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Rearing of the Larval Crab, *Portunus trituberculatus*, with the Artificial Microparticulate Diets

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Abstract

Attempts to rear the larval crab, *Portunus trituberculatus*, with the aritificial microparticulate diets, nylon-protein microencapsulated diet (MED), gelatin-arabic gum-MED, cholesterol-lecithin micro-coated diet (MCD), and carrageenan microbinded diet (MBD), were conducted. In experiment I, the feeding rate of the rotifers in the practical seed production of the crab was reduced by half and replaced with one of the above artificial diets during the period of $zoea_2$ - $zoea_4$ stages. The larval crabs receiving every artificial diet along with the rotifers by the above mentioned manner had 11-20% survival comparable to the survival rate of the control group receiving the live feeds such as the rotifer, *Artemia* nauplii, and the minced meat of a short-necked clam. In experiment II, the larval crab was reared with one of the artificial diets alone after hatching. Every artificial diet sustained growth of the larval crab from $zoea_1$ to juvenile stages, although the survival rates of the crab receiving the artificial diets were lower than that of the crab receiving the live feeds (control group). The results of the present study thus show the success in rearing the larval crab with the aritificial microparticulate diets.

As for the several species of fish[1] and the prawn, *Penaeus japonicus*[2], the technique of seed production using live food organisms has been established and used practically at the various Fish-Farming Centers and Fisheries Experimental Stations in Japan. The crab, *Portunus trituberculatus*, is one of the promising crustaceans in the field of aquaculture. Although the seed production of this crab has been conducted practically in several districts of Japan, it is still difficult to produce the seed stably due to many problems such as canibalism, high mortality, etc. during the larval period.

The present study is planned to rear the larval *P. trituberculatus* with the artificial micropaticulate diets. We believe that such an approach will give the track of resolving many problems in the seed production and clarifying the nutritional requirements of this crab. This paper shows the success in rearing the larval *P. trituberculatus* with the artificial diets from zoeal stages to juvenile crabs.

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Materials and Methods

Two feeding trials were conducted in the present study. $Zoea_1$ larvae of the crab, *P. trituberculatus*, were obtained from egg-bearing females and then divided into lots of 10,000 larvae in a 500-*l* polycarbonate tank containing 200 *l* of sea water for feeding trials. The feeding experiments were carried out at the Oyano Branch of the Fisheries Experimental Station of Kumamoto Prefecture under the conditions listed in Table 1.

Table 1. Rearing and feeding methods of the larval crab, *P. trituberculatus*.

Condition	Experiment 1	Experiment 2
Larvae used	$Zoea_1$ larvae	$Zoea_1$ larvae
Experimental period	12 days	16 days
Tank (capacity)	500 liters	500 liters
Number of the larvae	10,000	10,000
Water temperature (average)	30.7℃	28.5℃
Feeding frequency	6 times/day	6 times/day
Feeding level (% of body weight/day)		
Sum of food	200%	200%
Artificial diet	100%	100%

Table 2. Composition of the artificial diets*1

Principal ingredient*2	(%)
Casein	50.0
DL-Methionine	1.0
L-Tryptophan	0.5
Glucose	5.5
Sucrose	10.0
α-Starch	4.0
Glucosamine HCl	0.8
Sodium citrate	0.3
Sodium succinate	0.3
Pollack liver oil	8.0
Soybean phospholipids*3	3.0
Cholesterol	0.5
Mineral mixture	8.6
Vitamin mixture	5.4
α-Cellulose	2.1

*1 The artificial diets used in the present study are as follows: nylon-protein MED, gelatin-gum arbic MED, carrageenan MBD, and cholesterol-lecithin MCD. In addition to the principal ingredients listed in Table 2, the artificial diets contain the materials such as gelatin, carrageenan etc. for the forming and stabilization of diets.

*2 The composition of the principal ingredients was similar to that of the artificial diets for the prawn, *Penaeus japonicus*[10].

*3 Riken Vitamin Co. Ltd.

The sea water in the tanks was increased daily at the rate of 100 *l*/day for first 3 days and then renewed at the rate of 2000 *l*/day during the subsequent feeding period.

In the present study, the dietary value of 4 artificial diets was tested. Table 2 shows the principal composition of 4 artificial diets used : nylon-protein microencapsulated diet (MED), gelatin-gum arabic microencapsulated diet (MED), carrageenan microbinded diet (MBD), and cholesterol-lecithin micro-coated diet (MCD). The methods for the preparation of these 4 types of diets were described previously in detail[3,4].

Experimental groups of the larvae were fed the artificial diets and/or the live feeds as shown in Table 3. The larval crabs were given the artificial diets at the feeding rate of 200 % of their body weight daily. The artificial diets were supplied to the crab larvae

Table 3. Developmental stages of the crab, diets, and feeding rates*1.

Experiment	Group	Developmental stage					
		$Zoea_1$	$Zoea_2$	$Zoea_3$	$Zoea_4$	Megalopa	Juvenile
I	1 (Control)	Rotifer (5-10 ind./ml)					
					Artemia (1-100 ind./larva)		
					Short-necked clam (5-60 g/ton)		
	2	Rotifer (3-5 ind./ml)					
					Nylon-protein MED		
	3	Rotifer (3-5 ind./ml)					
					Cholesterol-lecithin MCD		
	4	Rotifer (3-5 ind./ml)					
					Gelatin-gum-arabic MED		
	5	Rotifer (3-5 ind./ml)					
					Carrageenan MBD		
II	6 (Control)	Rotifer (5-10 ind./ml)					
					Artemia (1-100 ind./larva)		
							Short-necked clam (5-60 g/ton)
	7	Nylon-protein MED					
	8	Cholesterol-lecithin MCD					
	9	Carrageenan MBD					

*1 The crab larvae were received the diets at the 200 % of their body weight daily. The body weights (mg) of the crab larva at each stage were as follows : $zoea_1$, 0.06 ; $zoea_2$, 0.14 ; $zoea_3$, 0.35 ; $zoea_4$, 0.75 ; megalopa, 1.65, juvenile, 3.17.

6 time a day (every 2 hours from 8 : 00 o'clock). One hour after supplying the diets, the sinked diets were floated by agitating the water with the air-lift. The control groups of the larvae were given the combination of the rotifers, *Artemia* nauplii at 11 : 30 o' clock, and a minced short-necked clam, *Tapes philippinarum*, at 13 : 30 o'clock. The population density of rotifers in the tanks was checked at 6 :30 and 18 : 00 o'clocks and adjusted to the desired density.

In experiment I, the larval crabs were reared with the rotifers during the period of $zoea_1$-$zoea_2$ stages and then received the live feeds (control group) or the combination of artificial diet and rotifer. In the groups of larvae receiving the artificial diets, the feeding rate of the rotifers in control diet was reduced by half and replaced with one of the artificial diets during the period of $zoea_2$-$zoea_4$ stages. In experiment II, attempts to rear the larval crab with the artificial diets alone during the period of hatching to juvenile crabs. Every day the survival raes and larval stages[5] were determined on 10 random samples in order to evaluate the dietary value of the diets used.

Results

The results of experiment I are given in Fig. 1 and Table 4. The $zoea_1$ larvae grew up to the $zoea_3$ stage in about 6 days in every experimental group. However, growth of the larvae during the period of $zoea_3$-juvenile crab stages differed with the types of diets, good growth being obtained in the groups receiving the carrageenan MBD (group 5) and control diet (group 1). In groups 1 and 5, more than half of the alive specimens after 12 days was the juvenile crabs (Table 4). Whereas, more than half of the alive specimens was still megalopa larvae after 12 days when received the nylon-protein MED (group 2), cholesterol-lecithin MCD (group 3), and gelatin-gum arabic MED (group 4). As for the survival rates after 12 days, the larval crabs receiving the nylon-protein MED, gelatin-gum arabic MED, or carrageenan MBD together with the rotifers had the survival rates (16.1-19.7 %) comparable to that (17.0 %) of the larvae receiving the control diet. In the case of the control group, the survival rates of larvae decreased steadily during the period of $zoea_1$-megalopa stages and markedly between the megalopa and juvenile crab stages. Interestingly, the larval crabs receiving the nylon-protein MED, gelatin-gum arabic MED, or carrageenan MED along with the rotifers had the higher survival rates until $zoea_4$ stages than the control group receiving the live feeds. However, the survival rates of the larval crab receiving 3 artificial diets were decreased markedly during the period of $zoea_4$-megalopa stages. As mentioned above, the results of experiment I show that 3 artificial diets, especially carrageenan MBD, were effective in sustaining growth of the larval crab.

The results of experiment II are shown in Fig. 2 and Table 4. The larval carbs receiving cholesterol-lecithin MCD or carrageenan MBD grew up to the juvenile crabs

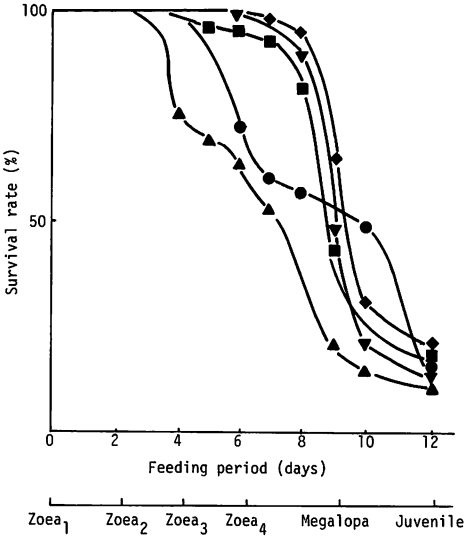

Fig. 1. Survival rates and larval stages of the crab larvae in experiment I.
● : Control
▼ : Rotifer+Nylon-protein MED
▲ : Rotifer+Cholesterol-lecithin MCD
◆ : Rotifer+Gelatin-gum arabic MED
■ : Rotifer+Carrageenan MBD

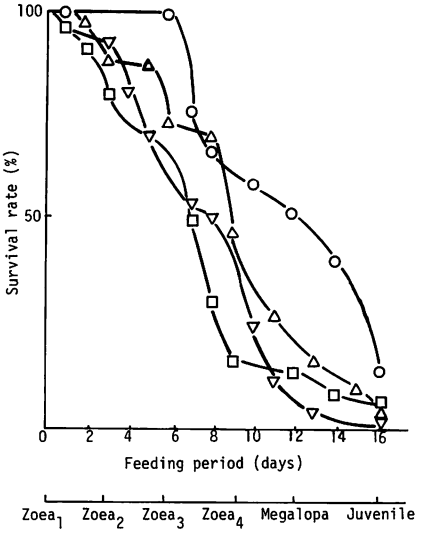

Fig. 2. Survival rates and larval stages of the crab larvae in experiment II.
○ : Control
▽ : Nylon-protein MED
□ : Cholesterol-lecithin MCD
△ : Carrageenan MBD

Table 4. Survival rates and larval stages of the crab larvae after 12 day-(experiment I) and 16 day-(experiment II) feeding trials.

Experiment	Group	Diet used	Number of larvae		Survival rate (%)
			Megalopa	Juvenile	
I	1	Control	10	1,690	17.0
	2	Rotifer + Nylon-protein MED	1,260	350	16.1
	3	Rotifer + Cholesterol-lecithin MCD	610	490	11.0
	4	Rotifer + Gelatin-gum arabic MED	1,670	300	19.7
	5	Rotifer + Carrageenan MBD	630	1,120	17.5
II	6	Control	18	1,682	17.0
	7	Nylon-protein MED	10	0	0.1
	8	Cholesterol-lecithin MCD	250	110	3.6
	9	Carrageenan MBD	19	291	3.1

with the survival rates of 3.1-3.6 %, whereas those receiving nylon-protein MED reached the megalopa stage with 0.1 % survival. However, growth and survival rates of the larval crabs on the artificial diets were inferior to those of the control group receiving the live feeds. Although it was possible to rear the larval crab, *P. trituberculatus*, with either cholesterol-lecithin MCD or carrageenan MED alone from the $zoea_1$ to juvenile stages, a notable decrease in the survival rates during the metamorphosis indicated the nutritional and/or physical incompleteness of the artificial diets.

Discussion

The seedlings of crustaceans such as the prawn, *P. japonicus*, have been practically produced by using the live feeds in Japan. On the other hand, there have been several attempts to rear crustacean larvae with the artificially prepared microparticulate diets[6-8]. We have also tried to rear *P. japonicus* larvae with several types of microparticulate diets, indicating the high dietary value of diets with zein as a coating[3,9]. Later, we have further reared the prawn larvae successfully with carrageenan MBD[4], pointing out the necessity of dietary sterols[10,11] and phosholipids[10,12] for their normal growth and survival. Thus, it now appears probable that growth of the prawn larvae is supported with the microparticulate diets instead of live food with high survival rates.

In the present study, the dietary value of 4 microparticulate diets, nylon-protein MED, gelatin-gum arabic MED, cholesterol-lecithin MCD, and carrageenan MED, for the larval crab, *P. trituberculatus*, was examined. As a result, the larval crabs receiving the carrageenan MBD together with the rotifers had good growth and survival as comparable to the control group receiving the live feeds. The carrageenan MBD also

supported growth of the crab larvae from $zoea_1$ to juveniles stages when supplied as a sole diet, but the survival rate with the carrageenan MBD was lower than that with the live feeds. As stated above, the present study proves the possibility of rearing the larval crab, *P. trituberculatus*, with the microparticulate dites alone, showing the high mortarity during the metamorphosis from $zoea_4$ to meagalopa and juvenile stages as also observed in the practical seed production of this crab using the live feeds.

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