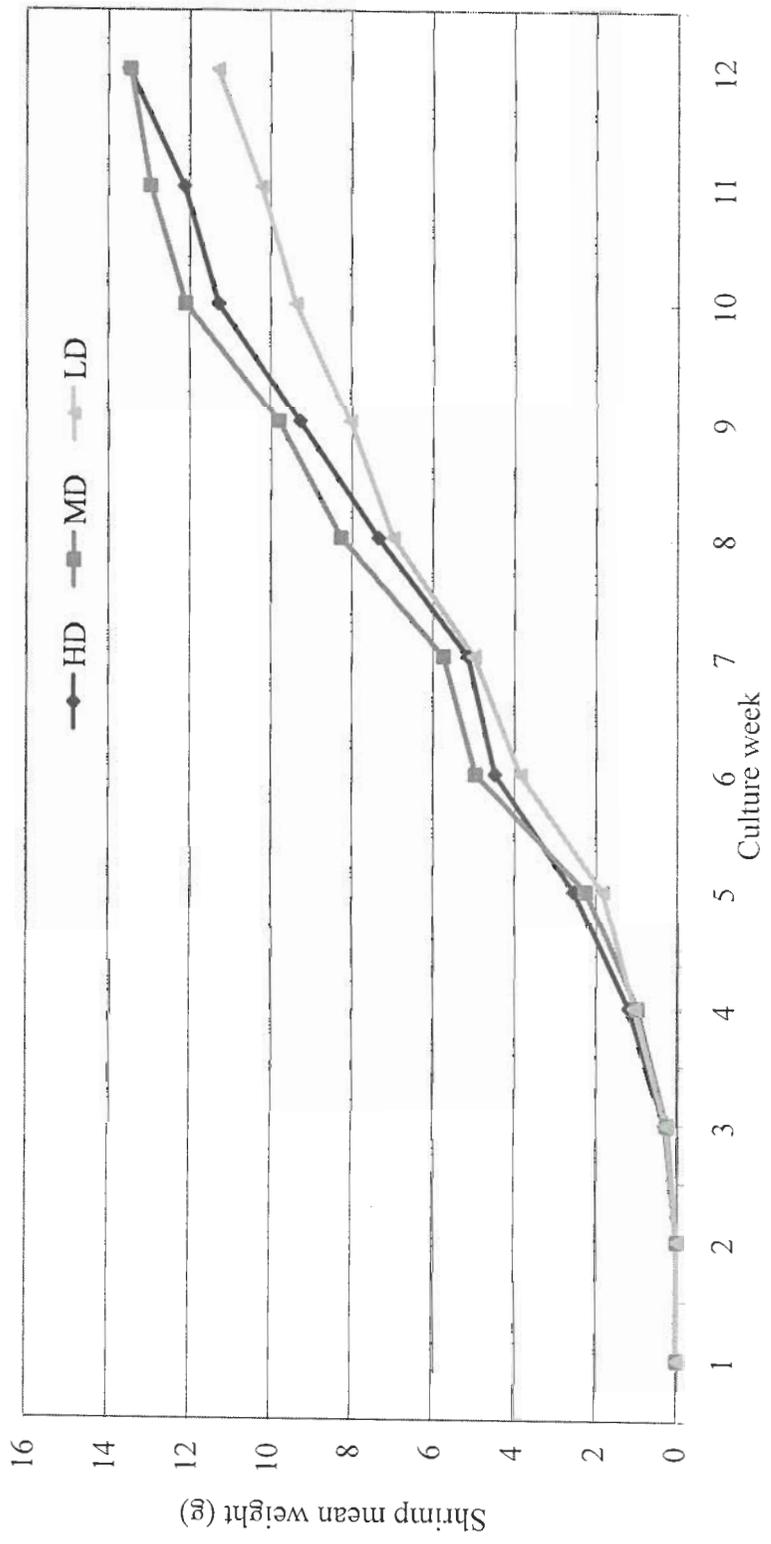
all tanks followed similar TAN dynamics, consistent with the water treatment schedule (Table 5). Concentrations of TAN increased and were greatest (around 1.6 mg/L) by day 16 and ended at a concentration below 0.2 mg/L (Table 5). The measured water quality parameters, were within recommended ranges for *L. vannamei* culture (Hanson and Goodwin 1977; Clifford 1985; Boyd 1989, and Brock and Main 1994).

Growout phase

At the conclusion of the week 12 of the culture period in ponds, mean average shrimp weights were 10.7, 13.6 and 12.5g; survivals were 83%, 68% and 89%; FCR were 2.1, 2.1 and 1.6; and average yields from the ponds were 2,914, 3,193 and 4,091 kg/ha, for LD, MD and HD treatments, respectively (Table 3). One pond D7 from the HD treatment was excluded from the study. Shrimp in this pond had much lower growth (7.1g average weight) and survival (68.5%) than the other ponds in the HD treatment. It was suspected, but not confirmed, that the lower performance of this pond was related to the incidence of the blue green algae *Microcystis sp.* which is known to produce microcystin toxin, which impairs shrimp growth (Boyd 1998). Significantly lower survival and greater shrimp average weight were found for shrimp of the MD treatment. Significantly greater yields were obtained in the HD treatment than in the other two treatments. The three treatments had similar growth pattern (Fig. 2).

Results of the water quality analysis from the growout phase are summarized in Table 6. Pond salinity was 17 ppt initially and ended in the 12 to 14 ppt range. Since pond management consisted of limited water exchange, salinity fluctuation was minimal.

Figure 2 . Weekly mean weights (n=4) of *Litopenaeus vannamei* stocked at a density of 35 shrimp/m² growout ponds and cultured for 12 weeks after a 21-day indoor nursery period at densities of 65 (HD), 38 (MD) and 25 (LD) PL/L.



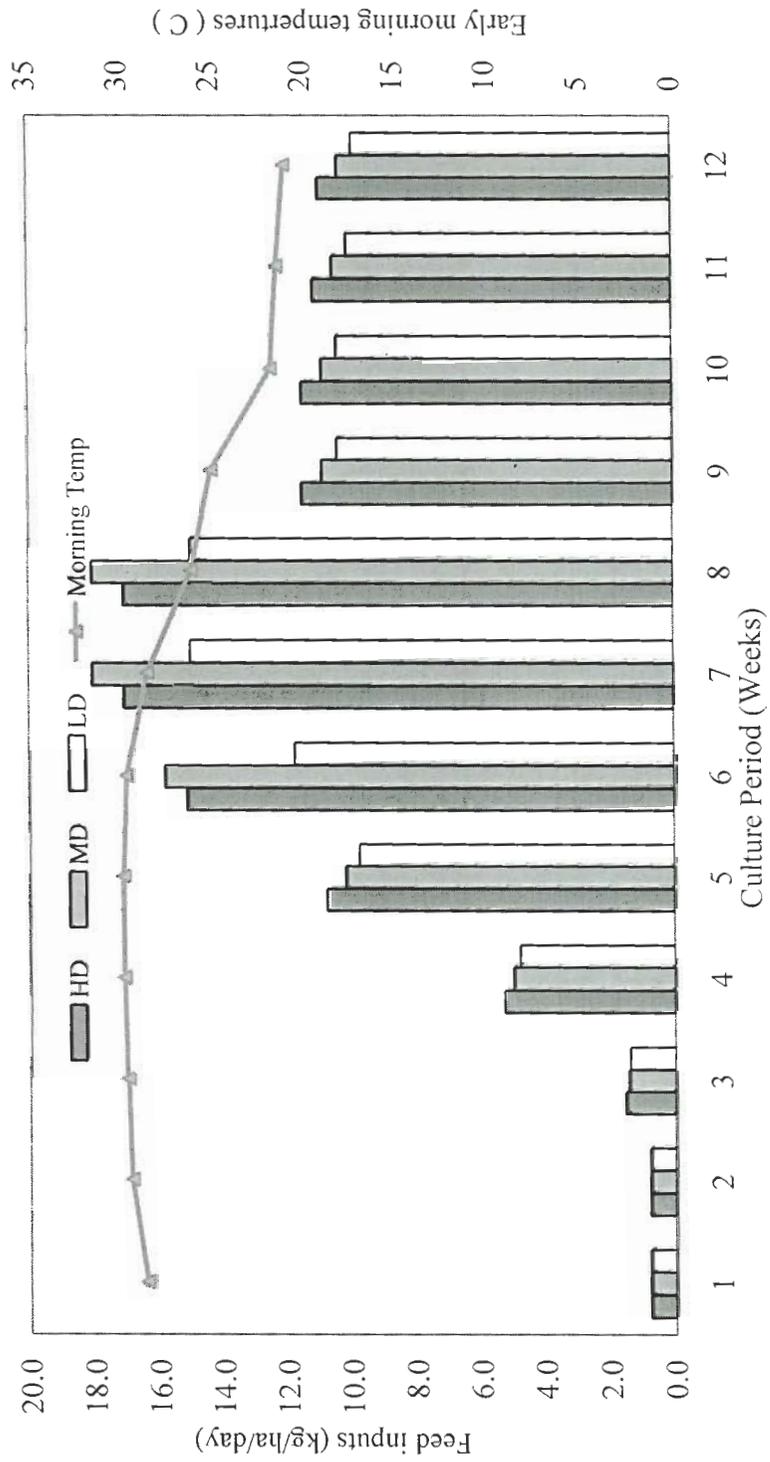
Pond water pH readings were taken from samples collected in the early morning, and were in the range of 7-7.8. For the three treatments, overall average of total ammonia-nitrogen was 1 mg/L. The highest observed TAN reading was 5 mg/L. This occurred while average pH and temperature were 7.33 and 26.2 C, respectively. The fraction of un-ionized ammonia out of the total ammonia-nitrogen reading was 0.015, resulting in an un-ionized concentration of 0.075 mg/L (Boyd 1998).

Average dissolved oxygen readings for the three treatments were 5 mg/L for early morning readings and 7 mg/L for night readings. Higher early morning dissolved oxygen readings were recorded from the 9th week of culture period and were related to lower temperatures of the fall season.

Average pond temperatures for early morning and late evening were 26.9 ± 3.7 C and 28.6 ± 3.5 C for the evening. In general the temperature trend through the production cycle was higher than 28 C until the 7th week but then gradually began to drop to 21 C during the last two weeks (Fig. 3).

Prior to fertilization, ponds had Secchi disk readings on the range of 80-110 cm. After a second applications of fertilizer, most ponds obtained plankton blooms. By the end of the production cycle all ponds had heavy blooms with secchi disk readings in the range of 10-30 cm. Temporal variability in secchi disk readings were the results of bloom and crash cycles that took place in different ponds during the production cycle. Whenever an algae die-off was noticed or forecast, at least two aerators were set to operate continuously for as long it took for a new bloom to develop and the oxygen cycle to return to a typical day-night cycle.

Figure 3. Feeding inputs (kg/ha/day) and early morning water temperatures of ponds stocked with *Litopenaeus vannamei* juveniles previously nursed for 21 days at densities of 65 (HD), 38 (MD) and 25 (LD) PL/L and stocked in ponds at 35 shrimp/m² and culture for 12 weeks.



Discussion

Findings of this experiment suggests that PL nursery densities in the range of 25 to 65 PL/L with good management practices have limited influence on subsequent growth and survival of shrimp during growout (Table 3). However, at higher density (65PL/L), improved feed and culture system utilization was obtained and a significantly greater biomass loading (1.3 kg/m^3) and survival (61%) was accomplished. The high density nursery treatment had a reduced tank water depth so the same blower pressure, in effect, provided more aeration. The higher aeration rate probably had a positive effect by increasing water circulation, which tended to keep particulate matter such as uneaten feed in better suspension. The tank environment was highly nutrient enriched when considering the water volume and feed inputs added to this treatment. The increased aeration had no effect on the dissolved oxygen levels in the water (Table 4) probably indicating all tanks were maintained at or near saturation. Since all treatments were stocked with PL of the same origin, genetic differences was not considered a possible cause of the observed differences in size distribution. Therefore, differences in growth were more likely to be related to the conditions of the treatment tanks. The disappearance of feed particles from the water column is a function of two things: the rate of consumption of the particles by the larvae, and the rate of settlement of the particles. It is inferred that, as the biomass loading in the tanks increased and more feed was supplied, an increase in water circulation was helpful to keep feed particles suspended. This assumes that an appropriate concentration of feed was used to allow the encounter rate to be high enough so that the PL ingested sufficient quantities with minimal effort.

However, in lower density tanks, aeration/circulation rates were lower so feed may not have been as evenly suspended. This may have resulted in some shrimp out competing others during their search for food and consequently some were able to grow faster. This study demonstrated that in these systems, good aeration/circulation is important to maintain feed in suspension and to remove waste from the water.

Over all nursery survivals (49-61%) were not as high as reported by Samocha and Lawrence (1992) and Samocha *et al.* (1993), however they are typical for this facility. Hydrogen sulfide concentrations may have had a negative effect on juvenile survival; upon finalizing the nursing period an inspection of the sand filter revealed an accumulation of detritus and a strong hydrogen sulfide smell. Any detectable concentration of hydrogen sulfide is considered undesirable, since a concentration of 0.01 to 0.05 mg/L of H₂S may be lethal to aquatic organisms (Vamos 1964, Brock and Main 1994, Ritvo *et al.* 2000) suggesting more frequent backwashes than once after 10 days may eliminate this problem.

A crucial aspect of PL quality was the level of variation within the size of the population (Fegan 1992). An increase in the variability of the PL size was noticed at the end of the nursery phase. The initial variation in size of individual shrimp prior to the nursery was already high, since CV greater than 30% are considered excessive (Brock and Main 1994). Uniformity did not improve in any of the nursery treatments. The HD treatment had the most uniform size juveniles (CV = 66%) when compared to the LD treatments (CV = 106.3%) and MD (CV = 156%). Taking into consideration the graphical pattern of size distribution (Fig. 4, 5) and the amount of variation in the size of individual shrimp as principle features in assessing size uniformity, the HD treatment

Figure 4. Coefficients of variation of individual weights of *Litopenaeus vannamei*, after being nursed for 21 days at 65 (HD), 38 (MD) and 25 (LD) PL/L and after a 12 week growout in ponds stocked at 35 shrimp/m². The initial CV from the hatchery was 67.3%.

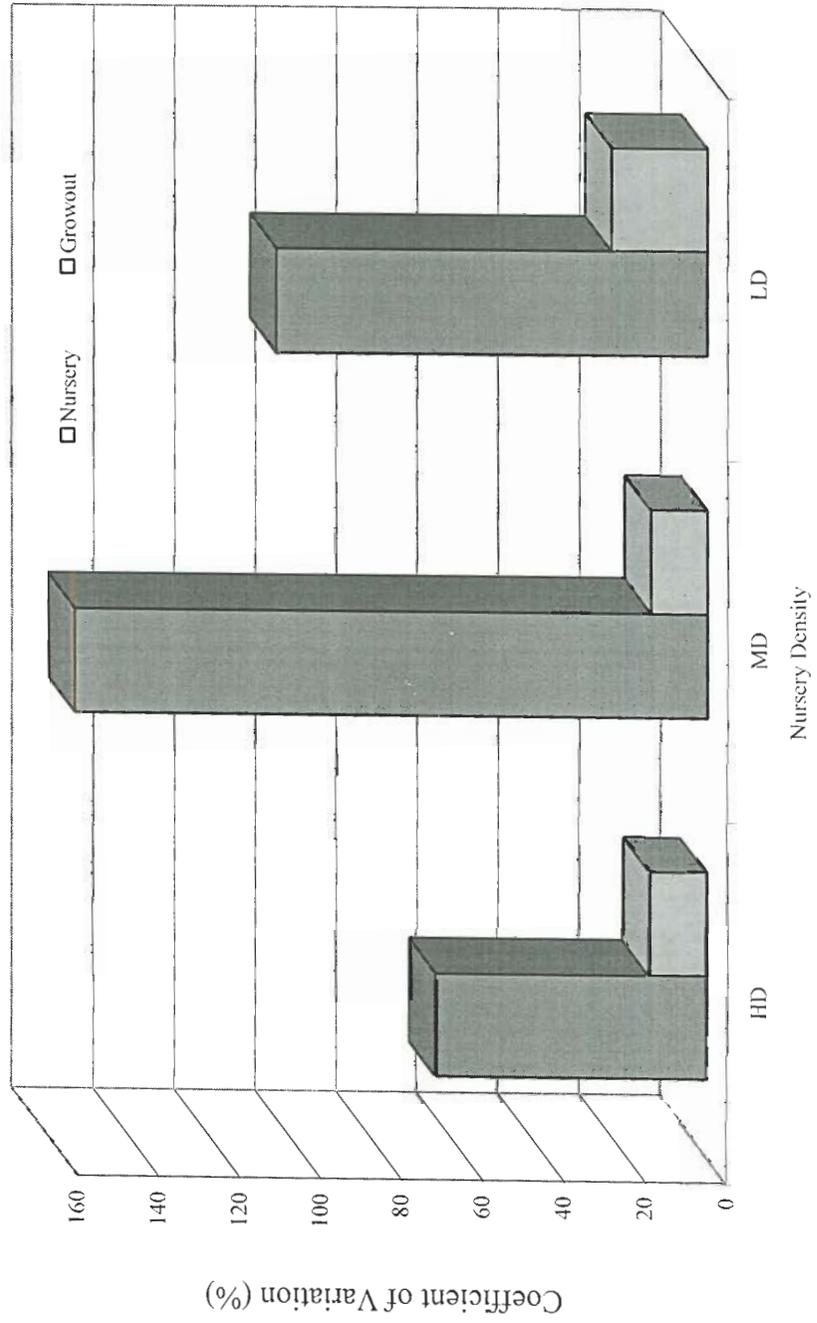
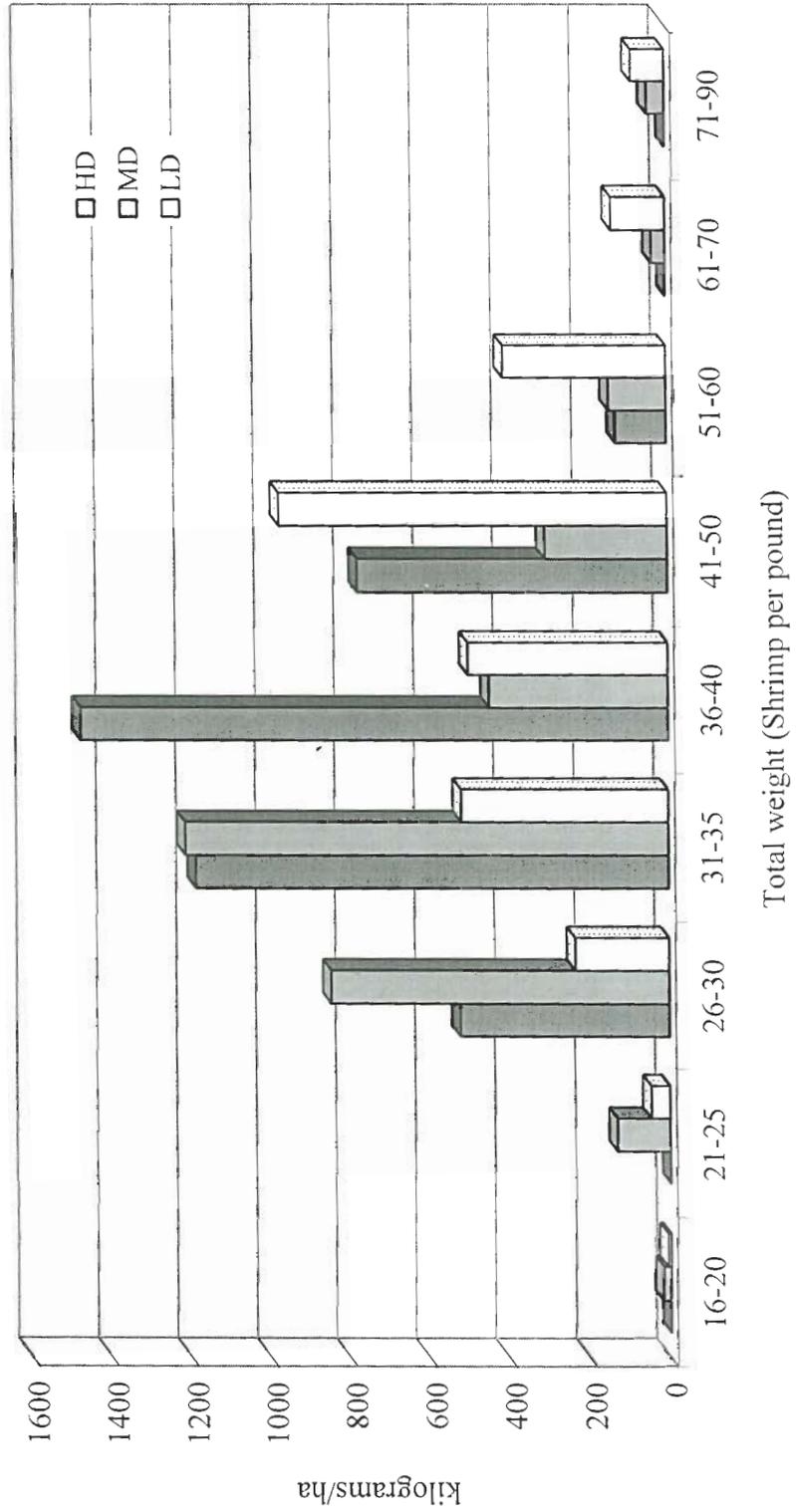


Figure 5. Total shrimp production as distributed across typical shrimp size classes. *Litopenaeus vannamei* juveniles were stocked in growout ponds at a density of 35 shrimp/m² and culture for 12 weeks after a 21-day indoor nursery period at densities of 65 (HD), 38 (MD) and 25 (LD) PL/L.



had better uniformity. After the growout phase, size distributions in all treatments were within normal ranges of 10-20% (Brock and Main 1994).

In previous research, there have been indications of improvement in size distribution of nursed shrimp (Stern and Letellier 1992, Garza, 2001); however, those studies did not include the initial and final coefficient of variation of individual weights, limiting the comparison with results of this study. Results indicate that regardless of the nursery stocking density, individual weight coefficient's of variation (CV), increased during the nursery, and then improved or became more uniform (lower CV) at the end of the growout phase (Fig. 2).

This study also revealed that juveniles from the best performing nursery treatment were most likely to result in higher yields and better size distribution in the growout phase. The observed mean individual weight variation for shrimp from the LD treatment (23%) was significantly greater than the other two treatments (14% and 13.9% for HD and MD respectively) which was consistent with results obtained during the nursery phase and that could have created a lagging effect in the LD treatment. Although growout pond survivals of LD (83%) were similar to HD (89%) yields were 28% less than in HD. This can be attributed to a more dispersed weight variation on the LD treatment, with observed lower percentage (45%) although not significantly different, of the shrimp population greater than the 36-40 count size than HD and MD (77.9% and 83.1%, respectively).

Therefore the results of this study demonstrate that while actual stock densities in nurseries may not directly affect growout, management practices during the nursery phase can affect later performance. Adequate feeding and strong aeration/circulation are

important to ensure PL at any density have sufficient food. Strong, healthy juveniles coming out of a nursery will continue to maintain good uniform growth during the following pond production phase.

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**III. INFLUENCE OF INDOOR NURSERY PERIOD AND DIRECT STOCKING
ON FINAL POND PRODUCTION AND SIZE DISTRIBUTION
OF THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*.**

Abstract

The objectives of this study were to evaluate the influence of nursery period on post larval shrimp survival, individual size and total production at the conclusion of the nursery phase and to evaluate the influence of PL (post larvae) age at stocking on the subsequent growout phase. The hypothesis was that a longer nursery period produces larger post larvae that would provide an advantage to shrimp performance during the growout phase. The experiment was conducted at the Claude Petet Mariculture Center, Gulf shores, Alabama. Six 4-m³ rectangular fiberglass tanks housed in a greenhouse were used for the nursery phase. Three tanks were stocked with white pacific shrimp *Litopenaeus vannamei* post larvae (PL) at a density of 30 PL/ L and nursed for 21 days (N21). A week later, a second group of three tanks were stocked with *L.vannamei* at the same density and nursed for 14 days (N14). Post larvae for both treatments were obtained from the same hatchery and at the same stage (PL 8) when stocked. Both nursery treatments were harvested at the same time. Mean shrimp survivals were 91.2% and 94.7% for N21 and N14, respectively. Mean harvest weights were 16.4 mg and 10.5 mg, FCR were 1.5 and 1.6, and biomass loadings were 0.8 kg/m³ and 0.4 kg/m³ for N21 and

N14 treatments, respectively. Except for biomass loading, no significant differences among treatments were found in any of the nursery results. Following the nursery phase, shrimp from the three replicated tanks of each nursery treatments were pooled and then stocked into four 0.1 ha ponds. Another four ponds were stocked directly (DS) with post larvae (PL 8). All 12 ponds were stocked at a density of approximately 35 PL/m², and culture over a 16 weeks pond production period. After harvest, mean average weights were 15.4, 16.9 and 14.9 g, survivals were 63%, 62% and 64%, FCR were 2.7, 2.5 and 2.7, average yields were 3,592 , 4,005 and 3,374 kg/ha, for N21, N14 and DS treatments, respectively. No significant differences were found among treatments in any of these results. Results indicate that regardless of nursing PL for 14 or 21 days, nursed juveniles did not differ significantly in production characteristics, from shrimp stocked directly from the hatchery. One benefit of head-starting PL was that it extended the period of time which PL could be acquired from a hatchery, avoiding the risk of a possible shortage in supply as the warmer season begins.

Introduction

The high demand and strong market value for shrimp have motivated the development of shrimp culture in the U.S. According to Fast (1991), shrimp imports into the U.S. now exceeds \$1 billion/yr, while the value of domestically produced shrimp is less than half of this amount. Domestic production from the wild is at maximum yield, while domestic consumption, per capita consumption, and imports have all continue to increase (Hopkins 1992, Keefe and Jolly 2002). The U.S. market outlook indicates that consumption for shrimp will continue to grow (United States Department of Commerce 1988).

As a result of these factors, expansion of shrimp culture is taking place with shrimp farms been built in the southeastern and western United States. From this expansion, economically successful farms have been characterized by Hopkins (1990, 1992) as those having good technology, business management, financial structure and marketing and selling to high value niche markets. Fast (1991) considered that the main constraint to their economic viability is in the costs of rearing shrimp to market size after they leave the hatchery.

Also these regions are limited in their culture period to the warm season because no areas of the continental U.S. (between 25° and 45°N latitude) are adequate for year-round shrimp production. Even at best only six to eight months per year are warm enough for shrimp culture in most regions (Sandifer *et al.* 1988, Hopkins 1992). With direct

stocking the growing season is too short for two crops to be economical (Griffin *et al.* 1981, Clifford 1985, Sadeh *et al.* 1986).

The inclusion of an early indoor nursery phase has been considered as a possible way to extend the growing season (Sturmer and Lawrence 1987, Lawrence *et al.* 1985, Sandifer *et al.* 1988). This could add one or two months for the first crop and might increase the potential for production of two crops per year. Some have suggested that nurseries also could improve survival, during growout which would allow more efficient managements of feed inputs.

Another natural resource that might aid U.S. shrimp culture is that about two thirds of the continental United States is underlain with saline water. Mariculture was suggested as means of utilizing these salt-affected lands (Feth 1970). This has encouraged the development of an inland shrimp culture industry in addition to the development of a coastal industry. The ability of shrimp to tolerate changes in salinity appears to be influenced in a large extent by age (Aquacop 1991, Samocha *et al.* 1998, McGraw *et al.* 2002). A nursing period to obtain older post larvae (PL) may be required to allow shrimp to be acclimated to lower salinities often found in underground water. McGraw *et al.* (2002) suggests that PL younger than 15 days old should not be acclimated to salinities below 4 ppt, whereas older PL can be acclimated to 1 ppt with good survival.

Current indoor nursery practice, normally involve the use of green house structures and tanks or raceways. Post larvae stocking densities vary between 2 and 70/L with survivals ranging from 41-100%, depending on age, size, and culture period (Stern and Letellier 1992, Samocha and Lawrence 1992, Stumer *et al.* 1992). Common feeding

practices include newly hatched *Artemia nauplii* for the initial phase, eventually replaced with high protein (45-50%) prepared diets (Fegan 1992). Specific algal blooms (preferably diatoms) are enhanced by fertilization and inoculation to increase natural productivity for optimal growth and water quality stabilization (Krom *et al.* 1985, Sturmer *et al.* 1992).

Implementing an indoor nursery phase also involves greater initial investment, higher operational costs and higher skilled labor. These additional costs can be justified if the nursery system increases yields or higher market value of the final product (Samocha and Lawrence 1992). Some studies have found an increase in cost of \$7.60 and \$9.46 per 1000 post larvae. These costs could be 67.3% of the total annual costs (Juan *et al.* 1988).

Although there are many suggested advantages of shrimp nurseries, there is little documented information on the effects on final growout from nurseries. The objectives of this study were to evaluate the influence of nursery period post larval shrimp survival, individual size and total production at the conclusion of the nursery phase and to evaluate the influence of PL age at stocking on subsequent growout phase. The hypothesis was that a longer nursery period produces larger post larvae that would provide an advantage to shrimp performance during the growout phase.

Materials and Methods

The study was conducted in the Claude Petet Mariculture Center, in Gulf Shores, Alabama. Three groups of *L. vannamei* PL (8 to 9 days old) were obtained from GMSB, Inc., Key West, Florida on April 9, 16 and 29 of 2002. The first two groups were stocked at densities of 34 and 31 PL/ L into replicate indoor tanks and nursed for 21 (N21) and 14

(N14) days, respectively (Appendix 4). The third group was received acclimated and stocked directly into ponds (DS) at the same time the nursery treatments were stocked into ponds.

Nursery

Post larvae were shipped from the hatchery in plastic bags at densities of 1,000-1,400 PL/L. Upon arrival, each batch of PL were acclimated and quantified. Prior to receiving the PL, a 940-L acclimation tank was filled with sea water and adjusted to the salinity of the shipping water. Upon arrival, the bags containing the post larvae were allowed to float in the acclimation tank until the temperature difference between the water in the tank and bags was within 0.5 C. A water sample was then collected from several bags and the water quality determined (Appendix 5). All post larvae were then released in the reception tank and their condition in terms of size, coloration, and activity, was evaluated as PL quality criteria. During acclimation, decapsulated and hatched *Artemia nauplii* (INVE Americas, Inc., Salt Lake City, UT, USA) were offered at a rate of 100 *Artemia*/PL (Treece and Yates 1990). Once the PL warmed up and their activity increased (approximately 1h), the PL were concentrated and quantified volumetrically.

To verify the quantity supplied and to allow an accurate distribution of PL among nursery treatment tanks or ponds a volumetric quantification (Hardin *et al.* 1985, Juarez *et al.* 1996) was done. Post larvae were concentrated into a 57-L tank, vigorously mixed by hand and sub-sampled with a 60-ml beaker to obtain an estimate of the total number. Means, standard deviation and coefficients of variation were recorded (Appendix 6). The

coefficients of variation of all counts were below 10% and considered acceptable population estimates for management decisions (Juarez *et al.* 1996).

The nursery phase of the experiment was conducted in six, fiberglass tanks (3.0 x 1.5 x 0.9 m) located inside a clear, polyethylene plastic quonset-style greenhouse. Tanks were set as a semi-closed recirculating system containing a common biological filter and a 7-lb. canister charcoal filter Ocean clear Model 320 (Red Sea, Houston TX, USA), a rapid-rate sand filter Model TR100 (AREA, Homestead, FL, USA) and a 60 gpm circulation pump (AREA, Homestead, FL, USA). Each tank was equipped with an aeration system supplied by a common 1hp regenerative blower, (Sweetwater, Lapwai, ID, USA) and six air stones running air-lifts installed evenly along the sides of each tank. The airlifts helped oxygenate and circulate the water from the bottom to the top and around the tank to help distribute the feed and the PL evenly throughout the tank. In each nursery tank, incoming water flow was adjusted to 5gpm by flow meters and was distributed along the bottom centerline through perforated openings spaced at regular intervals (2.5 cm) in a 2.5 cm pipe which also helped suspend feed particles. Water volumes were maintained by an internal 5-cm diameter stand pipe, 80 cm long and nested within a larger screen (250 μ m mesh). Water was drained from the opposite end of the incoming water. The biofilter was composed of six partitions of filter media (1.3 x 0.7 m) placed perpendicular in the tank. A week prior to the reception of each PL shipment, tanks were filled with full strength seawater, filtered through a sand filter for 24 hours to remove debris and organisms. The water was then disinfected by chlorination using sodium hypochlorite (approximately 10 ppm). Utensils needed in handling the PL were also immersed in the chlorinated water. Dechlorination was ensured by bubble

aeration for 4 days, then tested for chlorine residues. One day prior to PL stocking, tanks were seeded with algal paste, *Thalassiosira weissflogii* (Instant Algae, Reed Mariculture Inc, San Jose, CA, USA). A week after inoculation, algal counts were around 50,000 to 60,000 cells/ml.

Both nursed treatments pass through a water quality maintenance schedule that included a static period, circulation and filtration for 3 hours a day, water exchange at half the culture period, permanent circulation and filtration, finalizing with acclimation (Appendix 7). In order to eliminate settled waste products during water exchanges, the water level was lowered from the bottom. During the last four days of nursing, freshwater was added to the system to acclimate the juveniles to lower salinities. The sand filter was cleaned by backwashing every three days during the nursery period. Tank bottoms were inspected by submerging a PVC pipe with a clear bottom cap. Accumulated waste was siphoned as needed.

Four feedings were scheduled each day. The weighed dry feed was mixed with tank water to form a slurry which was distributed evenly around the tank. During the first three days, PL were offered PL Redi-Reserve (400-600mm) 50% protein diet (Zeigler, Gardners, PA, USA) and brine shrimp (52% protein, 200-300um) at a rate of 100 Artemia /PL/ day (INVE Americas, Inc., Salt Lake City, UT, USA). Thereafter, PL were fed with a combination of two, 45% protein commercial shrimp starter feeds; Rangen #0 (<0.6mm), #1 (0.6-1.0mm) and #3 (1.4-1.7mm) (Rangen Inc., Buhl, Idaho, USA). Random PL-samples were taken upon reception of PL and from nursery tanks every three days and at the conclusion of the nursery trial. Samples included at least 40 PL. Water excess was eliminated by collecting PL with a strainer, depositing them on an absorbent

paper cloth and then weighing individually ($0.1\text{mg} = 0.0001\text{g}$) on an analytical scale Model ER-182A (A&D Company, Milpitas, CA, USA). Feeding adjustments were made based on biomass determinations, starting with 50%, of the estimated biomass and gradually reduced to 15% of the estimated biomass for the last offering (Table 1). Before harvest, juveniles were acclimated from 31 ppt to 19 ppt over a 96 hours period, concentrated and quantified gravimetrically.

At the conclusion of the nursery phase data collected included, final average weight, survival, FCR, biomass loading and the coefficient of variation for individual weights.

Growout phase

Juveniles from each of the two nursed treatments were pooled within their treatment then stocked into 0.1-ha production ponds. Post larvae for the third treatment were received from the hatchery as PL8, acclimated and stocked directly into production ponds. All treatments were conducted in four replicate ponds, stocked at on the same day. All 12 ponds were stocked at a density of approximately 35 PL/m^2 and culture for 16 weeks.

Ponds used for the growout phase were approximately 0.1 ha in surface area, rectangular (46 x 20 m), equipped with a 20-cm diameter standpipe, a concrete catch basin (3.7 x 1.82 x 0.45 m) and lined with 1.52 mm thick high-density polyethylene (HDPE) sheeting (Grundle Lining System, Inc., Houston Texas, USA). The sloped pond bottoms were covered with a 25-cm deep layer of sandy-loam soil. Pond depths averaged 1.0 m.

Table 1. Feeding rates as percentage biomass and feed type utilized throughout the 14 and 21 days nursery period for *Litopenaeus vannamei* post larvae. Feed inputs were based on an assumed 100% survival, and sampled shrimp weights.

Days	% Biomass	Feed Type
1 to 3	50	^a PL Ready & 100 Artemia ^b / PL
4 to 6	35	PL ready
7 to 9	35	PL Ready & ^c Crumble # 0
10 to 12	35	Crumble # 0
13 to 15	30	Crumble # 0 & Crumle # 1
16 to 18	30	Crumble # 0 & Crumle # 1
19 to 21	20	Crumble # 1 & # 3

^a PL Ready 50% Protein, Zeigler Bross, Inc., Gardners, PA, USA.

^b INVE Americas, Inc., Salt Lake City, UT, USA

^c Rangen 45% protein, Rangen Inc., Buhl, Idaho, USA

Pond soils were dried for over two weeks and tilled with a rotor tiller prior to filling to allow for oxidation and mineralization of organic matter. Tilling blades dug the soil to a depth of 10-15 cm. Three weeks prior to stocking, the ponds were filled with water from the Intercostal Canal between Mobile and Perdido Bay. Fill water was filtered through a nylon filter sock (Domestic Lace Mfg., Inc., Style 8845230) to prevent the introduction of large predators, and minimize the introduction of larval fish and crabs, while allowing the introduction of small planktonic organisms. Two weeks before stocking, all ponds were fertilized with an application of liquid, inorganic fertilizers (10-34-0 and 32-0-0), applied at a ratio of 1:2 (N:P₂O₅), at 4 kg/ha N (Boyd and Tucker 1998). A mixture of 1.68 L (10-34-0) and 402 ml (32-0-0) and pond water was prepared in a 208-L container, then slowly dripped into the pond, while operating a paddlewheel aerator during a sunny day. Fertilization was used to maintain a minimum Secchi disk reading within the range of 25-40 cm. Depending on individual pond response to fertilization, a second application at half the initial rate was added two weeks after the first application. Twenty four hours before stocking, a 1:15 motor oil and diesel fuel mixture at a rate of around 9 L/ha, was applied evenly over each pond surface to reduce the number of air breathing insects.

Each pond had a 1-hp (0.75kW/ha) spiral paddlewheel aerator (Little John Aerator, Southern Machine Welding Inc., Quinton, AL) representing aeration capacity of 10 hp/ha. In emergency situations an additional 1 hp propeller aspirator aerator (Aire-O₂, Aeration Industries International, Inc. Minneapolis, Minnesota) was added to the ponds to maintain dissolved oxygen levels. When required aerators were operated during the night (8 hrs.) to maintain oxygen concentrations above a critical limit of 3 mg/L, for a

biological (Boyd 1989) and economical optimization (Griffin *et al.* 1981). Dissolved oxygen (DO) concentrations, were monitored with a YSI 85 or a YSI 556 meter, (Yellow Spring Instrument Co., Yellow Springs, OH, USA) twice a day, at sunrise (0500) and after dark (1900). Weekly water samples were taken in all ponds early in the morning with an 80-cm water column sampler (Boyd and Tucker, 1992) and analyzed for total ammonia-nitrogen measured using a spectrophotometer (Spectronic 20 Genesys, Spectronic Instrument Inc. Rochester, NY, USA) and the Nesslerization method (APHA 1989), pH and salinity with a YSI 556 salinity meter (Yellow Spring Instrument Co. Yellow Springs, OH, USA). Secchi disk visibility readings were taken once a week. Results of water quality determinations were averaged over time for each pond. Water was added to the ponds only to replace evaporation for 14 out of the 16 weeks of culture period. During the last two weeks of culture, water was exchanged for three consecutive days replacing an approximate total of 22% of the pond volume.

Shrimp were fed twice a day with a 35% protein pelleted feed (Burriss Mill & Feed, Inc., Franklinton, L.A, USA). Feeding took place in the morning and late in the afternoon. Feed adjustments were made weekly based on estimated shrimp biomass as well as observations from feed trays. Shrimp were sampled by seine for the third and fourth weeks and by cast net (monofilament net, 1.22 m radius and 0.95 cm openings) during the rest of the culture period. Weekly assessments included visual observation of appearance and average weight. Sampling took place in the early morning hours to reduce stress. Samplings did not allow for reliable estimates of shrimp survival, so an assumed 30% mortality for the 16-week culture period was utilized. Feed calculations also incorporated feed conversion rates ($FCR < 2:1$). Feed consumption was monitored

with feeding trays. Shrimp were sampled by seine for the third and fourth weeks and by cast net (monofilament net, 1.22 m radius and 0.95 cm openings) during the rest of the culture period. Weekly assessments included visual observation of appearance and average weight. Sampling took place in the early morning hours to reduce stress. For the first two weeks of the growout phase, ponds were fed at a rate of 8 kg/ha. Beginning the third week, feeding rates were based on 15% of the estimated biomass and then gradually reduced each week as the shrimp biomass increased (Table 2). Biomass was estimated based on the number of shrimp stocked, weekly sample weight and an assumed mortality of 30% fractionated over the 16-week growout period. Maximum feeding rates were set at 140 kg/ha. Because of warm water temperatures and higher standing crops, feeding rates were reduced during the last two weeks to 2.5 g/shrimp/wk. Feeding ceased three to four days prior to harvest.

Harvest took place after the 16th week of culture (Sept 27-29). Harvesting was accomplished by draining two thirds of the water from each pond during the night before harvest. Nightly aeration was provided during harvest, using only paddlewheel aerators. On the day of harvest, the rest of the water in the pond was pumped out through a hydraulic fish pump with a 25 cm suction pump (Aqualife-Life pump, Magic Valley Heli-arc and Mfg, Twin Falls, Idaho, USA). The pump was placed in the catch basin and shrimp were pumped out of the pond and dewatered as they were moved to the harvest truck. Shrimp were then taken to a wet lab to be washed and weighed to determine yields. During weighing, a random sample of 100 shrimp was collected and weighed individually from each pond to determine individual weight Coefficient of variation (CV), final mean weights, and count size distribution. Average number of shrimp per unit

Table 2. Feeding schedule for *Litopenaeus vannamei* reared in 0.1ha ponds over a 16 weeks culture period. Biomass estimates were based on weekly sampling for weight and an assumed survival of 70%.

Culture Week	Calculation based on	Daily Feedings ranges kg/ha
1	8 kg/ha	8
2	8 kg/ha	8
3	15% Biomass	14-15
4	14% "	48-52
5	10% "	98-100
6	6.5% "	110-150
7 & 8	5% "	140-180
9 & 10	4.5% "	110-115
11&12	4% "	100-110
13 &14	3.5% "	140
15 &16	2 g/shrimp/week	80

¹ Shrimp Feed 35% protein, Buris Mill & Feed Inc. Franklinton, LA

weight was determined for each pond to calculate the number of shrimp harvested from the total yield and estimate survival.

Data Analyses

The collected data was analyzed by a one-way Analysis of Variance using SAS program version 8.2 (SAS Institute Inc., Cary, NC). The Student-Newman-Keuls multiple comparison test was utilized to determine significant ($P \leq 0.05$) differences among treatment means.

Results

Nursery phase

After 14 and 21 days of nursery, mean average weight of juveniles were 10.5 and 16.4 mg/PL, survivals were 94.7% and 91.2%; FCR were 1.6 and 1.5; biomass loading's were 0.4 and 0.8 kg/m³, respectively (Table 3). One of the replicate tanks from the N21 treatment was excluded from the study (Tank 1). In this tank, the PL ended with an average mean weight of 35.9 mg, the survival was 68.5% and the Coefficient of Variation of the individual weights was 137.61%, all of these results varied by more than 50% from other replicates in the treatment. With the exception of biomass loading, no significant differences among treatments were found for final mean weight, FCR, survival or CV for individual weight. No treatment showed a growth decline during the nursing period (Fig. 1).

The nursery system water temperatures averaged 25.0 ± 2.6 C and 25.5 ± 2.7 C for early morning and late evening hours, respectively. Overall minimum and maximum temperatures were 20.0 and 30.0 C respectively. Dissolved oxygen readings in all tanks

Table 3. Production characteristics of *Litopenaeus vannamei* nursed for 21 (N21) and 14 days (N14) at 31-34 PL/L as well as the production of Nursed (N21 and N14) and direct stocked (DS)

Nursery Phase	Nursery length (Days)			<i>Pr</i> > <i>F</i>
	21	14	DS ¹	
Final average weight (mg)	16.4 ^a	10.5 ^a	1.23	0.1380
Survival (%)	91.2 ^a	94.7 ^a	-	0.6280
FCR ²	1.5 ^a	1.6 ^a	-	0.7190
Final biomass (Kg/m ³)	2.67 ^b	1.42 ^a	-	0.0110
CV (%) ³	70.8 ^a	53.8 ^a	58.5	0.0540
Grow out phase (16 weeks)				
Final average weight (g)	15.37 ^a	16.87 ^a	14.99 ^a	0.6800
Survival (%)	62.9 ^a	63.2 ^a	64.0 ^a	0.9290
FCR	2.7 ^a	2.5 ^a	2.7 ^a	0.6800
Yields (Kg/ha)	3,592 ^a	4,005 ^a	3,374 ^a	0.3550
CV (%)	17.3 ^a	16.8 ^a	18.2 ^a	0.6010

¹Means not sharing a common supercript within a row are significantly different

($P \leq 0.05$) based on Student-Newman-Keuls test.

²FCR = Total weight of feed given / Biomass increase

³CV= Standard deviation / mean * 100

Figure 1. Daily water temperature and mean weights of two populations of *Litopenaeus vannamei*

PL (stocked at 7 day interval) reared through a 14(N14) or 21 day (N21) nursery period .

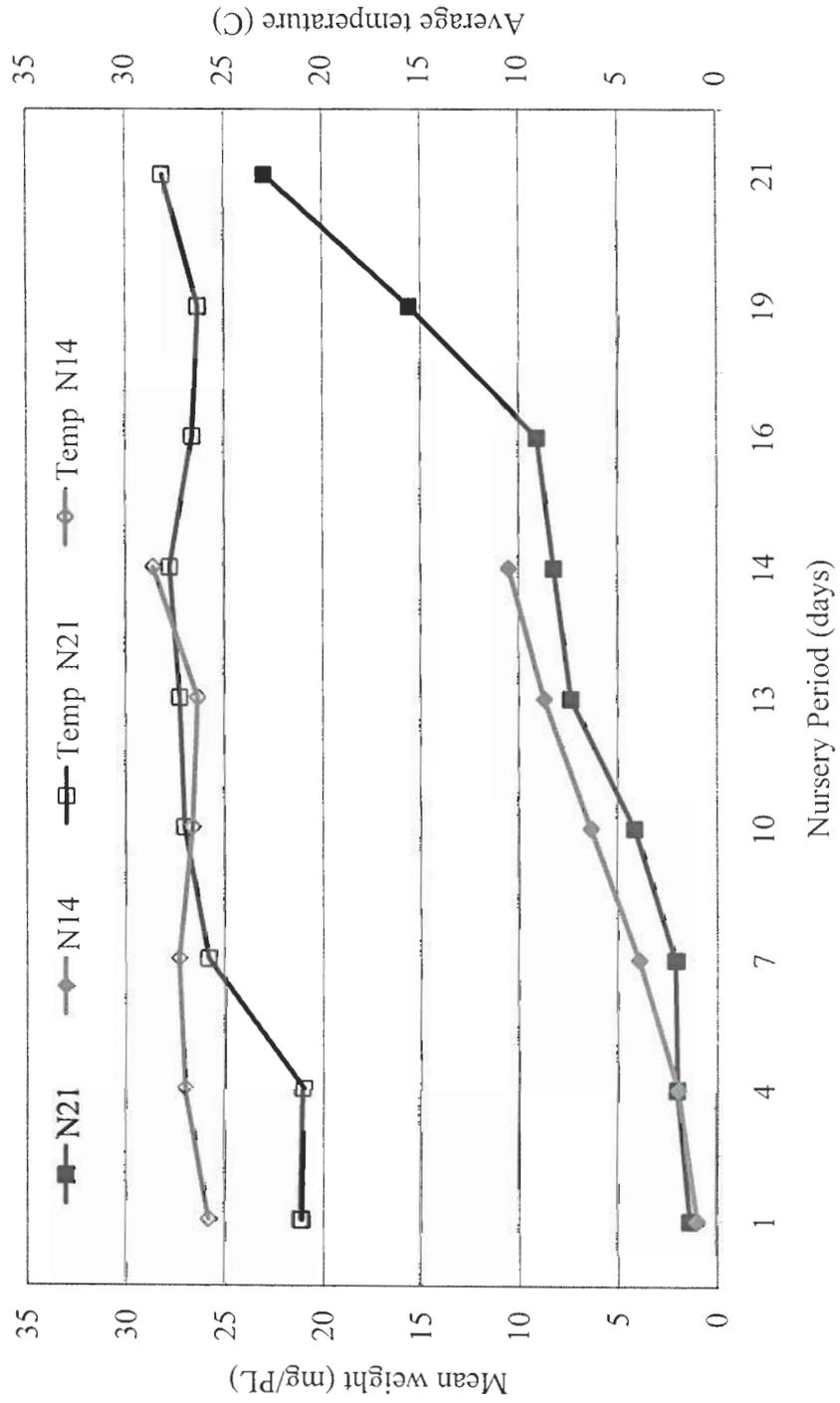


Table 4. Water quality parameters observed in nursery tanks over a 14 (N14) or 21 (N21) day of nursery period for *Litopenaeus vannamei* stocked at 33 PL/L. Figures are displayed as mean \pm standard deviation with maximum and minimum values in parentheses.

Treatment	pH	Salinity ppt	TAN mg/L	Oxygen mg/L
N21	7.84 \pm 0.28 (8.22, 7.47)	29.50 \pm 4.24 (31.0, 19.0)	1.32 \pm 1.05 (2.77, 0.15)	7.7 \pm 0.68 (8.8, 7.0)
N14	7.92 \pm 0.41 (8.34, 7.36)	28.0 \pm 6.0 (31.0, 19.0)	1.47 \pm 0.79 (2.52, 0.76)	7.3 \pm 0.41 (8.2, 7.0)

were consistently above 7.3 mg/L throughout the trial (Table 4). Minimum dissolved oxygen readings were near saturation (7.0 mg/L) and maximum readings of dissolved oxygen (8.8 and 8.2 mg/L) were likely related to photosynthetic activity of the algae which developed in the tanks. Salinity through most of the nursery was around 31 ppt then during the last three days, through a gradual acclimation, the salinity was reduced to 19 ppt. The pH generally fluctuated between 7 and 8. All tanks followed similar TAN dynamics, consistent with the water treatment schedule.

Overall TAN concentration averages were 1.32 and 1.47 mg/L for N21 and N14, respectively. Concentrations of TAN increased during the culture period and were greatest, around 2.77 and 2.52 mg/L (on the 17th and 10th nursing day) for N21 and N14 treatments, respectively. These occurred while average pH and temperature values were around 7.4 and 26.6 C, so the fraction of un-ionized ammonia was 0.015, or a concentration of 0.042 mg/L and 0.038 mg/L NH₃-N (Boyd and Tucker 1998). Water exchange at the midpoint of each nursing, was effective in lowering TAN concentrations. With the exception of the initial low water temperatures (21 to 23 C) during the first seven days of the nursery trial, all dissolved oxygen, salinities, pH, and TAN values were within recommended ranges for *L. vannamei* culture (Hanson and Goodwin 1977, Boyd 1989, Brock and Main 1994).

Growout stage

At the conclusion of the 16th week of culture, mean average weights of the harvested shrimp were 15.4, 16.9 and 14.9 g, survivals were 63%, 62% and 64%, FCR were 2.7, 2.5 and 2.7; and average yields were 3,592, 4,005 and 3,374 kg/ha, for N21,

N14 and DS treatments, respectively (Table 3). Although no significant differences were found among treatments means, greater observed yields occurred in the N14 treatment than in the other two treatments. The three treatments had similar growth patterns and none showed a growth decline (Fig.2).

Water quality analysis from the growout phase is summarized on Table 5. Pond salinity was 19 to 20 ppt initially and ended around 17 to 18 ppt. Salinity fluctuation was minimal since pond management consisted of no water exchange for most of the grow-out phase. Water addition included only evaporation compensation, and precipitation which was 46.2 cm during the culture period. Pond water pH, temperature and DO readings were recorded in the early mornings and late evenings. Overall average pH readings in the morning were around 7.5 (6.9 to 8.4) and evenings readings were around 8.0 (7.1-9.3). For the three treatments, overall average dissolved oxygen readings (DO) for early morning readings were around 4.0 mg/L, with the lowest readings in the range of 1.9 and 2.3 mg/L. Evening DO readings were around 6 mg/L and the lowest readings were between 2 to 2.6 mg/L. In general, early morning readings became lower as the cycle progressed and the standing crop increased. Also warmer early-morning water temperatures (30 to 31°C) occurred from the 12th week on, with even higher temperatures in the afternoon hours. Lowest DO concentrations in the evening were associated with continuous cloudy days. Average pond temperatures for early mornings and late evenings were 28.5 ± 2.5 C (32.6, 19.0) and 29.8 ± 2.7 C (34.5, 21.1), respectively (Table 5). In general, the temperature trend through the production cycle started with temperatures around 27.5°C that dropped to 23°C due to a cold front during the 3rd and 4th weeks and from there on increased to a range of 28-30 C (Fig.3).

Figure 2. Mean weight of *Litopenaeus vannamei* stocked at a density of 35 shrimp/m² in growout ponds for 16 weeks after a 21 (N21) and 14 (N14) days indoor nursery period or direct stocking (DS) as PL 8.

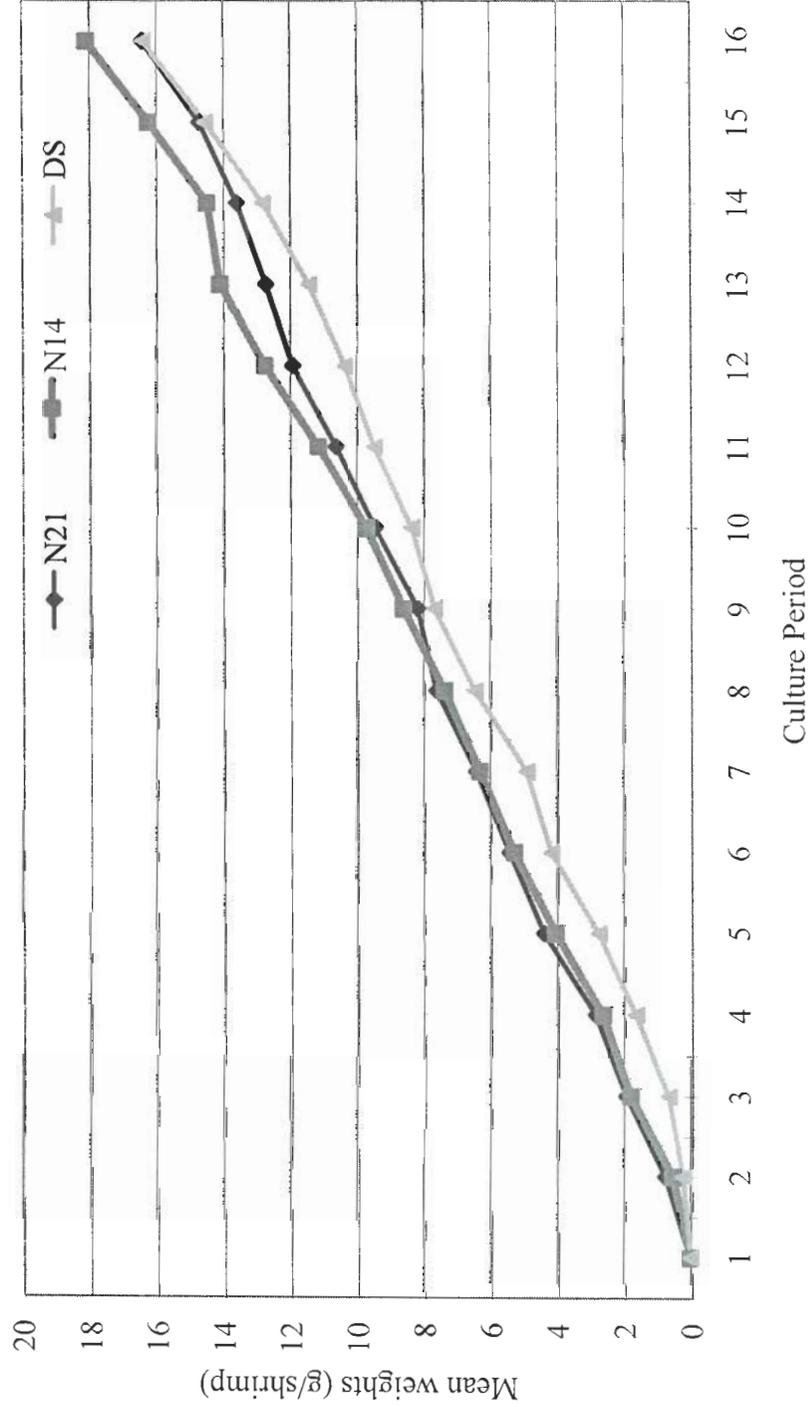
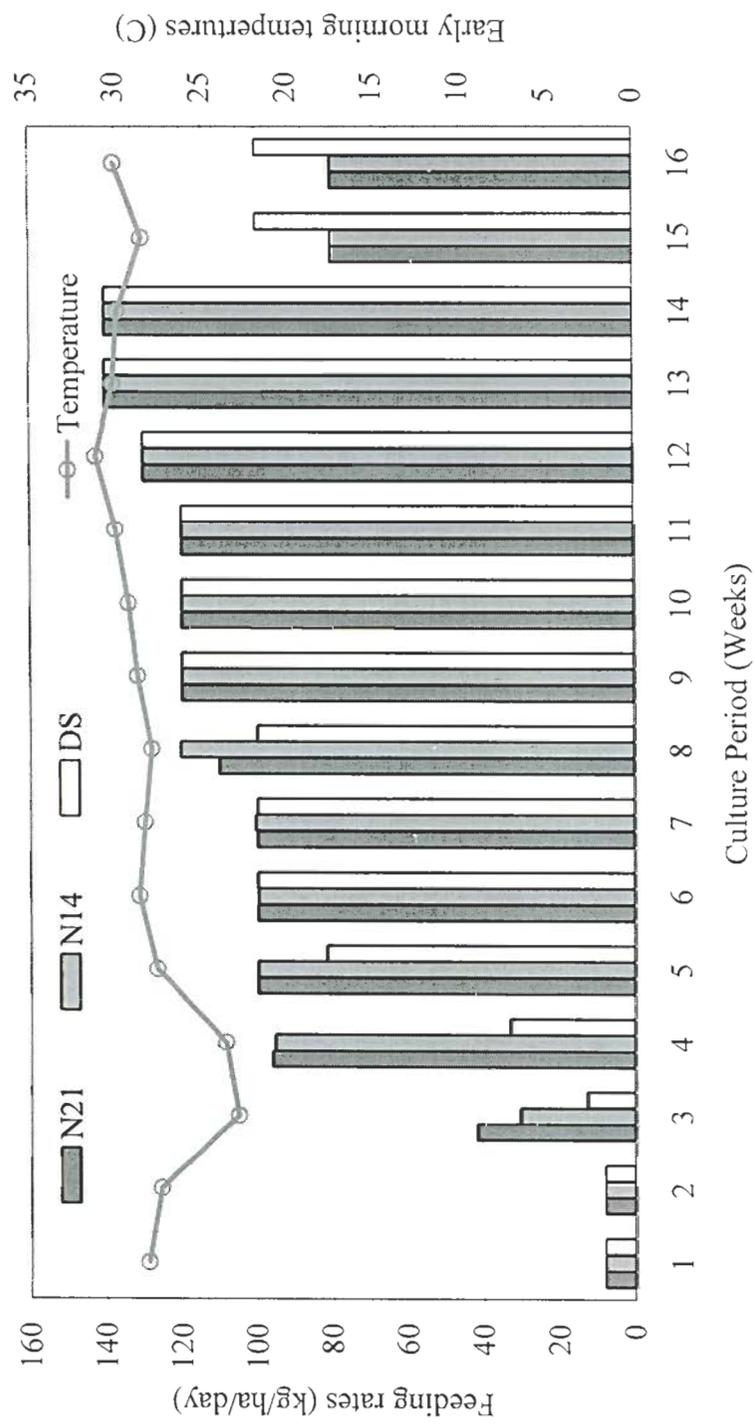


Table 5. Water quality parameters averaged by treatment over a 16-week growout period for *Litopenaeus vannamei* stocked at 35 shrimp/m² and previously nursed for 21 (N21) and 14 (N14) days at 31-34 PL/L or direct stocked (DS). Figures are displayed as mean \pm standard deviation

	N21	N14	DS
Morning pH	7.5 \pm 0.09 (8.4, 6.9)	7.4 \pm 0.3 (8.2, 7.0)	7.5 \pm 0.3 (8.4, 7.0)
Night pH	8.1 \pm 0.5 (9.3, 7.1)	8.2 \pm 0.5 (9.2, 7.2)	8.2 \pm 0.5 (9.1, 7.1)
Salinity (ppt)	21.87 \pm 2.46 (24.50, 17.33)	22.13 \pm 2.66 (25.37, 17.26)	21.54 \pm 2.42 (24.38, 17.16)
TAN (mg/L)	1.05 \pm 1.61 (6.18, 0.0)	0.74 \pm 0.87 (3.12, 0.0)	0.63 \pm 0.88 (2.93, 0.0)
Secchi (cm)	32 \pm 12 (56, 20)	28 \pm 7 (41, 20)	30 \pm 8 (48, 20)
Morning DO (mg/L)	4.61 \pm 1.02 (8.35, 2.29)	4.28 \pm 1.08 (8.50, 1.88)	4.55 \pm 1.08 (7.95, 2.21)
Night DO (mg/L)	6.11 \pm 2.41 (13.58, 2.29)	6.40 \pm 2.73 (14.45, 2.01)	6.44 \pm 2.55 (13.78, 2.58)
Morning Temp (C)	28.5 \pm 2.5 (32.6, 19.0)	28.5 \pm 2.5 (32.5, 19.0)	28.4 \pm 2.5 (32.4, 19.0)
Night Temp (C)	29.8 \pm 2.7 (34.5, 21.1)	29.8 \pm 2.7 (34.6, 21.1)	29.8 \pm 2.7 (34.5, 21.1)

Figure 3. Feedi inputs (kg/ha/day) and early morning water temperatures for ponds containing

Litopenaeus vannamei stocked at 35 PL/m² in growout ponds for 16 weeks after a 21 (N21) and 14 (N14) days indoor nursery period or direct stocking (DS) as PL 8.



For all three treatments, average total ammonia-nitrogen values were below 1 mg/L until the 8th week of the culture period. Total ammonia-nitrogen concentrations fluctuated and reached highest concentrations at different times in different ponds. The typical cycle was a high concentration during an algae die-off, followed by reductions in TAN during the following 3-4 days as new algae blooms developed. The highest TAN concentrations found during the experiment were 6.18, 3.12 and 2.93 mg/L for N21, N14 and DS treatments, respectively. This occurred while pH was in the range of 7.27 to 7.58 and temperature was 29.1 to 31.8 C. The decimal fraction of un-ionized ammonia was 0.020-0.031 or concentrations of 0.16, 0.10 and 0.16 mg/L NH₃-N (Boyd and Tucker 1998) with a decreasing trend on the following days. These concentrations were still within a suitable range for shrimp culture according to Chen and Chin (1988), who found toxic levels at 0.81 mg/L of un-ionized ammonia. Bray and Lawrence (1992) considered 1.29 mg/L NH₃ -N toxic values (48 hours LC₅₀), but also considered concentrations of 0.1 mg/L NH₃-N as maximum acceptable levels for culture.

Prior to fertilization ponds had Secchi disk readings in the range of 80 to 110 cm. Two weeks after the initial application most ponds had obtained plankton blooms, and had Secchi readings in the 35 to 40 cm range. By the end of the production cycle all ponds had heavy blooms with secchi disk readings in the range of 20 to 30 cm. Temporal variability in secchi disk readings were the results of bloom and crash cycles that took place in ponds during the production cycle. Whenever an algae die-off was noticed or suspected, at least two aerators were set to operate continuously for as long it took for a new bloom to develop. Water exchange during the last two weeks of the culture period helped to maintain Secchi readings in the range of 20 to 30 cm.

Discussion

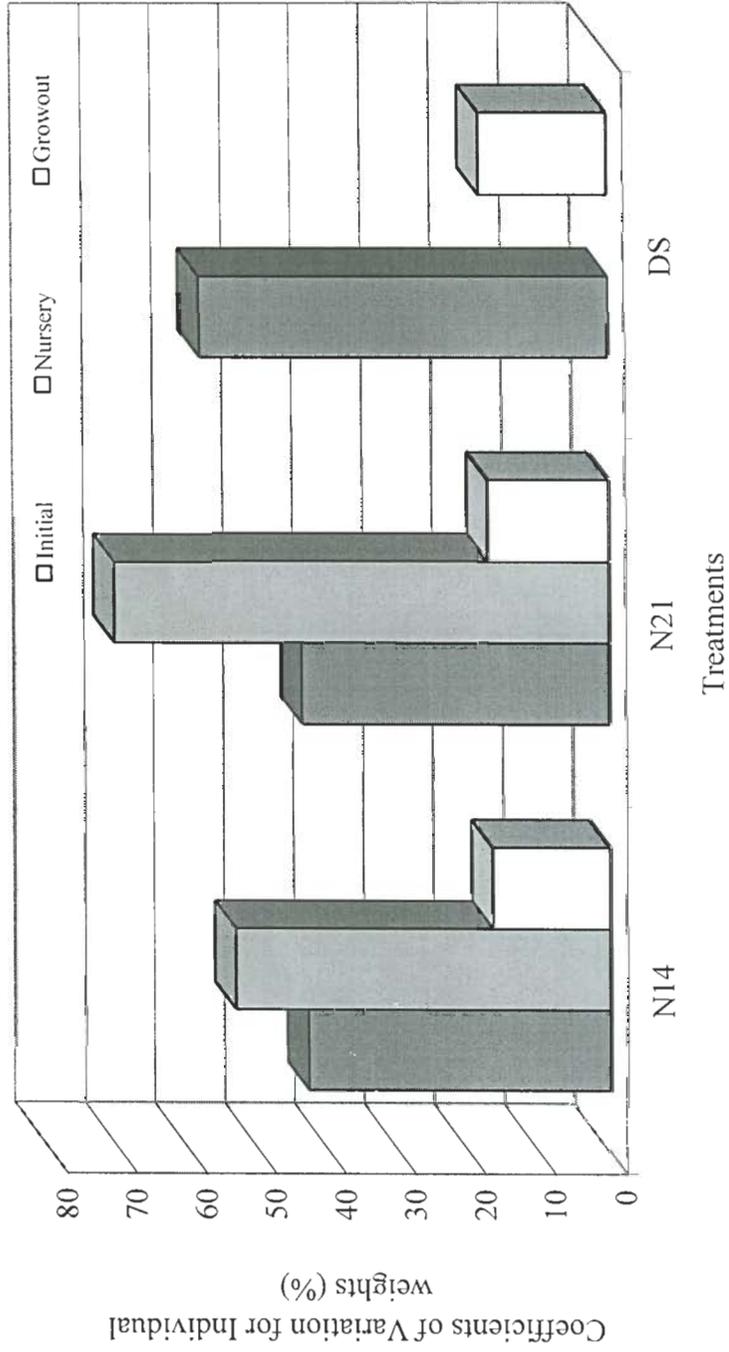
Both nursery treatments had good survivals (>90%). Both were better than some reported in other intensive nurseries studies (Sturmer and Lawrence 1987) and similar to results reported by Sturmer *et al.* (1992). Both nursery treatments ended up having similar FCR.

Post larvae for the three treatments had similar initial mean weights of 1.36 ± 0.59 , 0.96 ± 0.41 and 1.24 ± 0.72 mg/PL for N21, N14 and DS, respectively. When comparing nursery periods of 14 and 21 days (Table 3), larger juveniles were observed and significantly greater biomass loadings were found in the longer nursery period. Observed values for final average weight suggested treatment effects, but differences were not significant because of high variability between replicates. Also, at the time of stocking the N21 treatment and for the following six days of the nursery period, temperatures were around 21.7 C. In this period, growth of the N21 treatment was slow. Eventually (by the 10th and 4th nursing day for N21 and N14) temperatures reached a 26 to 27 C range. When comparing average weights after the same number of nursing days, although N14 initial weight (0.96 ± 0.41) was slightly lighter than N21 (1.36 ± 0.59), average weights for the 7th and 10th days were significantly greater in N14. This is most likely due to warmer temperatures (mean 26.7 C) during the first week of culture for the N14 group as compared to the temperatures (mean 21.7 C) during the first week of culture for N21 group. No significant differences between average weights were found at the 13th day. Although growth of the N21 treatment increased rapidly during the last

week (Fig.1), the initial lower temperatures may have influenced the feeding behavior and slowed metabolic processes (Lester and Pante 1992), consequently slowing its growth during the first week, this in turn, limited the average weight differences between the two treatments at the end of the nursery phase.

A crucial aspect of PL quality is the level of variation within the size of the population (Wyban and Sweeney 1991, Fegan 1992, Brock and Main 1994). Contrary to Garza (2001) and Wyban and Sweeney (1991) who reported that the Coefficient of Variation (CV) of individual weights of a healthy population declined during the nursery phase, results from the nursery phase of this study indicated that regardless of the length of nursing period, an increase in the variability of the PL individual weight was noticed at the end of the nursery phase relative to the initial CV of individual weights of PL from the hatchery (Fig.4), but after a pond growout phase the variation decreased. The DS treatment also showed a decrease in the CVs. For both nursery treatments, the CV for initial weights prior to the nursery period already was high. A CV greater than 30% is considered excessive (Wyban and Sweeney 1991, Brock and Main 1994). However, after the nursery, uniformity did not improve in any of the treatments. An increase of 27% and 10.4% in the CV of individual weights CVs relative to the initial variation, took place for the N21 and N14 treatments. Similar to the nursery treatments, the DS treatment PL had an initial CV that also was high (CV= 58.5%). However, the CV of final weights did not differ significantly between treatments at the conclusion of the pond growout. This suggests that regardless of PL age at stocking, an improvement in size variation takes place during the growout phase. Although three groups of PL were utilized in this trial, the lack of an improved CV during the nursery phase, was probably not related to

Figure 4. Coefficient of variation of individual weights of *Litopenaeus vannamei*. Values represent the initial population (initial), after the nursery period (Nursery) for 14 (N14) or 21 (N21) days and at the conclusion of the 16 week pond culture period (Growout). Nursery densities were 31-34 PL/L and 35 shrimp/m² in growout ponds.



differences in hatchery groups. Initial size variation from the hatchery were similar for all three groups; however, there was no significant difference in the size variation of the three treatments at the end of the growout phase.

The CV observed in this study were similar to CV of final weights (CV=66-156%) found in a 21 day nursery density study for which the PL had an initial CV of 67% (Chapter 2). In this study, the treatment that was highly nutrient enriched was the one with lower, almost negligible increase in the size variation. In the current study, the culture environment was less nutrient enriched (greater volume of water, lower food density) so the longer the PL were nursed, the greater dispersion was induced in the size distribution. Greater variation in weight distribution at the end of the nursery phase could be associated with some shrimp out competing others during their search for food and consequently some being able to grow faster. This indicates that appropriate concentrations of feed per unit of volume in the rearing unit are necessary to allow the encounter rate to be high enough so that the PL can ingest sufficient quantities with minimal effort (Kurmaly *et al.* 1988, Fegan 1992). However, at higher tank volumes and lower densities, greater feed quantity and aeration/circulation are required in order to maintain even suspension. This could be related to their natural behavior preferences, since white shrimp PL have been found in estuarine environments with nonvegetated bottoms and with large amounts of organic detritus (Williams 1955, Moriarty 1976, 1977).

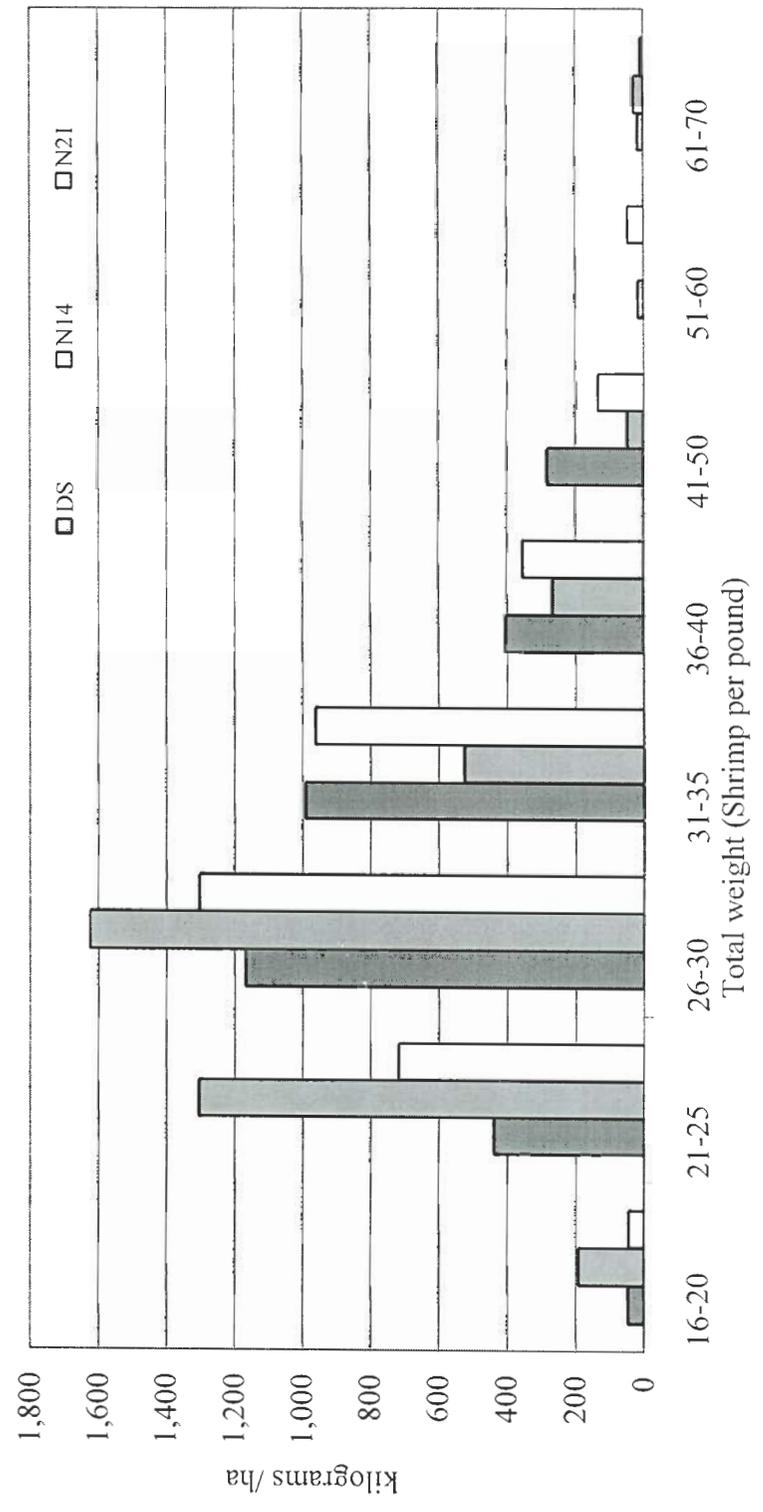
After the growout phase, individual weight variation in all treatments were within normal ranges of 10-20% (Brock and Main 1994). Similar pattern (weight variation increasing during the nursery and decreasing after the growout phase) were found in a

trial done the previous year (Chapter 2). Weight variation improvement after the growout phase may be related to the abundance and variety of natural food (Fegan 1992, Moss *et al.* 1992, Moss 1995).

During the culture period that followed, nursed juveniles did not differ significantly in production characteristics and population size distribution, from shrimp stocked direct from the hatchery. Similar to these results, Garza (2001), found no statistical differences in survival, yield or growth between nursed and non-nursed shrimp.

At the conclusion of the final growout phase, CVs individual weight were not significantly different among treatments (Table 3, Fig.4). However, it is important to consider the graphical pattern of size distributions (Fig.5) and the amount of variation in the size of individual shrimp as principle features in assessing effects on size uniformity. For all three treatments the predominant count size was 26-30 (average size of 16 g), however this count size accounted for 41.5%, 36.3% and 34.8% for N14, N21 and DS treatments, respectively. Count sizes that accounted for more than 1% of the population included six for N14 and DS and seven for N21. When considering the percentage of the population within the 26-30 counts and above, 77.75%, 49.75% and 55.75% were determined for N14, N21 and DS but no significant differences among treatments were found. Based on these observed results, the N14 treatment had better uniformity and size distribution at the end of the growout period which was consistent and possibly related to results obtained during the nursery phase; however, statistical evidence is limited.

Figure 5. Total shrimp production distributed across typical shrimp size classes at the conclusion of a 16 week of pond culture. Growout ponds were stocked at 35 shrimp/m² with *Litopenaeus vannamei* juveniles previously indoor nursed for 21 (N21) and 14 (N14) days or PL 8 direct stock (DS).



Recommendations

In temperate regions, implementing nurseries for head starting purposes could be useful if there is a possibility of two production cycles within the warmer period (Lawrence *et al.* 1985, Sturmer and Lawrance 1987, Sandifer *et al.* 1988). Wang and Leiman (2000) suggest using a two-stage production system consisting of a prolonged nursery stage followed by a grow-out stage. Although some studies (Juan *et al.* 1988) indicate that direct stocking growout ponds with PL and producing one crop per year is more profitable than stocking one gram juveniles and producing two crops per year. If only one production cycle is available, and PL supply is guaranteed from a hatchery and there are no biosecurity issues, results of this study indicate a nursery phase may not be justified. Instead it would be convenient to direct stock as pond temperatures reach safe readings and the production cycle could end as the fall season temperatures set in. From a biological and pond management perspective this could be more advantageous since small shrimp (< 1g) grow faster in warm water (Lester and Pante 1992) and medium (12g) and large shrimp (18g) grow faster at lower temperatures (common at the beginning of the fall) around 23-27°C (Sadeh *et al.* 1986, Wyban and Sweeney 1991).

If in the culturing site there is prevalence of viral diseases, then nurseries could be justified for biosecurity reasons. Viral diseases have been the most important cause of economic losses in many shrimp producing countries (Lightner 1992, 1996, Flegel 1997, Alday de Graindorge 1999). Farm operations have been recommended to minimize the risk of disease impact (Stumer *et al.* 1992, Fegan and Clifford 2001) by initially nursing shrimp in disease free environments (nurseries) and then stocking growout ponds with

larger and older juveniles that are thought to have better developed immune systems and therefore more resistance to both biological and abiotic threats (Villalon 1991, Prayinto and Latchford 1995) thus achieving higher survival rates (Samocha *et al.* 2000a, 2000b).

In either case (more production cycles or biosecurity reasons), when nurseries are run with the purpose of producing larger juvenile while having colder outdoor temperatures, results of this study indicate that if the indoor water temperature in the rearing units (of unheated facilities) are of 21C or lower temperatures, limited weight gain advantages would be achieved. In such cases heating systems could be considered or initiating the nursery later in the cold season when indoor temperatures (of the unheated facility) turn to be higher than 25 C. Although more advantages were expected, it is also important to point out that one advantage of the nursery was that in the case of not having a guaranteed PL supply from the hatchery, using an unheated greenhouse, PL could be head-started extending the period to acquire PL, possibly avoiding peak demand periods as the warmer season began.

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IV. INFLUENCE OF ARTEMIA AND ALGAL SUPPLEMENTS DURING NURSERY PHASE OF *Litopenaeus vannamei*

Abstract

A 21-day nursery trial was conducted to evaluate the influence of dried feed, algae and newly hatched Artemia on survival, growth and feed conversion ratio of post larval (PL) *Litopenaeus vannamei*. Sixteen round nursery tanks (795 L), were stocked at a density of 22 post larvae/L and four treatments evaluated. In three of the treatments, algae was supplemented every three days at (2.8×10^9 cells/tank) using a concentrated algae paste of the diatom *Thalassiosira weissflogii*. Within these three algae treatments, one was provided a commercial feed (F), one a commercial feed and Artemia every other day during the first seven days (FAr3) and in the third treatment, a commercial feed and Artemia were offered every day during the first seven days (FAr7). The fourth treatment was not supplemented with algae, relying only on autochthonously developed algae plus commercial feed. All treatments were offered equal quantities of the same type of dried feed. The nursery period lasted 21 days for all treatments. There were no significant differences in survival or FCR for PL nursed in the various treatments. However, PL receiving Artemia supplement for 3 days, were larger than those that did not. When comparing PL receiving Artemia for 3 or 7 days as well as those maintained with algae paste vs. autochthonously algae there were no significant differences between these pairs.

Results suggest an advantage of supplementing the dried feed with Artemia for 3 days and whereas the supplementation of algae paste did not improve results.

Introduction

As culture system intensity increases for marine shrimp, two-phase culture is becoming more common (Samocha and Lawrence 1992, 1998). The quantity and nutritional quality of the diet offered to shrimp post larvae is a determinant factor in its growth and survival. Proper feeding is a key to producing healthy juveniles that will do well once transferred to the growout system.

Post larvae shrimp, require different feeds according to behavior, morphology and nutritional needs throughout their various developmental stages (New 1976, Wickins 1976, Colvin and Brand 1977, Treece and Yates 1990, Wyban and Sweeney 1991). A combination of live feeds, such as micro algae and *Artemia*, with prepared high protein dried feeds is commonly used for the nourishment of post larvae reared in nursery systems (Fegan 1992). Hudinaga (1942) first recognized the need of an animal protein source as a component of the larval diet. Currently, *Artemia* is the food most commonly chosen in shrimp larvae culture to fulfill this nutritional need (Sorgeloos 1980, Fujita *et al.* 1980; Schaucer *et al.* 1980, Wilkenfeld 1992, Dhert *et al.* 1993, Navarro *et al.* 1991, Nelson *et al.* 2002, Shapawi and Purser 2003). In addition to protein, the level of essential polyunsaturated fatty acid (PUFA) content such as 20:5w3 (Leger *et al.* 1985 and Millamena 1988) plays a role in the nutritional quality and value of *Artemia* nauplii as crustacean larval food. Because of *Artemia* size (0.45mm of length, 0.17mm in width and 0.01 mg in weight) *Artemia* are ideal larval food, since shrimp larvae can handle a 0.5 mm size prey (Liao *et al.* 1993).

Commercialization of *Artemia* cysts has gradually increased since its nutritional value as food was first reported (Seal 1933, Hudinaga 1942). Demand for cysts soon exceeded commercial supplies (Sorgeloos *et al.* 1986) and as a result, a number of studies have been conducted in recent years on the optimum utilization and offerings of *Artemia* (Samocha *et al.* 1989, Liao *et al.* 1993). Although, there are numerous studies with regard to its use in larval culture, there is limited data on their use in Nursery systems.

Algae also plays an important role in shrimp larvae nutrition (Cockcroft and McLachlan 1986, Bailey-Brock and Moss 1992). After their egg and early naupliar stages, algae is a natural food for shrimp larvae. In indoor facilities where more algae development control is possible, species are selected for cultivation based on the criteria of ease and cost of culture as well as dietary value (Treece and Yates 1990). For these purposes, Diatoms have been found advantageous because of their low fiber content (Mann and Pruden 1988) and high concentration of polyunsaturated fatty acids (Phillips 1984). Penaeid shrimp have been found to consume diatoms in their natural environment (Gleason and Zimmerman 1984, Gleason and Wellington, 1988) and also in aquaculture ponds (Hunter *et al.* 1987, Bombeo-Tuburan *et al.* 1993, Moss and Pruder 1995) . However, Boyd (1998) neither supported nor disputed the relevance and advantages of diatoms in shrimp culture.

A number of products have been used with limited results as complete or partial replacements for live algae. These products include yeast, dried micro particulate feeds, and frozen, dried, and concentrated live algal pastes (Coutteau *et al.* 1990, Biedenbach *et al.* 1990). The advantages of such products include, stable nutritional composition,

reduction in both the labor and expense of maintaining a large live algal production facility, and the ability to harvest excess algal biomass for use at a later date, maintenance of the nutritional profile without requiring nutrient additions, ease of achieving higher concentrations (5.0×10^3 cells/ml) and ease of suspension in the water column with minimum circulation.

However, harvest and extended storage of algal materials can lead to cell wall disruption and nutrient loss. Of those products composed exclusively of algal material, concentrated live algal pastes and dried algae appear to hold the most promise for the future (Smith *et al.* 1993). In addition to their nutritional content microalgae, also plays a role in conditioning rearing water, contributing to dissolve oxygen levels and removal of toxic metabolites (Leger and Sorgeloos 1992).

Because live food is often expensive to produce and susceptible to variations in production and nutritional quality (Sorgeloos *et al.* 1983; Leger *et al.* 1986; Kuban *et al.* 1985) considerable work has been geared towards the development of dry diets to replace or supplement live feeds (Jones *et al.* 1987, Fegan 1992, Jones *et al.* 1993). Dry feeds are typically fortified with vitamins, minerals and have 10-30% higher protein content than feeds used in growout phase (Samocha and Lawrence 1992). Besides adequate nutritional content, artificial diets should meet certain minimum standards including adequate particles size, availability in the water at similar density to live feed, stability, minimal leach loss, high digestibility and long term storage capacity (Langdon *et al.* 1985). There are also economic challenges in dry diet manufacturing and usage. Most diets are formulated from natural products such as fish meals with a similar composition to that of phytoplankton or zooplankton (Jones *et al.* 1993). As both

ingredients and processing are costly, larval feed are expensive and may increase operational costs (Otoshi *et al.* 2001).

Even when total replacement of live feed (Jones *et al.* 1987) and algae (Kanazawa 1990) using microencapsulated feeds has been demonstrated both at laboratory and commercial levels, in most cases they are only used as partial (50-70%) replacement (Jones *et al.* 1993). On the other hand, it has also been demonstrated that growth may be significantly lower without live feed. The lack of water stability in most microparticulate diets has been found to cause leaching, bacterial development and water pollution (Liao *et al.* 1988; Jones *et al.* 1989). Therefore, there is still a predominant continuation of algae and Artemia usage to supplement larval diets (Wilkenfeld 1992).

Although several references support the individual usage of diet components, there is limited research related to shrimp growth response under different combinations and offering rates of those that are commercially available. Hence, the objective of this study was to evaluate the influence of dried feed combined with algae and newly hatched Artemia on post larvae shrimp survival, growth and feed conversion ratio during the nursery growout.

Materials and Methods

This research was conducted at the Claude Peteet Mariculture Center, Gulf shores, Alabama. (30 16.981 N, 087 39.914 W). The experimental design included four treatments, with four replicates each conducted over a 21-day nursery period. All treatment tanks were offered equal quantities of the same dried feed, combined with an additional diet component that varied according to treatments. In three of the treatments

algae was supplemented by using a concentrated algae paste (156×10^8 cells/ml) of the diatom *Thalassiosira weissflogii* (Instant Algae, Reed Mariculture Inc, San Jose, CA, USA). The algae was added before stocking and by subsequent applications of 2.8×10^9 million cells every three days (after water exchanges) to each of the assigned tanks throughout the nursery period. From these three treatments, one (F) received only commercial dried feed, in another one (F_{Ar7}), feed and Artemia was offered every day during the first seven days. In the third treatment (F_{Ar3}), feed and Artemia was offered at a ratio of 100 Artemia/post larvae every other day during the first seven days. The fourth treatment (FWA) was not supplemented with algae but relied only on autochthonous developed algae and the commercial feed. Cysts of Artemia from Great Salt Lake, USA (INVE Americas, Inc., Salt Lake City, UT, USA) were decapsulated with hypochlorite solution (Sorgeloos *et al.* 1977,1983,1986; Treece and Yates 1990), and hatched over a 24-hour period before offerings.

Litopenaeus vannamei PL (8/9 days old) were obtained from GMSB Inc. Key West, Florida on April 29 of 2002. Postlarvae were shipped at a density of approximately 1,400 PL/L. Water samples were collected to evaluate water quality of the shipping bags (Appendix 8). Initial weights and weight Coefficients of Variation (CV) were determined from samples collected from shipment bags by weighing at least 40 PL individually. Excess water was eliminated by collecting PL with a strainer, depositing them on an absorbent paper cloth and then weighing them individually (0.01 mg) on an analytical scale Model ER-182A (A&D Company, Milpitas, CA, USA).

Upon receipt, the PL were transferred into a 940 L acclimation tank filled with sea water and adjusted to the salinity that the PL were shipped in. The bags containing

the PL were allowed to float in the acclimation tank until temperatures between the water in the tank and bags were within 0.5 C. The PL were then released in the reception tank and their condition in terms of size, coloration and activity, was evaluated as a measure of PL quality. During acclimation, decapsulated and hatched Artemia nauplii (52% protein, 200-300 um) (INVE Americas, Inc., Salt Lake City, UT, USA) were offered at a rate of 100 Artemia/PL (Treece and Yates 1990). Artemia offerings were calculated based on the assumption of 250,000 Artemia cysts in each gram of Artemia cysts (Wyban and Sweeney 1991). Salinity of the acclimation tank was gradually adjusted to the salinity of the nursery tanks (from 30.6 to 21 ppt) over a period of eight hours. Once the PL warmed up and their activity increased, the PL were concentrated and quantified volumetrically. A volumetric quantification (Hardin *et al.* 1985, Juarez *et al.* 1996) was done, to verify the quantity supplied and to allow an accurate distribution of PL among nursery treatment tanks. Post larvae were concentrated into a 57-L tank, vigorously mixed by hand and subsampled with a 60-ml beaker to obtain the density for quantification purposes. Means, standard deviation and Coefficients of variation were recorded. Replicate nursery tanks were stocked at a density of 22 PL/L (total of 12,429 PL/tank) and the shrimp were nursed for 21 days.

Nursery System

Sixteen circular polyethylene round nursery tanks (0.85 m height x 1.22 m upper diameter, 1.04 m lower diameter, total volume of 795L), located under a plastic cover were used for the experiment. The nursery was set as a single pass flow through system, with a reservoir tank, a 3 kg canister charcoal filter (Ocean clear Model 320, Red Sea, Houston TX, USA) and a 1/3 hp (45gpm) pump (EBARA International Corp. Rock Hill

SC, USA) for water distribution. Each tank was equipped with two air stones connected to a common air supply from a 1 hp regenerative blower (Sweetwater Aquaculture Inc. Lapwai, ID, USA). Tanks had a center drainage with a stand pipe of 3.2 cm diameter and 75cm long, set to maintain water level at a height of 61 cm (570 L). Standpipes were surrounded by an external screened pipe (250 um mesh). One week prior to stocking the nursery system was filled with brackishwater (21 ppt) pumped from the Intracostal Canal between Mobile and Perdido Bay. Water was first pumped into a nearby raceway system for a sand filtration period of over 24 hours for removal of undesirable organisms, organic materials and other debris. A week prior to stocking the nursery system was filled. Water was added into the nursery system through the reservoir and distributed into the nursing tanks, pumped at 10.6 liters /tank / minute. Once the system was filled, water and nursery tanks were disinfected using sodium hypochlorite (approximately 10ppm chlorine). Dechlorination was ensured by bubble aeration for four days, and then tested for chlorine residues. To maintain good water quality, 50% of the water in each tank was exchanged every three days. First water was drained from the bottom of the tank until reaching the water column height of half its volume and then new water was added.

Feeding

Daily rations were determined based on estimated biomass and feed rates ranging from 50% to 30% of the estimated biomass (Table 1). Biomass was determined every three days from a random sample collected from each tank. At least 40 PL were

Table 1. Feeding rates as percentage biomass and feed type utilized through the 21 days nursery period for *Litopenaeus vannamei* post larvae. Feed inputs were based on an assumed 100% survival, and the mean shrimp weight.

Days	% Biomass	Feed Type
1 to 3	50	^a PL Ready
4 to 6	50	PL Ready
7 to 9	35	PL Ready / ^b Crumble # 0
10 to 12	35	Crumble # 0
13 to 15	30	Crumble # 0 & Crumble #1
16 to 18	30	Crumble #1
19 to 21	30	Crumble # 1

^a PL Ready 50% Protein, Zeigler Bross, Inc., Gardners, PA, USA

^b Rangen 45% protein, Rangen Inc., Buhl, Idaho, USA

collectively weighed to determine the average weight. Water excess was eliminated by concentrating PL through a strainer, and depositing them on a paper towel, then weighing them to the nearest 0.01mg on an analytical scale Model ER-182A (A&D Company, Milpitas, CA, USA) and counted.

Four feedings were scheduled each day. The weighed dry feed was divided into four containers. To facilitate feed immersion and distribution, feed in each container was mixed with tank water to form a slurry that was distributed evenly around the tank. During the first six days, PL were offered a 50% protein diet, PL Redi-Reserve (400-600 mm) (Zeigler, Gardners, PA, USA). Thereafter, PL were fed with a combination of two 45% protein commercial shrimp starter feeds, Rangen #0 (<0.6 mm) and #1 (0.6-1 mm) (Rangen Inc., Buhl, Idaho, USA).

Water quality analyses

Temperatures were recorded every two hours on a 24 hour basis with data a logger Boxcar version 3.7 (Onset Computer Corporation, Bourne, MA, USA). Dissolved oxygen was monitored daily in the morning hours using a YSI 85 and YSI 556 dissolved oxygen meter (Yellow Spring Instruments, Yellow Springs, OH, USA). Water quality parameters that were monitored every three days from samples collected from a randomly selected replicate from each treatment, included salinity, total ammonia-nitrogen (TAN) and pH. Salinity was monitored using a YSI 30 salinity meter (Yellow Springs Instruments, Yellow Springs, OH, USA). Total ammonia-nitrogen was monitored using a spectrophotometer Spectronic 20 Genesys (Spectronic Instrument Inc. Rochester, NY,

USA) and the Nesslerization method (APHA 1989), and pH measurement were taken using a Accumet pH meter (Fisher Scientific, Pittsburgh,PA, USA).

Data analyses

The data was analyzed by Analysis of Variance for juvenile's final average weight, survival, FCR, biomass loading and individual size coefficient of variation (CV). The Student-Newman-Keuls multiple comparison test was utilize to determine significant differences among treatments means ($P \leq 0.05$). Analyses were conducted using SAS program version 8.2 (SAS Institute Inc., Cary, NC).

Results

At the conclusion of the 21 day nursery trial, mean weight of juveniles were 27.1, 35.8, 47.0 and 29.4 mg/PL , FCR were 1.5, 1.6,1.4 and 1.2, survivals were 86.1%, 87.1%, 74.7% and 78.0%, biomass loading were 0.68, 0.91, 0.95 and 0.69 kg/m³, for low F, FAr7, FAr3 and FWA respectively (Table 2). No significant differences were found in FCR, survivals and coefficient of variation of individual weights among treatments. Average weight and biomass loadings were significantly greater in FAr3 than F and FAW but did not differ from FAr7. Although observed values for final average weight and biomass loading for FAr7 were numerically higher than those observed in F and FAW treatments, no significant differences were found. One of the replicate tanks from the FArt7 (Tank 11) and one from the FArt3 (Tank 13) treatments were excluded

Table 2. Production characteristics of *Litopenaeus vannamei* nursed for 21 days, stocked at 22 PL/L and fed with different diets combination. F is feed only. FAr7 is feed with Artemia fed for the first seven days. FAr3 is feed with Artemia fed every other day for 7 days. FWA is feed and naturally developed algae ¹.

	Treatments				Pr > F
	F	FAr7*	FAr3*	FWA	
Nursery Phase	F	FAr7*	FAr3*	FWA	Pr > F
Final average weight (mg)	27.1 ^a	35.8 ^{ab}	47.0 ^b	29.4 ^a	0.019
Survival (%)	86.1 ^a	87.1 ^a	74.7 ^a	78.0 ^a	0.362
FCR ²	1.5 ^a	1.6 ^a	1.4 ^a	1.2 ^a	0.149
Final biomass (kg/m ³)	0.679 ^a	0.910 ^{ab}	0.947 ^b	0.688 ^a	0.019
CV (%) ³	61.25 ^a	76.81 ^a	61.46 ^a	64.33 ^a	0.477

¹Means not sharing a common supercript within a row are significantly different ($P \leq 0.05$) based on Student-Newman-Keuls test.

²FCR = Total weight of feed given / Biomass increase

³CV= Standard deviation / mean * 100

from the study. In these tanks high mortalities were caused by a cold front that took place the last week of the nursing period. Survivals in these replicates were 13.4% and 38.9% while the rest of replicates averaged 87.9% and 74.7%, respectively.

The four treatments followed similar growth patterns, however treatments supplemented with Artemia (FAr7 and FAr3) had higher weights than the other two treatments during all sampling periods (Fig. 1). The gap between these two groups increased throughout the nursing period. No treatment showed a growth rate decline during the 21-d nursery period.

The CV for individual weights upon initiation of the growth trial was 44%. After the nursery CV ranged from 61-76.8% (Fig. 2). Although no significant differences were found among treatments, the highest size variation was observed in the FAr7 (CV = 76.8%).

Water temperatures throughout the nursery averaged 24.93 ± 2.86 and 25.05 ± 2.89 for day and night readings, respectively. However, on day 14 and then again during the last 4 days of the nursery period (days 18-21), two cold fronts on the area caused in the system low water temperatures that were in the range of 17.9-20 C and 18-19 C, respectively. Dissolved oxygen readings in all tanks were consistently in the range of 7.2 to 8.3 mg/L. Salinity throughout the experimental period ranged from 21 to 16.7 ppt. During the last three days, salinity was increased to 21 ppt due to salinity fluctuation of the water source. The pH generally fluctuated between 8.2 and 7.7. Highest detected TAN concentrations were 1.33, 2.25, 2.37 and 0.95 mg/L $\text{NH}_3\text{-N}$ for F, FAr7, FAr3 and FWA, respectively. Treatments FAr7 and FAr3 which were supplemented with Artemia,

Figure 1. Mean weight (n=4) of *Litopenaeus vannamei* post larvae (PL) at various days during the indoor nursery phase stocked at 22 PL/L and fed with different diets combination. F is feed only. FAr7 is feed with Artemia fed the first seven days. FAr3 is feed with Artemia fed every other day for 7 days. FWA is feed and naturally developed algae.

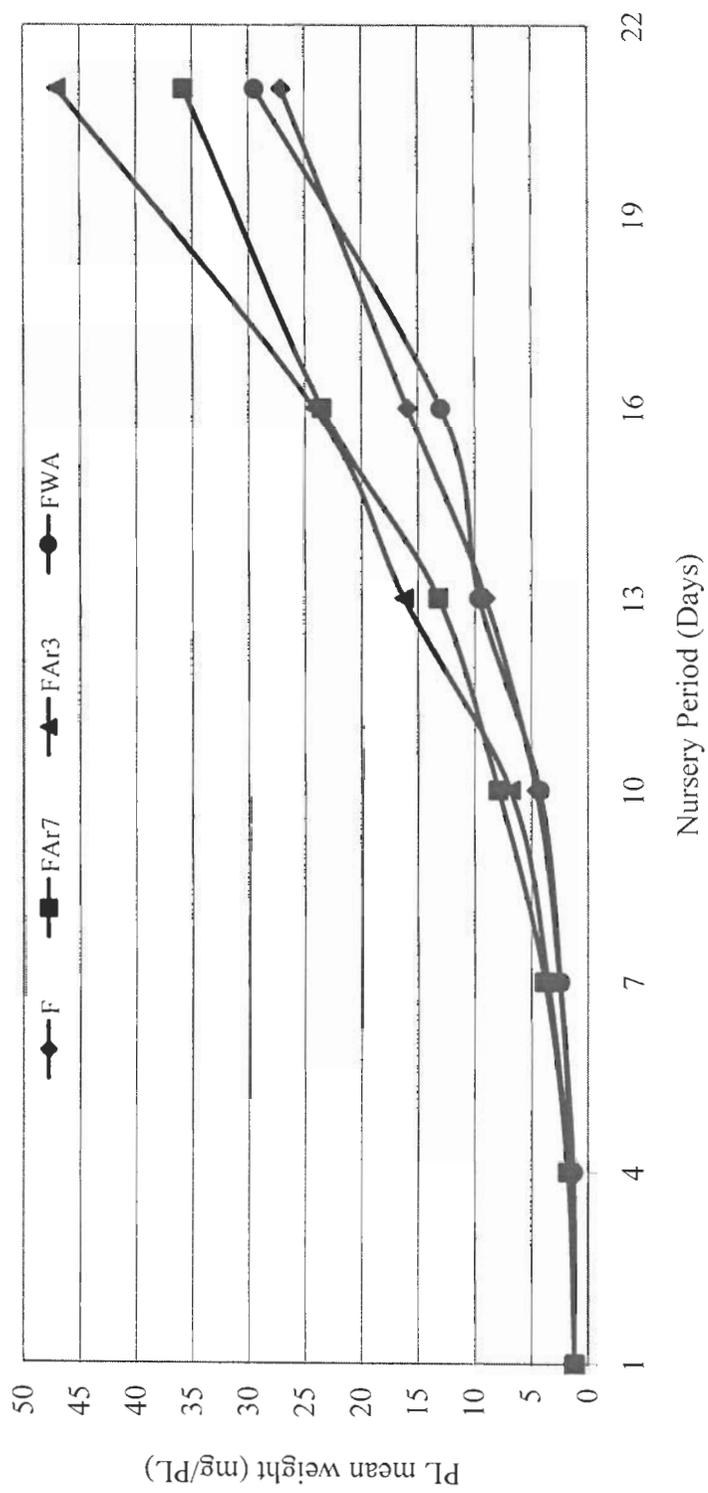


Figure 2. Coefficient of variation for individual weights of *Litopenaeus vannamei*. Values represent the initial population (initial) and after a 21 day nursery period with different diets combinations. F is feed only. FAr7 is feed with Artemia fed the first seven days. FAr3 is feed with Artemia fed every other day for 7 days. FWA is feed and naturally developed algae.

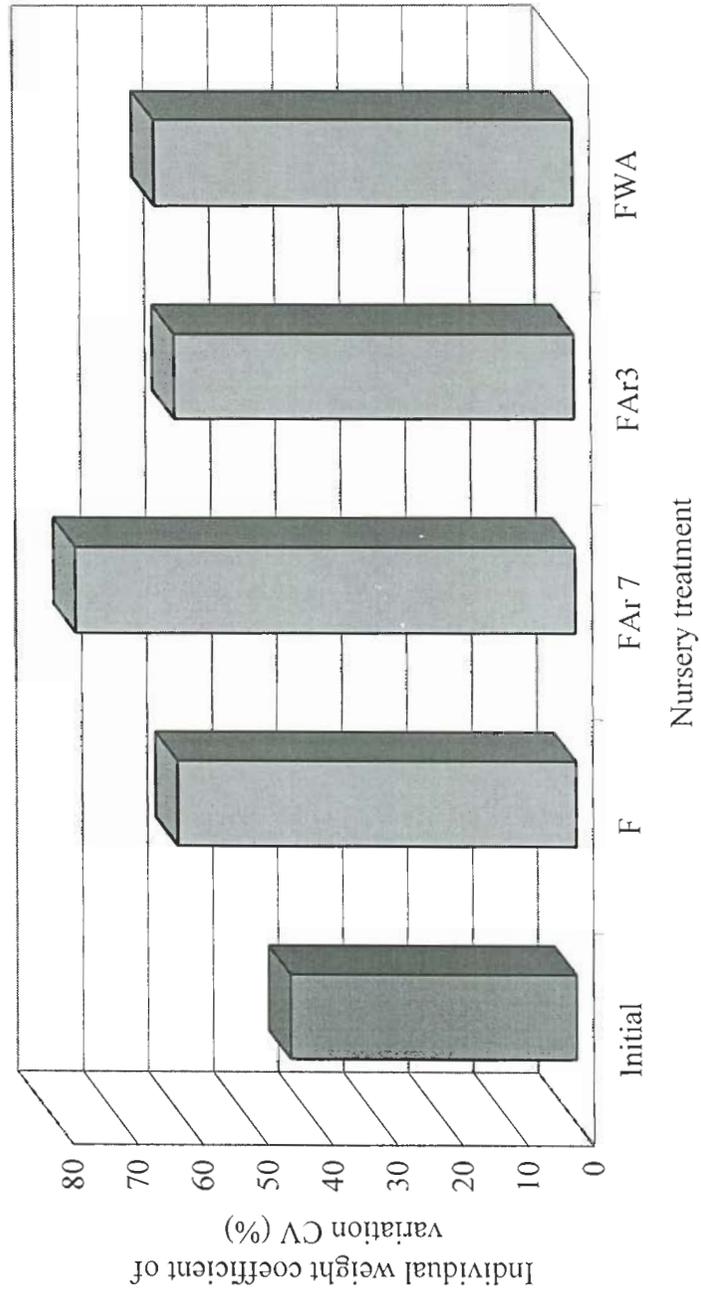


Table 3. Water quality parameters over a 21 day nursery period for *Litopenaeus vannamei* stocked at 22 PL/L and fed with different diets combination. F is feed only. FAr7 is feed with Artemia fed the first seven days.FAr3 is feed with Artemia fed every other day for 7days. FWA is feed and naturally developed algae. Figures are displayed as mean \pm standard deviation and maximum / minimum values in parentheses.

Treatment	pH	Salinity ppt	TAN mg/L
F	7.99 \pm 0.18 (8.22, 7.8)	19.3 \pm 2.33 (21.8,16.4)	0.76 \pm 0.50 (1.33, 0.126)
FAr7	7.96 \pm 0.19 (8.22, 7.7)	19.4 \pm 2.31 (21.8,16.5)	1.30 \pm 0.63 (2.25, 0.419)
FAr3	7.97 \pm 0.21 (8.24, 7.6)	19.4 \pm 2.31 (21.8,16.5)	1.13 \pm 0.81 (2.52, 0.76)
FWA	8.01 \pm 0.17 (8.23, 7.7)	19.4 \pm 2.31 (21.8,16.5)	0.57 \pm 0.35 (2.52, 0.76)

had TAN concentrations that were twice as high as treatments which did not receive Artemia (Table 3).

With the exception of the low water temperatures (17.9-20 C and 18-19 C) during day 14 and the last 4 days of the nursery period, temperatures, dissolved oxygen, salinities, pH, and TAN values were within recommended ranges for *L. vannamei* culture (Hanson and Goodwin 1977; Clifford 1985; Boyd 1989, and Brock and Main 1994).

Discussion

At the conclusion of the 21 day nursery trial, the various feeding protocols did not have a significant effect on survivals, FCR and final individual weight variation.

Survivals probably could have been higher than that which was observed as a number of dead shrimp were noticed during the cold period which presumably stressed the shrimp. Overall survivals and FCR were still good across all treatments, and were similar to results reported by Sturmer and Lawrence (1987), Sturmer *et al.* (1992), Samocha and Lawrence (1998) and Samocha *et al.* (1999). There are indications of treatment effects based on statistical differences found in final average weight and biomass loading.

Size variation is often considered a crucial aspect of PL quality (Fegan 1992). Wyban and Sweeney (1991) have reported that during the nursery phase the CV for individual weights declines. In this trial, the initial CV of individual PL weights prior to the nursery already was high (CV=44%), because CVs greater than 30% are considered excessive (Wyban and Sweeney 1991, Brock and Main 1994). At the conclusion of the nursery period CV increased in all the treatments ranging from 61.2- 76.8%. The final CV for other nursery trials (Chapter 2 and 3) ranged from CV=66-156% and CV=54-

71%, respectively. Greater individual weight variation at the end of the nursery phase could be associated with some shrimp out competing others during their search for food and consequently some being able to grow faster. High nutrient enrichment has been considered to playing a crucial role since appropriate feed concentrations are necessary to increase the encounter rate and minimize searching effort (Kurmaly *et al.* 1988, Fegan 1992). Also in their natural environment, white shrimp PL have been found in estuarine environments with nonvegetated bottoms and with large amounts of organic detritus (Williams 1955, Moriarty 1976, 1977). It has been suggested that size variation improves when the PL are transferred to pond conditions possibly due to the abundance and variety of natural food (Fegan 1992, Moss *et al.* 1992, Moss 1995). Such improvements in growout ponds also has been noticed in previous trials (Chapter 2 and 3) to this study.

It is relevant to emphasize that *Artemia* used in the experiment first was decapsulated and then it was hatched over 24 hours before being offered. Several advantages of *Artemia* decapsulation have been listed, including disinfection, facilitation of separating cysts shells from the hatched nauplii and the potential use of decapsulated cysts as direct food source for fish and crustacean larvae (Liao *et al.* 1993, GomezGil-RS 1994), also hatching rate improvement without causing significant individual naupliar dry weight variation (Sorgeloos 1977) and on an individual weight basis, the decapsulated cysts and nauplii of *Artemia* have been found with similar biochemical composition in all the major nutrients (Garcia-Ortega *et al.* 1998). Garcia-Ortega and Huisman 2001, have concluded that with regard to the amount of nutrients there is no difference in feeding *Artemia* cysts or nauplii to larvae.

Results indicate that there is an advantage in supplementing the dried feed with *Artemia nauplii* during the first week. Throughout the nursery period the two treatments supplemented with *Artemia* experienced greater growth and reach greater observed (FAR7) and significantly greater (FAR3) final average weight than the other two treatments (Fig. 1). Colvin and Brand (1977) and Akiyama *et al.* (1992) reported that the dietary protein requirement of early PL penaeid shrimp exceeds 40% crude protein, but decreases to less than 30% in the later life cycle stages. Leger *et al.* (1985) also suggest that highly unsaturated fatty acids, that have been found to significantly contributed in sustaining a faster PL growth. In this experiment, treatments without *Artemia* supplementation although fed with a high protein diet (50%) did not experience as good of growth as those supplemented with *Artemia* (Fig.1). This suggests that the nutritional advantage derived not only from its high protein content but also could be associated with other nutrients such as highly unsaturated fatty acids.

Although *Artemia* provide a good nutrient source, when offered in excess, they can create negative physical effects by limiting swimming and prey capture efficiency. Also uneaten *Artemia* could grow quickly and become invulnerable to predation and compete with larval shrimp for important resources, such as feed, algae and dissolved oxygen in the rearing unit and excrete metabolites contributing to water quality deterioration, affecting growth and complicating the rearing tank management (Samocho 1989, Liao *et al.* 1993). Because of this, it is important to determine optimal *Artemia* offerings into larval culture systems in order to avoid excessive quantities. Given the tanks volume (570 L) and the estimated amount of *Artemia nauplii* offered (1.2×10^5) offerings were about 100 *Artemia*/PL. This is similar to concentrations found to be

efficient by Samocha *et al* (1989) for larval rearing. He demonstrated that by increasing *Artemia nauplii* density in the culture medium up to 9 per ml (or 90 *Artemia*/PL in our trial conditions) and starting offering as early as Z2 dry weight increased, but further increasing *Artemia* offerings to 15 per ml (or 150 *Artemia*/PL) had no significant effect on growth. When comparing FAr7 and FAr3 to determine how to offer *Artemia* (daily or every other day) during the initial seven days of the nursery period. There was no significant difference between the two treatments. Hence, the use of *Artemia* every other day (FAr3) was found more advantageous as only half the amount of *Artemia* was used to achieve the same results.

In addition to *Artemia* providing a natural food source, the presence of algae may provide some advantage. However, results indicate that the use of diatoms fed through algal paste provided no advantage over algae that grew naturally in the nursery tanks. According to Liao *et al.* (1993) a minimum of 5×10^3 cells per ml is generally sufficient for rearing most shrimp larvae, but specific concentrations for *L.vannamei* are in the range of $30-100 \times 10^3$. The algae paste used was a non-viable product and was offered as fresh feed and not with the purpose to start a culture, however concentrations attained (based on ml of regular addition of algae paste and volumes in the tanks) were of 4.9×10^3 cells/ml, below the minimum recommended for *L.vannamei* larviculture. Algal concentrations found in the FWA treatments were much lower, around $1.5-2.0 \times 10^3$ cells/ml, because tanks were kept under an opaque plastic cover that minimized exposure to sunlight and may have limited natural development of algae. Because of the water exchange every three days, natural developed algae concentrations were lower and water quality was not an issue in any of the treatments, so there was not an opportunity to

notice the water quality stabilization function of live algae (FWA) over non-live algae paste. However, nursery period trends and overall averages TAN concentrations were greater in treatments supplemented with Artemia even after the suspension of Artemia offerings. Overall averages (Table 3) of FAr7 and FAr3 were 1.30 ± 0.63 and 1.13 ± 0.81 $\text{NH}_3\text{-N}$, respectively; concentrations that are between 1.5 and 2 times greater than average concentrations in F or FAW that had 0.76 ± 0.50 and 0.57 ± 0.35 overall averages, respectively.

In commercial operations, tanks are exposed to sunlight, that along with nutrients leached from dry diets and other feed sources support abundant algae development. Natural inoculation can easily take place even from other species than the ones initially inoculated; in some cases as many as 30 diatom species have been identified from a single culture tank (Krom *et al.* 1985, Sturmer *et al.* 1992, Liao *et al.* 1993). On previous nursery trials conducted in the same location (Chapter 2 and 3), experimental tanks were located inside a clear polyethylene plastic quonset-style greenhouse, only non-live algae paste was added. A week after initiating the nursery, naturally developed algae concentrations were in the $5\text{-}6 \times 10^4$ cells/ml range. Final average weight were similar to this study, ranging from 19-21.5 mg/PL (Chapter 2) to 10.5-16.4 mg/PL (Chapter 3) in the latter experiment. Lower weights were related to temperatures effects.

Although some advantages of algae over Artemia have been documented (Schauer *et al.* 1980; Leger *et al.* 1986; Navarro *et al.* 1991) mostly related to deficiency in docosahexaenoic acid [DHA, 22:6n-3] and low or variable in eicosapentaenoic acid [EPA, 20:5n-3] content (Watanabe *et al.* 1982, Rees *et al.* 1994, Narciso *et al.* 1999, Nelson 2002). In this experiment, decapsulated Artemia supplementation proved a

nutritional advantage by sustaining greater growth. Another advantage noted by Leger and Sorgeloos (1992) is the convenience in preparing Artemia instead of culturing algae. Despite the Artemia advantages mentioned, it is important to realize that there is a large variation in the nutritional quality of Artemia nauplii; their fatty acid profile have been found to vary geographically and also from year to year, or between different Artemia strains or even among batches of the same strain (Leger and Sorgeloos 1984, Leger *et al.* 1985, Millamena *et al.* 1988, Shapawi and Purser 2003).

Though there have been studies on alternative diets such as inert microalgal diets (Cordero and Voltolina 1996; Albentosa *et al.* 1997) or artificial diets (Langdon 1983), a complete substitution of live feed for larval rearing has not been achieved. In this study, although results indicate that the dry feed diet, regardless of supplementation with Artemia and algae, supported growth and PL survival, the shrimp performance still remain far behind than those in natural nutrient enriched environments. Some of the shrimp that came in the same shipment were direct stocked in growout ponds at the same time nursery tanks were stocked, and at 21 days their average weight (out of four replicate ponds) was 0.67 g, significantly different ($P < 0.001$) and between 14 and 25 times greater than those that were nursed for 21 days under the different treatments. This indicates that there still is a challenge in improving nursery feeds in terms of nutritional composition and feeding methods. Leger and Sorgeloos (1992) indicated that diet aspects that need to be improved before a complete live food replacement, includes nutritional composition and physical performance, especially regarding suspension in the water column and leaching. Greater feeding frequency has been proposed as a way of reducing

leaching from feed, since it has been shown that penaeid shrimp grow more rapidly and utilize feed more efficiently when fed more than once a day (Sedgwick 1979).

Although a clear advantage was found by supplementing the dried feed with *Artemia nauplii*, conducting nurseries with the diets evaluated and under current feeding methods still implies a much slower growth, demands greater management skills and result in little advantages over PL directly stock in ponds, making a nursery phase hardly justified by reasons other than biosecurity issues. Because the consistent trend of an increment in individual weights variation after a nursery period in previous trials (Chapter 2 and 3) and in this experiment further feeding evaluations are required to test the effect of various level of nutrient enrichment. Feeding should not only be based on the estimated biomass in culture but also requires to account for the volume of the rearing unit and target a suitable minimum feed concentration per unit of volume that increase the encounter rate, minimize searching effort, maximize growth and does not create water quality problems.

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V. EVALUATION OF FEED MANAGEMENT STRATEGIES FOR *Litopenaeus vannamei* UNDER POND PRODUCTION CONDITIONS

Abstract

This study was conducted with the objective of incorporating general aquaculture considerations into a management and feeding program and evaluated the production effect and economic implications of three feeding schedules used to grow marine shrimp in 0.1ha earthen ponds. All experiments were treated equally until the 7th week of culture when the shrimp had reached an average weight greater than 2 grams. At this point three feeding schedules were evaluated An early aggressive feeding schedule (EAF) which incorporated a high feed input during weeks 7-9 followed by restricted feed inputs. A late aggressive feeding schedule (LAF) which minimized feed inputs during weeks 7-9 followed by high feed inputs. An intermediate feeding schedule (IF) which utilized a more typical feeding strategy. At the conclusion of the 18th week of pond culture, the shrimp were harvested. Mean average shrimp weights were 15.2 g, 15.7 g, and 16.2 g, survivals were 81%, 79% and 78%, FCR were 1.5, 2.0 and 1.8, and average yields were 4,328, 4,384 and 4,398 kg/ha, for the EAF, LAF and IF treatments, respectively. No significant differences were found among treatments for survival, final weight, total yield or coefficient of variation of individual weights. The percentage of the population within

or above the 26-30 count, range were 55%, 67% and 72% for EAF, LAF and IF, however no significant difference were found among treatments. Gross income and returns above selected variable costs were not different among treatments. However, feed cost was significantly lower for the EAF treatment. Significant differences in FCR and consequently feed costs, strongly suggest an advantage of feeding aggressively at the beginning and more conservatively towards the end of the production cycle. This schedule reduces cost without affecting growth. Significant differences were found in FCR when comparing feed management based on feed tables against the target the FCR method. Using a targeted FCR to calculate feed inputs, reduced feed by 17% while achieving the same or greater yields.

Introduction

Feed management is one of the most important activities when considering water quality management (Boyd and Tucker 1998) and production economics (Jolly and Clonts 1993). It has been estimated that feed accounts for 55 to 60% of the operation cost in intensive systems and around 40% in semi-intensive systems (Chanratchakool *et al.* 1994, Lovell 1998). According to Wyban *et al.* (1989) survival and growth have the greatest impact on the economic performance of shrimp production, and adequate feeding is essential in attaining both of them. Adequate management should aim to optimized feed inputs, feed conversion ratio (FCR) and minimize the potential impact on effluents (Jory *et al.* 2001). Over the last decade there has been a tendency toward greater intensity in shrimp production, resulting in higher stocking densities and greater feed inputs which commonly result in a higher FCR (Peterson 1999, Peterson and Walker 2002). Most production failures, have been blamed on PL quality, feed, water quality and/or disease, although in most cases the origin of the problem is poor feed management (Cruz 1991, Piedad-Pascual 1993).

The need to improve feed management stems not only from economic considerations, but also from environmental issues. Shrimp pond water represents a potential environmental impact because only a portion of the nutrients in feed are consumed, assimilated and utilized for shrimp growth. A large unutilized fraction remains in the water column. Briggs and Funge-Smith (1994) estimated that in intensive systems, 95% of the nitrogen and 71% of the phosphorus was in the form of feed and

fertilizers, while harvested shrimp accounted for only 24% of the nitrogen and 13% of the phosphorus. Similar findings were reported by Green *et al.* (1995), who concluded that about 47 of the nitrogen and 55% of the phosphorus in semi-intensive shrimp ponds was derived from the feed and fertilizer but harvested shrimp accounted for only 37% and 20% of the applied nitrogen and phosphorus, respectively. The unutilized nitrogen and phosphorus in pond water promotes eutrophication in the pond system and in neighboring coastal waters where effluents are released (Boyd and Musig 1992, Dierberg and Kiattisimkul 1996, Boyd and Tucker 1998). When the environmental conditions in the area deteriorate, the effects are not isolated, but promote the outbreak of diseases and other water quality related problems. Which directly or indirectly affects production (Goddard 1996, Boyd and Tucker 1998, Jory *et al.* 2001, Samocha *et al.* 2001).

Traditionally, feed inputs are based on feeding tables which relate the feeding rates to percentages of the estimated biomass determined on a weekly basis (Villalon 1991, Wyban and Sweeney 1991, Brock and Main 1994, Jory 1995). Feed tables are the most commonly used method (Jory *et al.* 2001). Generally, the percentage used to calculate feed inputs decreases as the shrimp grow, but the total amount of feed increases proportionally with increasing shrimp biomass (Jory 1995). In most cases procedures for population assessments are applicable and economically justified in an attempt to narrow the error in the biomass determination. Even when population assessments are incorporated, accurate estimates are seldom obtained because procedures are complex and the estimates are often inaccurate (Hutchins *et al.* 1980). Part of the reason that feed management is a poorly understood “art” is because there are many other factors that play deterministic roles in the culturing process.

Feeding program

Good quality feed should be nutritionally adequate to meet metabolic requirements and support good growth. It is also important that there is a rapid and complete consumption of the feed to prevent waste (Goddard 1996). Ensuring adequate feed inputs and consumption for marine shrimp is complex and requires the consideration of a variety of factors. Because of the complexity, specific recommendations are difficult at best, but may even become impractical on a commercial scale. For best results, feeding procedures should be determined at each farm, for each feed type and culture species. Good feeding programs should be developed that account for seasonal and environmental factors (Jory 1995). Nevertheless, there are primary considerations that are applicable to most production systems. Important guidelines and considerations for feeding and pond management have been condensed into the following four points to be used in developing improved shrimp feeding programs:

1. Limit feed inputs.

Limiting the total amount of feed to be used during the weekly offerings and the total cycle will facilitate planning and ensure that feed is available when it is needed. The determination of an estimated total amount of feed to be used in the production cycle can be made by back calculating from a desired FCR. These calculations should take into account expected survivals and yields that are (based on historical records under similar pond management). Hence it is critical to maintain accurate production records which can then be used to predict average yields and survivals under standardized pond

management procedures, which then allow for the production forecasting. Once the total amount of feed for the cycle is determined, it should be divided between the weeks available for the culture period to determine amounts for weekly offerings. For example, a maximum limit for total weekly offerings could be established at 2 gram per shrimp per week. This is based on an average weekly shrimp growth of around 1 gram per week and a targeted FCR of 2 (Villalon 1991, Wyban and Sweeney 1991, Brock and Main 1994). Two grams of feed/shrimp/week offered to the estimated population should give a safe margin to avoid over or under feeding. In order to determine feed input one also needs to estimate the number of shrimp. The number of shrimp assumed alive should be adjusted on a weekly basis, taking into account typical mortalities during the production cycle (under similar management with similar type of feed and PL quality). Hence, using historical production a feeding program can be developed. Weekly weight monitoring under this type of management would help in determining general health conditions, but growth would not be the sole criteria to determine feed quantities. Weekly FCR should be calculated to better picture the feeding efficiency progress. In the case of a new production system for which there is no previous onsite record, references of production under similar conditions should be collected and cautiously used.

Another consideration is that daily feed inputs should not exceed the ability of the pond system to assimilate the byproducts and maintain a sufficient level of dissolved oxygen. When pond management is intensified and stocking and feeding rates are increased beyond the natural carrying capacity of the pond, water quality deteriorates (Cole and Boyd 1986). Low dissolved oxygen levels are usually the first indication that

the carrying capacity has been exceeded (Hopkins *et al.* 1992). In ponds without aeration, suitable feeding rates have been found to be around 25-30 kg/ha/day (Tucker *et al.* 1979).

Whereas with nighttime mechanical aeration it has been estimated that feeding rates can be sustain up to 100-120 kg/ha/day with (Boyd 1989, Boyd and Tucker 1998). Even if adequate aeration is provided to prevent dissolved oxygen depletion, other limiting factors such as high levels of ammonia may limit feed input and production (Boyd and Tucker, 1998). At excessive levels of aeration; other problems such as flow force and velocity can be created that can become detrimental to the shrimp (Peterson 1999; Peterson and Walker 2002).

Water exchange has also been used to minimize water quality problems encountered during shrimp production (Clifford 1992; Chien 1992; Hopkins 1993). This practice should be minimized since pond effluents constitutes a potential negative impact on receiving waters and will eventually inhibit production (Pruder 1992; Ziemman *et al.* 1992; Boyd and Musig 1992; Hopkins *et al.* 1995; Teichert-Coddington 1995; Teichert-Coddington *et al.* 1999; Boyd 2000; Lawrence *et al.* 2001).

2. Number of daily feedings.

Multiple daily feedings are desirable because shrimp eat slowly and more or less continuously (Lovett and Felder 1990; Lovell 1998). Disintegration of the feed particles and a loss of nutrients such as water soluble vitamins, free amino acids, some minerals and attractants can be expected when feed pellets are exposed to water for an extended period before consumption (Goddard 1996). This deterioration can be minimized through multiple daily feedings. Increased feeding frequency has the benefits of reduced nutrient

leaching and improves feed utilization efficiency (Wyban and Sweeney 1989; Villalon 1991; Robertson *et al.* 1993; Velasco *et al.* 1999; Nunes and Parsons 2000). Clifford (1992) suggests that the number of feedings should be based on the water stability of the feed used. That is, the less water stable the feed, the more frequent the feedings should be. Although increasing the number of feeding is often helpful, this increases labor costs and there is a limit to the number of feedings that are practical (Wyban and Sweeney 1989) who reported that six feedings per day did not improve growth. Moderate feed inputs of 2-4 times /day are often recommended.

3. Feed distribution.

Uniform distribution of feed throughout the pond also is important because shrimp do not move great distances to feed and they have territorial behaviors (Lovell 1998). Localized areas with anaerobic sediments where hydrogen sulfide and other toxic compounds are present. Areas immediately in front of aerators where flow is high should also be avoided (Jory 1995).

4. Environmental considerations.

Water temperatures and dissolved oxygen levels influence feeding activity, metabolism, and growth. Therefore these parameters should have a fundamental importance in the determination of both the types and quantities of feeds used (Goddard 1996). Cloudy days or prolonged cloudy conditions limit algae photosynthetic activity and consequently lowers DO concentrations. On the other hand extreme warm sunny and/or calm days may cause algal crashes, pond stratification, and high temperatures that

affect shrimp feeding activity. All these weather conditions should be noticed and taken into account daily for a good feed management schedule. Hence, it is critical to predetermine feed rates as guidelines but knowing that they must be adjusted based on the current situation.

Bottom soil conditions play a major role in influencing water quality and aquatic production in ponds (Hajek and Boyd 1994; Boyd 1995). Feed input is the major factor causing deterioration in pond bottom soils. Feces and unconsumed feed pellets settle to the bottom. When feed amounts are excessive, organic matter builds up on pond bottoms, and accumulates in the interstices between soil particles, limiting oxygen diffusion into the soil and generating anaerobic conditions (Boyd 1995, Lin 1995). Anaerobic soils lead to high concentrations of metabolites such as NH_3 , NO_2 and H_2S (Boyd, 1992; Fast and Boyd 1992; Hopkins *et al.* 1994.) which are toxic to shrimp (Boyd 1995; Muir and Owens 1997). Also sulfide oxidation demands higher dissolved oxygen. This increases the risk of shrimp mortalities if the soil-water boundary is disturbed. It also requires that the overlying water is maintained at 70% saturation (Ritvo *et al.* 2000). Deteriorated pond bottom conditions can be minimized by conducting periodic pond bottom assessments to detect the presence and build up of uneaten feeds followed by appropriate pond management.

Feeding early

It has been suggested that postlarvae utilize mostly natural feed for the first few weeks after stocking and therefore do not require any additional feed (Chanratchakool *et al.* 1994). There has been considerable research on the role of natural food organisms on shrimp production. Penaeid shrimp cultured in extensive aquaculture ponds, with annual

yields of several hundred kg/ha, feed almost totally on natural pond biota (Pruder 1987). Moss *et al.* (1992) consider that food items consumed by shrimp in these systems include detrital aggregates, microalgae, nematodes, copepods, amphipods, polychaetes, molluscs, and others. In more intensive ponds, with yields from several hundred to several thousands kg/ha annually, the contribution of natural pond biota to shrimp growth is still considered to be important, even though significant quantities of pelleted feed may be provided (Rubright *et al.* 1981). Anderson *et al.* (1987) demonstrated that 53-77% of *L. vannamei* growth in semi-intensive systems (20/m² in cages) was derived from the consumption of natural food organisms, while formulated feeds accounted for the remaining 30-40%. Bianchi *et al.* (1990) demonstrated that 70-80% of the weight gain of *L. vannamei* reared under laboratory conditions was attributed to the consumption of bacterial flock. Some experiments indicate that even under growout conditions, shrimp growth is enhanced by unknown growth factors produced autochthonously in the intensive shrimp pond (Leber and Pruder 1988; Moss *et al.* 1992; Moss 1995).

It has also been assumed by some farmers that shrimp cultured at high densities, acquire limited nutrition from natural pond biota and that primary production is to variable and is hence unreliable. Based on this, farmers assume that early feeding is beneficial and offer feed to shrimp in growout ponds, even when ponds have been recently stocked (Jory *et al.* 2001). There are few studies related to the proper feeding rate of shrimp from PL to juveniles in ponds, nevertheless the need of natural food supplementation with a formulated feed has been recognized (Rubright *et al.* 1981). Feed quantities used during this initial period commonly are small and the offering is usually justified because it acts as a fertilizer even if it is not consumed directly (Chanratchakool

et al. 1994). Initial feeding rates often range from 0.5- 4.5 kg feed per hectare (Jory *et al.* 2001).

Despite the importance of feed management there are few studies that have evaluated feeding strategies or feeding programs. The majority of the information available discusses the basic nutrient requirements of various shrimp species during their different stages. Few references are available that incorporate effective pond management practices to help develop feeding programs. Most feed management protocols are based on the use of feeding tables. This study was conducted with the objective of incorporating general aquaculture considerations into a management and feeding program. The three treatments included 1) More aggressive feeding at the beginning with reduced feed late in the cycle, 2) less aggressive feeding at the beginning with the most aggressive feeding late in the cycle and, 3) an intermediate feeding program which is more similar to commonly used programs on commercial farms.

Methods

Nursed juvenile shrimp were stocked into four replicate production ponds at a density of 35 shrimp/m². Ponds used for the growout phase were approximately 0.1 ha in surface area, rectangular (46 x 20 m), with a 1.0 m average depth. Each pond was equipped with a 20-cm diameter standpipe, a concrete catch basin, and lined with 1.52 mm thick high-density polyethylene sheeting (Grundle Lining System, Inc., Houston Texas, USA). The sloped pond bottoms were covered with a 25-cm deep layer of sandy-loam soil which was dried and tilled to a depth of 10-15 cm prior to filling. Ponds were filled with water from the Intracoastal Canal between Mobile and Perdido Bay three weeks

prior to stocking. Fill water was filtered through a nylon filter sock (Domestic Lace Mfg., Inc.) to prevent the introduction of large predators, and minimize the introduction of larval species while allowing the introduction of small planktonic organisms. Two weeks before stocking, all ponds were fertilized with an application of liquid, inorganic fertilizers, at a ratio of 1:2 (N:P₂O₅), and a rate of 4 kg/ha N (Boyd and Tucker 1998). A mixture of 1.68 L of 10-34-0 and 402 ml 32-0-0 and pond water was prepared in a 208 L container, and then slowly dripped into each pond while operating a paddlewheel aerator. Fertilization was used to maintain a minimum Secchi disk reading within the range of 25-40 cm. Depending on response of the algae in individual ponds, a second application at half the initial rate was added two weeks after the first application. Twenty four hours before stocking, a 1:15 motor oil and diesel fuel mixture at a rate of around 9 L/ha, was applied evenly over each pond surface to reduce the number of air breathing insects.

Each pond had a 1-hp (7.5kW/ha) spiral paddle wheel aerator (Little John aerator, Southern Machine Welding Inc. Quinton, AL) representing aeration capacity of 10 hp/ha. In emergency situations an additional 1-hp (7.5kW/ha) propeller aspirator aerator (Aire-O₂, Aeration Industries International, Inc. Minneapolis, Minnesota) was added to the ponds to maintain dissolved oxygen levels.

Aerators were operated during the night (8 hrs.) to maintain oxygen concentrations above a set limit of 3 mg/L. Dissolved oxygen (DO) levels, pH and salinity, were monitored with a YSI 556 DO meter, (Yellow Spring Instrument Co., Yellow Springs, OH, USA) three times a day, at sunrise (0500), at noon time(1200) and after dark (1900). Weekly water samples were taken in all ponds with an 80-cm water column sampler (Boyd and Tucker 1992) early in the morning and analyzed for total

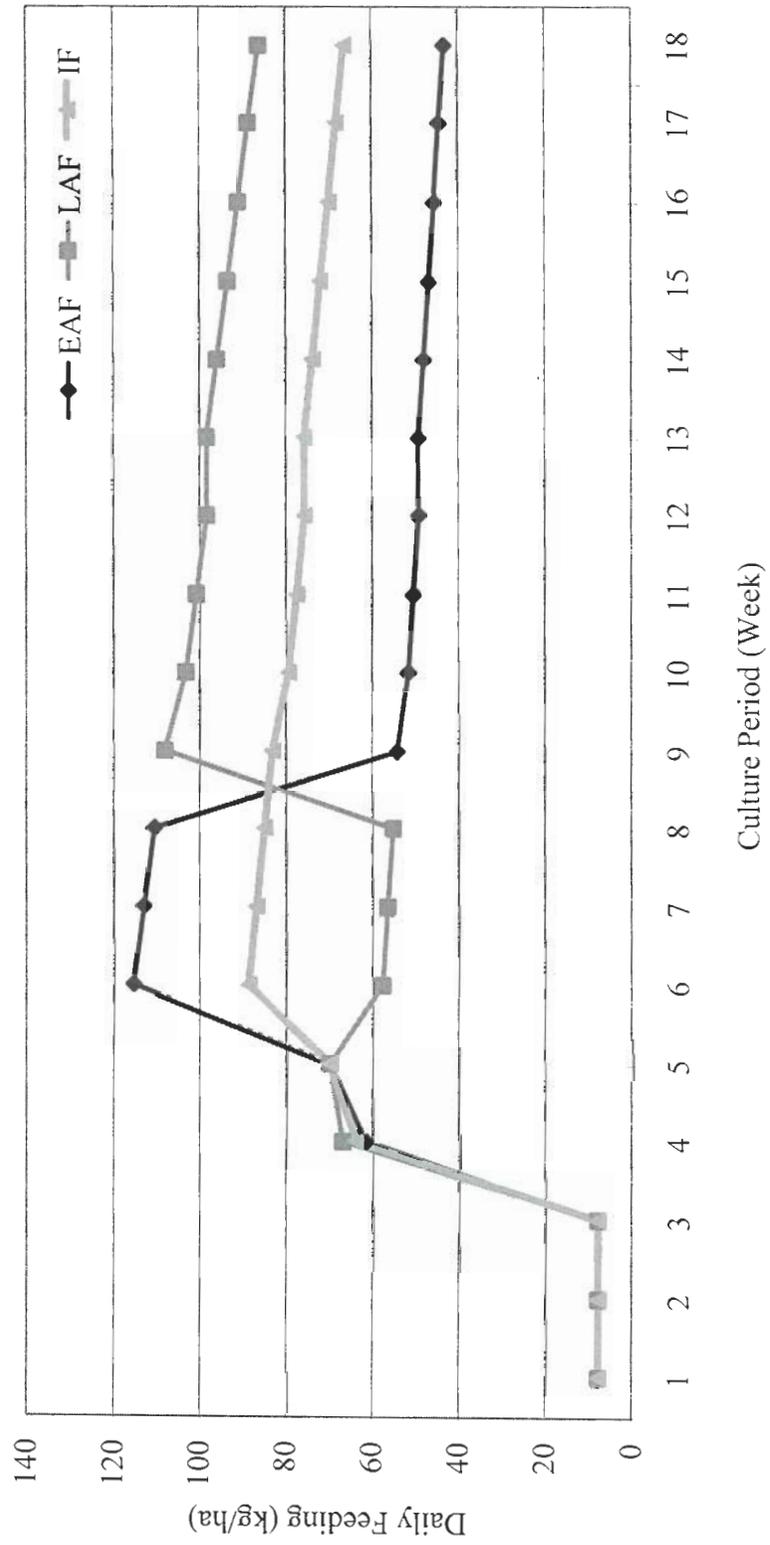
ammonia nitrogen measured with a spectrophotometer (Spectronic 20 Genesys, Spectronic Instrument Inc. Rochester, NY, USA) and the Nesslerization method (APHA 1989). Secchi disk visibility readings were taken once a week. Results of water quality determinations were averaged over time for each treatment. Water was added to the ponds primarily to replace evaporation losses. During the last two weeks of culture, water was exchanged at an approximate rate of 7% / day for three consecutive days, because it was the phase of highest shrimp standing crop. Water exchange was needed for the maintenance of desirable water quality, reduction of the phytoplankton densities and minimization of the risk of a sudden algae die-off.

The three feeding treatments evaluated were an early aggressive feeding schedule (EAF), a late aggressive feeding (LAF) schedule and an intermediate feeding (IF) schedule. Shrimp were fed twice a day with a sinking 35% protein (Burriss Mill & Feed, Inc., Franklinton, L.A, USA) pelleted feed (Appendix 1). Feeding took place in the morning and late in the evening. Daily feeding amounts were calculated based on a targeted FCR, adjusted for mortalities (30% mortality for the total 18-week culture period, adjusted weekly). All treatments were fed equally through the first five weeks of culture. The first two weeks shrimp were fed at a rate of 8 kg/ha/day. Beginning the third week, feed inputs were adjusted to 15% of the estimated biomass and then gradually reduced to 14% and 10% for the 4th and 5th week, respectively. From the 6th week on and until the end of the 8th week, treatments were fed, back calculating from targets FCR of 2.6, 1.3 and 2.0, respectively, for EAF, LAF and IF. And from the 9th week half and on of the culture period the FCR were interchange for the EAF and LAF treatment (Fig.1). The IF treatment was fed based on a targeted FCR of 2.0 for the whole culture period.

Shrimp were sampled by seine for the third and fourth weeks and by cast net (monofilament net, 1.22 m radius and 0.95 cm opening) during the remainder of the culture period. Weekly assessments included a visual observation of appearance and average weight. Sampling took place in the early morning hours to reduce stress. Feed calculations incorporated the adjusted population, size and the targeted feed conversion rates for each treatment. A random sampling technique consisted of cast netting in nine locations of each pond. Repeated sampling aimed at building a 95% confidence interval (of mean ± 2) for population estimates (Hutchins *et al.* 1980) was conducted close to harvest to evaluate the reliability of the procedures to estimate population.

Maximum feeding rates were set at 110.5 kg/ha/day, 108 kg/ha/day and 89 kg/ha/day for EAF, LAF and IF. Feeding ceased two days prior to harvest. Harvest took place upon finalizing the 18th week of culture period (Sept 3rd-5th). Harvesting was accomplished by draining two thirds of the water from each pond during the night before harvest. Aeration through the night was accomplished as needed, using only paddlewheel aerators to minimize erosion on pond bottoms. On the day of harvest, the remaining water in each pond was pumped out through a hydraulic fish pump with a 25-cm suction (Aqualife-Life pump, Magic Valley Heli-arc and Mfg, Twin Falls, Idaho, USA). The pump was placed in the catch basin and shrimp were pumped and dewatered as they were moved to the harvest truck. Shrimp were then taken to a wet lab to be washed and weighed. During weighing, a random sample of 100 shrimp was collected for individual weights. Individual weights were used to calculate mean weights, survivals and size distributions.

Figure 1. Daily feeding for *Litopenaeus vannamei* stocked at 35 shrimp/m² and culture in growout ponds for 18 weeks under three feeding strategies, EAF (Early aggressive feeding), LAF (Late aggressive feeding) and IF (Intermediate feeding).



Data Analyses

The data was analyzed by a one-way Analysis of Variance. The Student-Newman-Keuls multiple comparison test was utilized to determine significant differences among treatment means. Analyses were done using SAS program version 8.2 (SAS Institute Inc., Cary, NC). Significant differences among treatment means were determined at a probability level of $P \leq 0.05$.

Results

At the conclusion of the 18th week of culture, mean average shrimp weights were, 15.2 g, 15.7 g, and 16.2 g, survivals were 81%, 79% and 78%, FCR were 1.5, 2.0 and 1.8, and average yields were 4,328, 4,384 and 4,398 kg/ha, for EAF, LAF and IF treatments, respectively (Table 1). One pond C5 from the LAF treatment was excluded from the study. Survival in this pond was 59% and yields of 3,164 kg/ha were about 30% below the average of the rest of the replicates. In this pond, the lowest DO values were 2.54 mg/L, 3.48 mg/L and 3.04 mg/L. There was only one algal crash experienced in this pond on the 52 day. Since there was no clear reason for the low survival, it may be that the pond was under stocked and hence was excluded. No significant differences were found among treatments for survival, final weight or total yield. Significant differences for the three treatments were found for FCR; with the best FCR found in EAF and the poorest in the LAF.

Table 1. Production characteristics of *Litopenaeus vannamei* stocked at 35 shrimp/m² and culture for 18 weeks in growout ponds under three feeding management strategies; EAF (Early aggressive feeding), LAF (Late aggressive feeding) and IF (Intermediate feeding)¹. n = 4.

Treatment	Survival %	Mean Weight g	Mean Yields kg/ha	FCR ²	Size Distribution CV ³
EAF	81 ^a	15.2 ^a	4,328 ^a	1.5 ^a	15.0 ^a
LAF ⁴	79 ^a	15.7 ^a	4,384 ^a	2.0 ^b	15.4 ^a
IF	78 ^a	16.2 ^a	4,398 ^a	1.8 ^c	14.1 ^a
<i>P</i> value	0.739	0.388	0.859	0.001	0.7451

¹Means not sharing a common supercript within a column are significantly different ($P \leq 0.05$) based on Student-Newman-Keuls test.

²FCR = Total weight of feed given / Biomass increase

³CV= Standard deviation / mean * 100

⁴n=3

Results of water quality analysis from the growout phase are summarized in Table 2. Pond salinity was initially around 10-11 ppt and ended in the 7.6-7.9 ppt range. Pond water pH readings were taken at the same time dissolve oxygen reading were taken (early morning and late in the evening), and were around 7.0 for the morning reading and 8.0 for the evening hours. For the three treatments, overall averages of total ammonia-nitrogen were below 0.3 mg/L NH₃-N. Highest observed TAN readings were, 3.4, 1.9 and 1.5 mg/L NH₃-N for EAF, LAF and IF, respectively. The average pH and temperatures were 7-8 and 28-29 C, respectively. The fraction of un-ionized ammonia out of these TAN readings could have been as high as 0.075, resulting in an un-ionized concentration of 0.25, 0.22 and 0.11 NH₃-N for EAF, LAF and IF, respectively (Boyd and Tucker 1998).

Average dissolved oxygen readings for the three treatments were around 5 mg/L for early morning readings and around 6.0 mg/L for night readings. Average pond temperatures for early mornings and late evenings were 28.3 ± 2.2 C and 29.4 ± 1.9 C. Lowest temperatures registered were of 19.9 C and the highest readings were 32.7 C.

Initially pond water had Secchi disk readings in the range of 80-110 cm. After fertilizer application, ponds developed plankton blooms and by the 5th week, readings were in the 25-30 cm range. By the end of the production cycle all ponds had heavy blooms with secchi disk readings in the range of 10-25 cm. Weekly fluctuations in Secchi disk readings were the results of bloom and crash cycles that took place in different ponds during the production cycle (Appendix 9) . Whenever an algae die-off

Table 2. Overall pond water quality average by treatment over the 18-weeks growout period for *Litopenaeus vannamei* stocked at 35 shrimp/m², under three feed management protocols; Early aggressive feeding (EAF), Late aggressive feeding (LAF) and Intermediate feeding (IF). Figures are displayed as mean ± standard deviation and minimum/maximum values in parentheses.¹

	EAF	LAF ²	IF	P value
Morning pH	7.2 ± 0.3 ^a (8.41, 6.84)	7.3 ± 0.3 ^a (8.3, 6.9)	7.3 ± 0.3 ^a (8.5, 6.9)	0.5470
Night pH	8.1 ± 0.6 ^a (9.6, 7.1)	8.2 ± 0.6 ^a (9.6, 7.2)	8.2 ± 0.6 ^a (9.8, 7.1)	0.1990
Salinity (ppt)	7.6 ± 2.0 ^a (11.1, 4.5)	7.9 ± 2.0 ^a (11.6, 4.7)	7.7 ± 2.1 ^a (13.9, 4.6)	0.7620
TAN (mg/L)	0.26 ± 1.1 ^a (3.39, 0)	0.1 ± 0.8 ^a (1.92, 0)	0.1 ± 0.7 ^a (1.5, 0)	0.3810
Secchi (cm)	25.4 ± 7.4 ^a (43.8, 16.3)	26.8 ± 6.11 ^a (38.8, 18.0)	25.6 ± 6.43 ^a (37.5, 16.3)	0.4500
Morning DO (mg/L)	5.2 ± 1.5 ^a (11.4, 2.3)	5.0 ± 1.4 ^a (9.6, 1.5)	5.2 ± 1.4 ^a (9.4, 2.0)	0.6540
Noon DO (mg/L)	8.4 ± 3.1 ^a (18.8, 3.3)	8.5 ± 3.4 ^a (21.1, 2.6)	8.3 ± 2.9 ^a (16.6, 2.9)	0.7210
Night DO (mg/L)	6.8 ± 2.8 ^a (22.2, 2.5)	6.6 ± 2.6 ^a (19.2, 2.2)	6.6 ± 2.3 ^a (20.0, 2.1)	0.6270
Morning Temp (C)	28.2 ± 2.0 ^a (31.1, 18.4)	28.5 ± 1.5 ^a (31.4, 25.0)	28.2 ± 2.9 ^a (30.9, 6.0)	0.3810
Night Temp (C)	29.4 ± 1.7 ^a (32.7, 24.5)	29.4 ± 2.0 ^a (32.8, 20.0)	29.31 ± 2.12 ^a (32.6, 17.03)	0.5810

¹Means (n=4) not sharing a common superscript within a row are significantly different (P ≤ 0.05) based on Student Newman-Keuls test.

²n=3

was noticed or forecast, in anticipation at least two aerators were set to operate continuously for as long it took for a new bloom to develop and the oxygen cycle to return to a typical day-night cycle.

Discussion

In this trial, with a density of 35 postlarvae/m², using night time aeration (0.75 kW/ha) and limited water exchange, overall pond survivals ranged from 73-81%, yields from 4,300-4,400 kg/ha and FCR ranged from 1.5 to 2.0. On the same experimental site, and using similar aeration (0.75 kW/ha) and densities (33-35 PL/m²), McGraw (2000) reported his highest yields achieved were 3,975 kg/ha with 61% survival, and Garza (2001) when using a fixed-FCR feeding method reported 3,747 kg/ha with 77% survival. Other references, using greater aeration (3.7 kW/ha) but lower density (25 postlarvae/m²) obtained 2,852 kg/ha (Wyban *et al.* 1989) with an FCR of 3.2, and with considerably greater aeration (7.5 kW/ha), a higher density (45 postlarvae/m²), a culture period of 169 days and 17% water exchange, yields of 7,500 kg/ha have been obtained, but with an FCR as high as 2.5 (Sandifer *et al.* 1987).

The implementation of standard stocking procedures, standard management procedures and adjustments of the population on a weekly basis based on an average mortality from historical records, was effective in conducting a reliable management program. Overall yields and FCR were 9% and 6-10% better than estimated (Appendix 10 and 11). The population assessment procedure which was evaluated during the last week of the culture period was 30-50% below the actual population density as

determined at harvest. This range was considered too wide for effective managerial purposes (Appendix 12).

The three treatments had similar growth patterns and none showed a growth decline during the culture period (Fig 2). The initial coefficient of variation of individual shrimp weights (from the hatchery) was 43%, after the growout phase, size distributions in all treatments were within the 14-16% reported by (Brock and Main 1994). These values were also consistent with CVs observed in previous trials (Chapter 2 and 3). Although the final growout phase, CVs were not significantly different among treatments (Table 1), it is important to consider the graphical pattern of size distributions (Fig.3). Count sizes that accounted for more than 1% of the population included five for EAF and IF and six for LAF. For all three treatments the predominant count size was 26-30 per pound (average size of 16 g), however this count size accounted for 42.4 %, 43.7 % and 50.6 % for EAF, LAF and IF treatments, respectively. When considering the percentage of the population within the 26-30 counts and above, 55% , 67% and 72% were determined for EAF, LAF and IF, respectively (Table 3). Although the IF treatment based on this criteria had apparently better size distribution, with a greater fraction of its population in the largest count sizes, statistically no significant difference was found among treatments for total yields of 26/30 and larger counts.

The EAF feeding strategy incorporated a high feed input early in the cycle to maximize early growth of the shrimp with feeding rates reduced during the second half of the cycle when water quality could be more unstable. The LAF treatment minimized early feed inputs when natural productivity should be adequate and maximized feed

Figure 2. Mean weights of *Litopenaeus vannamei* stocked at 35 shrimp/m² and culture in growout ponds for 18 weeks under three feeding strategies, EAF (Early aggressive feeding), LAF (Late aggressive feeding) and IF (Intermediate feeding).

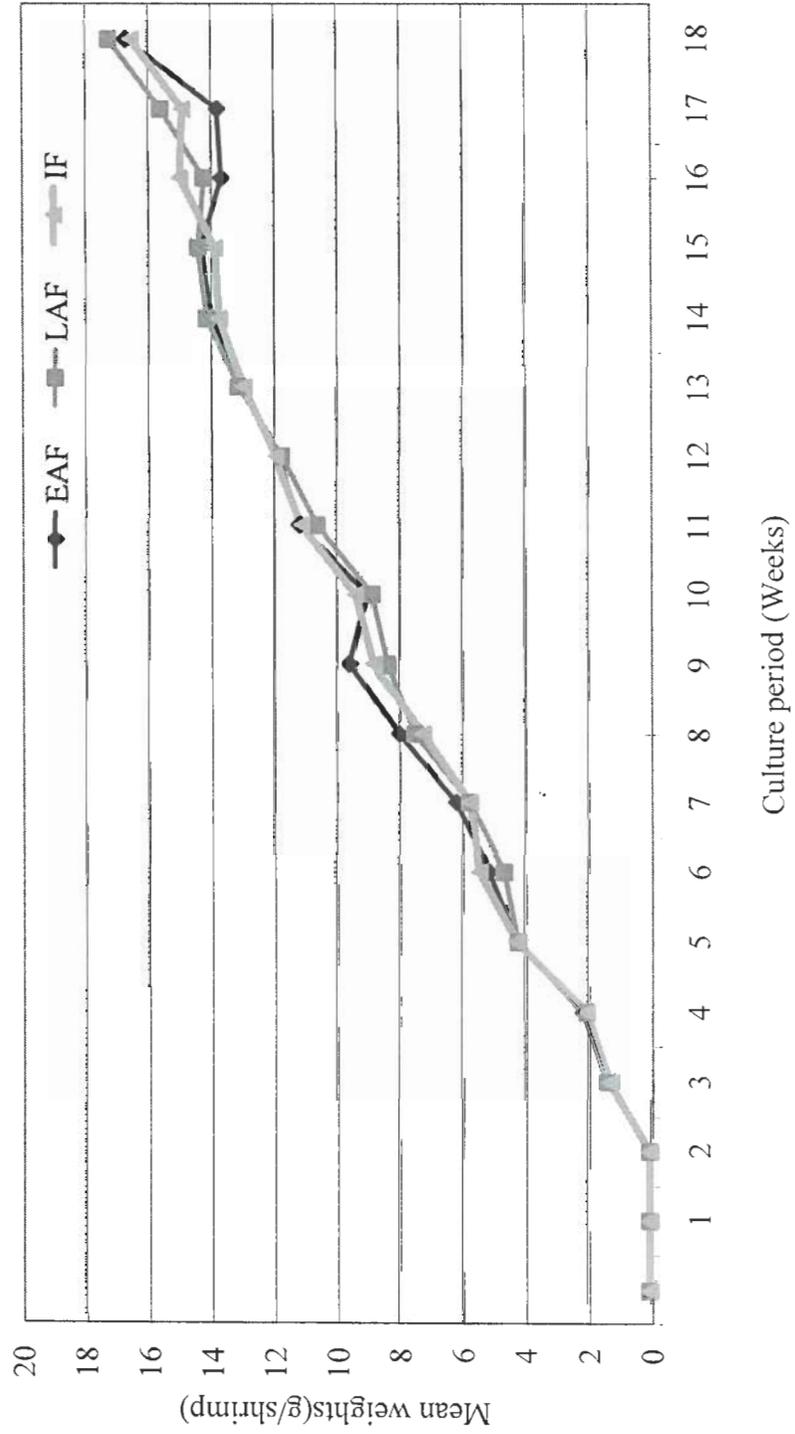


Figure 3. Population size distribution of total shrimp weights for *Litopenaeus vannamei* stock at 35 shrimp/m² for 18 weeks in growout ponds and managed under three feeding strategies, EAF (Early aggressive feeding), LAF (Late aggressive feeding) and IF (Intermediate feeding) and Control.

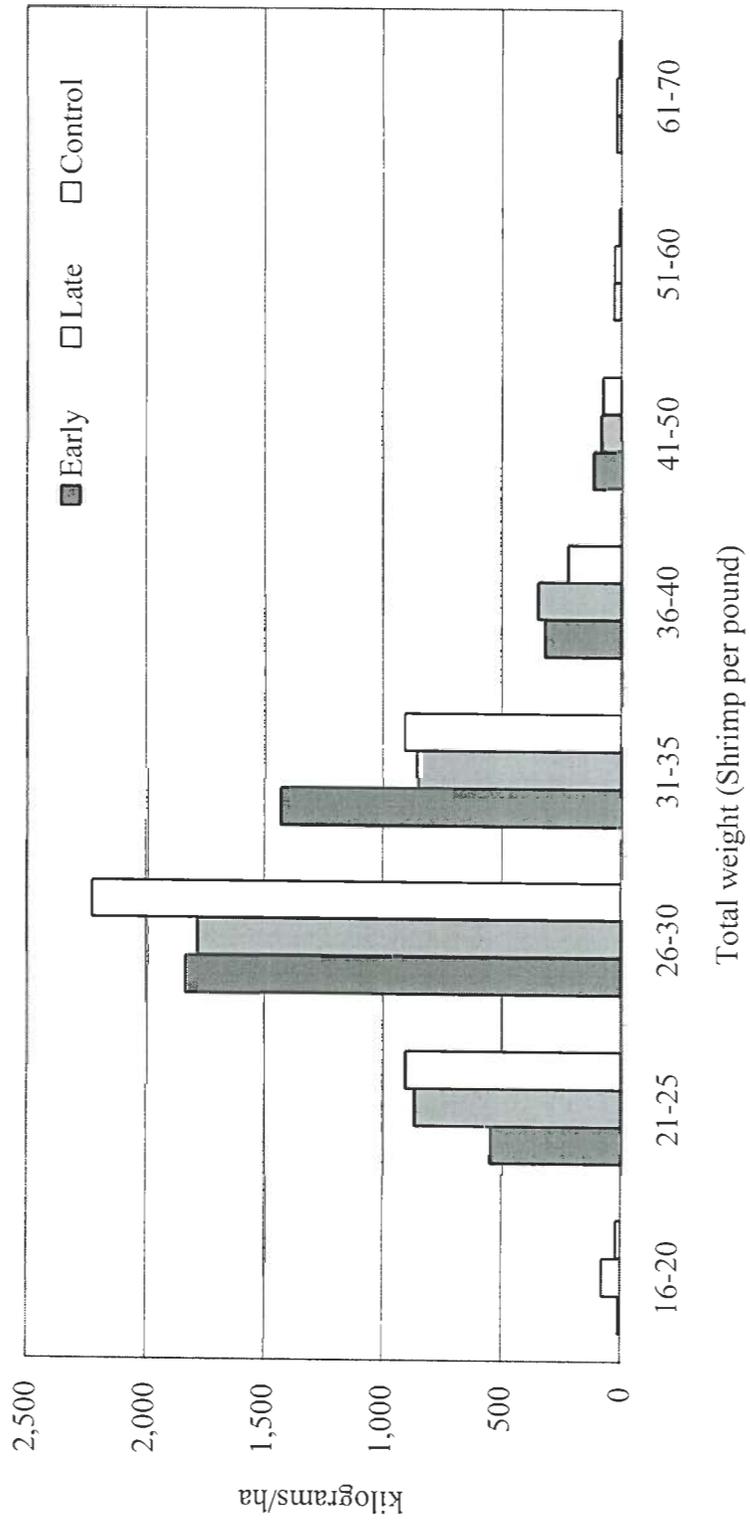


Table 3. Population percentages of a head-on count size of 26/30 and up of *Litopenaeus vannamei* stocked at 35 shrimp/m² and culture for 18 weeks under three feeding strategies; EAF (Early aggressive feeding), LAF (Late aggressive feeding) and IF (Intermediate feeding)¹. n = 4.

Treatments	Yields (kg/ha)	Above 26-30 (kg/ha)	Above 26-30 %
EAF	4,328 ^a	2,395 ^a	55
LAF ²	4,384 ^a	2,873 ^a	67
IF	4,398 ^a	3,151 ^a	72
<i>P</i> value	0.8592	0.2744	0.2196

¹Means not sharing a common supercript within a column are significantly different ($P \leq 0.05$) based on Student-Newman-Keuls test.

²n=3

inputs late in the cycle when natural productivity was more likely to be limited. The third treatment or intermediate feed strategy utilized a more typical feeding strategy between the first two treatments. Although it was observed that the EAF strategy had greater survival with a large CV, no significant differences were found among treatments for any of the production variables evaluated.

Salinity fluctuations were typical for this facility, with reduced values primarily caused by precipitation during the culture period. However, salinity fluctuations toward the end of the cycle could have been influenced by water exchanges. High algae concentrations took place due to a variety of reasons, and did not appear to correspond to a specific feeding protocol. Nine algal crashes were experienced during the production cycle. Observed algal crashes took place as early as the 52nd day in the LAF treatment with a feed input as low as 58 kg/ha/day, or as late as the 100th day in the EAF treatment which was receiving a feed input of 48 kg/ha/day (Appendix 9). Algal crashes appeared to occur without any relation to feeding level and were usually related to extremely warm sunny days continuous cloudy periods, heavy rains periods, or dense algal blooms (Secchi readings less than 15 cm). There are a number of ways to assess the algae conditions (e.g. cell counts of chlorophyll *a* determinations). The most practical methods to predict when an algal crash is about to occur was usually the observed color, especially color changes, shallow Secchi disk readings and low D.O readings.

In this study, early morning DO readings were useful to ensure that the night time operation of aerators was enough to maintain DO above 3.0 mg/L. However, to maximize energy efficiency usage, aerators in the morning normally were turned off. To reduce the risk of low daytime D.O., which may occur in the case of algae that is crashing, already

crashed or having low photosynthetic activity due to cloudy conditions, mid day D.O readings were also taken. Because it was noticed that pond stratification during the day could result in differences of 3-4 C and 4-5 mg/L of DO differences between the upper and lower water column, aerators were routinely operated for at least 15-30 minutes to mix the water column and reduce stratification just before noon. Benefits of circulation have been widely emphasized (Fast *et al.* 1983; Geiger 1983; Rogers 1989; Brune and Garcia 1991). The noon DO readings provided data on the algae's photosynthetic activity and was useful for determining if stratification was still taking place. These readings helped in identifying ponds with extremely high midday readings that more likely needed careful attention at night. Noon readings often identified daytime pond situations that could have turned catastrophic if actions were not taken early. Although continuous aerator operation has been reported in intensive shrimp production (Wyban *et al.* 1989, Hopkins *et al.* 1992; Hopkins *et al.* 1993), keeping aerators operating permanently was avoided as much as possible. Mid-day operation was limited to destratification purposes, ponds under an algal crash or under extreme weather conditions (continuous cloudy days). In the same experimental station, growout pond trials with *L. vannamei* using daytime continuous aeration, achieved poorer FCR and pond bottoms in general had much more deteriorated conditions (Garza 2001). Garza (2001) was feeding based on a fixed FCR of 1.6, but the achieved FCR at the end of the cycle was of 2.03 , or 21% off the desirable target, and with less days of feeding input than this trial (112 days vs. 130).

According to Peterson (1999) it is critical when using mechanical aeration, not to exceeded a maximum limit force of 0.01 N/m^2 shear stress (force applied on the area of sediment-water interface). In the same study it was determined to be better if sheer stress

is below 0.003 N/m^2 during feeding times. The theory that Peterson considered is that below 0.01 N/m^2 , the flow makes it possible to sort organic and mineral particles, leaving the latter ones undisturbed. At full speed paddlewheel and propeller aspirator aerators generally produce 10 kg per motor per horsepower which is equivalent to 0.01 N/m^2 (Peterson 1999) under the experimental conditions of this research (0.1 ha pond area with 1-2 hp/pond aeration) the force of the flow was between 10 or 20 times greater, or 0.1-0.2 N/m^2 , so limiting aeration to indispensable conditions was an important aspect in the management.

Comparison of feeding methods

Although survival and other factors may cause variation, in terms of total amounts of feed offered, it has been observed that when total feed offerings were 6,000-6,500 kg/ha with survivals of 68-88%, FCR average around 1.65-2.1 (Chapter 2), with feed offerings in the range of 9,000-9,600 kg/ha and survivals of 62-64% FCR average 2.5-2.7 in (Chapter 3), and in this trail with feed offerings of 6,500 (EAF)-7,960 (IF) kg/ha and survivals of 81-77.8% , FCR averaged 1.5-1.8. This was consistent with results in Chapter 3, when feed inputs were of 9,070 kg/ha (LAF) even with survivals of 80% FCR averaged 2.0. This suggests that even under close pond management and acceptable survivals, total feed offerings above 8,000 kg/ha tend to be detriment to FCR

Contrary to Garza (2001) who did not find significant differences between feeding tables and targeted FCR methods, the targeted FCR method under the implemented feeding program was useful in reducing feed by an average of 17% when compared to a previous study that was based on typical feed tables (Chapter 3).

Experimental ponds on both of these growout trials were stocked at the same density, however shrimp in the previous trial (Chapter 3) were cultured for 16 weeks as compared to the 18 in the present study. When analyzing the FCR of the two trials, significantly lower FCR ($P < 0.0001$) were found on the targeted FCR (mean FCR 1.77) and culture for 18 weeks, than those based on feeding tables (mean FCR 2.6) and culture for 16 weeks.

Economics

For management comparison, returns above selected variable costs (RASVC) were calculated to compare the three feeding strategies. Variable costs included: seed (PL cost), energy (kWh), and feed (kg). Since all ponds were stocked at the same density (35 PL/ m²) and PL were obtained from the same hatchery the total PL cost was the same for all three treatments. All ponds also were managed under the same protocol so other variable costs were considered among treatments.

It is beyond the scope of this research to discuss returns above total costs (variable and fixed) however calculations were made and none of the treatment generated positive returns, mainly because of limited economy of scale on human resource utilization and depreciation costs of the infrastructure and equipment, that are appropriate for research but more sophisticated than needed for average production conditions (Appendix 13, 14, 15).

Using off-vessel prices paid in the Gulf coast region according to the Alabama Marine Resources Division (AMRD 2003) and the yields per count size per treatment (Table 4), the gross income (\$/ha) was determined (Table 5). Shrimp smaller than a

Table 4. Overall yields (kg/ha) divided into production per count size for each feeding treatment. Prices are based on Alabama Gulf coast vessel prices (\$/Lb) coast off vessel white shrimp prices (\$/Lb) in 2003.¹

Counts	kg/ count			Price \$/lb ³
	EAF	LAF ²	IF	
16-20	11	14	21	2.34
21-25	549	894	905	1.92
26-30	1,833	1,966	2,225	1.92
31-35	1,431	975	909	1.53
36-40	322	393	226	1.42
41-50	117	85	79	N/A
51-60	32	30	12	N/A
61-70	22	28	11	N/A
71-90	0	0	0	N/A
91-100	0	0	12	N/A
101-110	11	0	0	N/A
110-120	0	0	0	N/A
Kg/ha	4,327 ^a	4,384 ^a	4,398 ^a	

¹Means not sharing a common supercript within a row are significantly different (P ≤ 0.05) based on Student-Newman-Keuls test.

² n = 3

³According to AMRD for 2003.

Table 5. Gross income for *Litopenaeus vannamei* stocked at 35 shrimp/m² and culture in growout ponds for 18 weeks using three feeding strategies. EAF (Early aggressive feeding), LAF (Late aggressive feeding) and IF (Intermediate feeding)¹.
n = 4.

Counts	Gross Income		
	EAF	LAF ²	IF
16-20	59	73	110
21-25	2,295	3,737	3,783
26-30	7,743	8,303	9,397
31-35	4,816	3,282	3,059
36-40	1,006	1,226	705
\$/ha	15,943 ^a	16,661 ^a	17,093 ^a

¹Means not sharing a common supercript within a row are significantly different (P ≤ 0.05) based on Student-Newman-Keuls test.

²n=3

36/40 count (11 g) were considered of negligible value and of no contribution to the gross income calculated. No significant difference was found among treatments for gross income. Related to the significant difference found in FCR, feed costs were also significantly different among the three treatments; EAF with the lowest and LAF with the highest costs. No significant difference was found for electricity costs among treatments. Returns above selected variable costs were \$6,559/ha, \$5,813/ha and \$6,685/ha for EAF, LAF and IF, respectively, but no significant difference was found among treatments (Table 6).

Although it appeared that a larger portion of the shrimp population was in the 26-30 size category in the IF treatment (72%), when compared to the EAF treatment (55%) and LAF treatment (67%), differences were not significant (Table 3). Gross income and RASVC were not significantly different among treatments, it is worth pointing out that feed cost were significantly lowered in the EAF treatment, indicating that by feeding aggressively at the beginning and more conservatively towards the end of the production cycle is possible to reduce cost without affecting growth.

Although no significant differences were found for the different variables analyzed to support the theory that EAF sustains less competitive individuals; significant differences in feed used and consequently its costs, are sufficient to indicate an advantage of an EAF treatment, since similar yields were obtained with less feed. The EAF treatment, with an initial abundance of feed, and reduced feed towards the end, avoided

Table 6. Returns above selected variable costs \$/ha for three feeding strategies used to culture *Litopenaeus vannamei* in ponds over a 18 week period. Feeding strategies included EAF (Early aggressive feeding), LAF (Late aggressive feeding) and IF (Intermediate feeding)¹. n = 4.

SVC	EAF	LAF ²	IF	P value
Seed	3,185	3,185	3,185	
Feed	3,345 ^a	4,626 ^b	4,064 ^c	0.0001
Electricity	2,854 ^a	3,037 ^a	3,159 ^a	0.6503
\$/ha	6,559 ^a	5,813 ^a	6,685 ^a	0.7724

¹Means not sharing a common supercript within a row are significantly different (P ≤ 0.05) based on Student-Newman-Keuls test.

²n=3

high inputs that may be unnecessary especially when the primary productivity is high enough to supplement the diet.

The management risk in the EAF also is lower. If early in the cycle feeding goes beyond the quantity really needed (which would never be known exactly) the excess quantity will still be well within the assimilative capacity of the pond. This will ensure the provision of plenty of feed, giving a better opportunity for the smaller, less aggressive shrimp to receive their share (Lovell 1998) and representing a minimum risk toward deteriorating the pond bottom condition.

On the other hand, if feeding is most abundant towards the end of the cycle and incurs in excess, the excess amount would more likely contribute to the pond bottom deterioration, especially after the pond has been receiving inputs for more than half the cycle. Based on the results of this study it did not caused a significant advantage in production characteristics over the other treatments.

When feed inputs are based primarily on feeding tables, an increment in the average weight can be misleading and result in an increase in the amount of feed offered. Ponds where pond bottoms have been deteriorated or any other management failure have taken place, may cause an unnoticed mortality. The sampled shrimp on which the mean weight was determined could be growing faster due to the lower density. Increasing the feed in this situation further deteriorates the pond bottom and further complicates management. This is one of the reason why more criteria is needed when doing feeding determinations, and pond bottom assessment giving a realistic feedback of the pond management. Even if no mortality has taken place, over feeding towards the end of the cycle represents a greater risk of deteriorating pond conditions, because the amounts of

feed offered tends to be greater making the potential margin of error larger. Under this feeding program, the equivalent of 19 feed offerings (two offerings per day) were suspended or modified for all treatments based on criteria specified in the implemented feeding program. No feedings adjustments was required because of bottom conditions, however the estimated adequate condition base on samplings were verified during the harvest with clean bottom conditions found in ponds. Because of the achieved survivals and yields, these feeding suspensions are considered appropriated at the time they were applied.

Conclusion

The relevance of effective feed utilization on management, economical and environmental success feeding, makes meritorious the incorporation of as many criteria as possible to reach efficiency and reduce overfeeding. The criteria enclose in the implemented feeding program proved to be useful for this purpose. And under this type of management, following a feeding schedule that is more conservative towards the end of the cycle proved to be convenient.

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VI. SUMMARY AND CONCLUSIONS

Nurseries

Findings of this experiment suggest that PL densities in the range of 25 to 65 PL/L, with good management practices, have limited influence on subsequent growth and survival of shrimp during growout. However, at the higher density (65 PL/L), improved feed and culture system utilization was obtained. The tank environment was highly nutrient enriched when considering the water volume and feed inputs added to this treatment. Differences in growth were more likely to be related to the conditions of the treatment tanks. As the biomass loading in the tanks increased and more feed was supplied, an increase in water circulation was helpful to keep feed particles suspended. Assuming an appropriate concentration of feed was used to allow the encounter rate to be high so that PL ingested sufficient quantities with minimal effort. This experiment also demonstrated that in these systems, good aeration/circulation is important to maintain feed in suspension and to remove waste from the water.

When comparing nursery periods of 14 and 21 days, except for biomass loading, no significant differences among treatments were found in any of the nursery results. Initial lower temperatures in N21 limited the average weight differences between the two treatments at the end of the nursery phase. Both nursery treatments had survivals greater than 90%.

Results of nursery diet experiments suggest that there is an advantage in supplementing dried feed with *Artemia nauplii*. Throughout the nursery period the two treatments supplemented with *Artemia* experienced greater growth during the nursing

period and reached significantly greater final average weight and biomass loading than the other treatments in which diets were not supplemented with Artemia.

Results of nursery diet experiments also suggest that the use of algal paste did not produce better results than algae that grew naturally in nursery tanks. Because of the water exchange every three days, water quality was not an issue and there was not an opportunity to notice the water quality stabilization function of live algae over non-live algae paste. However, nursery period trends and overall average TAN concentrations were greater in treatments supplemented with Artemia.

Although results indicate that the dry feed diet, regardless of supplementation with Artemia and algae, supported growth and PL survival, the shrimp performance still remained far behind those in pond environments. At 21 days pond nursed PL had average weights between 14 and 25 times greater than those that were nursed for 21 days under the nursery treatments. This indicates that there is still a challenge in improving nursery feeds in terms of nutritional composition and feeding methods.

Nurseries size variation

Size variation has been considered a crucial aspect of PL quality. Although references have reported that during the nursery phase, the Coefficient of Variation of individual weights (CV) declines, in these nursery trials the initial individual weight variation (CV=44-67) increased in all nursery treatments regardless of densities (CV=66-156%), nursery duration (CV=54-71%) and diets offered (CV=61-76). Among the nursery experiments, the treatment that was most highly nutrient enriched was the one with lower, almost negligible increase in the size variation. Greater individual weight

variation at the end of the nursery phase could be associated with some shrimp out competing others during their search for food and consequently being able to grow faster. High nutrient enrichment may play a crucial role since appropriate food concentration is necessary to increase the encounter rate and minimize search effort.

Pond-Nursery density

Juveniles from the best performing nursery treatments were most likely to result in higher yields and better size distribution during the growout phase. The observed mean size variation for shrimp from the Low Density treatment (23%) was significantly greater than the other two treatments (14% and 13.9%) for High Density and Mid Density. These results were consistent with results obtained during the nursery phase which apparently carried over into the growout phase. Although pond survivals for the Low Density treatment (83%) were similar to High Density treatment (89%) yields were 28% lower than in High Density treatment. This can be attributed to a more diverse size distribution in the Low Density treatment, with a lower percentage (45%) of the shrimp population greater than the 36-40 count size when compared to approximately 78% in High density treatment and 83% in the Medium Density treatment.

Therefore, the results of this study suggest that actual stocking densities in nurseries may not directly affect growout, however management practices during the nursery phase can have a significant effect on later performance. Adequate feeding and strong aeration/circulation are important to ensure postlarvae at any density have sufficient food. Strong, healthy juveniles coming out of a nursery will continue to maintain good uniform growth during the following pond production phase.

Pond-Nursery Duration

Results of the nursery duration experiment, demonstrated that nursed juveniles did not differ significantly in production characteristics and population size distribution from shrimp stocked direct from the hatchery.

Growout pond Feeding Management

Under the conditions of this study (35 postlarvae/m², nighttime aeration (0.75 kW/ha) and limited water exchange) some general trends were observed during the growout phase of the experiments. When total feed inputs were reduced, survivals went up and Feed Conversion Rates improved. In experiment 1, total feed offerings were 6,000-6,500 kg/ha, survivals 68-88% and FCR average around 1.65-2.1 (Chapter 2). In experiment 2, feed offerings were 9,000-9,600 kg/ha, survivals were 62-64% and FCR average 2.5-2.7 (Chapter 3). In experiment 3, feed offerings were again 6,500 -7,960 kg/ha, survivals were 81-77.8% and FCR averaged 1.5-1.8 (Chapter 5). This suggests that even under careful pond management and acceptable survivals, total feed offerings above 8,000 kg/ha tend to be detrimental to FCR.

Another observation was made in shrimp individual weight variation. In all experiments individual weight coefficient of variation increased in the nursery phase and decreased during the pond culture phase when there was an abundance of natural food in ponds. After the growout phase, individual variation weight, in all treatments were within ranges of 13-23%.

The implementation of standardized stocking procedures, management procedures and careful adjustments of the population on a weekly basis based on historical mortality records, was effective at forecasting FCR and yields closer to real ones. Overall real yields and FCR were 9% and 6-10% better than estimates respectively. Intensive sampling with cast nets were not as accurate in estimating population densities as the use historic survival data. Even the most careful cast net samples were 30-50% below the actual population densities.

In intensive shrimp ponds, algae bloom die-off were usually associated with weather conditions rather than feed inputs and/or time. Algae crashes occurred as soon as the 52nd day and as late as the 100 day of culture. Algae crashes were usually associated with extremely hot and sunny conditions, after continuous cloudy period, or periods of heavy rains that may cause significant change in salinity, or when the blooms became to dense (Secchi readings less than 15 cm . The most practical way to anticipate an algae crash is to observe color changes , Sechii disk readings and fluctuations in dissolved oxygen.

Intensive pond often stratified during mid-day and could reach more than 3-4 C and 4-5mg/L of D.O, of difference between the top and bottom layers of a 1m- deep pond. Mixing pond with aerators for 15-30 minutes during the middle of the day broke stratification and increased pond stability. Noon DO reading gave data that can be used to ensure algae population are undergoing active photosynthesis. Noon DO readings also helped in identifying those ponds with extreme high readings at noon and that more likely needed careful attention at night. The most serious problem could better be anticipated and corrective action taken before catastrophes occurred.

The FCR method improved feed efficiencies by 17% when compared with commonly used feeding tables (Chapter 3). The lowest FCR were found with an early aggressive feeding strategy. This strategy maximized early shrimp growth and reduced feed inputs during the second half of the cycle when water quality was least stable. Gross income tended to be higher in ponds feed with late aggressive feeding but cost were also higher. Return above seed, feed and electricity costs were similar for all treatments.

Although gross income and RASVC were not significantly different among treatments other benefits were realized. In the early aggressive feeding strategy, less feed was used during the last part of the culture cycle so there was less pressure on water quality. This means there was reduced management risk when using the early feeding strategy.

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VIII. APPENDICES

Appendix 1. Quantification of PL shipped from hatchery based on three concentrations and three counted samples on each concentration.

Concentrations	Mean	Standard Deviation	Coefficient variation
3	150,385	6,327	4.2
3	205,836	26,704	12.9
3	380,950	52,297	13.7
PL received	737,171		
Estimated shipped	550,000		

Appendix 2. PL stocking distribution per treatment, tank water column and volumes , and density achieved for a 21 day nursery phase of *Litopenaeus vannamei* stocked at three densities 25 (LD),38 (MD) and 65 (HD) PL/L .

Treatment	Tanks	PL/tank	Water column (in)/tank	Volume (m ³)/tank	Density (PL/L)
HD	1	146,153	19.00	2.22	65
HD	2	146,153	19.00	2.22	65
MD	3	118,136	26.00	3.04	38
MD	4	118,136	26.00	3.04	38
LD	5	100,369	34.00	3.97	25
LD	6	100,369	34.00	3.97	25
Total		729,317			

Appendix 3. Nutritional composition of Feed used in the trial. Buris Mill & Feed Inc. 35% protein Shrimp Feed.

Nutrient	Unit	Analysis
Weight	LB	1
Protein, Crude	PCT	35.065
Fat, Crude	PCT	7.0063
Fat,added	PCT	2.8486
Fiber, crude	PCT	2.5215
Ash	PCT	9.6791
Moisture	PCT	10.5402
Calcium	PCT	2.0018
Phosphorus, Tot	PCT	1.1202
Phosphorus, Avai	PCT	0.8504
Potassium	PCT	1.2019
Lysine	PCT	2.1891
Methionine	PCT	0.7316
Tryptophane	PCT	0.3652
Magnesium	PCT	0.3652
Manganese	PPM	87.5586
Zinc	PPM	200.388
Iron	PPM	280.099
Copper	PPM	27.4614
Starch	PCT	20.5586

Appendix 4 . *Litopenaeus vannamei* PL stocking distribution per treatment, tank water column and volumes, and density achieved for a nursery period of 21(N21) and N14 (N14) days.

Treatment	Tanks	Pls/tank	Water column cm / tank	Volume m3 / tank	Density PL/L
N21	1	114,981	73.7	3.39	34
	3	114,981	73.7	3.39	34
	5	114,981	73.7	3.39	34
N14	2	105,387	73.7	3.39	31
	4	105,387	73.7	3.39	31
	6	105,387	73.7	3.39	31
Total		661,104			

Appendix 5. Information on shipping conditions of *Litopenaeus vannamei* postlarvae received from the same hatchery during three shipments.

	Groups		
	1 for N21	2 for N14	3 for DS
PL Average Weight	1.36 ± 0.59	0.96 ± 0.41	1.24 ± 0.72
CV of Individual Weights	44.04%	43.24%	58.52%
Estimated Shipped	350,000	358,400	400,000
Counted Recieved	344,945	316,160	340,796
Temperature C	20.9	20.6	20.7
Salinity (ppt)	33.3	32.2	30.6
Oxygen (ppm)	8.2	8.2	8
TAN	0.0	0.09	0.169
pH	7.43	7.12	7.84

Appendix 6. Quantification of *Litopenaeus vannamei* PL shipped from a hatchery based on three concentrations and three counted samples on each concentration for a nursery period of 21 (N21) and 14 (N14) days.

Treatments	Sample <i>n</i>	Mean	SD	CV	PL quantified
N21	6	121.8	10.7	8.8%	115,710
	6	241.3	23.0	9.6%	229,235
	PL's received				344,945
	Estimated shipped				358,400
N14	6	70.3	5.92	8.42%	66,785
	6	262.5	11.64	4.43%	105,387
	PL's received				316,160
	Estimated shipped				300,000
*DS	5	149.4	8.7	5.8%	141,930
	15	209.3	23.0	18.5%	198,866
	PL's received				340,796
	Estimated shipped				400,000

* For treatment DS only 141,930 PL's were used to stocked 4 growout ponds at 35 shrimp/m², the rest were used for other experiments.

Appendix 7. Water Quality maintenance activities during a *Litopenaeus vannamei*

indoor experiment for 21(N21) and 14 (N14) days.

Treatment	Days	Operation
N21	1-4	Static
	5-9	Circulation & Filtration 3 hours
	10	Circulation for 24 hours
	11	Water Exchange of 50%
	12-13	Water recycling & Filtration for 8 hrs
	14-16	Water recycling and Filtration Permanently
	17-21	Acclimation from 31 to 19
N14	1-3	Static
	4-5	Recycling & 50% water exchange 5th day
	6-10	Water recycling and Filtration Permanently
	11-14	Acclimation from 31 to 19

Through the 21 days of usage, every three days the sandfilter was cleaned by backwashing. N14 was stocked in the tanks of the nursery system on the 7th day of N21. Both treatments were terminated at the same time.

Appendix 8. Information on shipping conditions of *Litopenaeus vannamei* postlarvae received from the same hatchery during three shipments.

PL Weight mg/PL	Coefficient of variation of Individual Weights	PL Shipped	PL Recieved	Temp C	Salinity ppt	Oxygen mg/L	TAN mg/L	pH
1.24 ± 0.72	58.52%	400,000	340,796	20.7	30.6	8.0	0.169	7.84

Appendix 9. Algae die-off on growout ponds stocked at 35 shrimp/m² and culture for 18 weeks under three feeding strategies; EAF (Early aggressive feeding), LAF (Late aggressive feeding) and IF (Intermediate feeding).

Treatments	Ponds	Dates	Culture day
EAF	D2	3-Aug	100
	D5	7-Jul	73
	C6	27-Jun	63
	C9		
LAF	D3	4-Jul	70
	D7	11-22 Jul	77, 88
	C7		
	C5	16-Jun	52
IF	D4		
	D6	5-Jul	71
	D8	5-Aug	102
	C8		

Appendix 10. Comparison of estimated and real FCR for *L.vannamei* stocked at 35 shrimp/m² and culture for 18 weeks under three feeding strategies; EAF (Early aggressive feeding), LAF (Late aggressive feeding) and IF (Intermediate feeding).

Treatment	Replicates	FCR			
		Estimated	Est. Avg.	Real	Real Avg.
EAF	1	1.6	1.6	1.4	1.5
	2	1.7		1.5	
	3	1.7		1.6	
	4	1.6		1.5	
LAF	1	2.2	2.2	2.0	2.0
	2	2.3		2.1	
	3	2.3		2.1	
	*4	2.0		2.9	
IF	1	2.0	2.0	1.9	1.8
	2	2.1		1.9	
	3	2.0		1.7	
	4	1.9		1.8	

* Replicate eliminated from the treatment average.

Appendix 11. Comparison of estimated and real yields (kg/ha) for *Litopenaeus vannamei* stocked at 35 shrimp/m² and culture for 18 weeks under three feeding strategies; EAF (Early aggressive feeding), LAF (Late aggressive feeding) and IF

Treatment	Replicates	Yields (kg/ha)			
		Estimated	Est. Avg.	Real	Real Avg.
EAF	1	4,026	3,881	4,573	4,327
	2	3,775		4,305	
	3	3,837		4,193	
	4	3,885		4,240	
LAF	1	4,028	3,888	4,647	4,384
	2	3,791		4,277	
	3	3,846		4,228	
	*4	4,356		3,164	
IF	1	3,921	3,842	4,308	4,398
	2	3,562		4,223	
	3	3,798		4,626	
	4	4,086		4,436	

* Replicate eliminated from the treatment average.

Appendix 12. Population assesment of L.vannamei conducted previously to harvest, after 18 weeks of culture period on 0.1 ha ponds and stocked a shrimp/m2 and fed under three feeding strategies.EAF (Early agressive feeding), LAF (Late aggressive feeding) and IF (Intermediate feeding).

Sampling Stations

- 1 Right front corner
- 3 Right rear corner
- 5 Left rear corner
- 7 Left Front Corner
- 8 Front center
- 9 Center

7	6	5
8	9	4
1	2	3

Front of pond>>>>

Pond area (m2) 1000
 effective Coverage 50%
 Net area 4.67
 Real coverage 2.34

Pond stations	EAF			LAF			IF			
	C6	C9	D5	D2	C5	D7	D3	C8	D8	D4
1	20	18	29	38	13	20	14	13	16	9
2	85	9	17	15	7	48	30	9	40	30
3	22	12	81	72	5	28	45	28	48	24
4	31	48	89	35	24	22	60	34	69	33
5	47	24	57	28	11	30	49	29	30	29
6	20	23	35	18	6	47	39	25	51	38
7	44	2	7	30	10	1	24	20	11	13
8	18	40	13	33	2	9	21	7	3	26
9	32	18	15	9	14	13	48	11	65	50
	9	9	9	9	27	9	9	9	9	9
n	35.44	21.56	38.11	30.89	10.22	24.22	36.67	19.56	37.00	32.67
Aveg	21.38	14.60	30.53	18.27	6.48	16.01	15.26	9.90	23.60	11.36
Stdev	14.25	9.73	20.35	12.18	2.49	10.68	10.18	6.60	15.73	7.57
CI (+/-)	15180	9232	16322	13229	4378	10374	15703	8375	15846	13990
Pop estimate	27362	28527	29860	28360	18,699	26070	28860	27816	31597	25,232
Real Population	45	68	45	53	77	60	46	70	50	45
% underestimate	64	67	70	66	44	61	67	65	74	59
# shrimp/ cast for real										
Average % off	53			63			55			
Pop estimate with CI	21283	13399	25038	18443	5445	14946	20061	11202	22584	17233
% underestimate	22	53	16	35	71	43	30	60	29	32
Average % off	32			50			39			

Appendix 13. Costs and returns of culturing *Litopenaeus vannamei* stocked at 35 shrimp/m² and fed under an EAF

(Early aggressive feeding) management.

Weight / Size distribution % / count	%	Kg/ count	Prices \$/ lb	Gross Income
16-20 (25.54)	0.26	1	2.34	5.89
21-25 (19.89)	12.69	55	1.92	231.92
26-30 (16.3)	42.36	183	1.92	774.27
31-35 (13.81)	33.07	143	1.53	481.65
36-40 (11.98)	7.44	32	1.42	100.59
Total Avg kg/ 0.1 ha		414.64	\$/ 0.1ha	1,594

Gross Receipt **1,594**

<u>Variable Cost/pond</u>	<u>Unit</u>	<u>\$ Cost/ unit</u>	<u>Quantity/pond</u>	<u>Cost</u>
Juveniles	1000	9.10	35	318.50
Feed	kg	0.51	656	332.29
Chemicals				
Fertilizer 32-0-0	Liter	0.271	0.402	0.11
Fertilizer 10-34-0	Liter	0.95	1.68	1.60
Labor (Part time: Stocking/harvest)	hrs	6.1	18.5	112.85
Electricity	Kwh	0.08	3,567	285.38
Gator wheeler (fuel, oil)	Gal	1.33	8.33	11.08
Total variable cost/ pond				1,061.80
Income above variable cost				4,247.20
Income above variable cost				532.51
Fixed Cost				
Manager and research student	Individual			8,784.00
Depreciation on Vehicle (gator)	Unit	\$		735
Depreciation on aerators	Unit	\$		320.00
Depreciation on D.O meter	Unit	\$		300
Depreciation on well pump	Unit	\$		750
Depreciation on lined ponds	Unit	\$		647.92
Total fixed cost / 0.1ha				11,536.92
Total of all specified expenses/ 0.1 ha				12,598.72
Net return above all specified expenses/ 0.1 ha				(11,004.41)

BREAKEVEN YIELD (kg/0.1ha) TOTAL TO COVER:
 VARIABLE COSTS 251.37
 ALL SPECIFIED COSTS 2,982.65

BREAKEVEN PRICE(\$/LBS.) TO COVER:
 VARIABLE COSTS 1.16
 ALL SPECIFIED COSTS 13.81

Appendix 14. Costs and returns of culturing *Litopenaeus vannamei* stocked at 35 shrimp/m² and fed under an LAF (Late aggressive feeding) management.

Weight / Size distribution % / count	%	Kg/ count	Prices \$/ lb	Gross Income
16-20 (25.54)	0.32	1	2.34	7.26
21-25 (19.89)	20.39	89	1.92	377.61
26-30 (16.3)	44.84	197	1.92	830.35
31-35 (13.81)	22.24	98	1.53	328.24
36-40 (11.98)	8.95	39	1.42	122.63
Total Avg kg/ 0.1 ha		424.15	\$/ 0.1ha	1,666

Gross Receipt **1,666**

<u>Variable Cost/pond</u>	<u>Unit</u>	<u>\$</u> <u>Cost/ unit</u>	<u>Quantity/pond</u>	<u>Cost</u>
Juveniles	1000	9.10	35	318.50
Feed	kg	0.51	907	459.51
Chemicals				
Fertilizer 32-0-0	Liter	0.271	0.402	0.11
Fertilizer 10-34-0	Liter	0.95	1.68	1.60
Labor (Part time: Stocking/harvest)	hrs	6.1	18.5	112.85
Electricity	Kwh	0.08	3.797	303.7
Gator wheeler (fuel, oil)	Gal	1.33	8.33	11.08
Total variable cost/ pond				1,207.38
Income above variable cost				458.70
Fixed Cost				
Manager and research student	Individual			8,784.00
Depreciation on Vehicle (gator)	Unit	\$		735
Depreciation on aerators	Unit	\$		320.00
Depreciation on D.O meter	Unit	\$		300
Depreciation on well pump	Unit	\$		750
Depreciation on lined ponds	Unit	\$		647.92
Total fixed cost / 0.1ha				11,536.92
Total of all specified expenses/ 0.1 ha				12,744.29
Net return above all specified expenses/ 0.1 ha				(11,078.21)

BREAKEVEN YIELD (kg/0.1ha) TOTAL TO COVER:

VARIABLE COSTS
ALL SPECIFIED COSTS

285.84
3,017.12

BREAKEVEN PRICE(\$/LBS.) TO COVER:

VARIABLE COSTS
ALL SPECIFIED COSTS

1.29
13.66

Sensitivity analysis

Appendix 15. Costs and returns of culturing *Litopenaeus vannamei* stocked at 35 shrimp/m² and fed under an IF (Intermediate feeding) management.

Weight / Size distribution % / count	%	Kg/ count	Prices \$/ lb	Gross Income
16-20 (25.54)	0.48	2	2.34	10.98
21-25 (19.89)	20.58	90	1.92	382.26
26-30 (16.3)	50.58	222	1.92	939.67
31-35 (13.81)	20.66	91	1.53	305.92
36-40 (11.98)	5.13	23	1.42	70.48
Total Avg kg/ 0.1 ha		428.53	\$/ 0.1ha	1,709

Gross Receipt **1,709**

Variable Cost/pond	Unit	\$		Cost
		Cost/ unit	Quantity/pond	
Juveniles	1000	9.10	35	318.50
Feed	kg	0.51	797	403.75
Chemicals				
Fertilizer 32-0-0	Liter	0.271	0.402	0.11
Fertilizer 10-34-0	Liter	0.95	1.68	1.60
Labor (Part time: Stocking/harvest)	hrs	6.1	18.5	112.85
Electricity	kWh	0.08	3,949	315.9
Gator wheeler (fuel, oil)	Gal	1.33	8.33	11.08
Total variable cost/ pond				1,163.78
Income above variable cost				545.52
Fixed Cost				
Manager and research student	Individual			8,784.00
Depreciation on Vehicle (gator)	Unit	\$		735
Depreciation on aerators	Unit	\$		320.00
Depreciation on D.O meter	Unit	\$		300
Depreciation on well pump	Unit	\$		750
Depreciation on lined ponds	Unit	\$		647.92
Total fixed cost / 0.1ha				11,536.92
Total of all specified expenses/ 0.1 ha				12,700.70
Net return above all specified expenses/ 0.1 ha				(10,991.40)

BREAKEVEN YIELD (kg/0.1ha) TOTAL TO COVER:

VARIABLE COSTS	275.52
ALL SPECIFIED COSTS	3,006.79

BREAKEVEN PRICE(\$/LBS.) TO COVER:

VARIABLE COSTS	1.23
ALL SPECIFIED COSTS	13.47