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Ingestion Rates of *Brachionus* sp. and *Artemia* sp. Nauplii by Blue Swimming Crab, *Portunus pelagicus* (Linnaeus, 1758) Larvae

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ABSTRACT

The experiment was conducted to determine the ingestion rate of Artemia sp. nauplii and Brachionus sp. by individual blue swimming crab, Portunus pelagicus larvae from zoea 1 until megalopa stages after 24 h. The study also to determined, if the presence of Brachionus sp. influences the ingestion of Artemia sp. nauplii by the individual P, pelagicus larvae. This involved three different feeding treatments, with Artemia sp. nauplii only (Treatment 1), Brachionus sp. only (Treatment 2) and with both Artemia sp. nauplii and Brachionus sp. (Treatment 3) in culture tank. Results indicates that ingestion rates of Artemia sp. nauplii and Brachionus sp. after 24 h by P. pelagicus larvae are 0 Artemia sp. nauplii and 35-36 Brachionus sp. for zoea 1, 1-2 Artemia sp. nauplii and 37-38 Brachionus sp. for zoea 2; 8-15 Artemia sp. nauplii and 38-40 Brachionus sp. for zoea 3; 12-18 Artemia sp. nauplii and 27-37 Brachionus sp. for zoea 4 stage; and finally 32-35 Artemia sp. nauplii and 16-30 Brachionus sp. for Megalopa stages. The individual P. pelagicus larvae ingested more Artemia sp. nauplii during the late larval stages (zoea 3, zoea 4 and megalopa stage) as compared to the initial larval stages (zoea 1 and zoea 2) but ingested more Brachionus sp. during the initial larval stages compared to the late larval stages. However, the presence of Brachionus sp. did not influence the consumption of Artemia sp. nauplii by the individual P. pelagicus larvae at each larval stage.

Key words: Artemia sp. nauplii, Brachionus sp., blue swimming crab, ingestion rate, Portunus pelagicus larvae

INTRODUCTION

Blue swimming crabs, *Portunus pelagicus* also known locally as 'ketam bunga' or 'ketam renjong' is an important source of income for fishermen in the Malaysia. The high price and increased demands among community contribute to over-exploitation in capture production of *P. pelagicus* (Sounndarapandian and Dey, 2008). This decline due to over-exploitation has an impact in the total productions of *P. pelagicus* in some Asian countries (Ikhwanuddin *et al.*, 2005). In Malaysia, statistics from Department of Fisheries (DOF) shows that the landings of *P. pelagicus* is 3514 tons in 2007 and increase to 4427 tons in 2008 but the landings are decreasing in 2009 with 3057 tons (Department of Fisheries, 2009). These declines of crab's production in Malaysia territorial water act as an early indication of deficiency in the future because increasing of fishing and coastal environmental damages.

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P. pelagicus is distributed and available throughout coastal waters of the tropical regions of the western Indian Ocean and the eastern Pacific (Xiao and Kumar, 2004; Bhat et al., 2011; Talpur et al., 2011) and also considered as an invasive swimming crab (Archdale et al., 2010). The culture and potential culture of the large Portunus species such as P. pelagicus and P. sanguinolentus (Soundarapandian and Singh, 2008; Soundarapandian and Raja, 2008), Charybdis feriatus and the more temperate P. trituberculatus are evaluated for stock enhancement potential criteria (Williams and Primavera, 2001). P. pelagicus is the most abundant species in Malaysia. However, P. pelagicus survival rate is very low and it about 10.1% until 1st day juvenile crab (Ikhwanuddin et al., 2005). For further aquaculture industry development, commercial seed production technology should be reviewed to increase survival rate, durability, quality and efficiency (Talpur et al., 2012). Study on water quality, phytoplankton and food organisms by Brick (1974), use of recirculation system (Heasman and Fielder, 1983), amount different diet combinations (Baylon and Failaman, 1999; Quinitio et al., 1999; Williams et al., 1999; Zeng and Li, 1999; Genodepa et al., 2006) and salinity tolerance (Parado-Estepa and Quinitio, 1999) have been conducted on a small-scale on portunid crabs but need further research to enhance the information in P. pelagicus. Many countries like Japan, Philippines, India, Indonesia, Thailand, Bangladesh, Vietnam, Australia and USA are actively involved in crab culture and research (Soundarapandian et al., 2007). Based on the previous research, most of these studies focused on basic of fecundity, embryology, survival rate and growth development of crab larvae in order to increase crab seed production (Arshad et al., 2006) but no information about ingestion of food especially for P. pelagicus larvae culture. Thus, more information on culture techniques is required to develop a suitable ingestion rate for larval rearing in P. pelagicus.

The previous studies have done on feeding of crab larvae with a combination of Brachionus sp. and Artemia sp. nauplii, Artemia sp. nauplii or Brachionus sp. alone (Baylon et al., 2004; Suprayudi et al., 2002; Baylon, 2009). Zoea I and II was fed with live food such as Chaetoceros sp. and Brachionus sp. (Ikhwanuddin et al., 2012). According to Ikhwanuddin et al. (2012), Brachionus sp. and Artemia sp. nauplii was given to zoea III and IV. The cladoceran, Moina micrura and prawn-egg custard as well as Artemia sp. nauplii and bivalve meat (Soundarapandian et al., 2007) was provided during Megalopa stage.

In order to determine the optimum ingestion rate and feeding regimes to maximize survival rate in the larval rearing, this study had the following objectives; to find out optimum density of *Brachionus* sp. for each larval stage and to find out optimum density of *Artemia* sp. nauplii for each zoea stages and megalopa stage. The use of *Brachionus* sp. and *Artemia* sp. nauplii in the larval diet can be minimized, then substantial savings in food cost can be achieved to produce mass production of *P. pelagicus* larvae.

MATERIALS AND METHODS

Broodstocks management: Gravid broodstocks were brought from Gelang Patah, Johor, Malaysia. Then, gravid broodstocks were placed inside 100 L fiberglass tank with 1 gravid crab per tank and was filled with filtered seawater. Constant aeration was supplied during rearing of gravid broodstock until crabs hatching. Three centimeters thick of sand in tray was placed at center part of culture tank for egg hatching. Filtered seawater was exchanged 50% in every gravid crab tank at every morning and water parameters was maintained at 30 ppt salinity, 27-28°C temperature, pH 7-7.9 and more than 5 ppm Dissolved Oxygen (DO). Gravid broodstocks were monitored daily. They were fed with fresh squid. Once the eggs were hatched, water in broodstocks tank was

reduced to 50 L. Immediately broodstock was separated from new hatched larvae and placed into another tank.

Larvae management: Actively swimming larvae accumulated near the water surface were siphoned out by plastic tube into larval rearing tank (10 L tank capacity) that filled with 7.5 L of filtered seawater. Constant and slow aeration rate was supplied inside larval rearing tank. Crab larvae were stocked at a density of 50 zoea L⁻¹ and placed inside larval rearing tank that covered with dark colour paint to avoid illumination. During larval rearing, every stages of crab larval must be observed under profile projector after daily fed larvae with *Brachionus* sp. as well as *Artemia* sp. nauplii.

Water quality management: Filtered seawater in 1 tons of tank was treated by using 30 ppm of Calcium hypochlorite and aerated within 24 h. After 24 h, seawater was neutralized with sodium thiosulphate at 15 ppm within 12-24 h. Then, treated seawater was siphoned into larval rearing tank for about 7.5 L for larval rearing. Water in plastic tube was monitored daily to measure pH, salinity, temperature and DO level of cultured water by using YSI Multi-parameter Probe (Model: YSI 556 MPS) and Hand Refractometer.

Experimental design: Three aquarium tanks (5 L) was set up in the experiment which the centrifuge tubes were placed where the larvae are fed with *Artemia* sp. nauplii only (Treatment 1/T1), *Brachionus* sp. only (Treatment 2/T2) and with both *Artemia* sp. nauplii and *Brachionus* sp. (Treatment 3/T3). Every aquarium tanks was equipped with 20 pieces of centrifuge tubes (ten replicates each for control and treatment). Small holes were perforated at the bottom side of aquarium tanks so that filtered seawater would enable engulfing 1/3 of bottom part of aquarium tanks. Figure 1 below shows the arrangement of the tubes and the 5 L aquarium tanks.

In the experiment, 50 mL of centrifuge tubes were filled with 40 mL of filtered seawater at salinity 28-30 ppt. One individual of crab larvae was taken from master culture tank and gently pipette into three treatments tubes; T1 (Brachionus sp.), T2 (Artemia sp. nauplii only) and T3 (Artemia sp. nauplii and Brachionus sp.). Ten control tubes in every tank are without crab larvae. The experiment was conducted from zoea I until megalopa stage. Twenty tubes in T1 were inoculated with Brachionus sp. (50 individual/tubes). For T2, 50 individual/tubes of Artemia sp. nauplii were inoculated in every ten centrifuge tubes. At the same time T3, 50 individual of Brachionus sp. and 50 individual of Artemia sp. nauplii were inoculated in ten centrifuge tubes of combination Artemia sp. nauplii and Brachionus sp. A number of live feed that was inoculated in all centrifuge tubes was remained from zoea I until Megalopa stages. A vigorous aeration was

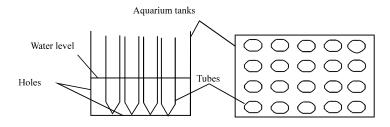


Fig. 1: Side view and upper view of the aquarium tank and the tubes

J. Fish. Aquat. Sci., 7 (6): 402-411, 2012

supplied in all centrifuge tubes. Water heater is used in batch culture system to control water temperature fluctuations (28-30°C). A number of preys ingested within 24 h were determined for each treatment. After 24 h, aeration in all centrifuge tubes was being stopped. All centrifuge tubes were brought to laboratory for calculation step. Seawater mixed with prey left in centrifuge tubes was filtered by using seine 40 µm (one by one). Immediately pipette filtered preys (*Brachionus* sp. or *Artemia* sp. nauplii) and all preys were placed on Sedgewick-Rafter counting chamber.

Brachionus sp. and Artemia sp. nauplii were first immobilized with iodine solution before being counted under the microscope. This number was labeled as final number of preys left in centrifuge tubes after 24 h. A possibility for unhatched Artemia sp. cysts to hatch and Brachionus sp. to reproduce might happen within 24 h of experimental period. Hence, the mean number of prey (Artemia sp. nauplii and Brachionus sp.) consumed by individual crab larvae was calculated by using the mean density of prey left in the control after 24 h as the initial number of prey organisms present in the experimental culture water. The formula shows as below:

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Mean No. of prey consumed by = Mean No. of prey Final No. of preys individual crab larvae after 24 h = (left without any crab larvae) - (left in centrifuge tubes after 24 h)
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This step was followed for all aquarium tanks of T1 (*Brachionus* sp. only), T2 (*Artemia* sp. nauplii only) and T3 (combination *Artemia* sp. nauplii and *Brachionus* sp.) in every larval stage (zoea I until Megalopa stages). Different stages of crab larvae were also observed under profile projector and described based on Arshad *et al.* (2006).

Statistical analysis: In the experiment, two statistical analyses were performed by using Microsoft Excel 2007 and Independent-Samples t-test analysis using SPSS version 16.0 was used to determine the comparison mean number of *Artemia* sp. nauplii ingested by individual crab larvae with and without *Brachionus* sp. in *Artemia* sp. nauplii only-combination *Artemia* sp. nauplii and *Brachionus* sp. All results will be presented as means±SD. The difference will be displayed as statistically significant when p<0.05.

RESULTS

The data on *Brachionus* sp. ingested by individual crab larvae in treatment 1 (*Brachionus* sp. only) tank has been showed on Table 1. During zoea 1, there were 34.8 individuals of *Brachionus* sp. ingested by individual crab larvae. One crab larvae able to consumer at mean 37 individual of *Brachionus* sp. during zoea 2 stage, 40 individuals of *Brachionus* sp. ingested at zoea 3 stage, 37.4 individuals of *Brachionus* sp. ingested at zoea 4 stage and 29.6 individuals at Megalopa stage.

For T2 (Artemia sp. nauplii only), during zoea 1, there was no individual of Artemia sp. nauplii ingested by individual crab larvae. For zoea 2, one crab larvae was able to consume about 1.8 individual of Artemia sp. nauplii. Meanwhile, during stage zoea 3, 14.8 individuals of Artemia sp. nauplii ingested by one crab larvae, 17.8 individuals of Artemia sp. nauplii on stage zoea 4 and 34.7 individuals of Artemia sp. nauplii on stage Megalopa has been showed on Table 2.

Based on Table 3 for T3 (combination *Artemia* sp. nauplii and *Brachionus* sp.), during zoea 1 stage, there were 0 individual *Artemia* sp. nauplii and 36.1 individuals of *Brachionus* sp. ingested by individual crab larvae, feeding with both *Artemia* sp. nauplii and *Brachionus* sp. For zoea 2, individual crab larvae consumes at about 0.9 individual *Artemia* sp. nauplii and 38.1 individuals of *Brachionus* sp., 8.1 individual of *Artemia* sp. nauplii and 38.3 individuals of *Brachionus* sp.

Table 1: Brachionus sp. ingested by individual crab larvae after 24 h from zoea 1 until Megalopa stages

| Table 1: Brach | <i>ionus</i> sp. | ıngested | l by ind | ividual cr | ab larva | e after 24 | h from | zoea 1 | until Me | egalopa sta | ages | | | | | |
|----------------|------------------|-------------------------------|----------|------------|------------------------|--------------------|--------|--------|----------|-------------|--------|-----|-------|--|--|--|
| | Con | Control (without larvae crab) | | | | | | | | | | | | | | |
| Larvae stage | R1 | R | 2 | R3 | R4 | R5 | Re | 3 | R7 | R8 | R9 | R10 | MC | | | |
| Zoea 1 | 51 | 4 | 4 | 51 | 54 | 52 | 54 | 4 | 48 | 48 | 49 | 48 | 49.9 | | | |
| Zoea 2 | 47 | 5 | 2 | 43 | 49 | 49 | 5 | l | 55 | 55 | 55 | 54 | 51.0 | | | |
| Zoea 3 | 53 | 5 | 3 | 51 | 48 | 50 | 54 | 4 | 54 | 45 | 51 | 46 | 50.5 | | | |
| Zoea 4 | 49 | 5 | 2 | 47 | 51 | 58 | 5 | l | 53 | 47 | 47 | 47 | 50.2 | | | |
| Megalopa | 55 | 5 | 3 | 51 | 55 | 48 | 4 | 5 | 49 | 46 | 49 | 48 | 49.9 | | | |
| | Treatn | nent 1 (E | 3rachio | Brace | chionus s _l | o. ingested/larvae | | | | | | | | | | |
| Larvae stage | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | МТ | | MC-MT | | | |
| Zoea 1 | 17 | 14 | 16 | 17 | 12 | 19 | 20 | 14 | 12 | 10 | 15.1 | | 34.8 | | | |
| Zoea 2 | 14 | 15 | 14 | 15 | 18 | 16 | 10 | 10 | 16 | 12 | 14.0 | | 37 | | | |
| Zoea 3 | 12 | 11 | 10 | 8 | 8 | 10 | 14 | 14 | 10 | 8 | 10.5 | | 40 | | | |
| Zoea 4 | 15 | 15 | 11 | 12 | 15 | 11 | 17 | 10 | 10 | 12 | 12.8 | | 37.4 | | | |
| Megalopa | 22 | 20 | 20 | 24 | 18 | 22 | 20 | 19 | 21 | 17 | 20.3 | | 29.6 | | | |

MC: Mean No. Brachionus sp. left in the control without any crab larvae after 24 h, -MT: Mean No. Brachionus sp. left in the treatment with individual crab larvae after 24 h

Table 2: Artemia sp. nauplii ingested by individual crab larvae after 24 h from zoea 1 until Megalopa stages

| | Control (without larvae crab) | | | | | | | | | | | |
|--------------|-------------------------------|----|----|----|----|----|----|----|----|-----|-------------------|--|
| | | | | | | | | | | | | |
| Larvae stage | A1 | A2 | А3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | MC | |
| Zoea 1 | 50 | 48 | 50 | 49 | 49 | 50 | 50 | 50 | 49 | 50 | 49.5 | |
| Zoea 2 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 49 | 49 | 50 | 49.8 | |
| Zoea 3 | 49 | 49 | 49 | 50 | 50 | 50 | 50 | 48 | 48 | 50 | 49.3 | |
| Zoea 4 | 49 | 50 | 47 | 50 | 50 | 50 | 50 | 49 | 49 | 47 | 49.1 | |
| Megalopa | 48 | 48 | 50 | 50 | 48 | 49 | 49 | 50 | 49 | 50 | 49.1 | |
| | | | | | | | | | | Ar | temia sp. pauplii | |

| | Treat | ment 2 (A | | ingested/larvae | | | | | | | | |
|--------------|-------|-----------|----|-----------------|----|----|----|----|----|-----|-------|-------|
| Larvae stage | A1 | A2 | Аз | A4 | A5 | A6 | A7 | A8 | A9 | A10 | MT | MC-MT |
| Zoea 1 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50.0 | 0.0 |
| Zoea 2 | 48 | 49 | 48 | 47 | 48 | 48 | 47 | 49 | 48 | 48 | 48.0 | 1.8 |
| Zoea 3 | 35 | 34 | 34 | 33 | 35 | 32 | 36 | 35 | 35 | 36 | 34.5 | 14.8 |
| Zoea 4 | 34 | 32 | 31 | 33 | 34 | 28 | 28 | 32 | 31 | 30 | 313.0 | 17.8 |
| Megalopa | 15 | 14 | 12 | 14 | 16 | 16 | 12 | 15 | 16 | 14 | 144.0 | 34.7 |

MC: Mean No. *Artemia* sp. nauplii left in the control without any crab larvae after 24 h, -MT: Mean No. *Artemia* sp. nauplii left in the treatment with individual crab larvae after 24 h

ingested by crab larvae during zoea 3. For zoea 4, 27.1 individuals of *Artemia* sp. nauplii and 27.1 individuals of *Brachionus* sp. ingested meanwhile 32.4 individuals of *Artemia* sp. nauplii and 16.4 individuals of *Brachionus* sp. during Megalopa.

Based on Table 3, individual crab larvae mostly preferred to ingest *Brachionus* sp. during initial life of larval stage while at the last stage of larval development, *Artemia* sp. nauplii was the most selected prey. Based on Table 3, individual crab larvae mostly preferred to ingest *Brachionus* sp. during initial life of larval stage while at the last stage of larval development, *Artemia* sp. nauplii was the most selected prey. Based on the Fig. 2, there was no significant (p>0.05) between the

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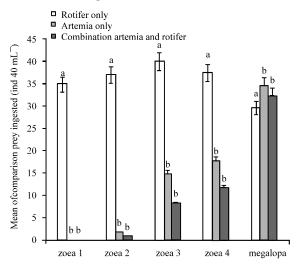


Fig. 2: Mean of comparison treatment 1 (*Brachionus* sp. only), treatment 2 (*Artemia* sp. nauplii only), treatment 3 (combination *Artemia* nauplii and *Brachionus* sp.) ingested by individual crab larvae after 24 h from zoea 1 until Megalopa stages which feeding with *Artemia* sp. nauplii and *Brachionus* sp.

Table 3: Brachionus sp. ingested by individual crab larvae after 24 h from zoea 1 until megalopa stages, feeding with both Artemia sp. nauplii and Brachionus sp.

| naupin and Discontinuous sp. | | | | | | | | | | | | |
|------------------------------|------|-----------|----------|----|----|----|----|----|----|-----|------|-------|
| Larvae stage | Trea | tment 3 (| combinat | , | | | | | | | | |
| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | мт | MC-MT |
| Zoea 1 | 13 | 17 | 15 | 12 | 15 | 13 | 12 | 16 | 13 | 12 | 13.8 | 36.1 |
| Zoea 2 | 16 | 14 | 13 | 10 | 13 | 10 | 15 | 14 | 13 | 11 | 12.9 | 38.1 |
| Zoea 3 | 14 | 11 | 12 | 12 | 11 | 13 | 14 | 13 | 12 | 10 | 12.2 | 38.3 |
| Zoea 4 | 20 | 22 | 24 | 25 | 24 | 22 | 25 | 20 | 24 | 25 | 23.1 | 27.1 |
| Megalopa | 35 | 34 | 32 | 30 | 34 | 36 | 34 | 33 | 35 | 32 | 33.5 | 16.4 |

MC: Mean No. Brachionus sp. left in the control without any crab larvae after 24 h, -MT: Mean No. Brachionus sp. left in the treatment with individual crab larvae after 24 h

Table 4: Artemia sp. nauplii ingested by individual crab larvae after 24 h from zoea 1 until megalopa stages, feeding with both Artemia sp. nauplii and Brachionus sp.

| Larvae stage | | Treat | tment 3 | ` | | _ | Artemia sp. nauplii ingested/larvae | | | | | |
|--------------|----|-------|---------|----|----|----|-------------------------------------|----|----|-----|--------|-------|
| | A1 | A2 | А3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | МТ | MC-MT |
| Zoea 1 | 50 | 49 | 49 | 50 | 50 | 50 | 50 | 50 | 50 | 49 | 49.7 | 0 |
| Zoea 2 | 49 | 48 | 50 | 50 | 48 | 49 | 49 | 48 | 48 | 50 | 48.9 | 0.9 |
| Zoea 3 | 43 | 41 | 42 | 39 | 39 | 42 | 42 | 44 | 40 | 40 | 41.2 | 8.1 |
| Zoea 4 | 37 | 38 | 39 | 39 | 38 | 35 | 38 | 36 | 40 | 35 | 37.5 | 11.6 |
| Megalopa | 19 | 16 | 16 | 17 | 14 | 15 | 15 | 18 | 18 | 19 | 16.7 | 32.4 |

MC: Mean No. Artemia sp. nauplii left in the control without any crab larvae after 24 h, -MT: Mean No. Artemia sp. nauplii left in the treatment with individual crab larvae after 24 h

mean numbers of T2 (Artemia sp. nauplii only) and T3 (combination Artemia sp. nauplii and Brachionus sp.) ingested by individual crab (p = 0.0622 for Z1; p = 0.312 for Z2; p = 0.4753 for

Table 5: Feeding regime for Brachionus sp and Artemia sp. nauplii in larvae rearing P. pelagicus

| | Food intake | | | | | | | | | | | | |
|----------|----------------|------------|----------------------------|------------|--|--|--|--|--|--|--|--|--|
| Larvae | Brachionus sp. | Ind/larvae | <i>Artemia</i> nauplii sp. | Ind/larvae | | | | | | | | | |
| Zoea 1 | 35-36 | 35.5 | 0 | 0.0 | | | | | | | | | |
| Zoea 2 | 37-38 | 37.5 | 1-2 | 01.5 | | | | | | | | | |
| Zoea 3 | 38-40 | 39 | 8-15 | 11.5 | | | | | | | | | |
| Zoea 4 | 27-37 | 32 | 12-18 | 15.0 | | | | | | | | | |
| Megalopa | 16-30 | 23 | 32-35 | 33.5 | | | | | | | | | |

Z3; p = 0.3463 for Z4). Meanwhile, there was no significant (p>0.05) between the mean numbers of T1 (*Brachionus* sp. only) ingested by individual crab feeding with T3 (combination *Artemia* sp. nauplii and *Brachionus* sp.) (p = 0.0561 for Z1; p = 0.6012 for Z2; p = 0.5753 for Z3; p = 0.47463 for Z4). The comparison was done for every zoea 1 until megalopa stages.

There was no significant different (p>0.05) in T3 where larval crab fed with both *Artemia* sp. nauplii and *Brachionus* sp. respectively as been showed in Table 4. The mean numbers of *Artemia* sp. nauplii ingested by individual crab larvae after 24 h are 0 individual/larvae, 0.9, 8.1, 11.6 and 32.4 individual/larvae for zoea 1, 2, 3 and megalopa stages, respectively in Table 4.

The number of *Brachionus* sp. ingested by the individual *P. pelagicus* larvae in each zoea stage are 35-36 individual/40 mL for zoea 1, 37-38 individual/40 mL for zoea 2 stage, 38-40 individual/40 mL for zoea 3 stage, 27-37 individual/40 mL for zoea 4 and 16-30 individual/40 mL for Megalopa has been showed on Table 5. The mean comparison for each treatment ingested by individual crab larvae after 24 h from zoea 1 until Megalopa stages are showed in Fig. 2. The amount of *Artemia* sp. nauplii to give feed for crab larvae at different larvae stages from zoea 1 until Megalopa stages are average 0 individual/40 mL for zoea 1 stage, 1-2 individual/40 mL for zoea 2 stage, 8-15 individual(s)/40 mL for zoea 3 stage, 12-18 individuals/40 mL for Z4 stage and 32-35 individuals/40 mL for Megalopa as shown in Fig. 2. The result showed that zoea 1 and zoea 2 ingested more *Brachionus* sp. rather than *Artemia* sp. nauplii and low ingestion of *Artemia* sp. nauplii found in zoea 3, zoea 4 and Megalopa stages. The introduction of both *Brachionus* sp. and *Artemia* sp. nauplii in feeding regime showed that the present of rotifer in cultured water did not influence the consumption of *Artemia* sp. nauplii from zoea 1 until Megalopa stages.

DISCUSSION

Individual ingestion of Artemia nauplii by larvae Scylla paramamosain found that, there was an average 15, 25 and 37 of Artemia nauplii consumed during Z3, Z4 and Z5 stages (Nghia, 2004). Study by Baylon et al. (2004), Artemia density of 2.5 mL⁻¹ was comparable with 5.0 mL⁻¹ by individual Z1, Z2 and Z3 stages of Scylla serrata. It shows that Artemia nauplii is ingested more Artemia nauplii in late larval stage compared to initial larval stage as same as these studies as shown in Table 5. An introduction of purely Artemia nauplii for about 4.0 individual/mL during zoea stage (zoea 1 until zoea 4 stages) increased the survival rate of crab larvae. According to Baylon (2009), until Z4 stage, more than 70% survival rate of S. tranquebarica after fed with purely Artemia nauplii. Most of Portunid crabs were unable to maintain their survival rate after been introduced with purely Brachionus sp. During initial life of crab (zoea stage), crab probably can survive but mortality occurred at the Megalopa stage (at the end of the larval stages). In the previous study, larvae of S. serrata was able to survive during initial zoea stage but later died

before reaching Megalopa stage (Baylon and Failaman, 1999; Zeng and Li, 1999). One of the problems is probably due to inadequate of nutrition content in *Brachionus* sp. According to McConaugha (1982), lower lipid content in *Brachionus* sp. that has been fed to *Rhithropanopeus harrisii* showed a difficult of metamorphosis process of crab larvae. The small size of *Brachionus* sp. (<100 µm) was disabling to be consumed until the whole life of crab's larval. This happened because, in larvae development, they are most likely attractive to active prey such as *Artemia* nauplii compare *Brachionus* sp. during increasing larvae stage. Hence, rotifer as the sole prey failed to maintain the survival rate of crab larvae as well as other cultured species.

The presence of both important lives feed such as Artemia nauplii and Brachionus sp. would gave a beneficial effect for the mass seed production of frog crab, Ranina ranina (Minagawa and Murano, 1993). In term of rapid development, studied on Callinectes sapidus larvae found that there was 30% of metamorphosis process to Megalopa stage after the introduction of both Artemia nauplii and Brachionus sp. (Sulkin, 1975). Furthermore, study by Godfred et al. (1997) shows that survival rate of Thalamita crenata Z1 until Z2 stages is higher when fed with Brachionus sp. alone while Z3 until Z5 stages fed with both Artemia nauplii and Brachionus sp. There was a significant reduction in the intake of Brachionus sp. with increasing consumption of Artemia in the early zoeal stages (Z1, Z2 and Z3 stages) but at later stages (Z4 and Z5 stages) the intake of Artemia was no longer affected by the presence of Brachionus sp (Baylon et al., 2004). The different size between Artemia nauplii and Brachionus sp. shows that, individual crab larvae was more prefer on Brachionus sp. because of small size when introduced during initial larval stage than Artemia nauplii. High mortality of individual crab larvae occurred on treatment feeding with Artemia only as compared to feeding with Artemia and Brachionus sp.. Different types of prey that was introduced during rearing larvae probably influence the survival rate of individual crab larvae. In T2 (Artemia only) tank, although crab larvae unable to fed the whole body of Artemia nauplii but under the microscope, there are some body parts was consumed especially the head and appendages. Furthermore, Artemia nauplii are the bigger size and higher swimming speed than Brachionus sp.

When introduced at the first zoea stage, crab larvae may unable to catch *Artemia* nauplii and facing difficult in holding the prey against the mouthparts because the abdomen part was not well developed especially at the beginning of zoea stage. The function of the abdomen was important in prey capture, holding the prey against the mouthparts and prey graze (eat slowly) also applied to other carnivorous zoea. The abdomen part helped in catching prey and presses them against the mouthpart by the zoea of *Scylla serrata* (Ong, 1964).

CONCLUSION

The study concluded that the initial larval stages ingested low *Artemia* sp. nauplii rather than *Brachionus* sp. and high ingestion of *Artemia* sp. nauplii found in the late larval stages.

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