PARTIAL REPLACEMENT OF LIVE ALGAE IN THE LARVICULTURE OF *Penaeus vannamei* WITH MICROENCAPSULATES AND SPRAY-DRIED ALGAE *Schizochytrium sp.*

P. Boeing
Pacific Seafarms International S.A. de C.V., Cerro Escondido #122, Lomas de Mazatlan, Mazatlan, Sinaloa, Mexico.

Introduction

Although the successful total replacement of live algae in the larviculture of penaeid shrimp has been reported (Ottogalli, 1991), there still remains the need to produce sufficient amounts of live algae for the average hatchery to produce good quality seed for most penaeid species. The routine production of this live algae is expensive, labour intensive and can be unpredictable. This study was designed to investigate how the amounts of live algae required can be reduced throughout the larval stages without affecting survival and metamorphic rate to the postlarval (PL) stage in *Penaeus vannamei*.

Materials and methods

Three experiments were carried out, the first was designed to establish if partial replacement of live algae with spray-dried heterotrophically grown *Schizochytrium* sp. was possible. The second experiment was designed to further reduce the amount of live algae required by using a single dose of live algae (SDLA) fed at the first feeding stage and then no more, only combinations of the dry diets. The third experiment was designed to test the *Schizochytrium* sp. at 100% replacement after the larvae had received the SDLA.

All experiments were conducted in 2 l round bottomed flasks containing larvae stocked at the zoea one stage (Z1) at a density of 100/l in triplicate. The seawater was filtered to 5 μm and was not changed during the experiment, salinity was 32 parts per thousand and the culture temperature was maintained at 28 ± 0.5 C by means of a water bath. Gentle aeration was provided through a fine bore (2 mm internal diameter) glass tube at the bottom of each flask. Previous experiments provided the optimum daily ration for the dry diets to be 6 mg/l/d for both CAR (INVE feeds) and Micro-Mac30 (Aquafauna Bio-Marine) and 8 mg/l/d for *Schizochytrium* sp. (Algamac-2000, Aquafauna Bio-Marine) or at their relative percentage substitutions. The diets were rehydrated into suspension using a glass homogeniser just prior to each feeding time, and supplied to the larvae in four equal doses per day up to mysis one (M1). All treatments received freshly hatched *Artemia* nauplii at 5/ml from M1 to PL1.

The larvae were counted upon reaching PL1 to assess the percentage survival and sub samples from each triplicate were measured for total length.

A comparison of the average survival rates and total length measurements between the live fed controls and the dry diet replacements was carried out for each experiment using ANOVA, results were considered significant at the $P < 0.05$ probability level.

Results and Discussion

The results from experiment one summarised in table 1. show that the percentage survival, metamorphic rates and total length measurements between the controls fed 50,000 cells/ml *Chaetoceros* sp. and a feeding regime of 25,000 cells/ml *Chaetoceros* sp. + 4mg/l/d Algamac was not significantly different $P<0.05$. The results also show that the larvae fed on 50,000 cells/ml *Tetraselmis suecica* or a mixture of 25,000 cells/ml *Chaetoceros* sp. and 25,000 cells/ml *T. suecica* were not able to survive, suggesting that the larvae at Z1 can not ingest the larger cell size of *T.suecica* or if it is ingested that it may not be digested. Hence demonstrating that a ration of 25,000 cells/ml *Chaetoceros* sp. alone is not sufficient to produce growth if not co-fed with the Algamac-2000.

Table 1. Average survival rates, metamorphic rates and total length measurements reached by the first postlarval stage in experiment one.

Feeding regime from Z1 to M1	Average survival rates from Z1 to PL1 (%)	Time (days) from Z1 to PL1	Average total length (mm)
Chaetoceros sp. 50,000 cells/ml	61[a]	11[a]	4.59[a]
Chaetoceros sp. 25,000 cells/ml + Algamac 4mg/l/d	71[a]	11[a]	4.74[a]
Chaetoceros sp. 25,000 cells/ml + *T. suecica* 25,000 cells/ml	0	n/a	n/a
T. suecica 50,000 cells/ml	0	n/a	n/a

n/a: not available
Values with the same superscript in the same column are not significantly different ($P<0.05$).

The results from experiment two summarised in table 2. show that there is no significant difference between the live fed controls and both of the treatments for either survival or metamorphic rate, but there was a significant difference in the total length. Both of the treatments given the SDLA followed by either of the microencapsulated diets in combination with the Algamac-2000 at 75%,25% levels respectively, were both significantly larger than the live fed controls.

Table 2. Average survival rates, metamorphic rates and total length measurements reached by the first postlarval stage in experiment two.

Feeding regime	Average survival	Time (days)	Average total

from Z1 to M1	rates from Z1 to PL1 (%)	from Z1 to PL1	length (mm)
Chaetoceros sp. 50,000 cells/ml + *T.suecica* 20,000 cells/ml	77[a]	10[a]	4.21[a]
SDLA + CAR 75% + 25%	78[a]	10[a]	4.76[b]
SDLA + Micro-Mac 30 S 75% + Algamac 25%	86[a]	10[a]	4.82[b]

Values with the same superscript in the same column are not significantly different (P<0.05).

The results from experiment three summarised in table 3. show that there was no significant difference between the average survival rates, metamorphic rates and total length between the live fed controls and the treatment of SDLA + 8mg/l/d Algamac-2000.

Table 3. Average survival rates, metamorphic rates and total length measurements reached by the first postlarval stage in experiment three.

Feeding regime from Z1 to M1	Average survival rates from Z1 to PL1 (%)	Time (days) from Z1 to PL1	Average total length (mm)
Chaetoceros sp. 50,000 cells/ml + *T.suecica* 20,000 cells/ml	94[a]	11[a]	4.75[a]
SDLA + Algamac 8mg/l/d	97[a]	11[a]	4.80[a]

Values with the same superscript in the same column are not significantly different (P<0.05)

Conclusions

The results of this study strongly suggest that the larviculture of *P. vannamei* from Z1 to PL1 is possible using only a minimal amount of micro algae fed to the first feeding stage, with subsequent and regular feeds of either well formulated microencapsulated diets or the spray-dried marine micro algae *Schizochytrium* sp.. Moreover, the addition of the SDLA to the culture medium may provide a beneficial bacterial population which assists in the use of the aforementioned dry diets and reduces the amounts of water exchange required (Freeman et al., 1996). The condition of very early postlarvae is often difficult to assess, but recent work by (Barclay and Zeller, 1996) has shown the docosahexaenoic acid (DHA) and eicosapentaenoic (EPA) rich *Schizochytrium* sp. was successfully used for the enrichment of *Artemia* nauplii with these highly unsaturated fatty acids. This suggests that the continued feeding of *Schizochytrium* sp. throughout the stages of penaeid development receiving *Artemia* nauplii, would enhance the nutritional value of the *Artemia* to the shrimp. It has been pointed out that resistance to stress is related to the presence of adequate levels of DHA and EPA in crustacean larvae (Dhert et al., 1992). This is in agreement with recent findings (Montaño and Navarro, 1996) that wild

post larvae contain higher levels of both DHA and EPA, and are in turn more resistant to handling stress, resulting in higher survival when introduced to the on-growing ponds.

References

Barclay W., S. Zeller. 1996. Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and *Artemia* nauplii by feeding spray-dried *Schizochytrium* sp. J. World Aqua. Soc. vol 27 no3 p314-322

Dhert P., P. Lavens, and P. Sorgeloos. 1992. Stress evaluation: a tool for quality control of hatchery produced shrimp and fish fry. Aquacult. Sci. 17:6-10

Freeman M., N. Misciattelli, Z. Che Cob, C.A. Martinez Palacios, A.O. Alab, and D.A. Jones. 1996. Preliminary trials demonstrating the effects of culture water conditioning on the use of microencapsulated diets in penaeid larval culture. (In press) Tercer Symposium Internacional de Nutricion Acuicola. 11 al 13 Noviembre de 1996 Monterrey, Nuevo Leon, Mexico.

Montaño M., J.C. Navarro. 1996. Fatty acids of wild and cultured Peneaus vannamei larvae from Ecuador. Aquaculture 142:259-268.

Ottogali L. 1991. Complete substitution of microcapsules for algae for *P.stylirostris* larval rearing in New Caledonia. J. World Aqua. Soc. 22:46A

Proximate Analysis

Protein	39%
Fat	32%
Carbohydrate	13%
Ash	12%
Moisture	3%

Sterols

Cholesterol (mg/g)	2.6
Brassicasterol (mg/g)	0.5
Stigmasterol (mg/g)	1.5

Other

Lecithin (mg/100g)	2860.0
Carotene (mg/lb)	22.5
Xanthophyll	6.3

Fatty Acid Profile

Fatty acid content (%w/w) 29.5%

Fatty acid (% of total fatty acids)

14:00	11.0
16:00	38.5
16:01	7.3
18:00	1.1
18:01	4.1
20:3w6	0.4
20:5w3	0.6
22:5w6	12.9
22:6 w3	24.0