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Effects of Feeding Regimes on Survival, Development and Growth of Blue Swimming Crab, *Portunus pelagicus* (Linnaeus, 1758) Larvae

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Abstract: A study was conducted with newly hatched larvae of blue swimming crab, *Portunus pelagicus* reared in six different treatments. Treatment 1 larvae was fed with rotifer only at zoea 1 to megalopa stages, Treatment 2 larvae was fed with *Artemia* only at zoea 1 to megalopa stages and Treatment 3 larvae was fed with rotifer at zoea 1 to megalopa stages with *Artemia* at zoea 2 to megalopa stages. Meanwhile in Treatment 4, rotifer was given at zoea 1 to megalopa stages with *Artemia* at zoea 3 to megalopa stages and in Treatment 5 larvae was fed with rotifer at zoea 1 to megalopa stages and *Artemia* at zoea 4 to megalopa stages for *Portunus pelagicus* larvae. In Treatment 6 larvae was fed with rotifer at zoea 1 to megalopa stages and *Artemia* at megalopa stages. Result shows that there was higher survival rate of zoea 2 stages of larval on the Treatment 1 in the diet whilst Treatment 2 suffered high mortality. The highest survival rate and growth rate was obtained in Treatment 3. There was no significant difference (P=1.0; P>0.05) between the mean values of development duration for all treatment except for Treatment 3 where the development from zoea 4 to megalopa stages was 1 day faster. Based on results, further experiments should be done to evaluate differential nutritional composition when larvae are reared in large quantity for mass seed production.

Key words: *Artemia* · *Brachious* sp., Feeding regimes · Larvae · *Portunus pelagicus*

INTRODUCTION

Blue swimming crab, *Portunus pelagicus* is distributed throughout the Indo-Pacific region [1, 2]. *P. pelagicus* is a commercially important species for aquaculture [3]. Crab culture gained its importance in the last few decades due to great demand of live crabs and crab products in the export market. However, crab culture is presently dependent on wild caught seeds that are not sufficient. Seed production in the hatchery is of primary importance for developing a comprehensive technology. The development of crab hatchery technology has been focused primarily on mass seed production, where low survival rate to juvenile stage remains to be a problem [4]. The main reason for this problem has not been fully understood; it is believed to be associated with inappropriate nutrition [5].

Rotifer and *Artemia* sp. are the two live feeds most commonly used in crab culture [3, 6]. These planktonic organisms tolerate a wide range of environmental conditions, can be cultured at high densities and can be easily enriched with nutritional supplements, antibiotics or probiotics [7]. Earlier workers used rotifer as feed for earlier zoea stages [3, 6]. Other experiments were conducted by given *Artemia* sp. as feed for late zoea stages [8, 9]. Natural diet or live food was the basic requirement in the feeding regimes; meanwhile the different or suitable introduction of live food such as rotifer and *Artemia* nauplii in larval rearing was the main problem in crab culture.

Thus, to develop seed production technology for increasing the survival rate for this species, a study was undertaken evaluating feeding regimes. The aim of this study was to assess the effects of feeding

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regimes on the larval survival rate, larval stage development and larval growth rate of blue swimming crab, *P. pelagicus*.

MATERIALS AND METHODS

Broodstock Crabs: *P. pelagicus* berried females used in the present study were caught from the wild using gill net around Johor coastal water (Johor, Malaysia) and were transported to the Marine Hatchery, Institute of Tropical Aquaculture, University Malaysia Terengganu. The berried females were kept in a circular fibreglass holding tank with stocking density of one crab per ton of water. Chopped fish meat was given once daily as food. When berried female has matured with black eggs, the crab was transfers to the hatching tanks (500 L) with stocking density of one crab per tank. After hatching, the aeration in the hatching tank was turn-off for 10 to 15 min to allow the vigorously swimming larvae to aggregate at surface, where they were collected.

Experimental Design and Setup: Newly one day hatched larvae were stocked at density 50 ind.L^{-1} in 24 units, 100 L aquarium tanks containing 90 L of treated water. Four replicate tanks were prepared for each treatment. 10% of water was changed daily for zoea 1 to zoea 4 and 50% daily for megalopa. Dead larvae and uneaten food were siphoned out from the tanks daily. Live foods were given at around 0800 hrs and 1600 hrs after the water exchange.

Newly molted megalopa were promptly collected and transferred to a different plastic aquarium. The experiment was terminated when all Zoea 4 stage larvae had molted to megalopa stage. Water quality parameters such as temperature, salinity, pH, dissolved oxygen and ammonia were recorded daily.

A total of six feeding regimes were planned for maintaining t he crab larvae for the present study (Table 1). The live food density given for each crab larvae stages of *P. pelagicus* are as in Table 2 according to [4].

Data and Statistical Analysis: The survival rate (%) at a particular larval stage was calculated as the number of larvae moulted successfully to the next stage divided by the initial number of larvae in each replicate.

The larval development rate was calculated using Larval Stage Index (LSI). To calculate the LSI, the larval stages were index as in Table 3. The larval stage was identified using dissecting microscope. The example to determine the LSI is as follows:

If there are 10 larvae been sampled from one treatment, 7 larvae are in Zoea 2 stage and 3 larvae are in Zoea 1 stage. So the LSI is as follows:

$$LSI = [(2 \times 7) + (1 \times 3)]/10$$
$$= 1.7 \tilde{} 2.0$$

Thus, the larvae are concluded in Zoea 2 stage.

Table 1: Six different feeding regimes throughout the study periods for *P. pelagicus* larvae.

T*	Feeding regimes
1	Rotifer only at Zoea 1 to Megalopa (RZ1-M)
2	*Artemia* only at Zoea 1 to Megalopa (AZ1-M)
3	Rotifer at Zoea 1 to Megalopa; *Artemia* at Zoea 2 to Megalopa (RZ1-M+AZ2-M)
4	Rotifer at Zoea 1 to Megalopa; *Artemia* at Zoea 3 to Megalopa (RZ1-M+AZ3-M)
5	Rotifer at Zoea 1 to Megalopa; *Artemia* at Zoea 4 to Megalopa (RZ1-M+AZ4-M)
6	Rotifer at Zoea 1 to Megalopa; *Artemia* at Megalopa (RZ1-M+AM)

*T=Treatment

Table 2: Live food density given for each crab larval stages of *P. pelagicus* [4]

	Food given	
Crab larval stages	Rotifer (individuals/larvae)	*Artemia* nauplii (individual (s) /larvae)
Zoea 1 (Z1)	50	0
Zoea 2 (Z2)	40	10
Zoea 3 (Z3)	30	20
Zoea 4 (Z4)	20	30
Megalopa (M)	10	40

Table 3: Crab larval stage with Larval Stage Index (LSI) used in the present study

Crab Larval Stage	Larval Stage Index
Zoea 1 (Z1)	1
Zoea 2 (Z2)	2
Zoea 3 (Z3)	3
Zoea 4 (Z4)	4
Megalopa (M)	5

Sampled crab larvae were put into disposal vials (Bjorn bottle), preserved in 10 % of formalin and the wet weight was measured with a microbalance. The mean wet body weights (BW) for each treatment for different larval stages were used to calculate the specific growth rate (SGR), according to the following formula:

$$SGR\ (\%) = \frac{\text{In (Final body weight)} - \text{In (Initial body weight)} \times 100\%}{\text{Culture period (day)}}$$

In the experiment, collected data were analyzing using SPSS for Windows version 16.0 software. One-way analysis of variance (ANOVA) was used to determine whether the significant variation between treatments existed. All results are presented as means ± SD. The differences between means were determined and compared by Tukey HSD test. The difference is displayed as statistically significant when $P<0.05$.

RESULTS

Larval Survival Rate: There was mean survival rate of up to 66.33% ± 0.09 of Z2 stages of 4 day after hatching (DAH) larval on the treatments that included rotifer, T1 in the diet whilst those fed purely with *Artemia* nauplii, T2 suffered high mortality at survival rate of 5.08% ± 0.16 are as in Table 4. In combination with rotifer, T3 which *Artemia* nauplii was present in the diet of Z2 have a better survival rate compare to the T4, T5 and T6 where *Artemia* nauplii was present at Z3, Z4 and M stages respectively. Based on the results as in Table 4, there were significant difference ($P=0.00$; $P<0.05$) in survival rate between T1, T2, T3, T4, T5 and T6.

The mean survival rate was done for every Z1 until M stages by days are as in Table 4. The highest survival rate (8.25% ± 0.02) from Z1 to M stages was obtained on a combination of rotifer (Z1 to M stages) and introduction of *Artemia* nauplii from Z2 to M stages. The mean survival rate for the other treatment using the combination of rotifer and *Artemia* nauplii for T4, T5 and T6 are 6.33% ± 0.02, 5.33% ± 0.01 and 4.08% ± 0.01 respectively as been showed in Table 4.

Larval Stage Development: The mean values of developmental duration for all larval stages under different treatment conditions are as in Table 5. There was no significant difference ($P=1.0$; $P>0.05$) between the mean values of development duration of LSI for all treatment except for T3, where the development from Z4 to M stages was 1 day faster respectively as been showed in Table 5.

Specific Growth Rate: The larval mean BW on 1 DAH in all treatments was 0.015±0.0008 mg. The highest SGR was obtained on T3, within 14 days, the mean BW increased

Table 4: Mean survival rate (%) of *P. pelagicus* larvae reared under different feeding regimes

T[1] / D[3]	T1	T2	T3	T4*	T5*	T6*
2	84.42% ± 0.12[b]	73.50% ± 0.12[b]	88.00% ± 0.08[a]	84.75% ± 0.10[b]	86.67 ± 0.12[b]	85.17% ± 0.08[b]
3	73.42% ± 0.10[b]	34.42% ± 0.16[b]	75.92% ± 0.09[b]	73.67% ± 0.10[b]	72.75% ± 0.07[a]	71.00% ± 0.04[a]
4	66.33% ± 0.09[b]	5.08% ± 0.17[b]	69.58% ± 0.07[a]	66.75% ± 0.08[a]	64.42% ± 0.09[a]	62.33% ± 0.09[b]
5	58.92% ± 0.09[b]	na[2]	62.00% ± 0.07[a]	59.08% ± 0.06[a]	57.00% ± 0.11[b]	55.17% ± 0.08[a]
6	51.83% ± 0.12[b]	na[2]	55.42% ± 0.09[b]	53.17% ± 0.04[a]	50.50% ± 0.14[b]	48.58% ± 0.04[a]
7	44.50% ± 0.11[b]	na[2]	48.58% ± 0.08[a]	47.00% ± 0.04[a]	44.67% ± 0.13[b]	42.58% ± 0.04[a]
8	38.33% ± 0.11[b]	na[2]	41.58% ± 0.10[b]	40.42% ± 0.03[a]	38.17% ± 0.13[b]	36.50% ± 0.03[a]
9	31.67% ± 0.12[b]	na[2]	35.58% ± 0.09[b]	33.42% ± 0.04[a]	32.00% ± 0.09[a]	30.92% ± 0.03[a]
10	25.92% ± 0.11[b]	na[2]	29.75% ± 0.05[a]	27.92% ± 0.04[a]	25.67% ± 0.10[b]	25.33% ± 0.01[a]
11	19.67% ± 0.08[b]	na[2]	24.17% ± 0.06[a]	22.17% ± 0.03[a]	20.67% ± 0.08[a]	20.00% ± 0.03[a]
12	14.67% ± 0.07[a]	na[2]	18.58% ± 0.04[a]	17.92% ± 0.03[a]	15.67% ± 0.07[a]	15.00% ± 0.01[a]
13	8.42% ± 0.03[a]	na[2]	13.25% ± 0.04[a]	11.83% ± 0.03[a]	10.58% ± 0.06[a]	9.25% ± 0.02[a]
14	3.42% ± 0.01[a]	na[2]	8.25% ± 0.02[a]	6.33% ± 0.02[a]	5.33% ± 0.01[a]	4.08% ± 0.01[a]

T[1]: Treatment; na[2]: not available because 100% mortality; D[3]: Days; T1: Rotifer only at Z1 to M stages; T2: *Artemia* only at Z1 to M stages; T3: Rotifer at Z1 to M stages and *Artemia* at Z2 to M stages; T4: Rotifer at Z1 to M stages and *Artemia* at Z3 to M stages; T5: Rotifer at Z1 to M stages and *Artemia* at Z4 to M stages; T6: Rotifer at Z1 to M stages; *Artemia* at M stages. Values in the same column (for each treatments) showing the different superscript are significantly different ($P<0.05$)

Table 5: Mean number of larval stage index (LSI) of *P. pelagicus* larvae reared at different feeding regimes

T[1]	Days Crab	1	2	3	4	5	6	7	8	9	10	11	12	13	14
T1	LSI[2]	1.0	1.0	1.0	1.6±0.4[b]	2.0	2.0	2.7±0.13[b]	3.0	3.0	3.7±0.12[b]	4.0	4.0	4.7±0.13[b]	5.0
	LS[3]	Z1	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z3	Z4	Z4	Z4	M	M
T2	LSI[2]	1.0	1.0	1.0	1.7±0.13[b]	2.0	na[4]	na[4]	na[4]	na[4]	na[4]	na[4]	na[4]	na[4]	na[4]
	LS[3]	Z1	Z1	Z1	Z2	Z2	na[4]	na[4]	na[4]	na[4]	na[4]	na[4]	na[4]	na[4]	na[4]
T3	LSI[2]	1.0	1.0	1.0	1.8±0.10[a]	2.0	2.0	2.7±0.15[b]	3.0	3.7±0.19[a]	4.0	4.0	4.7±0.05[a]	5.0	5.0
	LS[3]	Z1	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z4	Z4	Z4	M	M	M
T4	LSI[2]	1.0	1.0	1.0	1.7±0.19[b]	2.0	2.0	2.8±0.12[b]	3.0	3.0	3.7±0.12[b]	4.0	4.0	4.7±0.23[b]	5.0
	LS[3]	Z1	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z3	Z4	Z4	Z4	M	M
T5	LSI[2]	1.0	1.0	1.0	1.7±0.10[b]	2.0	2.0	2.8±0.13[b]	3.0	3.0	3.7±0.12[b]	4.0	4.0	4.7±0.23[b]	5.0
	LS[3]	Z1	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z3	Z4	Z4	Z4	M	M
T6	LSI[2]	1.0	1.0	1.0	1.7±0.10[b]	2.0	2.0	2.7±0.21[b]	3.0	3.0	3.8±0.17[b]	4.0	4.0	4.7±0.17[b]	5.0
	LS[3]	Z1	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z3	Z4	Z4	Z4	M	M

T[1]: Treatment; LSI[2]: Larval stage index; LS[3]: Larval stages; na[4]: not available because 100% mortality; T1: Rotifer only at Z1 to M stages; T2: *Artemia* only at Z1 to M stages; T3: Rotifer at Z1 to M stages and *Artemia* at Z2 to M stages; T4: Rotifer at Z1 to M stages and *Artemia* at Z3 to M stages; T5: Rotifer at Z1 to M stages and *Artemia* at Z4 to M stages; T6: Rotifer at Z1 to M stages; *Artemia* at M stages. Values in the same column (for each treatments) showing the different superscript are significantly different (P<0.05)

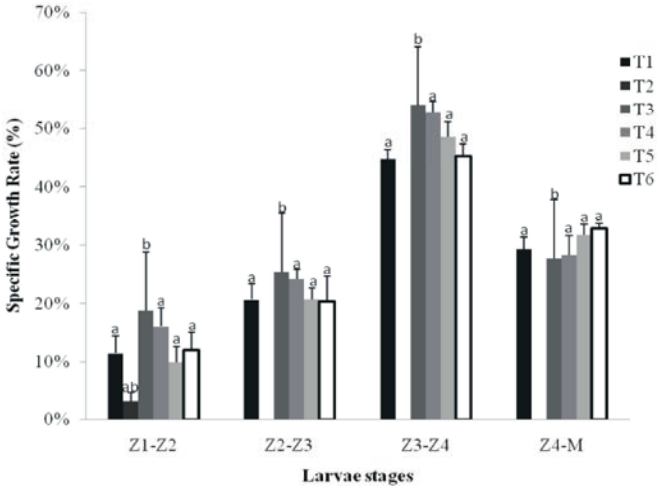

Fig. 1: Mean specific growth rate (%) of *P. pelagicus* larvae by larvae stages fed with different feeding regimes. T1: Rotifer only at Z1 to M stages; T2: *Artemia* only at Z1 to M stages; T3: Rotifer at Z1 to M stages and *Artemia* at Z2 to M stages; T4: Rotifer at Z1 to M stages and *Artemia* at Z3 to M stages; T5: Rotifer at Z1 to M stages and *Artemia* at Z4 to M stages; T6: Rotifer at Z1 to M stages; *Artemia* at M stages. The different letters indicates significant different (P<0.05) among treatment.

to 0.798 ± 0.028 mg with mean SGR of 30.57% ± 0.17 and the lowest mean BW in the T1 was 0.448 ± 0.028 mg with mean SGR of 26.13% ± 0.14 except on the larvae in the T2 where survived until Z2 stage. Further, there were no significant differences (P=0.347; P>0.05) among other treatments; SGR for T1, T3, T4, T5 and T6 were 26.13% ± 0.14, 30.57% ± 0.17, 29.59% ± 0.16, 27.35% ± 0.16 and 26.93 ± 0.15%, respectively.

Figure 1 shows the mean SGR of Z1-Z2, Z2-Z3, Z3-Z4 and Z4-M from larvae reared in different treatments. The mean SGR of crab larvae from Z1 to Z2 stage are 11% ± 0.03, 3% ± 0.014, 19% ± 0.033, 16% ± 0.032, 10% ± 0.26 and 12% ± 0.03 for larvae reared in T1, T3, T4, T5 and T6. The mean SGR of crab larvae from Z2 to Z3 stages for T3 produced the highest mean SGR of 25% ± 0.025 as compare to the other five treatments of T1, T4, T5 and T6 with mean SGR of 21% ± 0.027, 24% ± 0.016, 21% ± 0.019 and 20% ± 0.043 respectively. T1 and T6 produced the lower mean SGR of 45% ± 0.016 and 45% ± 0.021 from Z3 to Z4 stage as compare to another three treatment of T3, T4 and T5 with mean SGR of 54% ± 0.030, 53% ± 0.021 and 49% ± 0.026 respectively.

The result also shows that only T6 have a highest mean SGR from Z4 to M stage of 33% ± 0.008 as compare to the other five treatments of T1, T3, T4 and T5 with mean SGR of 29% ± 0.021, 28% ± 0.028, 28% ± 0.034 and 32% ± 0.018 respectively.

DISCUSSIONS

The current study investigates the feeding regime, which was the main factor of crab larval survival, rapid developmentsand growth.Conclusively, the highest survival, development rate and growth rate of the larvae were observed in T3. The results obtained were compared with previous studies, which have shown successful feeding combinations using *Artemia* nauplii and rotifer. The presence of live food such as *Artemia* nauplii and rotifer would provide a beneficial effect for the mass seed production of frog crab, *Ranina ranina* [10].

Further, previous work on *Thalamita crenata* showed higher survival rate in Z1 and Z2 stages when fed with rotifer and from Z3 to Z5 stages when fed with both *Artemia* nauplii and rotifer [11]. According to [5], more than 70% survival rate of mud crab, *Scylla tranquebarica* was achieved until Z4 stage after feeding with *Artemia* nauplii. The larvae of mud crab, *Scylla serrata* were reared purely on *Artemia* nauplii diet from stage Z2 onwards while Z1 survived on dinoflagellates, producing a better survival rate [12]. Studies on the ingestion of rotifer and *Artemia* nauplii by mud crab *S. serrata*, showed fourfold higher ingestion of the rotifers compared to ingestion of the *Artemi*a when the concentration of rotifer and *Artemia* was at the same level (20 individual/mL), during early zoea stages of *S. serrata* [8]. The portunid crab *Thalamita crenata*, Z1 and Z2 stages larvae had the highest survival, when fed with rotifer alone, but later stages (Z3 to Z5 stages) showed better survival and development on a combined diet (rotifer and *Artemia* nauplii) [11]. The mud crab, *S. serrata*, obtained highest megalopa production when fed on a diet of *Artemia* nauplii in combination with rotifer [6, 13]. For *Scylla paramamosain*, there was best zoea survival when rotifers were replaced by *Artemia* nauplii at Z2 or Z3 stages [14]. *Artemia* nauplii should be introduced before the end of the Z3 stage. The Z3 stage has been identified as a critical stage of nutritional vulnerability which if lacking in suitable live feed, it would compromise survival and development in *S. paramamosain* [14], *S. tranquebarica* [5], *S. serrata* [6, 8, 15] and other crab larvae [11].

In T1 of the present study, the high mortality occurred from Z2 to M stages and larval development was delayed up to one day. Previous work showed that the low survival rates were reported on other Portunid crabs, which unable to survive after continued presence of rotifers up to the last zoeal stage [5, 8, 11, 14]. In the previous work, larvae of *S. serrata* were able to survive during early zoea stages but mortality was observed before reaching megalopa stages [15, 16] and *S. tranquebarica* larvae also suffered high mortalities caused by the failure of Z5 stage larvae to completely moult to megalopa [5].

Furthermore, the high mortality occurred due to several factors, for example: the nutrients provided by rotifers are not good enough for larvae to maintain survival and inter moult duration. It was attributed to insufficient nutrition content of certain cultured rotifer species and low survival durations during the rearing period. The essential fatty acids could be obtained by the crab larvae through enrichment of prey organisms [5]. The high mortality in the inter moult duration on crab larvae caused by moult death syndrome and is believed to have been caused by the presence of bacteria in the rearing water [16] and high concentrations of the microalgae *Nannochloropsis* in the larval rearing tanks [17].

Even though, rotifers are generally considered as a more appropriate live feed for late larval stages of *P. pelagicus*, this gives further evidence that the diet should include *Artemia* nauplii [15, 18, 19] to provide all requirement nutrition in the crab larvae diet. In the present study, live feed did not enrichment and mean stocking density of rotifer was 33.4 individual/larvae. The zoea larvae of *Portunus trituberculatus* could be reared to megalopa stages with high survival rates when fed with rotifers at a higher density of 40 individual/mL [18]. The enrichment of rotifer should be a routine technique for larval reared [20]. In this study, if rotifer were enrichment than the results on survival, growth and development of larvae will change due to the addition of nutrients in rotifer.

The first zoea stage was introduced with *Artemia* nauplii only but the larval survival decrease and none survived at second zoea stage. The high mortality in the Z1 stage showed that *Artemia* nauplii was an inappropriate feed to early stage of crabs larvae. Furthermore, the survival rate of *P. pelagicus* larvae was compromised, when not provided with rotifers during Z1 and Z2 stages. However, the zoea larvae of *S. serrata* were unable to consume the entire *Artemia* nauplii but they were able to ingest bits and pieces of the body, specifically the head and the appendages [8].

Furthermore, *Artemia* nauplii contained nutrients higher nutrients (51 - 55 percent of protein, 14 - 15 percent of carbohydrate, 13 - 19 percent of fat and 3 - 15 percent of n-3 HUFA) compared to rotifer it was observed

essential to feed *Artemia* nauplii to fish larvae as soon as possible after hatching to take full advantage of the yolk and stored reserves found in freshly hatched nauplii. The high mortalities in Z1 stage and not survive at Z2 stage may indicate that the presence of rotifers in the early stages have fulfill the nutrients requirement of larvae for post moult. Meanwhile, introduction *Artemia* nauplii only in the early stage crab larvae were not recommended but it can introduce for the late zoea stage and megalopa.

CONCLUSIONS

Food type and feeding regimes influenced survival rate, larval development and specific growth rate of *P. pelagicus* larvae. The best survival, most rapid development and specific growth rate the highest number of megalopa produced were obtained when larvae was fed with a combination diet consisting of rotifers from stages Z1 to M stages and *Artemia* nauplii from last day of stage Z2 until stage M stages. The continued existence of rotifers in the diet was essential for metamorphosis to megalopa. Larvae fed with *Artemia* nauplii only died at Z2 stage while those fed exclusively with rotifer only were able to reach megalopa, but the number of megalopa produced was significantly lower than those raised on a mixed diet. High mortality of individual crab larvae occurred after sole feeding with *Artemia* nauplii only and rotifer only compared to feeding with combination of *Artemia* nauplii and rotifer. Different types of feed introduced during larval rearing probably affected the survival rate of crab larvae.

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