Rearing of the Larval Stages of Prawn, *Penaeus japonicus* BATE, Using Artificial Diet

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Abstract

Survival and growth rates of the zoeal and mysis stages of the prawn, *Penaeus japonicus* Bate, were studied using natural and artificial diets.

The highest survival rate, 34.2%, was obtained in the larvae fed with the artificial diet, Diet-B. Larvae fed with the diatom, *Chaetoceros gracilis*, plus *Artemia* nauplii did not give the best survival; however, growth was the fastest in this group. The larvae metamorphosed into the mysis$_3$ stage in 7 days. Comparable growth rate was obtained on larvae fed with Diet-B. The larvae metamorphosed into the mysis$_3$ stage in 8 days. The results thus seem to demonstrate that Diet-B is an effective diet for the early larval stages of the prawn, *P. japonicus*.

INTRODUCTION

Mass culture of *Penaeus japonicus* Bate during the larval stages, zoeal and mysis stages, has been based almost entirely on live planktonic food[1-4]. The diatoms, *Skeletonema costatum* (Grev.), *Chaetoceros* spp., *Nitzshia* spp., etc. and *Artemia salina* provide the food source. However, success of diatom culture is dependent on weather conditions, the diatoms growing well only in sunny weather. Also fluctuation in the quality of natural sea water has prevented reliable culture of the algae. SHIGENO[5] has stressed the difficulties of scaling up seed production, in particular the use of *Skeletonema* as food. Since then many shrimp culturists have improved rearing procedures. MOCK[6], BROWN[7], and FURUKAWA[8] proposed the use of preserved algae and yeast as supplemented food when living algae is scarce, and in Japan soybean cake has been used in emergencies[6,9]. However, a great deal of manual help and some equipment are necessary for the cultivation and concentration of the algae and yeast.

The use of *Artemia* as a nutritious and convenient food is restricted by its high cost and occasional variations in quality[10]. The large scale culture of living rotifers such as *Brachionus* spp. has been attempted as a possible economic alternative to *Artemia*[11].

ZEIN-ELDIN[12] and SHIGENO[13] have reported some success with penaeid larvae fed with a compounded diet. KANAZAWA *et al.*[14] have devised an artificial diet for the nutritional requirements of prawn. However, feeding trials on artificial diets have been

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conducted mainly on the juvenile stage of the prawn[14-18]. Now that ways of solving the physical problems of larval diet production have been suggested, the study of larval nutrition is just as important and should commence in earnest. Hence, the authors intended to investigate the possibility of using artificially prepared diets and some commercial feeds for larval rearing of *P. japonicus*. This study aimed to find the most suitable substitute for the live planktonic food for the mass culture of *P. japonicus* larvae.

MATERIALS AND METHODS

Larvae Larvae of *P. japonicus*, $zoea_1$ stage, hatched from Tarumizu Experimental Station, Kagoshima Prefecture were used in this experiment. The larvae were transferred from the hatching tank to round plastic aquaria at the end of the nauplius stage (V or VI). Each aquarium was filled up to the 5-liter level with sea water filtered through a plastic screen of 10 micron mesh size. A stocking rate of 50 larvae per liter of sea water was used, each aquarium therefore containing 250 larvae. The larvae were reared from $zoea_1$ to $mysis_3$ stages using the four different feeds or diets shown in Table 1. Each treatment was replicated twice in a randomized block design.

Table 1. Feeds and feeding rates used for rearing $zoea_1$ to $mysis_3$ stages of *P. japonicus* larvae

Treatment	Feeds	Feeding rate
1	Diatom, *Chaetoceros gracilis* + *Artemia* nauplii	50-100 × 10^3 cells/ml 50 nauplii/larva
2	Artificial diet, Diet-B	0.16 mg/larva/day
3	Short-necked clam meal	0.16 mg/larva/day
4	Mysid meal	0.16 mg/larva/day

Preparation of the diets and feeding methods The diatom, *Chaetoceros gracilis*, was cultured in separate aquaria using 150 mg/liter KNO_3 and 15 mg/liter KH_2PO_4 as enrichment[19]. Besides these nutrients, 30 mg/liter Clewat-32 and 10 mg/liter Clewat-Ca were added as inorganic nutrients. The density of diatoms in the larval rearing water was initially adjusted to 50-100 × 10^3 cells per ml, the concentration suggested by HUDINAGA and MIYAMURA[2]. The diatoms did not grow in the rearing water and the density fell to less than 20 × 10^3 cells per ml during the rearing period. Newly hatched *Artemia* nauplii were fed to the larvae during the mysis stages in addition to the diatoms.

The composition of the artificial diet, Diet-B, devised and modified by KANAZAWA *et al.*[14,16], for the nutritional requirements of prawn is shown in Table 2. The addition of mineral mixture (8.6%) and vitamin mixture (2.7%) was found to give superior growth[16]. Agar was used as binder of the artificial diet. The ingredients were thoroughly mixed with 130-135 ml of water per 100 g of dry diet. After adjusting to pH 6.8, the diet was heated at 100 ℃ in an autoclave for 20 min and sealed in a Kurehalon tube, and heated at 100 ℃ for 10 min. After cooling in tap water, it was

Table 2 Composition of the artificial diet, Diet-B

Ingredient	% in dry diet
Glucose	5.5
Sucrose	10.0
Starch	4.0
Glucosamine	0.8
Casein (Lipid and vitamin free)	50.0
Na-citrate	0.3
Na-Succinate	0.3
Pollack residual oil*1	8.0
Cholesterol	0.5
Mineral mixture*2	8.6
Vitamin mixture*3	2.7
Cellulose powder	9.3
Agar	3.0
Water	130-135 ml

*1 Residual oil obtained by distilling away vitamin A from pollack liver oil

*2 K_2HPO_4 2.000, $Ca_3(PO_4)_2$ 2.720, $MgSo_4 \cdot 7H_2O$ 3.041 and $NaH_2PO_4 \cdot 2H_2O$ 0.790 g / 100 g dry diet

*3 *p*-Aminobenzoic acid 10.00, Biotin 0.40, Inositol 400.00, Nicotinic acid 40.00, Ca-Pantothenate 60.00, Pyridoxine-HCl 12.00, Riboflavin 8.00, Thiamine-HCl 4.00, Menadione 4.00, β-Carotene 9.60, α-Tocopherol 20.00, Cyanocobalamine 0.08, Calciferol 1.20, Na-Ascorabate 2000.00, Folic acid 0.80, and Choline chloride 120.00 mg/100 g of dry diet

stored in the refrigerator. Then the diet was cut into small pieces and dried at 37 ℃ for 3 days. After drying the mixed diet was reduced into powder and passed through a sieve mesh with 10-50 μm mesh size.

The other two test diets, short-necked clam and Mysid meals, are commercial powdered diets and therefore received no treatment except sieving as above. The diets were then stored in the refrigerator until use. Diets were fed at a feeding rate of 0.16 mg / larva / day, following the rate used by HIRATA *et al.*[9] using soy-cake particles for the zoea stage. Diatom density was monitored daily using a Thoma hemacytometer.

Growth, temperature and pH Growth, measured in terms of the developmental stage of the larvae, was recorded daily by taking a random sample of 10 larvae from each treatment and examining them under the microscope following Hudinaga's classification[1]. Growth of the larvae is given by the following formula:

$$\text{Growth (Developmental stage)} = \frac{A}{10}$$

where A = absolute value × no. of larvae

Stage	Absolute Value
Zoea-1	1
2	2
3	3
Mysis-1	4
2	5
3	6

Temperature was recorded twice daily while pH was measured only once daily. Survival and growth rate of the prawn lervae were recorded at the end of the rearing period.

RESULTS AND DISCUSSION

Filtration of the sea water through a 10-micron screen mesh was assumed to have eliminated most of the plankton. Evidence of actual feeding on the artificial diets was shown by the presence of long faeces trailing from behind the larvae. Survival and growth of *P. japonicus* larvae fed with diatoms plus *Artemia* and artificial diets are shown in Table 3. Number of feeding days varied due to low survival and slow

Table 3 Growth and survival of *P. japonicus* larvae fed with diatoms plus *Artemia* and artificial diets

	Diatom + *Artemia*	Diet-B	Short-necked clam meal	Mysid meal
Days of feeding	7	8	9	8
No. of larvae before feeding	250	250	250	250
Survival rate (%)	21.6	34.2	12.4	3.0
Developmental stage (Growth)	6.0	6.0	4.7	4.4
Body length (mm)	4.1	3.7	3.8	2.8
Water temperature ℃				
Mean	28.0	28.1	28.1	28.0
Range	26.8-30.5	26.2-30.6	26.4-30.0	26.0-30.6
pH (range)	7.67-8.40	8.05-8.40	7.55-8.40	7.77-8.40

development in larvae fed with short-necked clam and Mysid meals. Feeding with Diet-B resulted in the highest survival of 34.2%. With diatoms plus *Artemia*, a survival rate of 21.6% was obtained. The survival rates of larvae receiving short-necked clam and Mysid meals, on the other hand, were extremely low, 12.4% and 3%, respectively. The lower survival rate obtained from diatoms plus *Artemia* may be attributed to the low feeding density. As mentioned above, HUDINAGA and MIYAMURA[2]

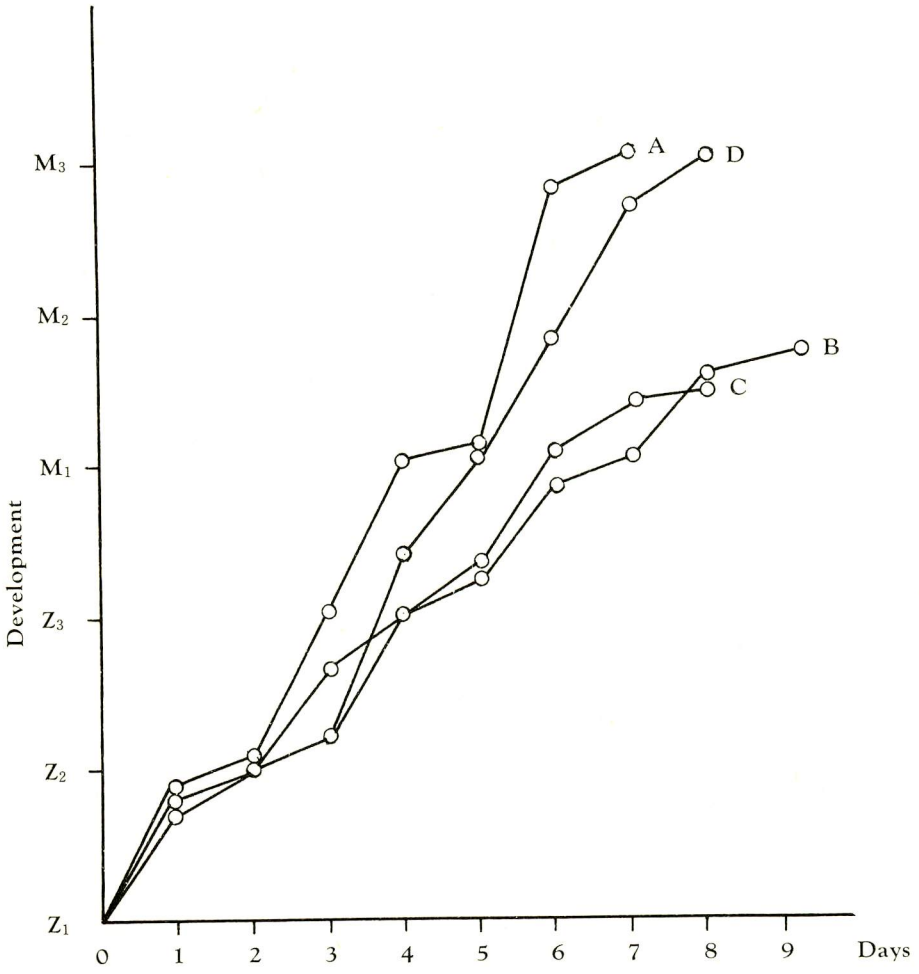

Fig. 1 Growth of *P. japonicus* larvae on different diets A : *Chaetocros gracilis* and *Artemia*, B : Short-necked clam meal, C : Mysid meal, D : Diet B

suggested a feeding density of 50-100 $\times$ 10^3 cells per ml as best for mass culture of *P. japonicus* larvae. In the present study, difficulties were encountered in maintaining this density and resulted in a diatom density of less than 20 $\times$ 10^3 cells per ml during the rearing period.

Fig. 1 shows the development of the larvae maintained on different diets. Diatoms plus *Artemia* feeding gave the fastest development and highest gain in length. Larvae metamorphosed into the mysis$_3$ stage in 7 days of rearing period. Diet-B feeding resulted in comparable development, the larvae reaching the mysis$_3$ stage in 8 days. On the other hand, with short-necked clam and Mysid meals feeding, the larvae metamorphosed up to the mysis$_{1\&2}$ stages only after 9 days of rearing period.

Growth, measured in terms of development, obtained in this study using Diet-B was better than that obtained by HIRATA *et al.*[9], using soybean cake particles. They obtained a zoeal period (zoea$_1$-zoea$_3$ stages) of 7 days using soybean cake particles compared to 8 days from zoea$_1$ to mysis$_3$ stages using Diet-B in the present study.

Recently, we have suggested the preparation of microencapsulated diet for the zoeal and mysis stages of *P. japonicus*, and the possibility of rearing of prawn larvae using pariculate diet such as Diet B[20]. The results obtained in the present study demonstrate that the zoea and mysis stages of *P. japonicus* can survive and grow on artificially prepared diet, Diet-B, which was comparable to that of the natural food, phytoplankton and zooplankton. It is suggested that Diet-B alone can sustain the rearing of the zoea and mysis stages of *P. japonicus*. However, feeding trials on a large scale using Diet-B warrant further study. Also, diet-B may be used as a test diet for the nutritional requirements of prawn larvae.

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