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Research Article
Microalgal Preference and Feeding Density of Selected Microalgae Diets by Blue Swimming Crab *Portunus pelagicus* (Linnaeus, 1758)

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Abstract

Background and Objectives: Blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) are economically important sources of soft-shell products for export demand. However, increasing over-exploitation and pollution towards the sea resources affected the production of the *P. pelagicus*. The study aimed to determine the microalgal preference and feeding density of the monospecific (*Nannochloropsis oculata*, *Chlorella vulgaris*, *Isochrysis galbana* and *Chaetoceros wighamii*) by individual *P. pelagicus* larvae. **Materials and Methods:** In the microalgal preference study, one polystyrene tray with 100 holes was set up in the experimental tank (200 L) to place the 50 mL centrifuges tube. For feeding density study, the survival rate, Specific Growth Rate (SGR), Body Weight (BW) and Larvae Stage Index (LSI) of each larvae stage were collected from Zoea 1 (Z_1) till megalopa. **Results:** Results for mean microalgal preference of individual larvae preferred most *Chlorella vulgaris* during Zoea 1 and 2, the larvae consumed the highest of *Nannochloropsis oculata*. For Zoea 3 and 4, the highest preference is *Isochrysis galbana*. For feeding regime, Zoea 1, larvae fed rotifer, *Artemia* and *Chlorella vulgaris* (T_3), was the highest survival rate, Zoea 2 was larvae fed rotifer, *Artemia* and *Nannochloropsis oculata* (T_5) and both Zoea 3 and Zoea 4, larvae fed rotifer, *Artemia* and *Isochrysis galbana* (T_4) was the highest survival rate. The highest SGR and LSI were obtained in T_4 cultured in 14 days. **Conclusion:** In conclusion, the microalgal preference and feeding density of the selected microalgae are different depending on each larvae stage.

Key words: Live food, survival, ingestion rate, feeding regime, aquaculture, portunid crab, specific growth rate, larval stage index

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Blue swimming crab, *Portunus pelagicus* are economically important sources of soft-shell products for export demand[1]. These species are distributed throughout the Indo-Pacific region includes Asian countries. *P. pelagicus* is most abundant in Southeast Asia and one of the fishermen's economic sources[2,3]. However, increasing over-exploitation and pollution towards the sea resources affected the production of the *P. pelagicus*. Over-exploitation in capture production of *P. pelagicus* occurred due to the high price and demand among the community[4]. Further study related to *P. pelagicus* in a hatchery and improving the culture techniques will increase the production of the *P. pelagicus* larvae rearing to fulfil the market demand[5].

Artemia itself is an incomplete food source due to the lack of certain important components in its composition, such as the Polyunsaturated Fatty Acids (PUFAs) n3 and n6, which are frequently needed for the success of crustacean larvae production[6]. Microalgae are considered as one of the most important factors affecting the early development of fish and shellfish during the early culture stages[7-10]. Live food is still a major problem in crab hatchery cultures due to the difficulty of culture, high cost of maintenance and acts as a disease vector[11]. Based on previous studies, the individual larvae of *P. pelagicus* ingested more *Artemia* sp. during the late larval stages and consumed more *Brachionus* sp. during initial larval stages[12]. The significance of this research is the most preferable microalgae feed by *P. pelagicus* larvae during their life cycle can be revealed. The information from the research can be used to develop a suitable feeding density by using selected microalgae, which are *Nannochloropsis oculata*, *Chlorella vulgaris, Isochrysis galbana* and *Chaetoceros wighamii* for each of the larval stages to maximize the survival rate in larval rearing and minimize food cost can be achieved to produce mass production of *P. pelagicus* larvae.

Microalgae are required for larvae nutrition during rearing, either for direct use in the case of molluscs and penaeid shrimp or indirectly as food for live prey such as small fish larvae[13]. Microalgae have a beneficial impact on the growth rate and their elevated triglycerides are correlated with the physiological status of aquatic organisms, assist protein deposition on muscle and enhanced disease tolerance, decreased environmental nitrogen output and increased content of omega-3 fatty acids, physiological activity and carcass quality[14,15].

The study by Araujo and Garcia[16] stated that, *C. wighamii* size 2-20 µm suitable food for marine planktonic larvae. On the other hand, *N. oculata*, have a diameter of 2-3 µm[17]. It's had higher proportions of EPA and AA[18,19]. It is also used as a main energy source for crustacean larvae and rotifer. For *I. galbana* it has 2-4 µm wide[20]. High in protein and another essential nutrient, *C. vulgaris* becomes an attractive food source. It also can produce natural antioxidants and is used as protein-rich food for sewage oxidation[21].

Based on the previous review, most studies focus on behavioural, survival rate, hepatopancreas, gonad, fatty acid, glucose and energy density of crab broodstock to increase crab seed production[22-25]. But lack of information about the microalgal preference of microalgae especially for *P. pelagicus* larvae that may lead to the waste in food cost and lower survival rate during the early larval stages culture.

The objectives of this study were to determine the microalgal preference of the selected microalgae of *N. oculata*, *C. vulgaris, I. galbana* and *C. wighamii* by individual *P. pelagicus* larvae from Zoea 1 until Zoea 4 stages after 24 hrs and to develop a feeding density of this selected microalgae in each larval stage.

MATERIALS AND METHODS

Study area: The study was carried out at the Hatchery and Laboratories of the Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu (UMT), Malaysia from August, 2013-September, 2014.

Broodstock management: The berried female crabs with orange and greenish in the colour of eggs were selected and placed in hatching tanks. The berried female crabs were placed inside a 100 L fibreglass tank with 1 berried female crab per tank and were filled with treated filtered seawater. Three centimetres thick of sand in trays were placed in the centre part of the culture tank for egg hatching. Constant aeration was supplied during the rearing of berried female crabs until crabs hatched. Filtered seawater was exchanged 50% in every berried female crab tank every morning and water parameters were maintained at 30 ppt salinity, ±28°C temperature, pH±7.9 and more than 5 ppm Dissolved Oxygen (DO). The berried female crabs were monitored daily. The fresh squid was fed to the berried female and reared until the eggs hatching. The tanks were checked daily for the appearance of larvae. Once the egg hatched, water in the berried female crab's tank was reduced to 50 L and immediately transferred into the larval rearing tank. The aeration in the hatching tank was stopped for 10-15 min to allow the swimming larvae to aggregate at the surface, where they were collected. Broodstock management was based on the previous methods by Azra *et al.*[26].

Larval management: After hatching, the crab larvae were transferred to a culture tank (500 L). Estimation of the larvae stocked in the culture tank was done to know the number of larvae produced. Active swimming larvae accumulated near the water surface were siphoned out by a plastic tube into the larvae rearing tank (500 L tank capacity) filled with 350 L of treated seawater, constant aeration was supplied inside the larval rearing tank. Aeration was used to maintain dissolved oxygen concentration and help to spread particles of food suspended in the water column. Crab larvae were stocked at a density of 50 larvae L^{-1} and placed in a larval rearing tank that was covered with a dark colour cover to avoid illumination. During larval rearing, the stages of crab larvae were observed under profile projector after daily fed with microalgae, *Artemia* sp. and *Brachionus* sp.

Microalgae preparation: The seawater was autoclaved 0.45 um filtered and UV-treated. Microalgae were cultivated using conway nutrient medium. Meanwhile, *Chaetoceros wighamii* were cultivated using F/2 nutrient medium at 30 PSU and was supplemented with a silicate solution (30 mg L^{-1}) for optimal growth[2]. The cultures of microalgae were supplied with continuous aeration and were subjected in a culture chamber to a 12:12 hrs light: Dark photoperiod and 22±1°C temperature. The number of cells per mL was estimated daily using a particle counter machine (Model: SLS 2000, Colorado, USA) and cultures were preserved at 10^6 cells per mL. Once cell populations entered the exponential growth period (i.e., 6 days from inoculation), the culture was used to feed the larvae. Throughout the experimental phase, the ratio of microalgae to larvae was constantly maintained[19].

Water quality management: Filtered seawater in 1 t of tanks was treated by using 30 ppm of calcium hypochlorite and aerated within 24 hrs. After 24 hrs, seawater was neutralized with sodium thiosulphate at 15 ppm within 12-24 hrs. Water in centrifuge tube was monitored daily to measure pH, temperature, salinity and DO level by using YSI Multi-parameter Probe (Model: YSI 556 MPS, YSI Incorporated, Yellow Springs, Ohio, the USA-imported).

Experimental design of feeding density of *P. pelagicus* larvae: One polystyrene tray with 100 holes was set up in the experimental tank (200 L) to place the 50 mL centrifuges tube for this study. There were 4 treatments involved in this study. The larvae were fed with *Chaetoceros wighamii* (T_1), *Chlorella vulgaris* (T_2), *Isochrysis galbana* (T_3) and *Nannochloropsis oculata* (T_4) (Table 1). The feeding densities for the 4 treatments are 10^6 cell mL^{-1}. Eighty pieces of centrifuge tubes, 10 replicates each for control (without larvae) and treatment (with larvae) were prepared. The polystyrene tray was floated on the surface of the water so that filtered seawater would enable to submerge 1/3 of the bottom part of centrifuge tubes. The seawater was used in this study act as a water bath. A heater was placed at the bottom of the tank to maintain the suitable temperature. In this study density of microalgae was measured 3 times by using particle counter machine (Model: SLS 2000, Colorado, USA) to get the initial mean that was recorded as IC_1 for control and IT_1 for treatment. Centrifuge tubes with a capacity of 50 mL were filled with 40 mL of microalgae for 20 pieces. One individual crab larval was taken from the culture tank and gently pipetted into 10 treatment tubes. Ten control tubes were conducted without the presence of crab larvae. The study was left for 24 hrs. Mild aeration was supplied in all of the centrifuge tubes. The study was conducted from Zoea 1 until 4 stages. *Artemia* sp. and *Brachionus* sp. were given as a continuous feed in the culture tank and not in the 24 hrs study period. After 24 hrs, aeration was stopped, all centrifuge tubes were brought to the laboratory to calculate the final density of the microalgae. The larvae were pipetted out of the centrifuge tubes. The densities of microalgae were measured by using a particle counter machine. The final number of microalgae for each treatment and control were calculated and labelled as FT_2 and FC_2, respectively. The number of microalgae ingested by an individual larvae crab was calculated by using the following formula[2,19]:

Table 1: Dietary treatments applied in ingestion rate experiment from Zoea 1 stage till Zoea 4 stage of *P. pelagicus* larvae

Treatments	Types of microalgae used
T_1	*Chaetoceros wighamii*
T_2	*Chlorella vulgaris*
T_3	*Isochrysis galbana*
T_4	*Nannochloropsis oculata*

Feeding density, 10^6 cell mL^{-1} (Ikhwanuddin *et al.*[28])

$$AC_3 = FC_2\text{-}IC_1$$
$$AT_3 = (IT_1+AC_1)\text{-}FT_2$$

IC_1 = Mean initial number of microalgae in control before 24 hrs

FC_2 = Mean final number of microalgae in control after 24 hrs

AC_3 = Mean actual number of microalgae increment in control after 24 hrs

IT_1 = Mean initial number of microalgae in treatment before 24 hrs

FT_2 = Mean final number of microalgae in treatment after 24 hrs

AT_3 = Mean actual number microalgae ingested by individual larvae after 24 hrs

The procedures were repeated 3 times. There were possibilities of the microalgae to reproduce within 24 hrs, that why AC_3 was taken into account. Different stages in crab larvae were observed under the profile projector (Model: ISP-Z2510, Insize Co., Ltd., Zafar, Tehran, Iran) and morphological description described based on Arshad *et al.*[27].

Experimental design of hatchery trial to develop feeding density: A hatchery trial was done to develop a feeding density for *P. pelagicus* in early larval stages. Propose feeding density of selected microalgae consume by individual larvae from the first experiment introduced in this experiment were shown in Table 2. The survival rate, Specific Growth Rate (SGR), Body Weight (BW) and Larvae Stage Index (LSI) of each larvae stage were collected during the experiment from Z_1 till megalopa. Microalgae that produce the highest survival rate, SGR and fast LSI showed the most suitable microalgae for larvae rearing.

Survival rate: Initial density larvae in each treatment tank were calculated. After the larvae reach Zoea 4 and the initial stages of the megalopa, the density of larvae left was calculated as the final amount. The survival rate (%) at a particular treatment was met by dividing the final stocking density with initial stocking density. Each treatment was replicated 3 times to get the mean value. The following formula[2,19] was used to calculate the survival rate:

$$\text{Survival Rate} = \frac{\text{Final density}}{\text{Initial density}} \times 100$$

Specific growth rate: Sampled crab larvae of each treatment (Z_1) and (Z_4) were put into disposal scintillation vials (Bjorn bottle) and then preserved in 10% of formalin (to make it and after that body weight was taken by used the microbalance (Model: Sartorius SE2 Microbalance, Bradford, Massachusetts, USA). The mean BW of larvae for each treatment for different larvae stages was used for calculating a specific growth rate (%). The data recorded and expressed as a Specific Growth Rate (SGR). The following formula[2,19] was used to calculate the Specific Growth Rate (SGR):

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

Larval stages index determination: The larval stage was determined using a dissecting microscope to ensure that the larvae reach the stage in the period. The larval stage can be differentiated by identifying its telson, eye-stalked, abdomen segmented, spine and setae. The development stages of the larvae were observed under a microscope and classified according to Arshad *et al.*[27]. The feeding density for rotifer and *Artemia* used in this work are based on the study by previous studies[28,29]. The feeding densities for the microalgae are based on the results from the previous microalgal preference experiment.

Table 2: Feeding regime applied in the hatchery trial from Zoea 1 stage till megalopa stage of *P. pelagicus* larvae

Treatments	Types of live food used
T_1	Rotifer and *Artemia*
T_2	Rotifer, *Artemia* and *Chaetoceros wighamii*
T_3	Rotifer, *Artemia* and *Chlorella vulgaris*
T_4	Rotifer, *Artemia* and *Nannochloropsis oculata*
T_5	Rotifer, *Artemia* and *Isochrysis galbana*

Feeding density, Rotifer: 32.0-39.0 ind crab larvae^{-1} (Ikhwanuddin *et al.*[28]), *Artemia*: 1.5-15.0 ind crab larvae^{-1} (Ikhwanuddin *et al.*[28]) and Microalgae: Based on the results from experiment 1

Statistical analysis: The data from the experiment were performed by using SPSS version 20.0 to determine the comparison of the mean number of the ingested microalgae. All results were present as Mean±SD. The differences were displayed as statically significant when $p<0.05$. Independent-sample t-test analyses were used to analyze the significant differences in final survival and SGR value.

RESULTS

Microalgae preference of *P. pelagicus* larvae: The data showed the mean microalgae preference by individual *P. pelagicus* larvae after 24 hrs. The mean number of preference microalgae without larvae (control) and the mean preference microalgae with larvae (T_1, T_2, T_3 and T_4) were as in (Table 3). In Zoea 1, the highest microalgae preference were *Chlorella vulgaris*, followed by *Isochrysis galbana*, *Chaetoceros wighamii* and *Nannochloropsis oculata*, with a mean of 1,169.72, 365.01, 231.65 and 215.91 cells (Fig. 1). For Zoea 2, the highest was 2,722.38 cells of *Nannochloropsis oculata*, preferred by the *P. pelagicus* larvae, followed by 1,505.82 cells of *Isochrysis galbana*, 1,342.25 cells of *Chlorella vulgaris* and 475.61 cells of *Chaetoceros wighamii*. Besides, the highest mean preference by individual larvae of Zoea 3 was *Isochrysis galbana* that recorded a mean of 2,561.53 cells followed by 1,636.24 cells of *Chlorella vulgaris*, 741.05 cells of *Nannochloropsis oculata* and the fewer microalgae consumed by the larvae was *Chaetoceros wighamii* mean 126.34 cells. Meanwhile, during Zoea 4, same as the Zoea 3, *Isochrysis galbana* recorded the highest microalgae species preferred by the larvae of mean 2,530.80 cells, followed by 441.57 cells of

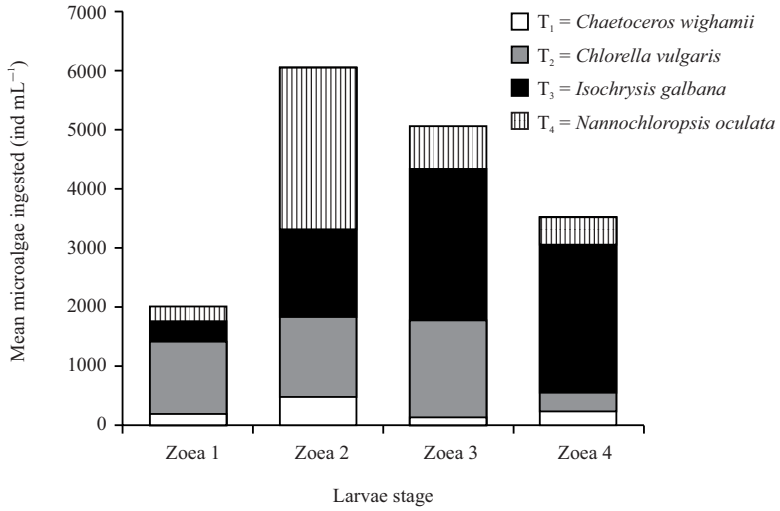

Fig. 1: Mean of selected microalgae consumed by individual larvae of blue swimming crab, *Portunus pelagicus* L. in centrifuges tube for Zoea 1 till Zoea 4

Table 3: Mean microalgae preferred by individual crab larvae after 24 hrs for Zoea 1 till Zoea 4 of experiment 1, *Chaetoceros wighamii*, *Chlorella vulgaris*, *Isochrysis galbana* and *Nannochloropsis oculata*

	IC_1/IT_1		FC_2		FT_2			
	Mean±SD	Mean	Mean±SD	Mean	Mean±SD	Mean	$AC_3 = FC_2-IC_1$	$AT_3 = (IT_1+AC_3)-FT_2$
Chaetoceros wighamii								
Z_1	41541.1±547.83	41541.1	42,061.15± 331.54	42061.15	41,829.5±651.87	41,829.50	520.05	231.65
Z_2	38,905.77±318.03	38,905.77	40,039.11±351.45	40,039.11	39,563.50±905.22	39,563.50	1,133.34	475.61
Z_3	11,868.03±110.80	11,868.03	13,336.77±978.74	13,336.77	13,210.43±802.76	13,210.43	1,468.74	126.34
Z_4	11,878.03±110.80	11,878.03	13,526.77±1059.79	13,526.77	13,262.00±758.05	13,262.00	1648.74	264.77
Chlorella vulgaris								
Z_1	19,861.23±402.71	19861.23	24,638.00± 2473.27	24638	23,468.28±1174.44	23468.28	4,776.77	1,169.72
Z_2	26,636.03±130.89	26636.03	28,738.98±387.87	28738.98	27,396.73±961.14	27396.73	2,102.95	1,342.25
Z_3	18,184.77±102.22	18184.77	19,663.22±715.35	19663.22	18,028.98±1,533.05	18028.98	1,478.45	1,634.24
Z_4	18376.11±331.12	18376.11	19,507.24± 522.35	19507.24	19,224.22±763.50	19224.2	1,131.13	283.04
Isochrysis galbana								
Z_1	39,120.9±396.81	39120.9	41,894.97±340.49	41894.97	41,529.96±450.19	41529.96	2774.07	365.01
Z_2	39,532.05±69.84	39,532.05	39,637.87±1371.27	39637.87	38,132.05±1775.445	38,132.05	105.824	1,505.82
Z_3	41,003.67±70.05	41003.67	43,092.22±1,491.22	43092.22	40,530.69±1,423.74	40530.69	2088.55	2,561.53
Z_4	41,541.2±60.84	41541.2	43,229.86±670.85	43229.86	40699.06±271.14	40699.06	1688.66	2,530.80
Nannochloropsis oculata								
Z_1	12,537.57±113.63	12537.57	17,714.01±2288.79	17714.01	17,498.10±1127.74	17498.1	5176.44	215.91
Z_2	16,680.33±130.62	16680.33	16,786.70±719.40	16786.7	14,064.32±3922.83	14064.32	106.37	2,722.38
Z_3	12,569.33±68.89	12569.33	14,149.47±1,288.81	14149.47	13,408.42±637.48	13408.42	1580.14	741.05
Z_4	12670.113±54.1	12670.11	14,273.18±1329.08	14273.18	13831.61±1362.25	13831.61	1603.067	441.57

Z1- Z4: Zoea 1-4, FC_2: Mean final number of microalgae after 24 hrs in control, FT_2: Mean final number of microalgae after 24 hrs in treatment, IC_1: Mean initial number of microalgae before 24 hrs in control, FC_2: Mean final number of microalgae after 24 hrs in control, AC_3: Mean actual number of microalgae increment after 24 hrs, IT_1: Mean initial number of microalgae before 24 hrs in treatment, FT_2: Mean final number of microalgae after 24 hrs in treatment and AT_3: Mean actual number of microalgae ingested by individual larvae after 24 hrs

Nannochloropsis oculata, 283.04 cells of *Chlorella vulgaris* and 264.77 cells of *Chaetoceros wighamii* (Fig. 1). The data of Table 4 showed the mean of selected microalgae preference by early larvae stages from Zoea 1 until Zoea 4 of *P. pelagicus* larvae. For Zoea 1, the proposed microalgae species for the crab larvae is *Chlorella vulgaris* means 29.24 cell mL^{-1}. About 68.06 cell mL^{-1} of *Nannochloropsis oculata* is proposed as live food for the crab larvae during Zoea 2. During Zoea 3 and 4, *Isochrysis galbana* can be used as live food for the larvae of *P. pelagicus* larvae with mean 64.04 and 54.76 cell mL^{-1}, respectively. The results illustrated that the early larval stages of *P. pelagicus* from Zoea 1 until 4 are capable of consuming

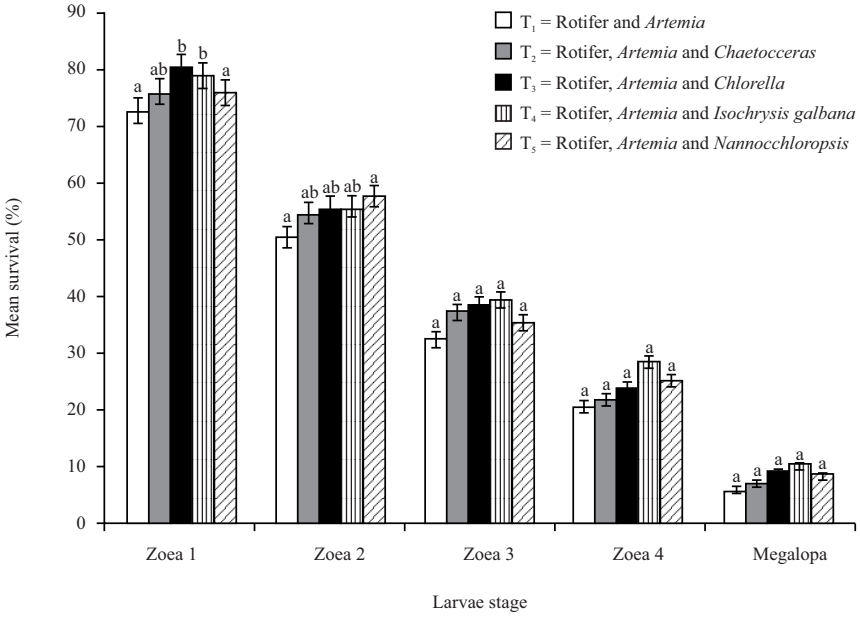

Fig. 2: Mean survival rate (%) of *Portunus pelagicus* larvae fed with selected microalgae for feeding density
SE: Standard error, the different letters, [a,b]Significant differences among treatments (i.e., treatment)

Table 4: Mean microalgae ingested by selected microalgae at different stages of *P. pelagicus* larvae

Larval stages	Microalgae species	Mean microalgae ingested (individual 40 mL^{-1})	Mean microalgae ingested (cell mL^{-1})
Zoea 1	*Chlorella vulgaris*	1,169.72	29.24
Zoea 2	*Nannochloropsis oculata*	2,722.38	68.06
Zoea 3	*Isochrysis galbana*	2,561.53	64.04
Zoea 4	*Isochrysis galbana*	2,530.80	54.76

all 4 microalgae. The result showed that the most preferred microalgae during Zoea 1 are *Chlorella vulgaris* which easily to catch by individual larvae compared to others. For Zoea 2, the most microalgae preferred is *Nannochloropsis oculata*. The small sizes of cells and developed morphological characteristics of the larvae can help it to ingest the food available. During Zoea 3 and 4, *Isochrysis galbana* is most ingested by individual larvae compared to others.

The survival rate from hatchery trial: The survival rate of *P. pelagicus* larvae in both control and treatment was significantly ($p<0.05$) during the day's culture. During day 3, the higher survival rate was recorded in larvae fed rotifer, *Artemia* and *Chlorella vulgaris* (T_3) with 79.96±0.11%. On the same day, the lowest survival rate recorded in larvae fed rotifer and *Artemia* only (T_1) with 72.34±0.03% as in Fig. 2. Based on Fig. 2, there was a significant difference ($p<0.05$) in the survival rate of the mean number of larvae fed rotifer and *Artemia* only (T_1), larvae fed rotifer, *Artemia* and *Chaetoceros wighamii* (T_2), larvae fed rotifer, *Artemia* and *Chlorella vulgar* and larvae fed rotifer, *Artemia* and *Nannochloropsis oculata* (T_5) between larvae fed rotifer, *Artemia* and *Isochrysis galbana* (T_4). At the megalopa stage, the highest survival rate from this experiment was obtained from T_4 with 10.21%±0.45 followed with T_3, T_5, T_2 and T_1 with 8.93±0.34%, 8.35±0.11%, 6.96±0.87% and 5.45±0.33%, respectively has been illustrated in Fig. 3.

Larval stages index (LSI): There was no significant ($p>0.0_5$) relationship between the mean values of cumulative development duration of LSI for all treatments. The LSI for T_1 was 1.5±0.15 (day 4), 2.5±0.21 (day 7), 3.5±0.53 (day 10) and 4.5±0.14 (day 13). Whereas, LSI for T_2 was 1.7±0.13 (day 4), 2.6±0.11 (day 7), 3.6±0.19 (day 10) and 4.6±0.17 (day 13). LSI for T_3 was 1.9±0.12 (day 4), 2.7±0.13 (day 7), 3.7±0.41 (day 10) and 4.7±0.13 (day 13). However, LSI for T_4 was 1.6±0.32 (day 4), 2.7±0.18 (day 7), 3.9±0.14 (day 10) and 4.9±0.15 (day 13). Lastly, LSI for T_5 was 1.6±0.25 (day 4), 2.9±0.18 (day 7), 3.7±0.13 (day 10) and 4.7±0.18 (day 13) has been shown in Table 5.

Body weight (BW): The mean larvae dry BW on day 1 (1 DAH) in all treatments was 0.018±0.0009 mg. The data of Fig. 4 shows the mean BW of the newly moults M stage from larvae reared in different treatments. The highest

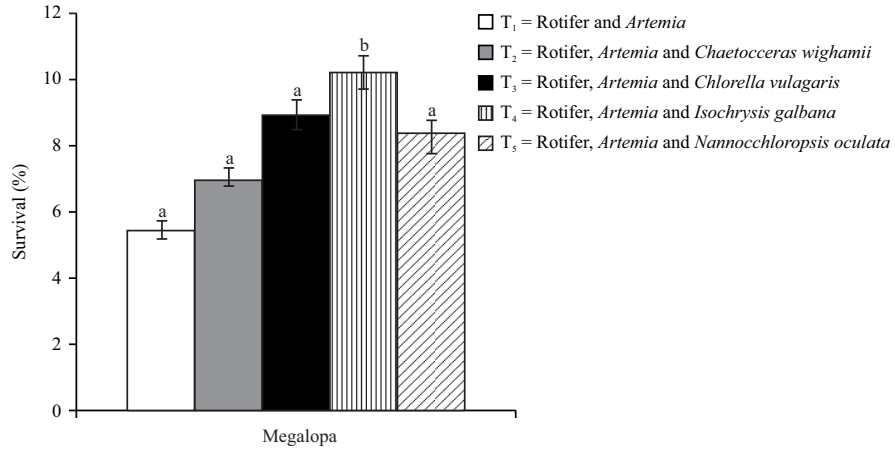

Fig. 3: Final survival rate (%) of *Portunus pelagicus* larvae at (megalopa stage) fed with selected microalgae for feeding density
Different letters (a, b) in each treatment shows that there are significant differences between treatments (p<0.05)

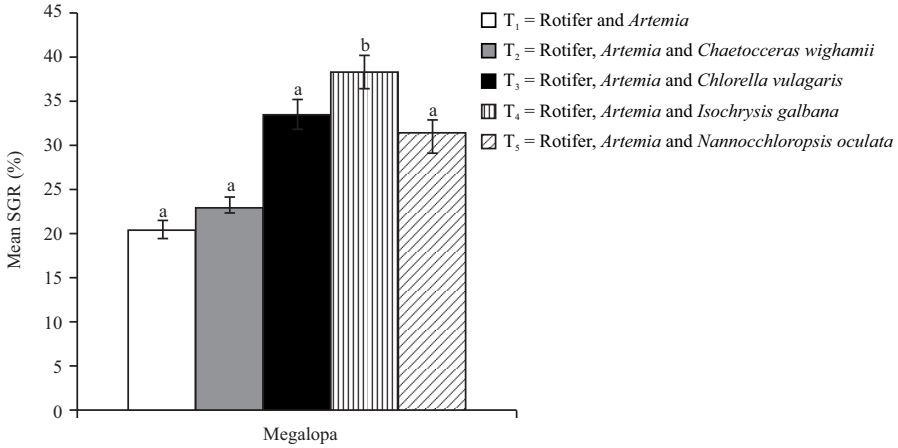

Fig. 4: Mean specific growth rate (SGR) of *Portunus pelagicus* larvae fed with selected microalgae for feeding regimes base on the larvae stages
Different letters (a, b) in each treatment showed significant differences between treatments (p<0.05)

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

	T_1		T_2		T_3		T_4		T_5	
Day	LSI	LS	LSI	LS	LSI	LS	LSI	LS	LSI	LS
1	1.0	Z_1	1.0	Z_1	1.0	Z_1	1.0	Z_1	1.0	Z_1
2	1.0	Z_1	1.0	Z_1	1.0	Z_1	1.0	Z_1	1.0	Z_1
3	1.0	Z_1	1.0	Z_1	1.0	Z_1	1.0	Z1	1.0	Z_1
4	1.5±0.15	Z_1/Z_2	1.7±0.13	Z_1/Z_2	1.9±0.12	Z_1/Z_2	1.6±0.32	Z_1/Z_2	1.6±0.25	Z_1/Z_2
5	2.0	Z_2	2.0	Z_2	2.0	Z_2	2.0	Z_2	2.0	Z_2
6	2.0	Z_2	2.0	Z_2	2.0	Z_2	2.0	Z_2	2.0	Z_2
7	2.5±0.21	Z_2/Z_3	2.6±0.11	Z_2/Z_3	2.7±0.13	Z_2/Z_3	2.7±0.18	Z_2/Z_3	2.9±0.18	Z_2/Z_3
8	3.0	Z_3	3.0	Z_3	3.0	Z_3	3.0	Z_3	3.0	Z_3
9	3.0	Z_3	3.0	Z_3	3.0	Z_3	3.0	Z_3	3.0	Z_3
10	3.5±0.53	Z_3/Z_4	3.6±0.19	Z_3/Z_4	3.7±0.41	Z_4	3.9±0.14	Z_3/Z_4	3.7±0.13	Z_3/Z_4
11	4.0	Z_4	4.0	Z_4	4.0	Z_4	4.0	Z_4	4.0	Z_4
12	4.0	Z_4	4.0	Z_4	4.0	Z_4	4.0	Z_4	4.0	Z_4
13	4.5±0.14	Z_4/M	4.6±0.17	Z_4/M	4.7±0.13	Z_4/M	4.9±0.15	Z_4/M	4.7±0.18	Z_4/M
14	5.0	M	5.0	M	5.0	M	5.0	M	5.0	M

LSI = 1, Z_1 : LSI = 2, Z_2 : LSI = 3, Z_3 : LSI = 4 and Z_4 : LSI = 5, M stages: LS = Larvae stages and DAH = Day after hatching, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3 and T_4 = Treatment 4

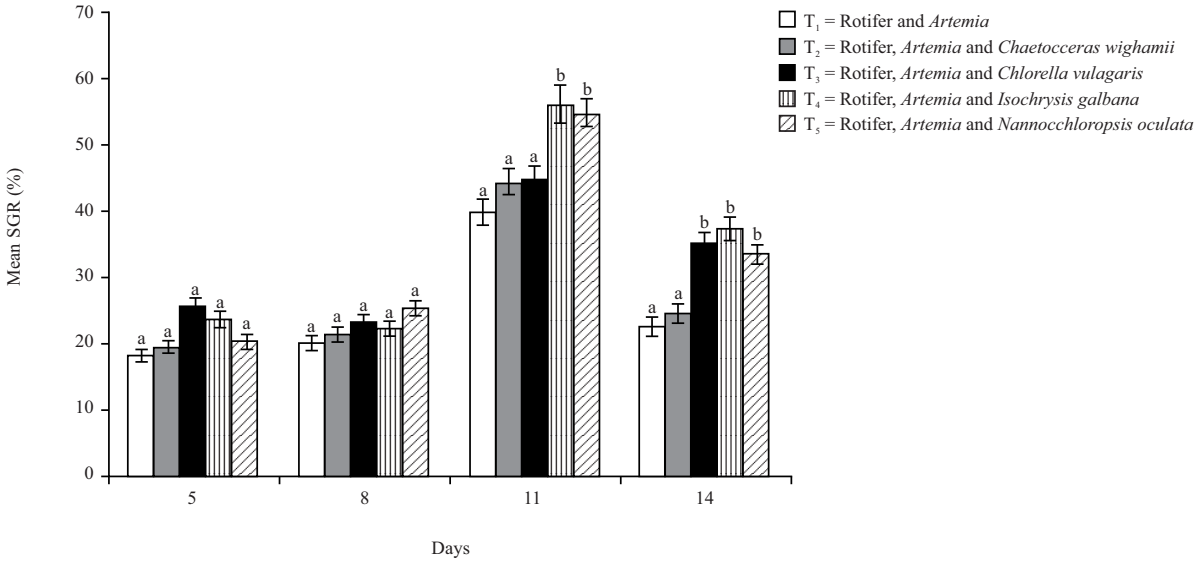

Fig. 5: Mean specific growth rate (SGR) of *Portunus pelagicus* larvae fed with selected microalgae for feeding density based on the culture period (days)

SE: Standard error, the different letters, [a,b]Significant differences among treatments (i.e., treatment)

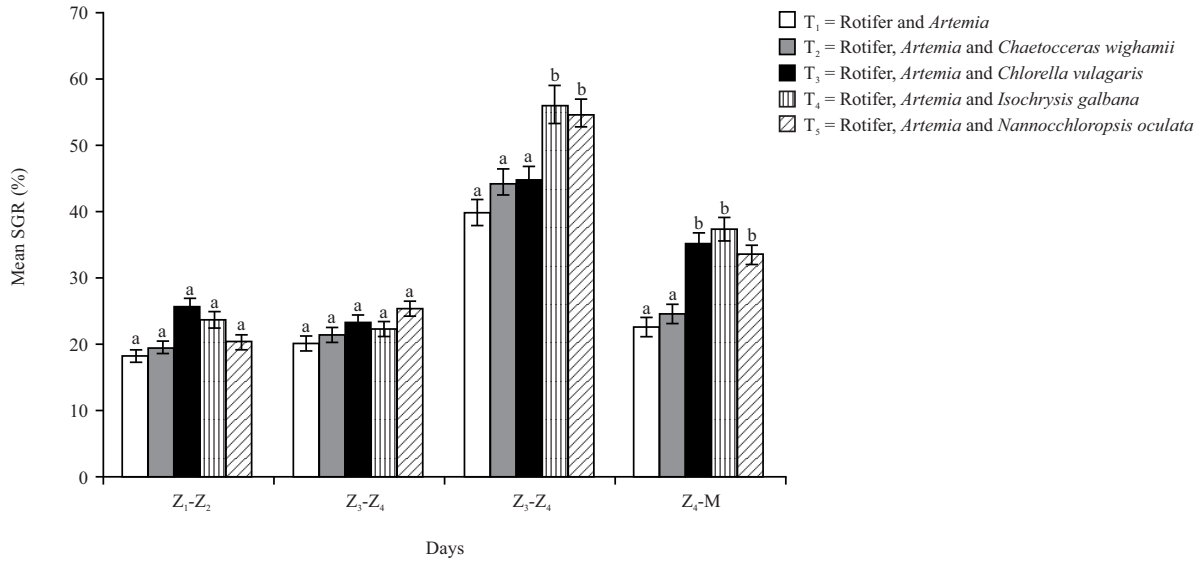

Fig. 6: Mean specific growth rate (SGR) of *Portunus pelagicus* larvae fed with selected microalgae for feeding regimes base from larvae stages to the next larvae stages

SE: Standard error, the different letters, [a,b]significant differences among treatments (i.e. treatment)

Specific Growth Rate (SGR) of the megalopa stage was obtained in T_4 cultured in 14 days. The mean dry BW increased to 3.43±0.07 mg with a mean SGR of 37.50±0.02% and the lowest mean dry BW in T_1 was 0.42±0.02 mg with mean SGR 22.50±0.01%. There were significant differences ($p<0.05$) in were detected among treatment T_3, T_4 and T_5 between treatment T_1 and T_2. The data of Fig. 5 showed the mean SGR of *P. pelagicus* larvae from day 5, 8, 11 and 14 with different treatment. The result of Fig. 6 showed the mean SGR of Z_1-Z_2, Z_2-Z_3, Z_3-Z_4 and Z_4-M from base larvae stages to the next larvae stages. The highest mean SGR of crab larvae from the Z_1-Z_2 stage was T_3 with 25.72±0.23% followed with T_4, T_5, T_2 and T_1 with 23.74±0.29%, 20.39±0.31%, 19.42±0.47% and 18.20±0.00%, respectively. For Z_2 to Z_3 stage, the highest

mean SGR was recorded in T_5 followed with T_3, T_4, T_2 and T_1 with 25.35±0.01%, 23.21±0.21%, 22.43±0.24%, 21.54±0.18% and 20.34±0.29%, respectively. T_1 produced the lowest mean SGR of 39.96±0.82% in Z3-Z4 as compared to T_4, T_5, T_3 and T_2 with 56.37±0.21%, 54.47±0.14% and 44.85±0.13% and 44.30±0.24%, respectively.

DISCUSSION

Larvae of *P. pelagicus* passed through four Zoea stages (Zoea 1 until Zoea 4) before metamorphosed to the megalopa stage. All 4 types of microalgae used in this study have a size at a range of 3-16 µm which are suitable as food for early larval. Work from Epelbaum and Borisov[30] stated that a reasonably wide range of particle sizes, from 100-2 mm, can be captured and preferred by Zoea. From the previous study, the larvae of *Litopenaeus vannamei* fed with *I. galbana* exhibited the highest survival from the larval stages of protozoal 1 to post-larvae 1[31]. *Isochrysis galbana*, *Pavlova lutheri* and *N. oculata* are commonly fed to the larval stages of crustacean and fish larvae[19]. The previous study also showed that Zoea 1 and 2 were fed with live food such as *C. wighamii* and rotifer[32]. From the measurement of microalgae size, the smallest microalgae are *I. galbana* about 3 µm, followed by *N. oculata* (4 µm), *C. vulgaris* and *C. wighamii* are about 5-7 µm. The sizes of microalgae used are capable to ingest by individual larvae at the early stages of Zoea 1 until Zoea 4. The microalgae used for larvae rearing need to be of the cor and have a digestible cell wall to be ingested to have high nutritional quality[33]. The *P. pelagicus* larvae able to consume the much larger microalgae species may be influenced by the visibility of the algae in the water and the digestible cell wall of the algae[33]. Baylon *et al.*[34] showed that the early larvae stages of mud crab *S. serrata* were unable to consume entire *Artemia* nauplii, but they were able to ingest bits and pieces of the body. *Isochrysis galbana* cell has no distinct cell wall as confirmed by Lora-Vilchis *et al.*[35] and only possess a plasma membrane. *Isochrysis galbana* can produce polyunsaturated fatty acid (DHA) and one of the n-3 fatty acids that are essential for the growth and development of marine culture organisms[35]. From the previous study, it is recognized that the addition of *N. oculata* into rearing water is useful to increase the activity and survival of larval fish[20,36].

The most frequently used species of microalgae in the aquaculture hatchery are *Chlorella vulgaris*, *Tetraselmis suecica*, *Isochrysis galbana*, *Pavlova lutheri*, *Phaeodactylum tricornutum*, *Chaetoceros wighamii*, *Nannochloropsis oculata*, *Skeletonema costatum* and *Thalassiosira weissflogii*. The selected microalgae of *Nannochloropsis oculata*, *Chlorella vulgaris, Isochrysis galbana* and *Chaetoceros wighamii* used in this study possess the significant nutrient value in the larvae rearing. The high nutritional requirement makes microalgae are known for their excellent nutrient properties[37]. Microalgae like *C. vulgaris*, *I. galbana*, *N. oculata* and *C. wighamii* were used to increase PUFA level in the larval diet[10,19]. For this analysis, microalgae selected were characterized by their high levels of amino acids (e.g., *C. wighamii* and *N. oculata*), lipids (e.g., *I. galbana*) and PUFA (e.g., *N. oculata*, *I. galbana* rich in DHA[33]. Variability and complexity of live food are essential for an animal's growth rate and survival. The supply of microalgae may have several beneficial effects on the nutritional state of larvae, one of which is that the presence of microalgae stimulates larval digestive enzyme production and colonization of the digestive tract bacteria[7-10]. From this present study after 5 days, the highest survival, growth and development rate of larvae was observed in larvae fed *C. vulgaris* (T_3). For day 8, larvae *N. oculata*. (T_5) was significantly higher compared to other treatments. This result similar to Ikhwanuddin *et al.*[28] stated that a combination of instant frozen *N. oculatar* gives a better survival rate till 1st-day juvenile crabs. However, in Zoea 3 and 4, *I. galbana* showed the highest survival, growth and development rate of larvae. These results were supported with the first experiment to determine the ingestion rate of *P. pelagicus* larvae. Following the results stated by Lora-Vilchis *et al.*[35], treatment on *C. wighamii* did not develop faster (in terms of SGR) than those fed on *I. galbana* after day 14. After 14 days, the number of dead larvae in the control tanks (*Artemia* only) became conspicuously higher than that in the other treatment tanks which are only 5.45±0.33% of survival rate. Moreover, water pollution was a probable factor inducing larval mortality. In the stud from Tamaru *et al.*[36], inorganic nitrogen (NH_4^- N, NO_2^- and NO_3^- N) and phosphate (PO_4^- P) concentrations increased both in tanks with microalgae and without microalgae. One of the reasons for the differences in concentrations of nitrogen and phosphate in the rearing water between tanks with microalgae and without microalgae seemed to algal nutrient absorption. Microalgae absorbed dissoluble nitrogen and phosphate as nutrients in the tanks. Additionally, many larvae died and their bodies, before being removed each day would also deteriorate the quality of the rearing water. Thus, water pollution seemed to be accelerating in a short term. From this point of view, using microalgae was effective. It has been reported that microalgae added to the culture water seemed to have a 'beneficial' effect in larval fish culture in terms of survival by releasing oxygen into and

removing certain metabolites like ammonia, from the culture medium[34]. It was even suggested that microalgae also release an antibiotic substance into the culture medium[34]. On the other hand, due to lack of highly unsaturated fatty acid such as eicosapentaenoic acid and docosahexaenoic acid cause high mortalities in crustacean larval rearing[32].

The implication of a combination between microalgae and culture species demonstrates the reducing effect of harmful microbes on crab culture. The non-specific immunity behind these phenomena is still unclear and need some more investigation in future. Further research should be conducted to reveal scientific insight into how the microalgae can produce their antibiotic effect. Therefore, critical studies must be conducted by taking into account those subject matters. The immunological effect at different species of microalgae and on species-specific is another aspect to be considered. This can be done between different commercialized aquatic lives. Lastly, identification of specific microalgae that preferable to be used as crab feeding is very crucial for the industrial application.

CONCLUSION

The microalgae preference of *C. wighamii, C. vulgaris*, *I. galbana* and *N. oculata* by *P. pelagicus* are different depending on each larvae stage. The study concluded that the individual *P. pelagicus* ingested more *C. vulgaris* for Zoea 1, *N. ocula* and *I. galbana* for Zoea 3 and 4, respectively. The feeding density can be concluded based on survival, growth and larval development. For Zoea 1, larvae fed *C. vulgaris* (T_3) is the best. However, in Zoea 2, larvae fed *N. oculata* (T_5) was the highest. For Zoea 3 and Zoea 4, larvae fed *I. galbana* (T_4) was dominant. In conclusion, microalgae type influenced survival rate, larvae development duration and specific growth rate of *P. pelagicus* larvae.

SIGNIFICANCE STATEMENT

This study discovers the potential of microalgae as additional feed for portunid crab in the nursery phase. The finding of this study is a new finding that can be beneficial for farmers to increase survival, improve growth and increase the immunity of portunid crab culture. This study will help the researcher to uncover the critical areas of high mortality during the planktonic larval phase that many researchers were not able to explore. Thus, a new theory on the antibiotic substance in microalgae maybe soon explore.

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REFERENCES

1. Tavares, C.P.D.S., U.A.T. Silva, L.A. Pereira and A. Ostrensky, 2018. Systems and techniques used in the culture of soft shell swimming crabs. Rev. Aquacult., 10: 913-923.
2. Taufik, M., Z. Bachok, M.N. Azra and M. Ikhwanuddin, 2014. Identification and determination of the fatty acid composition of *Portunus pelagicus* in Setiu Wetland Areas, Terengganu, Malaysia by GC-MS. Middle-East J. Sci. Res., 21: 1908-1915.
3. Azra, M.N., M.A. Aaqillah-Amr, M. Ikhwanuddin, H. Ma and K. Waiho *et al*., 2020. Effects of climate-induced water temperature changes on the life history of brachyuran crabs. Rev. Aquacult., 12: 1211-1216.
4. Syafaat, M.N., T. Muhammad, A.B. Abol-Munafi and M. Ikhwanuddin, 2019. Data on population density, growth, survival, water quality, larval stage index and ingestion rate of selected microalgae of portunids crabs at different feeding regimes. Data Brief, Vol. 26. 10.1016/j.dib.2019.104438.
5. Abol-Muna , A.B. and M.N. Azra, 2018. Climate change and the crab aquaculture industry: Problems and challenges. J. Sustainability Sci. Manage., 13: 1-3.
6. Sorgeloos, P., P. Dhert and P. Candreva, 2001. Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. Aquaculture, 200: 147-159.
7. Ahmad, M.T., M. Shariff, F.M. Yusoff, Y.M. Goh and S. Banerjee, 2018. Applications of microalga *Chlorella vulgaris* in aquaculture. Rev. Aquacult., 12: 328-346.
8. Rosas, V.T., L.H. Poersch, L.A. Romano and M.B. Tesser, 2019. Feasibility of the use of *Spirulina* in aquaculture diets. Rev. Aquacult., 11: 1367-1378.
9. Hossain, M.S., S. Koshio and P. Kestemont, 2020. Recent advances of nucleotide nutrition research in aquaculture: A review. Rev. Aquacult., 12: 1028-1053.
10. Wan, A.H.L., S.J. Davies, A. Soler-vila, R. Fitzgerald and M.P. Johnson, 2019. Macroalgae as a sustainable aquafeed ingredient. Rev. Aquacult., 11: 458-492.
11. Azra, M.N. and M. Ikhwanuddin, 2016. A review of maturation diets for mud crab genus *Scylla broodstock*: Present research, problems and future perspective. Saudi J. Biol. Sci., 23: 257-267.

12. Azra, M.N. and M. Ikhwanuddin, 2015. Larval culture and rearing techniques of commercially important crab, *Portunus pelagicus* (Linnaeus, 1758): Present status and future prospects. Songklanakarin J. Sci. Technol., 37: 135-145.
13. Muller-Feuga, A., 2000. The role of microalgae in aquaculture: Situation and trends. J. Appl. Phycol., 12: 527-534.
14. Becker, E.W., 2013. Microalgae for Aquaculture: Nutritional Aspects. In: Handbook of Microalgal Culture: Applied Phycology and Biotechnology, Richmond, A. and E.Q. Hu (Ed.)., John Wiley and Sons, Ltd., United States, ISBN-13: 9780470673898, Pages: 21.
15. Brown, M.R. and S.I. Blackburn, 2013. Live Microalgae as Feeds in Aquaculture Hatcheries. In: Live microalgae as feeds in aquaculture hatcheries, Allan, G. and G. Burnell (Ed.)., Elsevier, Amsterdam, Netherlands, ISBN-13: 978-0-85709-119-2, pp: 117-156.
16. de Castro Araújo, S. and V.M.T. Garcia, 2005. Growth and biochemical composition of the diatom *Chaetoceros* cf. *wighamii* bright well under different temperature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids. Aquaculture, 246: 405-412.
17. Kandilian, R., E. Lee and L. Pilon, 2013. Radiation and optical properties of *Nannochloropsis oculata* grown under different irradiances and spectra. Bioresour. Technol., 137: 63-73.
18. Martínez-Fernandez, E., H. Acosta-Salmon and P.C. Southgate, 2006. The nutritional value of seven species of tropical microalgae for black-lip pearl oyster (*Pinctada margaritifera*, L.) larvae. Aquaculture, 257: 491-503.
19. Taufik, M., Z. Bachok, M.N. Azra and M. Ikhwanuddin, 2016. Effects of various microalgae on fatty acid composition and survival rate of the blue swimming crab *Portunus pelagicus* larvae. Indian J. Geo-Mar. Sci., 45: 1512-1521.
20. Jalal, K.C.A., A.A. Shamsuddin, M.F. Rahman, N.Z. Nurzatul and M. Rozihan, 2013. Growth and total carotenoid, chlorophyll a and chlorophyll b of tropical microalgae (*Isochrysis* sp.) in laboratory cultured conditions. J. Biol. Sci., 13: 10-17.
21. Wu, H.S., R.S. Hseu and L.P. Lin, 2001. Identification of *Chlorella* spp. isolates using ribosomal DNA sequences. Bot. Bull. Acad. Sin., 42: 115-121.
22. Taufik, M., I. Shahrul, A.R.M. Nordin, M. Ikhwanuddin and A.B. Abol-Munafi, 2020. Fatty acid composition of hepatopancreas and gonads in both sexes of orange mud crab, scylla olivacea cultured at various water flow velocities. Trop. Life Sci. Res., 31: 79-105.
23. Taufik, M., A. Amin-Safwan, A.R.M. Nordin, I. Shahrul, A.B. Abol-Munafi and M. Ikhwanuddin, 2020. Experimental data on glucose and energy levels of orange mud crab, *Scylla olivacea* at different water velocities. Data Brief, Vol. 29. 10.1016/j.dib.2020.105232.
24. Taufik, M., H. Manan, S. Ismail, M.N.A. Rahman, M. Ikhwanuddin and A.B. Abol-Munafi, 2019. Locomotor, escaping activities and fatty acid composition of mud crab, *Scylla olivacea* at different water velocities. J. Teknologi, Vol. 82. 10.11113/jt.v82.13861.
25. Muhammad, T., S. Ismail, M. Ikhwanuddin and A.B. Abol-Munafi, 2019. Experimental data on behavioral, hepatosomatic, gonadosomatic indeces and total lipid of mud crab, *Scylla olivacea* at different velocity levels. Data Brief, Vol. 25. 10.1016/j.dib.2019.104205.
26. Azra, M.N., J.C. Chen, M. Ikhwanuddin and A.B. Abol-Munafi, 2018. Thermal tolerance and locomotor activity of blue swimmer crab *Portunus pelagicus* instar reared at different temperatures. J. Thermal Biol., 74: 234-240.
27. Arshad, A., Efrizal, M.S. Kamarudin and C.R. Saad, 2006. Study on fecundity, embryology and larval development of blue swimming crab *Portunus pelagicus* (Linnaeus, 1758) under laboratory conditions. Res. J. Fish. Hydrobiol., 1: 35-44.
28. Ikhwanuddin, M., M.N. Azra, Y.Y. Sung, A.B. Abol-Munafi and M.L. Shabdin, 2012. Live foods for Juveniles' production of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1766). J. Fish. Aquat. Sci., 7: 266-278.
29. Ikhwanuddin, M., M.N. Azra, A. Redzuari, Z.A. Aizam and A.B. Abol-Munafi, 2012. Ingestion rates of *Brachionus* sp. and *Artemia* sp. nauplii by blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) larvae. J. Fish. Aquat. Sci., 7: 402-411.
30. Epelbaum, A. and R. Borisov, 2006. Feeding behaviour and functional morphology of the feeding appendages of red king crab *Paralithodes camtschaticus* larvae. Mar. Biol. Res., 2: 77-88.
31. Nunez, M., C. Lodeiros, M. de Donato and C. Graziani, 2002. Evaluation of microalgae diets for *Litopenaus vannamei* larvae using a simple protocol Aquacult. Int., 10: 177-187.
32. Redzuari, A., M.N. Azra, A.B. Abol-Munafi, Z.A. Aizam, Y.S. Hii and M. Ikhwanuddin, 2012. Effects of feeding regimes on survival, development and growth of blue swimming crab,*Portunus pelagicus* (Linnaeus, 1758) larvae. World Appl. Sci. J., 18: 472-478.
33. Guedes, A.C. and F.X. Malcata, 2012. Nutritional Value and Uses of Microalgae in Aquaculture. In: Aquaculture, Muchlisin, Z. (Ed)., IntechOpen Limited, London, ISBN-13: 978-953-307-974-5, pp: 59-78.
34. Baylon, J.C., A.N. Failaman and E.L. Vengano, 2001. Effect of salinity on survival and metamorphosis from zoea to megalopa of the mud crab *Scylla serrata* Forskal (Crustacea: Portunidae). Asian Fish. Sci., 14: 143-151.
35. Lora-Vilchis, M.C., B. Cordero-Esquivel and D. Voltolina, 2004. Growth of *Artemia franciscana* fed *Isochrysis* sp. and *Chaetoceros muelleri* during its early life stages. Aquacult. Res., 35: 1086-1091.
36. Tamaru, C.S., R. Murashige and C.S. Lee, 1994. The paradox of using background phytoplankton during the larval culture of striped mullet, *Mugil cephalus* L. Aquaculture, 119: 167-174.
37. Hatton, A.D. and S.T. Wilson, 2007. Particulate dimethylsulphoxide and dimethylsulphoniopropionate in phytoplankton cultures and Scottish coastal waters. Aquat. Sci., 69: 330-340.