

Determination of Live Prey Ingestion Capability of Blue Swimming Crab, *Portunus pelagicus* (Linnaeus, 1758) Larvae

¹M. Ikhwanuddin, ¹T. Nor Adila, ¹M.N. Azra, ²Y.S. Hii, ^{1,3}A.D. Talpur and ¹A.B. Abol-Munafi

¹Institute of Tropical Aquaculture, Universiti Malaysia Terengganu,
Kuala Terengganu, Terengganu, Malaysia

²Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu,
Kuala Terengganu, Terengganu, Malaysia

³Department of Fisheries, Government of Sindh, Pakistan

Abstract: Numbers of trials were conducted to determine the ingestion rate of live prey by *Portunus pelagicus* zoea larvae, at each zoeal stage one larvae was reared individually in 50mL centrifuge tube with *Artemia*, rotifers and both as a co-feed. The average number of ingested live prey over 24 h was measured. Each treatment group was inoculated with live prey as *Artemia* only (AO), rotifer only (RO) and *Artemia* + rotifer (AR) at density of 30 individual tubesG¹, 60 individual tubesG¹ and 30 individual tubesG¹ + 60 individual tubesG¹ respectively. The study shows that the individual *P. pelagicus* larvae ingested more rotifer after 24 hours during the initial zoeal stage as compared to the late zoeal stage with mean ingestion rate ranges of 32 individual, 32 individual, 30 individual and 28 individual for Zoea 1, Zoea 2, Zoea 3 and Zoea 4 stages respectively. The study conclude that the individual *P. pelagicus* larvae ingested more *Artemia* after 24 hours during the late zoeal stage as compared to the initial zoeal stage with mean ingestion rate ranges of 3 individual, 3 individual, 5 individual and 6 individual for Zoea 1, Zoea 2, Zoea 3 and Zoea 4 stages respectively. Based on results, further experiments should be done to illuminate the effect of prey density on the survival and larval development when larvae are reared in large quantity.

Key words: *Artemia* % *Portunus Pelagicus* % Prey % Zoea

INTRODUCTION

Portunus pelagicus also known as a blue swimming crab are found throughout the Indo-Pacific region [1, 2]. *P. pelagicus* is a commercially important species in Malaysia, for local consumption or for culture is caught from the sea. In Malaysia, effort to produce mass crab seed of *P. pelagicus* at hatchery is still experimental and aquaculture is completely dependent on wild crab seed. Live prey is vital source as first feeding in hatcheries. Thus, the development of a hatchery technology for this species, basic information on food preferences and feeding is incredibly important. Previous studies on larval rearing of crabs showed rotifer and *Artemia* as acceptable and convenient live prey from zoeal up to the megalopa stage [3, 4]. Prey allowance is one of the factors influencing larval feeding success for all aquaculture

species, which have hefty cannibalistic behaviour at larval stages in particular the brachyuran species. The effect of prey density on larval survival and development has been studied in several brachyuran species, such as *Scylla serrata* [5, 6].

The rotifer, which feeds on microalgae *Nannochloropsis* or *Chlorella*, can be easily maintained in the hatchery on one hand. On the other hand, *Artemia* cysts are commercially available and convenient to hatch but very expensive. In fact, economic analysis showed that it accounts for more than 50% of the expense entailed in the operation of a crab hatchery [7]. To minimize food waste, unnecessary feeding and pollution of culture water and maximize survival in the larvae rearing, the daily ingestion of the *P. pelagicus* larvae fed rotifer and *Artemia* should be known. This study was designed to determine individual ingestion by

the *P. pelagicus* larvae in each zoeal stage fed with *Artemia*, rotifer allowance or both as co-feeding on daily ingestion rate. Obtained information should provide basic knowledge to further develop an optimal live prey feeding regime (rotifer and *Artemia*) in larval rearing system of *P. pelagicus* on one hand. On the other hand, the use of rotifer and *Artemia* in the larval diet can be minimized, then substantial savings in food cost, microalgae production, floor space and labor can be achieved.

MATERIALS AND METHODS

Water for Broodstock and Larvae Rearing: Filtered seawater stored in one tone tank was treated by using 30 ppm of calcium hypochlorite, after 24 hours, seawater was neutralized with sodium thiosulphate (15 ppm). Water parameter in larval rearing tank such as pH, salinity, temperature and DO was monitored daily using YSI 556 MPS (USA) and Refractometer.

Broodstock: The brood stock was placed at 1 gravid crab per tank of 200 L fiberglass tank capacity for hatching provided with 3 cm thick beach sand. Tank was filled with 100 L filtered sterilized seawater. The water parameters were maintain at 30 ppt for salinity, 27-28°C for temperature, 7-7.9 for pH, more than 5 ppm for DO and 50% water exchanged daily until hatching. Once female hatched, it was moved out from the tank, aeration of water in hatching tank was stopped and actively swimming larvae accumulated at the surface of water body were siphoned by plastic tube to other tank (100 L tank capacity) that filled with 75 L of filtered sterilized seawater and supplied with aeration. Larvae were acclimated and collected for the study.

Experimental Design: Three water bath culture systems were set up in the experiment. Each water bath culture systems was comprised of an aquarium tank of 5 L filled with water and equipped with a water heater so as to maintain temperature between 28-30 °C and six centrifuge tubes (50 mL) were placed inside (three replicates each for control and treatments). Three different feeding patterns were tested such as *Artemia* only (AO), rotifer only (RO) and *Artemia* + rotifer (AR).

Centrifuge tubes were filled with 40 mL of filtered sterilised seawater at salinity 28-30 ppt each tube was supplied with gentle aeration. One crab larvae was tenderly pipette into treatment tubes for AO, RO and AR and each treatment was replicate. Three control tubes were without crab larvae. For the study Z1 to Z4 were

used and each trial was conducted for 24 hours. For the feeding experiment Z1 were 2-3 days after hatching (DAH), 5-6 DAH for zoeal 2 stage, 8-9 DAH for zoeal 3 stage and 11-12 DAH for zoeal 4. In AO tanks were inoculated with *Artemia* nauplii at density of 30 individual tubes^G. For RO tank, 60 rotifers individual tubes^G were inoculated in every centrifuge tubes. However, 60 individual of rotifer and 30 individual of *Artemia* nauplii were inoculated to centrifuge tubes for AR. Control against each was without crab larvae and inoculated with *Artemia* nauplii, rotifers and both at same concentration as in treatment tubes. A number of preys ingested within 24 hours were determined in every treatment centrifuge tubes (AO, RO and AR) discarding the aeration. Seawater mixed with prey left in centrifuge tubes was filtered by using sieve 20µm (one by one). Immediately pipette filtered prey *Artemia* naupli were placed on Sedge wick - Rafter counting chamber. Rotifer and *Artemia* naupli were first immobilized with 50% alcohol before being counted under the microscope. However, for rotifers 1 mL of seawater mixed with prey left in centrifuge tubes from each treatment/control was placed on Sedge wick - Rafter counting chamber and counted under microscope. This practice was exercised for five times (5mL from each tube) and average was meant out. The available number of live prey was labeled as number of preys left in centrifuge tubes after 24 hours.

Hence the mean number of preys (*Artemia* or/and rotifer) ingested by individual crab larvae after 24 hours was calculated from mean number of preys left in the treatment minus by mean number of preys found in the control without any crab larvae after 24 hours.

Identification of Larvae Stages: To determine the different crab larvae stages, all the crab larval stages from zoeal 1 till zoeal 4 stages were observed under profile projector based on morphological characters.

Data Analysis: Two statistical analyses were performed by using Microsoft Excel 2007 and Independent - Samples T Test analysis using SPSS version 16.0 for windows. All results were presented as means ± Standard deviation (SD). The difference was displayed as statistically significant when $P < 0.05$.

RESULTS

Water Quality Parameter: The results shows that the mean ± SD of water quality parameters during the crab larvae culture period were remain as 28.73°C ± 0.22 for

Table 1: Mean of water parameters in batch culture system containing three aquaria tanks during 12 days culture period of the larvae rearing.

	Temperature (°C)	Salinity (ppt)	pH	Dissolved oxygen (ppm)
Mean	28.73	29.46	7.52	5.70
Max	29.05	29.79	7.70	5.90
Min	28.40	29.05	7.30	5.35
SD	0.22	0.22	0.12	0.17
n	12	12	12	12

- Max: maximum, Min: minimum, SD: standard deviation, n: number

Table 2: Mean *Artemia* nauplii ingested by individual crab larvae after 24 hours from Z1 until Z4 stages, feeding with *Artemia* nauplii only.

Larval stages	Control				Treatment				Mean	
	R1	R2	R3	Mean±SD (MC ¹)	R1	R2	R3	Mean±SD (MT ²)	(MC-MT) ³	RU ⁴
Z1	33	29	30	30.67±2.08	29	27	27	27.67±1.15	3.00	3
Z2	28	29	31	29.33±1.53	23	27	28	26.00±2.65	3.33	3
Z3	32	33	30	31.67±1.53	26	28	27	27.00±1.00	4.67	5
Z4	33	30	34	32.33±2.08	26	27	25	26.00±1.00	6.33	6

-R: Replicate

-MC¹: Mean number of *Artemia* left in the control without any crab larvae after 24 hours.

-MT²: Mean number of *Artemia* left in the treatment with individual crab larvae after 24 hours.

-(MC-MT)³: Mean number of *Artemia* ingested by an individual crab larvae after 24 hours.

-RU⁴: Round up to the nearest one decimal place for mean number of *Artemia* ingested by an individual crab larva after 24 hours.

Table 3: Mean rotifer ingested by individual crab larvae after 24 hours from Z1 until Z4 stages, feeding with rotifer only.

Larval stages	Control				Treatment				Mean	
	R1	R2	R3	Mean±SD (MC ¹)	R1	R2	R3	Mean±SD (MT ²)	(MC-MT) ³	RU ⁴
Z1	59	53	56	56.00±3.00	22	25	25	24.00±1.73	32.00	32
Z2	54	56	58	56.00±2.00	26	24	23	24.33±1.53	31.67	32
Z3	57	58	60	58.33±1.53	27	29	29	28.33±1.15	30.00	30
Z4	59	62	60	60.33±1.53	32	34	31	32.33±1.53	28.00	28

-R: Replicate

-MC¹: Mean number of rotifer left in the control without any crab larvae after 24 hours.

-MT²: Mean number of rotifer left in the treatment with individual crab larvae after 24 hours.

-(MC-MT)³: Mean number of rotifer ingested by an individual crab larvae after 24 hours.

-RU⁴: Round up to the nearest one decimal place for mean number of rotifer ingested by an individual crab larva after 24 hours.

Table 4: Mean *Artemia* nauplii ingested by individual crab larvae after 24 hours from Z1 until Z4 stages, feeding with both *Artemia* nauplii and rotifer.

Larval stages	Control				Treatment				Mean	
	R1	R2	R3	Mean±sd (MC ¹)	R1	R2	R3	Mean±sd (MT ²)	(MC-MT) ³	RU ⁴
Z1	32	30	30	30.67±1.15	28	29	28	28.33±0.58	2.34	2
Z2	30	32	31	31.00±1.00	28	28	29	28.33±0.58	2.67	3
Z3	30	29	33	30.67±2.08	26	28	27	27.00±1.00	3.67	4
Z4	29	33	33	31.67±2.31	27	27	26	26.67±0.58	5.00	5

-R: Replicate

-MC¹: Mean number of *Artemia* left in the control without any crab larvae after 24 hours.

-MT²: Mean number of *Artemia* left in the treatment with individual crab larvae after 24 hours.

-(MC-MT)³: Mean number of *Artemia* ingested by individual crab larvae after 24 hours.

-RU⁴: Round up to the nearest one decimal place for mean number of *Artemia* ingested by an individual crab larva after 24 hours.

Table 5: Mean rotifer ingested by individual crab larva after 24 hours from Z1 until Z4 stages, feeding with both *Artemia* nauplii and rotifer.

Larval stages	Control				Treatment				Mean	
	R1	R2	R3	Mean±Sd (MC ¹)	R1	R2	R3	Mean±SD (MT ²)	(MC-MT) ³	RU ⁴
Z1	60	60	59	59.67±0.58	25	25	25	25.00±0.00	34.67	35
Z2	61	62	60	61.00±1.00	27	29	30	28.67±1.53	32.33	32
Z3	60	62	59	60.33±1.53	30	33	31	31.33±1.53	29.00	29
Z4	61	58	59	59.33±1.53	32	35	33	33.33±1.53	26.00	26

-R: Replicate

-MC¹: Mean number of *Artemia* left in the control without any crab larvae after 24 hours.

-MT²: Mean number of *Artemia* left in the treatment with individual crab larvae after 24 hours.

-(MC-MT)³: Mean number of *Artemia* ingested by individual crab larvae after 24 hours.

-RU⁴: Round up to the nearest one decimal place for mean number of *Artemia* ingested by an individual crab larva after 24 hours.

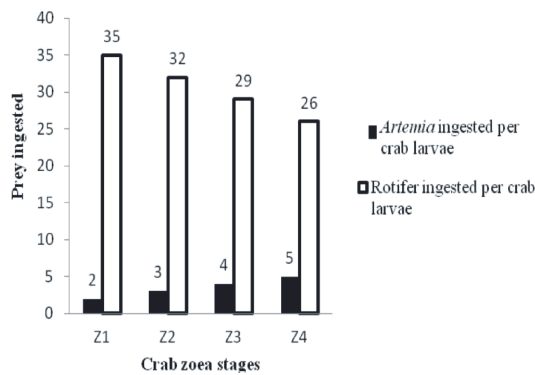


Fig. 1: Mean *Artemia* nauplii and rotifer ingested by individual crab larva after 24 hours from Z1 until Z4 stages, feeding with both *Artemia* nauplii and rotifer.

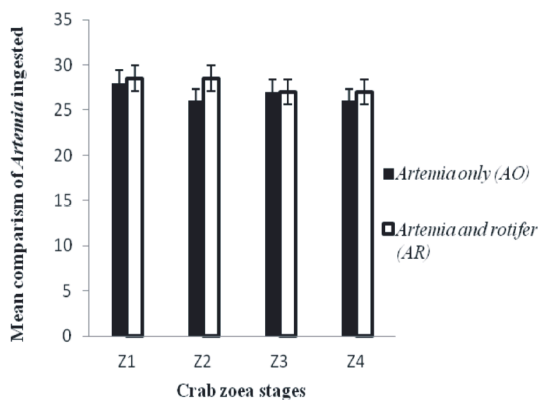


Fig. 2: Mean comparison of *Artemia* nauplii ingested by *P. pelagicus* larvae, feeding with *Artemia* and Rotifer and *Artemia* only.

temperature, salinity 29.46 ppt ± 0.22, pH 7.52 ± 0.12 and dissolved oxygen 5.70 ppm ± 0.17 respectively showed in Table 1

Ingestion Rate

***Artemia* Ingestion Rate:** The results show that 3 individual of *Artemia* nauplii were ingested by Z1 stage feeding with *Artemia* only (AO) daily. Z2 stage consumed also 3 individual of *Artemia* nauplii. Meanwhile, Z3 stage ingested 5 individual of *Artemia* nauplii and 6 individual of *Artemia* nauplii were ingested by Z4 stage respectively showed in Table 2.

Rotifers Ingestion Rate: The Z1 stage ingested 32 individual of rotifer and Z2 consumed 32 individual of rotifer. However, 30 rotifers were ingested by Z3 stage and 28 rotifers were ingested by Z4 stage respectively showed in Table 3.

***Artemia* and Rotifers Ingestion Rate:** In *Artemia* + rotifers feeding pattern, in the Z1 stage, ingested 2 *Artemia* nauplii and 35 rotifers and Z2, consumed 3 *Artemia* nauplii and 32 rotifers. However, 4 *Artemia* nauplii and 29 rotifers were ingested by Z3 and 5 *Artemia* nauplii and 26 rotifers were taken by Z4 after 24 hour feeding respectively showed in Table 4 and Table 5.

The results shows that the individual crab larva more preferred to ingested rotifer during initial zoeal stages while in the later zoeal stages, *Artemia* nauplii has been favorite as the most selected prey. Mean *Artemia* nauplii and rotifer ingested by Z1 until Z4 stages are respectively showed in Figure 1.

The data analyses shown that there was no significant ($P > 0.05$) relationship between the mean numbers of *Artemia* nauplii ingested by individual crab larvae, feeding with AR and feeding with AO for every zoeal stages ($P = 0.0504$ for Z1; $P = 0.8185$ for Z2; $P = 0.6756$ for Z3; $P = 0.3943$ for Z4).

However, comparatively the rotifer were ingested a bit higher by crab larvae feeding with both *Artemia* and

rotifer compared to those feeding with rotifer only for all zoeal stages except for zoeal 3 respectively showed in Figure 2.

Identification of Larval Stages: The identification of larvae from Z1 to Z4 was observed similar to Ramano and Zeng, [1]. No change in morphometry and zoeal structures were observed.

DISCUSSION

The results of the study showed that Z1 larvae of *Portunus pelagicus* are capable of consuming *Artemia* nauplii as sole diet and that can uphold their survival and growth. From the study it was observed that an increase in ingestion of *Artemia* by the individual crab larvae with increasing larval growth. This was perhaps based on the postulation that with growth, the larvae require more food to meet their metabolic requirements. It has been reported by Baylon *et al.* [4] that an increased ingestion by mud crabs, *Scylla serrata* larvae of *Artemia* with increasing larval development. The ability of the larvae to capture the prey could also be attributed to their highly sensitive mechanoreceptors located on the cuticle of the antennae and/or the prey size [8]. The low ingestion of *Artemia* at early zoeal stages could be attributed to inefficiency in capturing the prey, which most likely improved as the larvae grew. The present study is in agreement of previous research.

Harvey and Epifanio [8] observed that in the edible portunid crab *Thalamita crenata* and the common crab *Portunus herbstii*, early-stage larvae showed a strong preference for rotifers, while late-stage larvae showed a strong favorite for *Artemia*. However, the small size of rotifer and their slow swimming movement makes them easy prey for the early zoeal stages to capture and to consume, compare with the larger *Artemia* [9]. An increase in daily ingestion of *Artemia* naupli before metamorphosis to the next developmental stage and a decrease in rotifer ingestion after molting were demonstrated in all zoeal stages except at Z3. The gastric mill of the digestive system also starts to develop during these stages and an increase in the size of hepatopancreas occurs. It was reported by Ong [10] that at Z1, Z2 and Z4 *S. serrata* larvae crab stages undergo an increase in size after molting to the next stage, but it is at the Z3 stage where the larvae undergo changes such as the development of an additional abdominal segment from five segments in Z2 to six segments in Z3. Perhaps these major morphological and physiological changes weakened the larvae and affected their ingestion rate.

The increase in ingestion before moulting suggests that *Artemia* naupli are the source of nutrition to build up tissues required by the larvae for them to develop successfully to the next stage. After moulting, larvae probably weak with mouth parts still soft that could not yet handle the *Artemia* efficiently, hence the rotifer that can be easily consumed provided them the nutrition necessary for survival. Our results support the larval rearing procedure in the seed production of common mud crab *P. herbsti* [8] and the portunid crab [9], where early-stage larvae showed a strong choice for rotifers, while late-stage larvae showed a strong liking for *Artemia*. A diet of rotifer in the early zoeal stages is vital for the successful hatchery of mud crab, *Scylla* species [4]. In other work Baylon and Failaman [12] reported that for *Scylla serrata* larvae, rotifers resulted in a higher survival from Z1 to megalopa when fed with a combination of *Artemia* and compared to only *Artemia* or rotifers. The results of present study support the previous outcomes.

Since early zoeal stages seem unable to capture and consume larger *Artemia* nauplii as effectively as rotifers, perhaps using *Artemia* strains with smaller nauplii may result in higher survival. Live food organisms in the culture tanks compete with the larvae for space and available dissolved oxygen as well as contribute metabolites, which can foul the water causing stress and mortalities of the crab larvae. In particular, food shortage usually results in cannibalism in crab larvae cultures [6, 13]. In the present study single larvae rearing system was applied that did not enunciate difference in survival rate.

No change in morphometric characteristics was observed in larvae during the present study were similar to those observed by Arshad *et al.* [14].

This is the first ever study to determine the ingestion rate after 24 hours of *Artemia* and rotifer by individual *P. pelagicus* larvae in every zoeal stage. Further studies are needed to explore the larvae for further feeding at more density for optimal feeding regime in hatchery trials larvae rearing system.

CONCLUSION

The study concluded that the individual *P. pelagicus* larvae ingested more *Artemia* after 24 hours during the late zoeal stage as compared to the initial zoeal stage. Contrary the larvae ingested more rotifer after 24 hours during the initial zoeal stage as compared to the late zoeal stage. The introduction of both rotifer and *Artemia* naupli in feeding regime showed that the presence of rotifer do not influence the consumption of *Artemia* naupli by the individual crab larvae from Z1 till Z4 stages.

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