Available online at www.sciencedirect.com

SCIENCE DIRECT®

Aquaculture 249 (2005) 431–437

Aquaculture

www.elsevier.com/locate/aqua-online

Survival, growth and feeding efficiency of *Litopenaeus vannamei* protozoea larvae fed different rations of the diatom *Chaetoceros muelleri*

Pablo Piña[a], Mario Nieves[a], Luis Ramos-Brito[a], Cesar O. Chavira-Ortega[b], Domenico Voltolina[c,*]

[a]*Universidad Autónoma de Sinaloa, Facultad de Ciencias del Mar, Paseo Claussen s/n, Mazatlán, Sinaloa, Mexico*
[b]*Maestría Regional en Acuicultura, Departamento de Investigación Científica y Tecnológica de la Universidad de Sonora, Rosales y Niños Héroes s/n, Hermosillo, Sonora, Mexico*
[c]*Centro de Investigaciones Biológicas del Noroeste, Laboratorio UAS-CIBNOR, P.O. Box 1132, Mazatlán, Sinaloa, Mexico*

Received 15 February 2005; received in revised form 15 April 2005; accepted 18 April 2005

Abstract

The survival, development and growth of *Litopenaeus vannamei* protozoea (PZ) larvae were evaluated in three experiments during which they were fed five different rations of the diatom *Chaetoceros muelleri*, from emerging PZ I larvae until the day of change to mysis I. The total amount of food and energy supplied in 5 days to each larva ranged from 85.5 to 223.2 μg larva^{-1} (ash-free dry weight) and between 2.25 and 5.88 J larva^{-1}. After 2 days, the development index (DI) showed that some of the larvae fed the highest ration had reached the stage of PZ III. At the end of the experiment the mean DI ranged from 2.9 ± 0.2 to 3.2 ± 0.3 and the differences among treatments were not significant. The mean percentages of survival ranged from 35.8% to 51.7% and were not statistically different, but total length was progressively higher with increasing ration. The final values were 1.91 ± 0.54, 2.10 ± 0.53; 2.34 ± 0.36; 2.45 ± 0.37 and 2.55 ± 0.42 mm and were all statistically different with the exception of treatment 4, that was intermediate between treatments 3 and 5. However, the highest final organic weight (26.0 ± 1.7 μg larva^{-1}) was treatment 4, which was different from the values obtained with the two lower rations. Treatments 3 and 5 had intermediate values. The percentages of the food ingested did not vary with the rations supplied or with the age of the larvae and ranged from 74% to 86%. Food ingestion (F_i) was a function of the food supplied (F_s), according to the equation $F_i = 0.848F_s - 4.025$ ($R^2 = 0.992$; $P < 0.001$). Using the amount of food supplied and ingested with each feeding regime and the respective organic weight gains, it was calculated that the percentages of F_s and F_i used for body growth were 14.4% and 17.9% with the lowest ration and 6.9% and 8.3% with the highest, whereas the three intermediate rations gave similar efficiencies (9.8% to 10.2% of F_s and 11.8% to 12.5% of F_i). However, the weight and energy gains obtained with the highest food utilization

* Corresponding author. Tel./fax: +52 669 982 40 09.
E-mail address: microalgas@mzt.megared.net.mx (D. Voltolina).

doi:10.1016/j.aquaculture.2005.04.037

efficiency were lower than those obtained with the higher rations, indicating that cautious overfeeding is a more convenient alternative for *L. vannamei* PZ culture.

Keywords: *Litopenaeus vannamei*; Protozoea; Larval feeding; Growth; Food utilization

1. Introduction

Shrimp larvae are usually fed live feeds and although the protozoea stages of several species may ingest small zooplankters such as rotifers or *Artemia* nauplii (Yúfera et al., 1984; Kurmaly et al., 1989; Jones et al., 1997), the food used in most hatcheries for these larvae consists of one or more microalgae species supplied at different concentrations and with different feeding routines, depending on the shrimp species, on the larval stage and also on the personal experience of the operators in charge of each hatchery (Aguirre-Hinojosa et al., 1999).

A survey in approximately 40% of the 33 hatcheries registered in Mexico in 1999 showed that most use the diatom *Chaetoceros muelleri* as the only feed for the protozoea stages of *Litopenaeus vannamei*. This is supplied with an initial concentration of 40–50 · 10^3 cells ml^{-1} when the larvae in the last nauplius stage (N V) are close to moulting to protozoea I (PZ I). The daily ration is increased to 80–100 · 10^3 cells ml^{-1} day^{-1} for PZ I, and the final varies between 120 and 150 · 10^3 cells ml^{-1} day^{-1} until the larvae reach the first mysis stage (M I), which is fed *Artemia* nauplii and 20–50 · 10^3 cells ml^{-1} day^{-1} of *C. muelleri* or of the green flagellates *Tetraselmis suecica* and occasionally *Dunaliella tertiolecta* (López-Elías et al., 2003).

There is information on the ingestion and growth response to different food densities of the larvae of some penaeid species (Emmerson, 1980; Loya-Javellana, 1989), but the effect of lower or higher rations than those used in commercial hatcheries has not been studied with the protozoea stages of the most important species for aquaculture in Latin America, that is the Pacific white shrimp *L. vannamei*.

In this work we evaluated the survival, development and growth, as well as the food conversion efficiency of *L. vannamei* protozoea larvae fed five different rations of the microalga *C. muelleri* until the date of change from PZ III to M I.

2. Materials and methods

Three batches of *L. vannamei* larvae, obtained from two commercial hatcheries at the stage of N III were used for an equal number of experiments. The larvae were maintained under controlled conditions (temperature 29 °C; salinity 34 g l^{-1}; O_2 concentration close to saturation maintained by profuse aeration, 50 · 10^3 cells ml^{-1} day^{-1} of *C. muelleri* starting at the stage of N V), until >50% of the larvae reached the stage of PZ I.

On that date, 450 larvae were placed in each of 20 3-l containers (4 for each treatment). The initial concentration (150 larvae l^{-1}) was maintained constant until the end of each experiment, adjusting the volume of each culture after the daily evaluation of survival and of food consumption.

This was done immediately before total water exchange, counting all the larvae of each container concentrated with a submerged sieve in 500 ml of recently filtered seawater, that were restored to the original aquarium with the volume of 1-μm filtered seawater necessary to maintain the concentration at 150 larvae l^{-1}. The amount of algae consumed was calculated as the difference between those supplied the previous day and those remaining in each aquarium, evaluated with direct counts with a hemacytometer of three 10 ml samples concentrated to 1 ml to improve the precision of counting. These data were corrected for microalgae growth in two controls for each ration (Marin et al., 1986).

The larvae of four containers chosen at random at the beginning of each experiment were fed with one of five feeding routines, that ranged from 40 to 120 · 10^3 cells ml^{-1} day^{-1} for the stage of PZ I–PZ II, 80 to 200 · 10^3 cells ml^{-1} for PZ II–PZ III and 120 to 300 · 10^3 cells ml^{-1} day^{-1} for PZ III–M I (Table 1).

All daily rations were supplied in two equal portions at 12 h intervals and were changed to that of the

Table 1
Feeding regimes for the larval cultures of *L. vannamei* protozoea larvae (PZ), in 10^3 cells ml^{-1}

Stages	Treatment				
	1	2	3	4	5
PZ I–PZ II	40	60	80	100	120
PZ II–PZ III	80	100	120	150	200
PZ III–MI	120	150	200	250	300

Larval concentration: 150 larvae l^{-1}.

following stage of development in all containers, when the larvae of the four replicates of at least one of the treatments had started to moult to that stage.

Samples of 15–20 larvae were obtained every 24 h from each container before water exchange, fixed with the solution suggested by Correa-Sandoval and Bückle-Ramírez (1993) and their total length (TL) was measured under a dissecting microscope with a calibrated eyepiece. Additional samples of the same size, obtained every 6 h, were used to determine in vivo their stage of development. These larvae were restored to their original container to reduce mortality due to sampling. The development index (DI) was calculated as:

$$DI = n^{-1} \sum_i n_i$$

where I = absolute value for each larval stage (N V = 0; PZ I to III: 1 to 3; M I = 4); n_i = number of larvae of each stage; n = number of larvae in the sample (Villegas and Kanazawa, 1979).

All experiments ended when the mean DI was >3.1 in all the replicates of at least one treatment, indicating that at least 10% of the larvae had reached the stage of M I, when their main diet consists of *Artemia* nauplii.

After obtaining the samples for TL and the evaluation of final survival, the larvae remaining in each container were concentrated in triplicate precalibrated GF-C Whatman glass fiber filters, washed with 5–6 ml of a 4% solution of ammonium formate to eliminate sea salt, dried to constant weight (total dry weight = TW) at 60 °C for 48–60 h and ashed in a muffle furnace at 475 °C to obtain their inorganic content (AW). This was used to calculate by difference their ash-free dry weight (AFW = TW − AW).

The experiment was repeated three times with four replicates for each treatment, with a total of 12 replicates in three blocks. Therefore, the data of survival (corrected for mortality due to sampling), DI and TL obtained on the days in which the larvae started to change from PZ II to PZ III and from PZ III to M I (DI >2 and >3), as well as the final AFW, were compared with one-way block ANOVA or Friedman tests with $\alpha = 0.05$, depending on the results of the tests of normality and equal variances. The differences between treatments were identified with the multiple comparison tests of Tukey or Nemenyi (Zar, 1996).

Finally, the data of food supplied and ingested (F_s and F_i) and the initial and final individual AFW (W_i and W_f) were used to calculate the food conversion index (FCI) and the efficiency of food conversion (K_1), using the traditional equations:

$$FCI = F_s(W_f - W_i)^{-1} \text{ and } K_1 = 100(W_f - W_i) \cdot F_i^{-1}$$

These two indicators may be compared directly using the equation $K_0 = 100 \cdot FCI^{-1}$, where K_0 is the percentage of the food available used for biomass growth.

3. Results

The mean TW of *C. muelleri* was 62.3 ± 12.5 pg $cell^{-1}$, with an average organic content of 57.2% (35.6 ± 3.6 pg $cell^{-1}$). Protein, carbohydrate and lipid, determined with the methods of Lowry et al. (1951), Dubois et al. (1956) and Pande et al. (1963) as in Lora-Vilchis and Doktor (2001) were 34.47%, 9.15% and 13.55%, respectively, of TW. Using the caloric equivalents of Gnaiger (1983), the energy content of the biomass was calculated as 15.7 mJ μg^{-1} of TW (26.35 mJ μg^{-1} AFW).

The experiments lasted 5 days and the daily rations were changed on days 2 and 4. The total amount of food supplied to each larva ranged from 149.6 to 390.5 μg TW $larva^{-1}$ and from 85.5 to 223.5 μg AFW $larva^{-1}$. The energy available varied from 2.25 to 5.88 J $larva^{-1}$ (Table 2).

By the end of the second day the larvae fed the highest cell concentration were beginning to change from PZ II to PZ III (DI >2), whereas with the rest of the rations most larvae were still between the stages of PZ I and PZ II. By the end of the experiments only the mean DI of treatments 4 and 5 were >3 and there was a tendency to progressively higher values with in-

Table 2
Range of larval stages in at least one of the treatments before the change of daily ration, days of supply and μg of food supplied to each larva during the feeding experiments of *L. vannamei* protozoea larvae, in μg total dry weight larva^{-1} (A) and in μg ash-free dry weight larva^{-1} (B)

Stages	Days	Treatment				
		1	2	3	4	5
(A)						
PZ I–PZ II	2	33.2	49.9	66.5	83.1	99.7
PZ II–PZ III	2	66.5	83.1	99.7	124.6	166.2
PZ III–M I	1	49.9	62.3	83.1	103.9	124.6
Σ	5	149.6	195.3	249.3	311.6	390.5
(B)						
PZ I–PZ II	2	19.0	28.5	38.0	47.5	57.0
PZ II–PZ III	2	38.0	47.5	57.0	71.2	95.0
PZ III–M I	1	28.5	35.6	47.5	59.4	71.2
Σ	5	85.5	111.6	142.5	178.1	223.2
Σ*J*	5	2.25	2.94	3.76	4.70	5.88

Σ=total amount supplied. Σ*J*=Total energy supplied, in J larva^{-1}.

creasing food availability, but the differences were not significant (Table 3A).

The daily handling needed for a precise evaluation of survival, that was necessary to maintain constant the individual rations in all aquaria, caused a low survival in all cases. This tended to be higher with the higher rations, but the differences were not significant in either date (Table 3B), whereas the tendency to progressively higher TL with increasing rations caused significant differences on both dates (Table 3C).

The mean initial AFW was 8.6 μg larva^{-1} and the final ranged from 20 to 26 μg larva^{-1}. The only significant difference was for these two values, obtained with the larvae fed the second lowest and the second highest rations, showing a non-linear response of weight growth to the amount of food available (Table 4).

The mean daily ingestion of the larvae ranged from 74% to 86% of the microalgae supplied with the daily rations, without any significant difference related to the age or to the amount of food available or to their interaction (two-way ANOVA, $P>0.05$ in all cases). Thus, the equation relating the food available to that ingested may be used to evaluate the amounts of food and energy ingested and the respective percentages used for individual growth.

Organic weight gains ranged from 11.4 to 17.4 μg larva^{-1} and food ingestion increased continuously in parallel with its availability according to the linear model $F_i=0.848F_a-4.025$ ($R^2=0.992$; $P<0.001$). Comparing growth to the amounts of food supplied and ingested the lowest ration was the most efficient, because 14.4% of the food available and 18% of that ingested was used for weight growth, the respective values for the highest ration were 6.9% and 8.3% and the three intermediate rations seemed to be equally efficient, with between 10.2% and 9.8% of the food available and 11.8% and 12.5% of the food ingested used for body growth.

The increase in biomass of the three PZ stages of *L. vannamei* follows a linear rather than an exponential trend, although the correlation between weight and age is highly significant with both models (linear model: $r=0.988$; exponential model: $r=0.981$; $P<0.001$ in both cases, Piña-Valdez, 2004).

In this case, the linear equations corresponding to the five treatments calculated with the initial and final

Table 3
Mean and standard error ($n=12$ in all cases), of the development index (A), survival in percentage (B) and total length in mm (C), obtained after 2 and 5 days, equivalent to the days of change from PZ II to PZ III and from PZ III to M I

	Day	Treatment				
		1	2	3	4	5
A	2	1.42 a ± 0.09	1.73 b ± 0.11	1.88 bc ± 0.04	1.94 bc ± 0.03	2.06 c ± 0.07
	5	2.89 a ± 0.05	3.01 a ± 0.07	3.04 a ± 0.07	3.18 a ± 0.08	3.17 a ± 0.08
B	2	46.8 a ± 5.9	48.8 a ± 5.3	47.2 a ± 5.1	57.7 a ± 5.3	55.5 a ± 5.3
	5	35.8 a ± 5.2	35.4 a ± 5.5	38.3 a ± 5.1	42.5 a ± 7.1	51.7 a ± 7.2
C	2*	1.17 a ± 0.06	1.24 a ± 0.07	1.41 b ± 0.05	1.43 b ± 0.07	1.56 c ± 0.08
	5*	1.91 a ± 0.16	2.10 b ± 0.15	2.34 c ± 0.10	2.45 cd ± 0.11	2.55 d ± 0.12

Equal or common letters indicate lack of significant difference (one-way ANOVA, $\alpha=0.05$). a<b<c.
* Nonparametric tests.

Table 4
Mean and standard deviation of initial weight (W_i; $n=3$) and mean values and standard error of final weight (W_f; $n=12$), mean weight gain (WG), amounts of food supplied and ingested (F_s and F_i), all in μg larva^{-1} of ash-free dry weight

	Treatment				
	P_1	P_2	P_3	P_4	P_5
W_i (μg)	8.62 ± 3.87	8.62 ± 3.87	8.62 ± 3.87	8.62 ± 3.87	8.62 ± 3.87
W_f (μg)	20.90 ± 0.64a	19.95 ± 0.97a	23.00 ± 0.67ab	26.01 ± 0.50b	23.96 ± 1.06ab
WG (μg)	12.3	11.4	14.4	17.4	15.4
F_s (μg)	85.5	111.6	142.5	178.2	223.3
F_i (μg)	68.8	91.8	114.8	147.0	186.1
K_0 (%)	14.4	10.2	10.1	9.8	6.9
K_1 (%)	17.9	12.4	12.5	11.8	8.3

K_0 and K_i=percentages of food supplied and ingested used for weight growth. Equal or common letters indicate lack of significant difference among final weights (one-way ANOVA, $\alpha=0.05$). a≤ab≤b and a≤b.

mean AFW obtained in each replicate of each treatment were used to calculate the individual AFW of the larvae after 2 days of culture and these values, as well as those of final AFW obtained at the end of the experiment, were transformed into the respective energy contents.

On the date of change from N V to PZ I the mean energy content of *L. vannamei* larvae is 18.85 ± 0.50 mJ μg AFW^{-1} (Angulo-Escárcega, 2005), whereas that of the feeding stages remains practically unchanged throughout the larval development (Piña-Valdez, 2004). The mean values for PZ II–PZ III determined in separate experiments by direct calorimetry by Chavira-Ortega (2003) ranged from 20.54 to 22.36 mJ μg AFW^{-1} and the global mean was 21.91 ± 0.48 mJ μg AFW^{-1}, that was used to calculate the energy content of the larvae on days 2 and 5.

Table 5
Initial energy content (E_0) and values calculated after 2 days and 5 days of culture of *L. vannamei* protozoea larvae (E_2 and E_5) and amounts of energy ingested from days 0 to 2 (I_2), 3–5 (I_5) and 0 to 5 (I_t), in J larva^{-1}

	Treatments				
	1	2	3	4	5
E_0 (J)	0.163	0.163	0.163	0.163	0.163
E_2 (J)	0.296	0.288	0.315	0.341	0.323
E_5 (J)	0.458	0.437	0.504	0.570	0.525
I_2 (J)	0.318	0.531	0.743	0.955	1.174
I_5 (J)	1.380	1.751	2.647	2.812	3.607
I_t (J)	1.698	2.282	3.390	3.767	4.781
K_{0-2} (%)	41.8	23.5	20.5	18.9	13.6
K_{3-5} (%)	11.7	8.5	7.1	8.1	5.6
K_{0-5} (%)	17.4	12.0	10.0	10.8	7.6

K_{0-2}, K_{3-5} and K_{0-5}: efficiency of utilization in % of the energy ingested used for body growth in the intervals 0–2 days, 3–5 days and 0–5 days.

The energy equivalent of the food ingested, the initial energy of the emerging PZ larvae and of those at the stage of change from PZ II to PZ III and from PZ III to M I were used to calculate the efficiency of conversion of the ingested food into energetic content.

In the first 2 days, this efficiency was close or higher than 20% for the three intermediate rations, it reached >40% for the lowest and was <14% for the highest, whereas during the three following days all efficiencies were lower and ranged between less than 6% and 11.7%. The global values calculated for the 5 days of duration of the experiments for the three intermediate rations varied between 10% and 12% and the respective values for the lowest and the highest ration were 17.4% and 7.6%, indicating that food absorption varied depending on the amount ingested, which was particularly evident during the first 2 days, with the lowest and the highest rations (Table 5).

4. Discussion

Preliminary experiments indicated that the daily handling needed for a precise evaluation of survival may increase the mortality by 15% to 20%, but that this effect is independent from the experimental treatment. Therefore, it was not considered a significant source of variation, leading to the conclusion that survival was not affected by the size of the daily ration.

Several authors pointed out that when there are no differences in mortality, the evaluation of rearing regimes for shrimp larvae should consider growth in body weight rather than rates of development or increases in length (Kuban et al., 1983, 1985; Wilkenfeld et al., 1984), although Lora-Vilchis and Voltolina (2003) found that the survival of *Artemia franciscana* was not affected by a particularly poor diet whereas development and total length yielded similar results than weight increase. Similar results were obtained with the same species fed two microalgae that are considered of high food value for filter feeders, but gave significant differences in rates of development and different sizes, as well as different body weights (Lora-Vilchis et al., 2004).

Scope for growth has been used successfully as an indicator of food suitability in aquaculture, but may not be considered a reliable estimator of body growth, because the percentages of assimilated energy that go into secondary production vary widely according to the species in culture and even for the same species, depending on the experimental conditions (Beiras et al., 1993, 1994; Saoud and Anderson, 2004).

Artiles-Rodriguez (2000) found a negative effect of overfeeding on the larval survival of *Litopenaeus schmitti*, that was tentatively explained as the effect of toxic metabolites of microalgae or of impaired swimming caused by the excessive length of fecal filaments. Our data show that there was no toxic effect because there were no significant differences in mortality and survival tended to be higher with increasing food concentrations, whereas the second problem, that was observed in some of our earlier larval cultures, was corrected in these and in other later experiments using profuse aeration that breaks up fecal filaments. Confirming the results of Kuban et al. (1983, 1985) and Wilkenfeld et al. (1984), survival and development were not affected by the size of the ration. In addition, an evaluation based on the increase in size would have been misleading, because there were some significant differences in final length that were not confirmed by the respective weight gains.

From the practical point of view, the amounts of food supplied that are ingested and those that are used for weight gain are useful indicators of the suitability of a diet or of the daily ration, but the gain in weight is also of utmost importance in larval rearing, because of the amount of energy needed for the successive changes of larval stages.

In this case, the percentages of food ingested were similar with all rations and although the lowest gave the better efficiency of food utilization, it was conducive to a significantly lower final weight and to the lowest weight and energy increases. Therefore, a ration aiming to improve the efficiency of food utilization should not be considered a viable option, whereas cautious superfluous feeding would seem an appropriate strategy for culture of the PZ larvae of *L. vannamei*.

Acknowledgements

The first author acknowledges a PROMEP scholarship for his Ph.D. studies at the Posgrado Interinstitucional en Ciencias Pecuarias of the University of Nayarit. Work supported by CONACYT grant 28232 B and CIBNOR Project AC1.4. Alejandra Medina and Martin Guerrero helped in the laboratory work. Beatriz Garcia typed the manuscript. The observations of two anonymous reviewers helped to improve the original version of the manuscript.

References

Aguirre-Hinojosa, E., López-Torres, M.A., Garza-Aguirre, M.C., 1999. Cultivo larvario de camarones peneidos. In: Martínez Córdova, L.R. (Ed.), Cultivo de camarones peneidos. AGT Editor, México, D.F., pp. 67–104.

Angulo-Escárcega, C.G., 2005. Consumo de reservas durante el desarrollo embrionario y larvario de larvas lecitotróficas de *Litopenaeus vannamei* (Boone, 1931). M.Sc. Thesis. Universidad de Sonora. Departamento de Investigaciones Científicas y Tecnológicas. Hermosillo, Mexico, 40 pp.

Artiles-Rodriguez, M.A., 2000. Efecto de la concentración de microalgas (*Chaetoceros gracilis*) sobre la supervivencia larval en el cultivo del camarón blanco (*Litopenaeus schmitti*). Bol. Cent. Invest. Biol. 34, 21–31.

Beiras, R., Perez-Camacho, A., Albentosa, M., 1993. Influence of food concentration on energy balance and growth performance of *Venerupis pullastra* seed reared in an open-flow system. Aquaculture 116, 353–365.

Beiras, R., Perez-Camacho, A., Albentosa, M., 1994. Comparison of the scope for growth with the growth performance of *Ostrea edulis* seed reared at different food concentrations in an open-flow system. Mar. Biol. 119, 227–233.

Chavira-Ortega, C.O., 2003. Evaluación de *Brachionus plicatilis* como alimento vivo para larvas de Litopenaeus vannamei.

M.Sc. Thesis. Universidad de Sonora. Departamento de Investigaciones Científicas y Tecnológicas. Hermosillo, Mexico, 66 pp.

Correa-Sandoval, F., Bückle-Ramírez, L.F., 1993. Morfología y biometría de cinco poblaciones de *Artemia franciscana* (Anostraca: Artemiidae). Rev. Biol. Trop. 41, 103–110.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Emmerson, W.D., 1980. Ingestion, growth and development of *Penaeus indicus* larvae as a function of *Thalassiosira weissflogii* cell concentration. Mar. Biol. 58, 65–73.

Gnaiger, E., 1983. Calculation on energetic and biochemical equivalents of respiratory oxygen consumption. In: Gnaiger, E., Forstner, H. (Eds.), Polarographic Oxygen Sensors. Springer, Berlin, pp. 337–345.

Jones, D.A., Yule, A.B., Holland, D.L., 1997. Larval nutrition. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition. Advances in World Aquaculture. The World Aquaculture Society, Baton Rouge, Louisiana, U.S.A., pp. 353–389.

Kuban, F.D., Wilkenfeld, J.S., Lawrence, A.L., 1983. Survival and growth of *Penaeus setiferus* L. and *Penaeus aztecus* Ives larvae fed *Artemia* beginning at the protozoea-two substage versus the mysis-one substage. J. World Maric. Soc. 14, 38–48.

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

Kurmaly, K., Yule, A.B., Jones, D.A., 1989. Comparative analysis of the growth and survival of *Penaeus monodon* (Fabricius) larvae, from protozoea 1 to postlarva 1, on live feeds, artificial diets and on combinations of both. Aquaculture 81, 27–45.

López-Elías, J.A., Voltolina, D., Chavira-Ortega, C.O., Rodríguez-Rodríguez, B.B., Sáenz-Gaxiola, L.M., Cordero-Esquivel, B., Nieves, M., 2003. Mass production of microalgae in six commercial shrimp hatcheries of the Mexican northwest. Aquac. Eng. 29, 155–164.

Lora-Vilchis, M.C., Doktor, N., 2001. Evaluation of seven algal diets for spat of the Pacific scallop *Argopecten ventricosus*. J. World Aquac. Soc. 32, 228–235.

Lora-Vilchis, M.C., Voltolina, D., 2003. Growth and survival of *Artemia franciscana* (Kellogg) fed *Chaetoceros muelleri* (Lemmermann) and *Chlorella capsulata* (Guillard). Rev. Investig. Mar. (La Habana) 24, 241–246.

Lora-Vilchis, M.C., Cordero-Esquivel, B., Voltolina, D., 2004. Growth of *Artemia franciscana* fed *Isochrysis* sp. and *Chaetoceros muelleri* during its early life stages. Aquac. Res. 35, 1086–1091.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Loya-Javellana, G., 1989. Ingestion saturation and growth responses of *Penaeus monodon* larvae to food density. Aquaculture 81, 329–336.

Marin, V., Huntley, M.E., Frost, B., 1986. Measuring feeding rates of pelagic herbivores: analysis of experimental design and methods. Mar. Biol. 93, 49–58.

Pande, S.V., Khan, R.P., Venkitasubramanian, T.A., 1963. Microdetermination of lipids and serum total fatty acid. Anal. Biochem. 6, 415–423.

Piña-Valdez, P., 2004. Balance energético de los estadios larvarios de camarón blanco (Litopenaeus vannamei) con una dieta tradicional y una no tradicional. Ph.D. Thesis. Postgrado Interinstitucional en Ciencias Pecuarias, Universidad de Nayarit. Tepic, Mexico. 120 pp.

Saoud, I.P., Anderson, G., 2004. Using scope for growth estimates to compare the suitability of feed used in shrimp aquaculture. J. World Aquac. Soc. 35, 523–528.

Villegas, C.T., Kanazawa, A., 1979. Relationship between diet composition and growth of zoeal and mysis stages of *Penaeus japonicus* (Bate). Fish. Res. J. Philipp. 4, 32–40.

Wilkenfeld, J.S., Lawrence, A.L., Kuban, F.D., 1984. Survival, metamorphosis and growth of penaeid shrimp larvae reared on variety of algal and animal foods. J. World Maric. Soc. 15, 31–49.

Yúfera, M., Rodríguez, A., Lubián, L.M., 1984. Zooplankton ingestion and feeding behavior of *Penaeus kerathurus* larvae reared in the laboratory. Aquaculture 42, 217–224.

Zar, J.H., 1996. Biostatistical Analysis, 3rd ed. Prentice-Hall, Englewood Cliffs, NJ (662 pp.).