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Evaluation of Different Larval Feeds for Survival and Development of Early Stage Mud Crab (*Scylla olivacea*)

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ABSTRACT

The effects of early feeds and feeding regimes on survival and development of mud crab (*Scylla olivacea*) larvae were studied in two experimental phases ; phase 1 from zoea 1 to zoea 5 and phase 2 from megalopa to the first crab stage. In the first phase trial, the treatments were characterized by feeding regimes of two types of feeds of which the first feed was applied from outset zoea 1 and later when entering zoea 2, the first feed was partially substituted by the second feed. The treatments of aboved regimes were as followed: 1. rotifer and artemia 2. rotifer and frozen copepod 3. artemia and rotifer 4. artemia and frozen copepod 5. microencapsulated feed and artemia and 6. microencapsulated feed and frozen copepod. It was shown that the survival rate of zoea when developed till zoea 5 were 13.89, 4.00, 2.50, 10.33, 0.11 and 0.00% for treatments 1 to 6, respectively. The survival rates for treatments 1 and 4 were higher ($P<0.05$) than those of the other treatments. The average time for the development of zoea into zoea 5 were less ($P<0.05$) in treatments 1 and 4 which were 20.67 and 21.67 days, respectively.

The second phase aimed to study the effects of feed on the development of megalopa to the first crab stage. The experiment was randomized into five treatments for the following feeds: 1. artemia 2. chopped mussel 3. artemia and larval shrimp feed 4. artemia and chopped mussel 5. larval shrimp feed and chopped mussel. Survival rates of megalopa developed to the first crab stage were 75.00, 16.44, 63.89, 58.33 and 47.22% for treatments 1 to 5, respectively. There were no significant differences ($P>0.05$) in survival rates for treatments 1, 3 and 4 and these were higher ($P<0.05$) than that of treatment 2. The average development time of megalopa to the first crab stage in treatments 1 to 5 were 12.05, 15.22, 10.77, 12.15 and 10.99 days, respectively, which were not significantly different ($P>0.05$) among treatments.

Key words: *Scylla olivacea*, larval feed, zoea, megalopa, crab

INTRODUCTION

Mud crabs (*Scylla olivacea*) commonly found along the coast of Gulf and Andaman sea of Thailand, contribute to well-being and socio-economics of coastal fisherfolks. In 2002, Thailand exported over 3,900 metricton of mud crab, generated significant income to the fishers and culturists . However, the population of mud crab is under threatened and has continuously declined over years due to many reasons such as the destruction and polluting of mud crab habitats, the over-exploitation and undersized catching of mud crab (Ronquillo *et al.*, 1998). The concerns on the decline of crabs have brought about the attempts in conservation of wild crab stocks and increase

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population through aquaculture. For the latter, although the success in mass propagation of mud crab seeds is achieved, high mortality usually occurs at the stage of zoea and megalopa. Many causes of mortality are speculated and one of the most concern is focused on the proper use of larval feed and feeding management in the early stage of mud crab. In normal practice,rotifer and artemia are usually used as early feed for mud crab (Treece and Davis, 2000). These zooplankton are well nutritional defined for fish and crab larvae. However, the preparation process and cost are usually not attractive so the proper choices and feeding management may be the key success for larvae nursing.

The purpose of this study was to evaluate the effects of various early feed for mud crabs either feeding solely or in combination of two types on the survival and development of mud crab in zoea and megalopa stage. The result from this study would provide the information for the improvement of early feeds and feeding management for larval crab which may contribute to the reduction of mortality of mud crab larvae.

MATERIALS AND METHODS

The studies were conducted into two experiments. Experiment 1 was to evaluate the effects of different early feed on survival and development of zoea 1 to zoea 5. Experiment 2 was carried out for the same objective from megalopa stage to the first crab stage.

Experiment 1

Preparation of experimental larval crab

An ovigerous female crab obtained from a private farm was brought to the hatchery of the Ranong Coastal Aquaculture Station and acclimatized in a 50-l plastic tank containing 30 ppt sea water with aeration. The crab was daily fed with chopped trash fishes and squids at 0700 and 1800 hours and raised until maturation and spawned. Hatched zoea 1 (Z_1) were collected and randomly used for further experiments.

Experimental feed and sea water

Larval feeds used in the experiment were rotifer (R), artemia (A), copepod (C), and microencapsulate feed (M). Rotifer was prepared by cultivation with chorella in a 200-l fiberglass tank at 30 ppt salinity . Artemia obtained from hatching of commercial artemia cyst, copepod used was the thawed copepod. Microencapsulated feed was from commercially available.

Natural raw sea water of 26-28 ppt salinity was obtained from a private hatchery. It was stocked in a 20-ton concrete tank and treated with 30 ppm calcium hyperchlorite and allowed three days for the chloride evaporation before utilization. Salinity of treated sea water was adjusted to 30 ppt by using NaCl .

Experimental management

The experiments were carried out in 18 stylofoam boxes of $31.0 \times 43.5 \times 29.5$ cm^3,each containing 15-l of prepared sea-water. The boxes were randomly assigned to six treatments with three replications each. The treatments were different in either larval food type, their combination and/or sequence of feeding as shown in Table I.

Three allotments of 100 zoea 1 mud crab were randomly stocked in each stylofoam box to contain 300 zoeae. Throughout the experiment 1, zoea 1 were fed once a day in the morning according to the designed schemes. At stage of zoea 1, all treatments were fed with the different single early feeds till the development to stage of zoea 2. Then the combination of the feeds used in zoea 1 and another type of early feeds were applied for respective treatments till the larval crabs developed into zoea 5.

Rotifer, artemia and microencapsulate feed were fed to zoea 1 at the rate of 30 individual/ml, 10 individual/ml and 4 mg/l, respectively. During

Table 1 Treatments assigned for the treatment 1.

Treatment	Crab stage				
	Z_1	Z_2	Z_3	Z_4	Z_5
1	Rotifer	← Rotifer + Artemia →			
2	Rotifer	← Rotifer + Copepod →			
3	Artemia	← Artemia + Rotifer →			
4	Artemia	← Artemia + Copepod →			
5	Microencapsulate	← Microencapsulate + Artemia →			
6	Microencapsulate	← Microencapsulate + Copepod →			

zoea 2 - zoea 5, where the combination of feeds were used, the amount of early feeds were 15 individual/ml, 5 individual/ml, 5 individual/ml and 2 mg/l for rotifer, artemia, copepod and microencapsulate, respectively. Therefore, the amounts of previous feed used in zoea 1 were reduced to half when used in combination.

During daily water exchange, live zoea were siphoned from the stylofoam boxes to the containers. The boxes were cleaned and refilled with sea water. Zoea were then picked up from the containers by a wide-bore pipette, the number were recorded and returned to their respective boxes.

Survival rate of zoea (%) and the developmental period (days) from zoea 1 to zoea 5 were determined and subjected to statistical analysis.

Experiment 2

The experiment 2 was to study the influences of feed on the development of megalopa to the first crab stage . Five treatments set up with different types of feed were used for the experiment as follows

Treatment 1: newly hatched artemia (A)

Treatment 2: finely ground mollusc meat (GM)

Treatment 3: newly hatched artemia + black tiger larval shrimp feed (A+F)

Treatment 4: newly hatched artemia + finely ground mollusc meat (A+GM)

Treatment 5: black tiger larval shrimp feed + finely ground mollusc meat (F+GM)

Experiment 2 was carried out in 15 stylofoam boxes as in the experiment 1. A piece of foam with twelve cut holes of 8 cm in diameter was floated within each box. This foam was for holding twelve punched-plastic cups, each containing only a megalopa to prevent cannibalism. Each box contained 20 liter of 30 ppt salinity sea water. The boxes were randomly allocated to each of five treatments with three replications.

Megalopa were daily fed with their respective treatments right after daily water change. Artemia, finely ground mollusc meat, black tiger larval shrimp feed were fed to megalopa at the rate of 10 individuals/ml, 0.1g/megalopa and 0.1 g/ megalopa, respectively. For those treatments with the combination schemes, the quantities of food were reduced to half for each item. Survival rate (%) and developmental period (days) of first crab of all treatments were analyzed for statistical difference.

Data analysis

Survival rate (%) and developmental period (days) influenced by different food types in both experiments were analyzed with one-way analysis

of variance. Mean differences were determined by Duncan's new multiple range test at 95% confident level. Statistical analysis was performed using SPSS software.

RESULTS

Table 1 and Figure 1 showed the influences of different larval foods on survival rate of zoea in each stage. Survival rates of zoea 1 which developed to zoea 2 were not signficantly different (P>0.05) among treatments 1, 2, 3, 4 and 5. For treatment 6, zoea 1 survived to zoea 2 at lower percentage than those in other treatments. Accumulated high mortality in almost all treatments were observed during the development of zoea 1 to zoea 3. Zoea 3 where treatments 1, 2 and 4 survived only 46, 40 and 43%, respectively, but still were higher (P>0.05) than those of other treatments. It was obvious that zoea1 raised on treatment 6 was hardly survived to zoea 3 as its survival rate was only 0.45%. The survival rate of zoea1 developed to zoea 4 were 16, 11, 4, 12, 0.11 and 0% for treatments 1, 2, 3, 4, 5 and 6, respectively. The survival of zoea 5 developed from zoea 1 were only 14% and 10% for those fed with treatments 1 and 4, while the survival to zoea 5 in the other treatments were almost none.

The developmental period from zoea 1 to zoea 5 in treatments 1, 2, 3, 4 and 5 were 20.67, 28.00, 25.50, 21.67 and 29.00 days, respectively (Table 2). For treatment 6, zoea 1 failed to develop to zoea 5 since 100% mortality was observed in zoea 4.

Table 3 and Figure 2 showed the result of the second experiment. The survival rate of the first crab stage developed from megalopa was the highest (75%) for those fed on artemia. This survival rate was not significantly different (P>0.05) from those given artemia in combination with black tiger larval shrimp feed (treatment 3) or finely ground mollusc meat (treatment 4). The lowest survival rate (19%) was found in megalopa fed solely on finely ground mollusc meat (treatment 2).

Development periods for megalopa developed to the first crab stage were observed at the range of 11 to 15 days. These periods were not significantly different (P>0.05) among treatments.

DISCUSSION

It was clearly indicated in the experiment 1 that live feeds (rotifer and artemia) were superior to microencapsulate feed (processed feed) for the development of zoea. The survival rate of these zoea raised on live feeds were higher probably because the opportunity of zoea to come across live feed was higher than that to processed feed. Zoea at its early life stage has not yet fully developed swimming appendices so that it is unable to approach microencapsulated feed. The advantage of live feed is that they are freely dispersed in water made them readily available for zoea which taking feed randomly at this stage (Warner, 1977; Hill, 1979). Although microencapsulate feed is efficient for supporting survival of mysis shrimp up to 70% (Teshima *et al*., 1982), but in this study microencapsulated feed is not suitable for larval crab. This result agreed with the study by Quinitio *et al*. (1999) who reported high mortality of crab zoea nursed on microencapsulated feed, due to the zoea was unable to take on bottom spreading feed.

Regardless of feed types given to zoea, the overall survival from zoea 1 to zoea 2 was higher than 65%. This could attribute to the fact that at the early stage of zoea, yolk sac might play an important role on providing nutrient to zoea and contribute to the survival rate (Tseng, 1987).

During the developement of zoea 2 to zoea 3, the survival rate of zoea among treatments were quite low for some treatments. This was probably because yolk sac in zoea 2 was completly absorbed and no longer available for zoea. Therefore, zoea were fed on proper larval foods could survive at

Table 1 Mean percent survival of zoea 1 to zoea 5 (Z_1 - Z_5) as influenced by feeding of different larval feeds.

Larval feed	Z_1	Z_2	Z_3	Z_4	Z_5
1. R-(R+A)		81.45±0.69^{a}	45.56±13.83^{a}	15.56±8.33^{a}	13.89±7.03^{a}
2. R-(R+C)		85.22±3.35^{a}	39.89±8.44^{a}	11.00±6.33^{ab}	4.00±2.18^{bc}
3. A-(A+R)		86.17±4.95^{a}	20.50±10.61^{b}	4.00±4.24^{bc}	2.50±2.59^{c}
4. A-(A+C)		86.56±6.20^{a}	43.33±13.05^{a}	11.89±6.26^{ab}	10.33±5.51^{ab}
5. M-(M+A)		72.78±8.06^{ab}	12.22±6.85^{bc}	0.11±0.19^{c}	0.11±0.19^{c}
6. M-(M+C)		65.89±12.04^{b}	0.45±0.39^{c}	0.00^{c}	0.00^{c}

Means within the same column followed by the same letter are not significantly different (P>0.05)
note: Data are expressed as mean±SD

1. R-(R+A) = Rotifer - (Rotifer+Artemia)
2. R-(R+C) = Rotifer - (Rotifer+Copepod)
3. A-(A+R) = Artemia - (Artemia+Rotifer)
4. A-(A+C) = Artemia – (Artemia+Copepod)
5. M-(M+A) = Microencapsulate – (Microencapsulate+ Artemia)
6. M-(M+C) = Microencapsulate – (Microencapsulate+ Copepod)

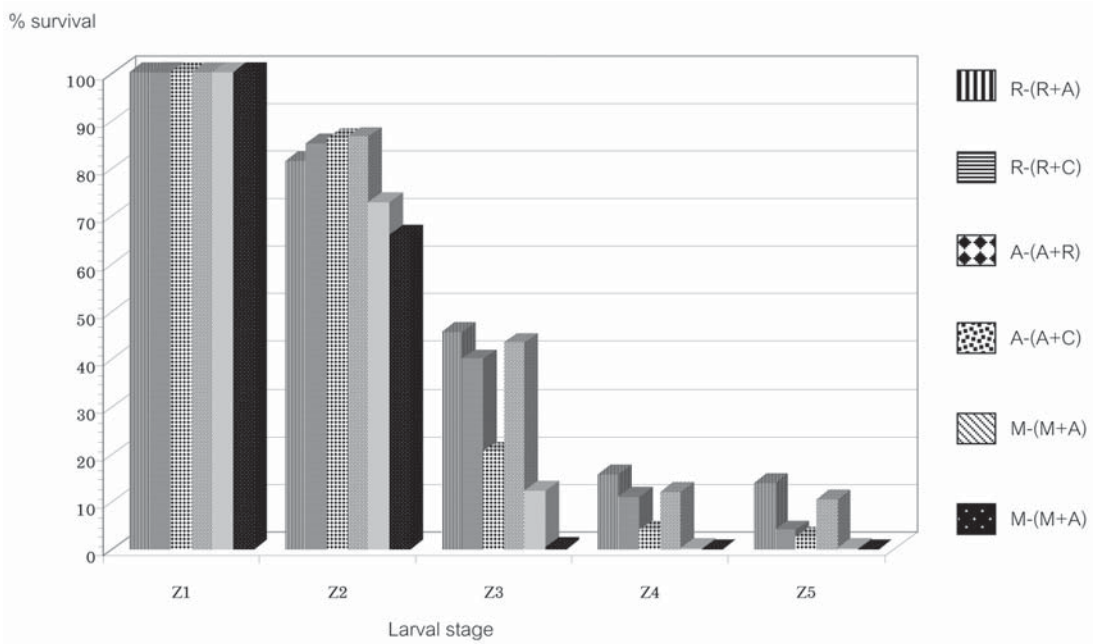

Figure 1 Percentage of zoea surviving reared by various feeds.
Note: R-(R+A) = Rotifer - (Rotifer+Artemia)
R-(R+C) = Rotifer - (Rotifer+Copepod)
A-(A+R) = Artemia - (Artemia+Rotifer)
A-(A+C) = Artemia – (Artemia+Copepod)
M-(M+A) = Microencapsulate – (Microencapsulate+ Artemia)
M-(M+C) = Microencapsulate – (Microencapsulate+ Copepod)

higher rate. The finding showed better results in treatments 1, 2 and 4 which were the combination of rotifer and artemia, rotifer and copepod , artemia and copepod, respectively. Live feeds in general is superior to processed feed due to its proper size, movability and completed nutrient compositions including free amino acid (Warner, 1977; Lebour, 1928; Tungkerkoran, 1999). However, the reason for low survival of zoea 2 developed to zoea 3 under feeding on artemia and rotifer (treatment 3) was that zoea was first fed on artemia (500 μ) which was much larger in size than rotifer (200 μ) . Therefore, zoea were accustomed to large food size and when fed rotifer at later stage, it was perhaps, difficult for zoea 2 to take rotifer of smaller size or otherwise zoea had to

Table 2 Developmental periods (days) of zoea 1 to zoea 5 as influenced by feeding of different larval feeds.

Larval feed	Developmental period (days)
1. R-(R+A)	20.67±0.58^{a}
2. R-(R+C)	28.00±1.41^{c}
3. A-(A+R)	25.50±2.12^{bc}
4. A-(A+C)	21.67±3.79^{ab}
5. M-(M+A)	29.00±0.00^{c}
6. M-(M+C)	-

Means within the same column followed by the same letters are not significantly different (P>0.05)
Note: Data are expressed as mean±SD

Table 3 Survival rates (%) and the developmental periods (days) of megalopa to the first crab stage influenced by feeding of different larval feeds.

Larval feed	Survival rate	Developmental period
1. A	75.00±8.33[a]	12.05±0.65[a]
2. GM	19.44±9.62[c]	15.22±3.27[a]
3. A+F	63.89±12.73[ab]	10.77±1.45[a]
4. A+GM	58.33±0.00[ab]	12.15±1.87[a]
5. F+GM	47.22±20.97[b]	10.99±0.35[a]

Means within the same column followed by the same letter are not significantly different (P>0.05)
note: Data are expressed as mean±SD
A = Artemia
GM = Finely ground mollusc meat
A+F = Artemia+ Black tiger larval shrimp feed
A+GM = Artemia+ Finely ground mollusc meat
F+ GM = Black tiger larval shrimp feed +Finely ground mollusc meat

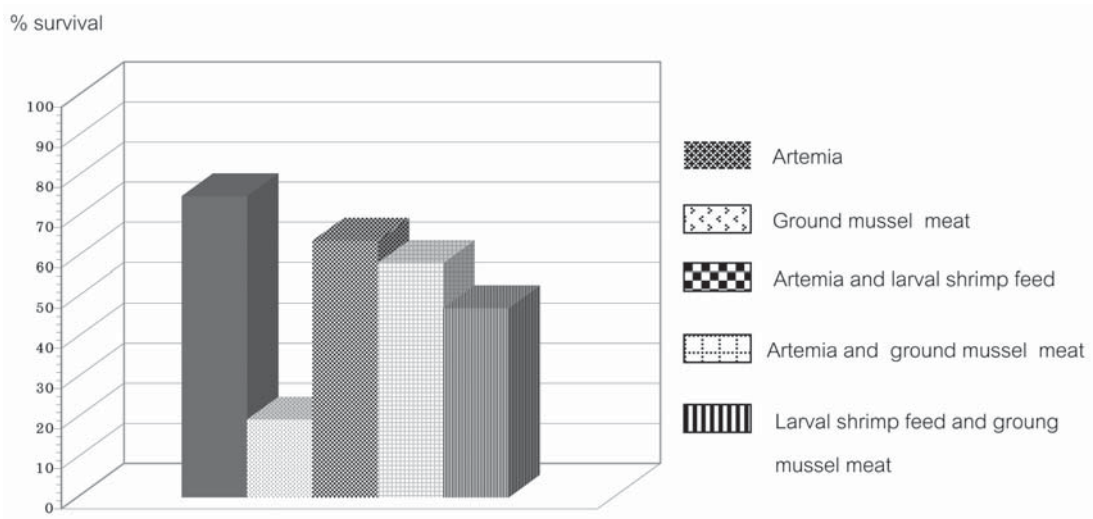

Figure 2 Percentage of megalopa reared by various feed.

spend more energy to fill up the stomach and hence resulted in poor performance (New, 1987 ; Herper, 1988 ; Lovell, 1989).

Zoea 3 was kept dying when developed to zoea 4. Only zoea which nursed on lived feeds in treatments 1, 2 and 4 survived 11-16% while those fed on microencapsulated feed died almost completely (0-4% survive). It was quite clear in this study that highly mortality of zoea occurred during zoea 2 developed to zoea 4 although it had higher survival rate in treatments 1, 2 and 4. The reasons were that during this peroid, gland filter and hepatopancreas were well developed meanwhile swimming and walking legs were still developing (Li *et al*., 1995) and therefore nutrients and energy were required. From zoea 4 to zoea 5, the rates of mortality declined in all treatments due to their digestive system and morphology being

much more stable.

The developmental period from zoea 1 to zoea 5 were the shortest (21-22 days) in treatments 1 and 4 for those fed on the combination of rotifer and artemia, artemia and copepod. This was consistent with the other studies that zoea 1 of *Scylla olivacea* and *Scylla serrata* nursed on rotifer and artemia took 18-25 days for development to zoea 5 (Pripanapong,1999; Heasman and Fielder,1983).

Results from the experiment 2 showed that newly hatched artemia was more suitable food for megalopa than mollusc meat and shrimp feed. This was indicated by high survival rate of megalopa feeding on artemia. Baylon and Failaman (1999) reported that megalopa of *scylla serrata* had high survival rate (72.22%) when fed on artemia, but survival rates decreased to 50.00, 38.89 and 33.33% when artemia mixed with worm, shrimp meat and squid meat were used, respectively. It was suggested that artemia was the suitable feeds for megalopa as artemia contained high protein (65.6%) and its movement facilitated the aggressive predation of megalopa (Warner, 1977; Williams *et al*.,1999).

The developmental period from megalopa to the first crab stage was not influenced by types of feed in this study. However, the shorter developement period was resulted from treatments contained shrimp feed. It might be postulated that well-balanced nutrients in black tiger larval shrimp feeds might affect period for the development of megalopa to a certain extent.

CONCLUSION

The influences of six various feed on survival rate of zoea, indicated that live feeds, rotifer and artemia were superior to microencapsulate feed for the development of zoea 1 to zoea 2. For the combinations of feed used for zoea 2 to zoea 5, the combination of either rotifer and artemia or artemia and copepod revealed high survival rate of zoea 5. Duration for development of zoea 1 to zoea 5 were 18-25 days. Zoea fed on rotifer and artemia satisfied the shortest time for development. For the experiment 2, the megalopa fed on artemia solely had higher survival rate than those fed on mollusc meat solely or combination with ground mollusc meat or larval shrimp feed. The developmental period of the first crab from megalopa was not influenced by types of feed and ranged from 11 to 15 days.

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