Mem. Fac. Fish. Kagoshima Univ.,
Vol. 50, pp. 27～29 (2001)

Larval Rearing of the Swimming Crab *Portunus trituberculatus* in the Japanese Summer Season Using *Brachionus rotundiformis* Cultured with *Nannochloropsis oculata*[*1]

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Keywords : *Portunus, Brachionus, Nannochloropsis, Tetraselmis,* Larval rearing, Summer season

Abstract

Nannochloropsis oculata was cultured in the Japanese summer season and tested for larval growth of the swimming crab *Portnus tritberculatus.* Three treatments (1, 2 and 3) were prepared: treatment 1; feeding of *Artemia* nauplius and the rotifer cultured with *N. oculata,* treatment 2; feeding of *Artemia* nauplius and the rotifer cultured with *T. tetrathele,* and treatment 3; with neither *Artemia* nor rotifer. The number of crab larvae over 11 days till megalops stage decreased gradually from 35 to 0 - 7 in treatment 1, and 1 - 4 in treatment 2. In tratment 3, the number of survival in the larvae decreased rapidly to zero in few days. The low content of n-3 highly unsaturated fatty acids (HUFA) in *N. oculata* might have caused the low survival.

The rotifer *Brachionus rotundiformis* and *Artemia* nauplii are fed on larval rearing of the swimming crab *Portunus trituberculatus*[1]. Unicellular alga of *Nannochloropsis oculata* is commonly used as a food for rotifer culture. The optimum temperature for *N. oculata* culture ranges from 20 to 25 ℃[2]. Its culture is not stable in the summer season of Japan. Therefore, the seed production of *P. trituberculatus* is usually conducted before the summer season.

We then tested on larval rearing of *P. trituberculatus* with *N. oculata* in the Japanese summer season from 4th to 15th of July in order to develop a rearing technique applicable in tropical areas, i.e. the Philippines. Rearing with *Tetraselmis tetrathele* which grows well under a wide temperature range was also tested.

Gravid females of *P. trituberculatus* were obtained from Matsushima Fishermen's Cooperative in Kumamoto Prefecture, Japan, and were transported live in a tank with aeration to our laboratory (Faculty of Fisheries, Kagoshima University). It took 3.5 h to transport them, while the water temperature was maintained at about 19 ℃ to prevent eggs from hatching as pre-zoea stage larvae. In addition, formalin was added in the seawater to prevent from fungal infection. On arriving, the crab was transferred into a 500-L tank filled with seawater of 28 ℃. Z1 larvae, hatched from 22:00 to 23:00 on that day, were used for the culture experiment. From the larvae gathering near the surface after stopping aeration, 35 individuals were pipetted into 1-L glass beakers. Three treatments were prepared: treatment 1; feeding of *Artemia* nauplius and the rotifer cultured with *N. oculata,* treatment 2; feeding of *Artemia* nauplius and the rotifer cultured with *T. tetrathele,* and treatment 3; with neither rotifer nor *Artemia.* Each treatment was carried out in triplicate. Aeration was supplied at 110 mL/min into all rearing water. This larval culture was conducted at room temperature.

*1 This research was supported by JSPS.

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Prior to the larval rearing experiment, *N. oculata* and *T. tetrathele* were cultured outdoor in 3 plastic tanks with a capacity of 500-L. *N. oculata* or *T. tetrathele* cultured with 3-L vessels at an aeration rate of 650 mL/min in an incubator (temp. 25±1 ℃; photoperiod, 15L: 9D; light intensity, 160 μmol/m^2/s) was inoculated into a 500-L tank at a density of 0.6 $\times 10^6$ or 0.05$\times 10^6$ cells/mL. At the end of exponential phase, these cells were inoculated into new culture media. For the preparation of culture media, seawater was filtered through a 45-μm opening plankton net and enriched with agricultural fertilizers including ammonium sulfate (150 mg/L), calcium superphosphate (30 mg/L), and Clewat 32 (15 mg/L, a mixture of trace metals and EDTA, Teikoku Kagaku Sangyo Co., Ltd, Osaka). These culture media were aerated at 16 L/min.

The rotifer was cultured in the room where the crab larvae were reared. Phytoplankton at the end of exponential phase were used as food. The cell densities ranged from 20$\times 10^6$ to 35$\times 10^6$ cells/mL for *N. oculata* and from 0.2$\times 10^6$ to 0.5$\times 10^6$ cells/mL for *T. tetrathele*, respectively. Thirty liters of tanks were used for the culture of rotifer which was fed on each phytoplankton. In each tank, aeration was provided at 2.8 L/min. The rotifer densities were controlled to maintain the phytoplankton density higher than 0.1 $\times 10^6$ cells/mL for *N. oculata* and 0.05$\times 10^6$ cells/mL for *T. tetrathele*. The rotifer was harvested everyday with a 45-μm pore size plankton net.

Artemia (China strain) cysts were incubated with aeration at 14L/min in a 3-L Ehrenmeyer flask with 2.7 L of seawater at room temperature.

The nauplii hatched by 14-17 h after initiation of incubation were collected with a siphon, and concentrated with a plankton net. Feeding rates of these *Artemia* naupii and the rotifer were 2.0 and 20 indiv./mL for Z1, 3.0 and 30 indiv./mL for Z2, 4.0 and 30 indiv./mL for Z3, and 6.0 and 30 indiv./mL for Z4, respectively. These food organisms were transferred to new rearing seawater at 09:00 every morning after counting the larval survival number. The new rearing seawater was supplied from a 30-L reserve container in which aeration was provided at 3 L/min. Feeding was done once a day. The rearing water was gently agitated every 2 h after feeding until evening everyday throughout the experimental period.

Temperature and pH in the larval rearing water were determined by counting the larval number with a portable pH meter (Yokogawa PH81). The measurements were also conducted in outdoor cultures of *N. oculata* and *T. tetrathele*.

The survival number of the crab larvae is shown in Table 1. There was observed a little change in larval numbers within a few days after initiation of rearing. Thereafter, the larval number decreased rapidly to zero in treatment 3. Its final developmental stage was Z2. On the other hand, the survival number to give the final number of megalops stage decreased gradually from 0 to 7 in treatment 1, and from 1 to 4 in treatment 2.

The standard deviation and range of water temperature were 28.1±0.6 and 27.1-28.7 ℃ in treatment 1, 28.1±0.5 and 27.1-28.7 ℃ in treatment 2, and 27.9 ±0.6 and 27.1-28.5 ℃ in treatment 3. Similarly, these physical parameters were 26.9±1.2 and 25.1-29.1 ℃ for *N.* oculata, and 27.1±1.4 and 25.1-29.5 ℃ for *T. tetrathele*. The pH values of all larval rearing water of the crab were kept at 7.8-8.2. In cultures of *N. oculata* and *T. tetrathele*, pH ranged from 8.9 to 7.0 and from 8.8 to 6.2, respectively. Both cultures showed lower pH values at late stationary phase.

It has been reported that n-3 highly unsaturated fatty acids (HUFA) are essential for the larval growth of *P. trituberculatus*[1]. Although the n-3 HUFA content of *N. oculata* is as high as 40% of the

Table 1 The survival number of swimming crab larvae reared in three different treatments

Treatment	Rearing day											
	0	1	2	3	4	5	6	7	8	9	10	11
	35	35	28	9	2	2	2	2	0			
1	35	33	33	24	20	19	19	16	10	8	7	7
	33	33	28	20	15	9	6	6	5	4	2	1
	35	33	28	24	19	18	14	11	2	2	1	1
2	35	35	34	30	23	19	16	12	5	4	4	4
	35	34	28	24	22	11	7	5	3	3	1	1
	35	29	29	24	8	1	0					
3	35	28	28	25	12	0						
	33	33	33	29	14	0						

total lipid at 14.7 ℃, its content decreases rapidly as temperature increases[3]. For example, the content of eicosapentaenoic acid in the total fatty acids reduces lower than half of the highest as temperature increases to 28.0 ℃. The same phenomenon was recognized in the field during warm season for neo- and micro-phytoplankton[4]. On the other hand, the n-3 HUFA content of *T. tetrathele* is lower than 14% even at lower temperatures[5]. It is considered that deficiency of n-3 HUFA is a possible reason for the low survival of crab larvae in the present study. Accordingly, the enrichment of n-3 HUFA to the food organism seems to improve the larval survival.

The health condition of newly hatched *P. trituberculatus* larvae in this experiment was assumed to be normal, because of the high larval survival rate without feeding few days after initiation of rearing.

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