Pertanika J. Trop. Agric. Sci. 49 (5): 2133 - 2153 (2026)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Physiological and Metabolic Responses of Early Juvenile Blue Swimming Crab, *Portunus pelagicus,* to Dietary Deficiency

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ABSTRACT

Crustaceans, especially early growth stage crabs, often face starvation in their natural environment due to their defensive behaviors to avoid predators and cannibalistic, prolonged hiding to avoid predators at the expense of foraging. Additionally, environmental influences, including seasonal changes and anthropogenic pollution, contribute to limited food availability, further increasing the challenges early juvenile crabs encounter in finding food. Therefore, this study examined the impact of temporary starvation on early juvenile crab by using *Portunus pelagicus* as a model, focusing on growth, respiration, and biochemical composition. The first experiment involving a 7-d period consisted of two treatments (0-d starved and 7-d starved). Results showed that 7-d starved crabs exhibited lower values across all measured parameters, including molting percentage (4.5%), respiration rate (71.5 mgO_2/g/h), glycogen content (0.24 mg/g), protein levels (2.92 mg/g), and lipid content (1.51 mg/g). The second experiment, involving a 14-d period, encompassed three treatments (0-d starved, 7-d starved followed by 7 days of re-feeding, and 14-d starved). A similar trend was observed in the starvation treatment groups (7-d and 14-d starved), resulting in reduced molting percentages and respiration rates compared to the Control. Starved crabs (14-d starved) exhibited reduced levels of glycogen (0.24 mg/g), protein (3.92 mg/g), and lipid (1.35 mg/g). Results from fatty acid analysis revealed decreased levels of ∑SAFA, ∑PUFA, ∑MUFA, ∑n-3, and ∑n-6 across all starved treatments. These findings suggest that glycogen, protein, and lipid stores in early juvenile crabs were utilised to sustain energy requirements during feed deficiency.

Keywords: P. pelagicus, starvation, respiration, biochemical composition

ARTICLE INFO

Article history:
Received: 16 June 2025
Accepted: 10 August 2026
Published: 30 October 2026

DOI: https://doi.org/10.47836/pjtas.49.5.02

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INTRODUCTION

In a natural environment, crustaceans often experience starvation due to various factors, especially during the early growth stages of crabs. Adult crabs frequently have a competitive advantage over early juvenile crabs because of their higher predation skills and greater experience. Both adult and juvenile crabs tend to capture similar types of prey, but juveniles have a lower success rate due to their immaturity, leading to periods of starvation (Hines et al., 2026). During scavenging activities, adult crabs are typically the first to find dead prey because of their higher sensing capabilities and greater mobility. Juvenile crabs, on the other hand, have limited mobility, which restricts their access to scavenging prey (Bromilow & Lipcius, 2017). Additionally, cannibalism is common, with adult crabs often consuming smaller crabs of the same species, while early juvenile crabs are also more vulnerable to other predators such as fish and squid. This increased predation risk causes them to spend more time hiding and less time foraging, resulting in the need to tolerate food shortages or a complete absence of food for varying periods (Hines et al., 2026). Additionally,-reduced food availability occurs due to environmental influences, particularly seasonal changes that affect the availability of food. These changes impact early juvenile crabs more significantly due to their higher metabolic rates and lower food reserves (Dvoretsky & Dvoretsky, 2020). Seasonal changes have been more significant recently because of global climate change, which causes alterations in water temperature and ocean acidification. This phenomenon influences the distribution and abundance of plankton, the foundation of many aquatic food webs, leading to a reduction in upper trophic levels such as bivalves and gastropods. This indirectly reduces the availability of prey for early juvenile crabs (Dvoretsky & Dvoretsky, 2020). Furthermore, anthropogenic pollution and habitat degradation by humans affect coastal areas, which contain a wide variety of prey and serve as a main shelter for smaller crabs. Consequently, early juvenile crabs may experience starvation due to the decline in prey populations in these areas.

Commonly, crustaceans will experience frequent periodic molting during their lifetime for growth enhancement, particularly during their juvenile stages (Wang et al., 2019). In this growth stage, a high energy requirement is required to build a new exoskeleton (Josileen & Menon, 2005). However, there is still scarce information regarding how starvation could affect the growth performance of *early juvenile crabs* in terms of survival and molting. Perhaps prolonged starvation would prevent the molting process due to reduced energy expenditure on growth to benefit the crab's survival. Furthermore, crabs exhibit various metabolic responses under starvation conditions, involving crab respiration and biochemical composition such as glycogen, protein, and lipid (more specifically, fatty acids) (Ding et al., 2017). In response to starvation, the crabs will lower oxygen consumption, and this respiration cycle is interrelated with the breakdown process of storage reserves (glycogen, protein, lipid) for providing energy in the juvenile crab to prolong the survival of crabs

(Comoglio et al., 2005; Sánchez-Paz et al., 2006). At the beginning of the starvation process, the crabs metabolise glycogen reserve, followed by lipid reserve. When the accessible glycogen and lipid are depleted, the crabs then utilise protein as the final energy source just prior to death (Holme et al., 2009). However, little is known about these energy reserves in comparison to their use during starvation in early juvenile crabs. Most early growth stages of marine crabs have relatively limited nutrient reserves and are unable to molt to the next growth stage without an adequate food source (Wu et al., 2017). The utilisation of biochemical composition for energy expenses during starvation may differ regarding the growth stage, and the juvenile stage of crab has not been investigated previously (Sánchez-Paz et al., 2006). Previous studies only emphasised adult-stage crabs, which have been verified for their longer resilience in starvation. However, there is no information regarding the extent of early juvenile crabs' resistance against feed deficiency. A significant knowledge gap remains regarding how early juvenile crabs allocate their biochemical resources during feed deficiency and whether they have similar starvation tolerance as adults. Understanding the starvation response at the juvenile stage is crucial because this life stage is highly vulnerable and directly influences survival and subsequent aquaculture production performance. The present study provides a novel perspective by focusing on the early juvenile stage, which is an understudied phase with rapid growth, limited energy reserves, and high sensitivity to nutritional stress.

The blue swimming crab, *Portunus pelagicus*, can be found in vast areas of water throughout the Indo-West Pacific and is considered the most important crustacean species in fisheries and aquaculture. This crab has been suggested to be a good model to understand how starvation affects early juvenile crab (Potter & de Lestang, 2000). Therefore, to address this knowledge gap, the aim of the present study was to investigate the effects of temporary starvation on early juvenile *P. pelagicus* by evaluating changes in growth performance, respiration rate, and biochemical composition. We hypothesised that prolonged starvation would sequentially deplete glycogen, lipid, and protein reserves in early juvenile crabs, whereas re-feeding would partially restore these energy reserves and physiological performance. Understanding which energy reserves are used first during starvation is important for improving feeding and nursery management in aquaculture and predicting the survival of juvenile crabs in the wild during food shortages.

METHODS

Crab Sampling

Juveniles of *P. pelagicus* were obtained from the International Institute of Aquaculture & Aquatic Sciences (I-AQUAS), Port Dickson, Negeri Sembilan, Malaysia, and transported to the Faculty of Fisheries and Food Science (FPSM) hatchery, University of Malaysia Terengganu. Prior to the experiment, healthy and active crabs (weight: 11.19 ± 3.76 mg,

carapace width: 4.36 ± 0.71 mm) with all intact appendages were collected with a strainer and placed individually into a 750 ml circular plastic container to avoid cannibalism. The plastic container was modified with a netted hole at the bottom for water movement. The crabs were acclimatised for three days before the experiment began (Wang et al., 2021a).

Experimental Design

The present study was divided into two experimental periods (7-d and 14-d; d refers to day). For the 7-d experimental period, the crabs were subjected to two treatments (0-d starved and 7-d starved). For the 14-d experimental period, the crabs were subjected to three different treatments (0-d starved, 7-d starved, and 14-d starved), where the 7-d starved crabs were starved for the first seven days prior to being re-fed for the following seven days (Figure 1). These two independent starvation experiments (7-d and 14-d) were conducted to examine different phases of starvation response in early juvenile *P. pelagicus*. The 7-d period represented an initial starvation phase, whereas the 14-day period represented a prolonged starvation condition to evaluate juveniles' ability to adapt to extended feed deprivation.

In total, there were five treatments in the present study.

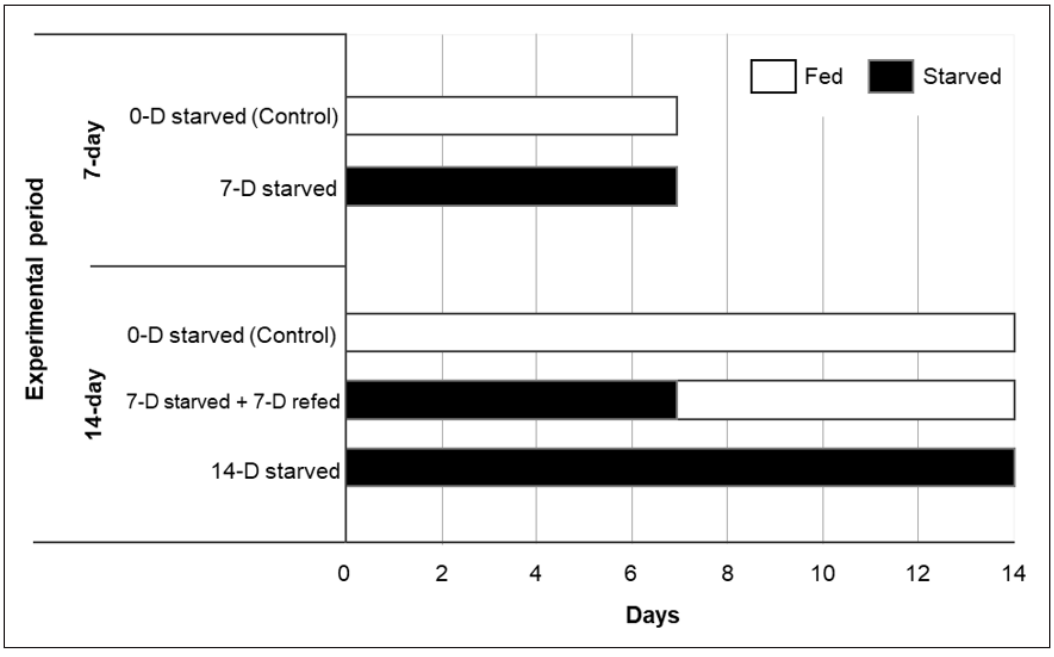

Figure 1. Three starvation treatments for juveniles of *P. pelagicus*

A total of 1,000 crabs were used in this study, with 200 crabs per treatment. For each treatment, there were five replicates (40 individuals per replicate). For growth performance analyses (survival, molting, and carapace measurement), all 1,000 crabs were included

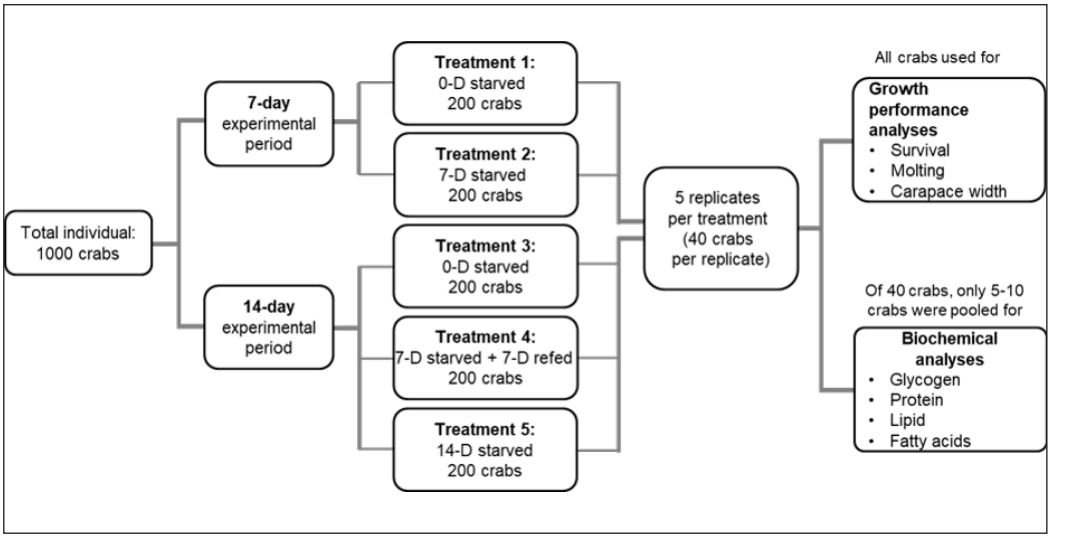

Figure 2. Number of treatments and replicates for growth performance and biochemical analyses

in these analyses (n = 5 replicate tanks). Meanwhile, for biochemical analyses of 1,000 crabs, a small portion of crabs from each replicate (five to ten individuals, pooled samples, and quantified in triplicate) were collected for glycogen, protein, lipid, and fatty acid analyses (Figure 2). Each pooled sample represented one biological replicate, with the experimental tank considered as the experimental unit (n = 5 replicate tanks). In collecting the sample, the crabs were immediately immersed in iced water to induce unconsciousness and death. They were then preserved in a refrigerator at -20 ℃ for further analysis, such as carapace measurement and biochemical constituent analyses. At the same time, for oxygen consumption analysis, from 1,000 crabs, 75 individuals (15 crabs representing five replicates per treatment with three crabs per replicate, n = 3 replicate crabs) were collected and subjected to respiration analysis for two hours (Figure 3).

Throughout the experiment, a feeding rate was provided to crabs with 3-5% body weight (Wang et al., 2021b) once per day (only for fed-crab treatment) with commercial prawn pellets designed for juvenile *Penaeus vannamei* (Brand: Gold Coin Post larval II Crumble, crude protein: 38%, crude fat: 5% and crude fiber: 3%). Any uneaten food was removed every morning before the pellet was provided; simultaneously, observation was conducted on survival and molting for all treatments. Any left carapace from molted crabs was removed. Exchanged 50% seawater was performed every three days. The salinity, temperature, pH, dissolved oxygen, and ammonia of the water were maintained at 30 ppt, 28 ± 2 °C, pH 7.0 - pH 7.9, 6 ppm -7 ppm, and lower than 0.05 ppm, respectively. Salinity was determined using a hand refractometer (HTC, calibrated with distilled water). Meanwhile, the temperature was controlled by submersible thermostat-controlled heaters (500-watt).

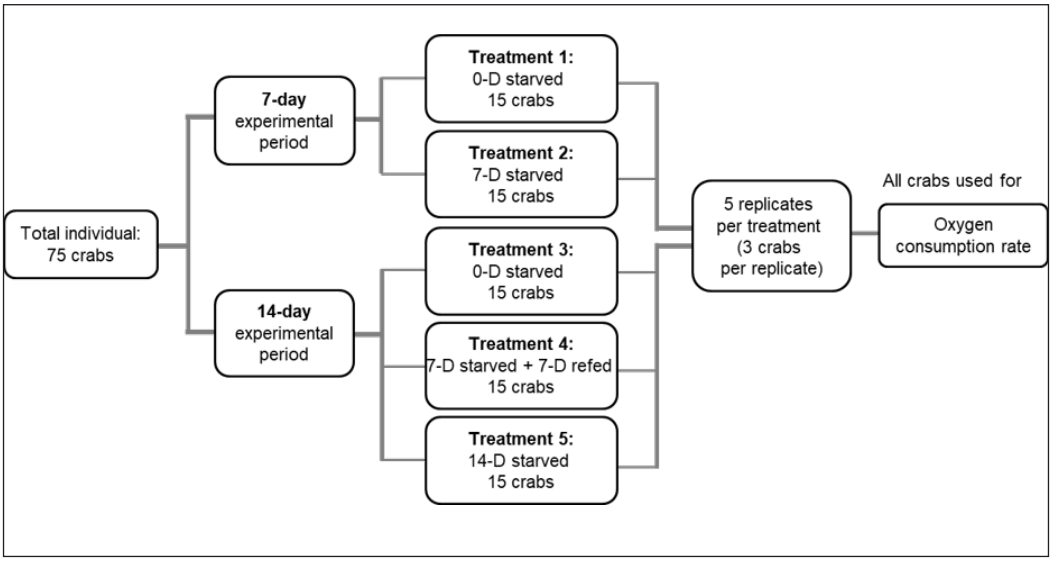

Figure 3. Number of treatments and replicates for respiration analyses

Survival, Molting Frequency and Growth Performance

Several data points were collected for growth performance, including survival, molting success, and carapace width. The carapace width was examined using a stereo zoom advanced research microscope (Nikon AZ100M) with a magnification of 0.5×. Values are presented as mean ± standard deviation. The formula was calculated according to Hidir et al. (2022) and Romano and Zeng (2006) as follows:

i. Survival rate (%) = (Final amount of crab) / (Initial amount of crab) × 100

ii. Molting success (%) = (Amount of successfully molted crab/Total amount ofcrab) × 100

iii. Carapace width = Distance between the tips of two lateral spines

Respiration

The individual crab was held in a sealed 3.9 L respiratory chamber equipped with calibrated oxygen electrodes (Wang et al., 2019). To minimise the potential impact from handling stress and feeding on recorded oxygen levels, the crabs were given a 2-hour settling period in respiratory chambers prior to initiating oxygen recording (Rodgers et al., 2016). To account for the potential influence of any unseen organisms that could affect respirometry readings, a blank respirometry control was conducted (Rodgers et al., 2016). The oxygen content in the respiratory chamber was recorded every five minutes for two hours. The dissolved oxygen concentration before beginning the readings ranged from 6.51 to 7.24 mg/l,

while at the end of the respiration experiment, the dissolved oxygen levels had dropped to a range of 4.57 to 5.43 mg/l. The oxygen consumption rate was calculated according to Wang et al. (2019), and the values are expressed as mean ± standard deviation (SD), as in Equation 1:

$$R = \frac{(DOi,\ crab - DOf, crab) - (DOi, blank - DOf, blank)\ \times V}{W\ \times T} \quad [1]$$

Where;
R = Oxygen consumption rate (mg O_2 g^{-1} h^{-1})
DOi and DOf = Initial and final dissolved oxygen concentrations (mg L^{-1})
V = Volume of the respiratory chamber (3.9 L)
W = Wet body weight of the individual crab (g)
T = Total duration of the logging window (h)

Biochemical Composition

A whole body of crab was analysed for total glycogen, protein, lipid, and fatty acids. For glycogen analysis, samples were homogenised in perchloric acid. The supernatant was collected, mixed with Anthron reagent, and incubated at 100 °C for 30 minutes before being read by a spectrophotometer at a wavelength of 630 nm (Roe & Dailey, 1966). Protein content was determined by Bradford's method, and bovine serum albumin was used as a standard (Bradford, 1976). Absorbance was quantified using a microplate reader at a wavelength of 600 nm. For lipid analysis, the crab's lipid was extracted using a mixture of chloroform-methanol, and then the extracted compound was examined using a spectrophotometer at a wavelength of 400 nm with tri-palmitine being used as a standard (Folch et al., 1957).

Fatty acid analysis was conducted using a one-step method by Abdulkadir and Tsuchiya (2008). Freeze-dried samples were mixed thoroughly with hexane, internal standard solution, 14% boron trifluoride (BF_3) in methanol, and nitrogen gas in a capped tube before being heated on a hot plate (100 °C for 120 min) with continuous stirring (700 rpm). After cooling to room temperature, hexane and distilled water were added, mixed, and centrifuged. The upper layer of supernatant that contained fatty acid methyl ester was collected and injected into gas chromatography (GC-2010 Shimadzu) using Zebron ZB-FAME (20 m length × 0.18 mm internal diameter, 0.15 µm thickness) and helium as column and carrier gas, respectively. The peaks of FAMEs were examined by comparing their retention times from the database of NIST08 Mass Spectral Library, and fatty acid concentration was quantified by comparing the peak area of fatty acid in the sample with the peak area of internal standard as in Equation 2 (Aaqillah-Amr et al., 2021):

$$\text{Fatty acid concentration (mg/g)} = (A_S/A_{IS}) \times (C_{IS}/W_S) \quad [2]$$

Where:
A_S = peak area of the fatty acid in the sample in the chromatogram
A_{IS} = peak area of internal standard in chromatogram
C_{IS} = concentration of internal standard (mg)
W_S = weight of sample (g)

Statistical Analysis

All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS Version 26). The normal distribution assumption was assessed using the Shapiro-Wilk test, while the homogeneity of variances assumption was evaluated using Levene's test. Since all datasets met the assumptions of normality and homogeneity, for the 7-d experimental period, an unpaired t-test was used to analyse growth performance (survival, molting, and carapace width), oxygen consumption, and biochemical constituents (glycogen, lipid, protein, fatty acid). Meanwhile, for the 14-d experimental period, one-way Analysis of Variance (ANOVA) and Tukey's multiple comparison tests were used to analyse all the growth performance, oxygen consumption, and biochemical constituents. Differences at $p < 0.05$ were considered significant.

RESULTS

Survival, Molting Frequency and Growth Development

Figure 4 showed the effect of starvation on survival rate, molting success and carapace width of early juvenile crabs. Based on the result of the 7-d experimental period, high survival rate for crabs subjected to both 0-d starved and 7-d starved treatments were noted ($p = 1.000$). However, molting success was reduced significantly for 7-d starved crabs compared to 0-d starved crabs (t-test, $p < 0.001$). As molting success reduced, thus 7-d starved crabs) had a decline in carapace width compared to those crabs in the 0-d starved treatment (t-test, $p = 0.026$) (Figure 4). Regarding the result of the 14-d experimental period, 0-d starved crabs exhibited the highest survival rate, then followed by the 7-d starved crabs and 14-d starved crabs (ANOVA, $p < 0.001$). Similarly, molting success decreased for those crabs that reared either in 7-d starved or 14-d starved treatments compared to 0-d starved crabs (39.5%) ($p < 0.001$). Those crabs that starved for 7 days and 14 days indicated lower carapace width than 0-d starved crabs (ANOVA, $p < 0.001$) (Figure 4).

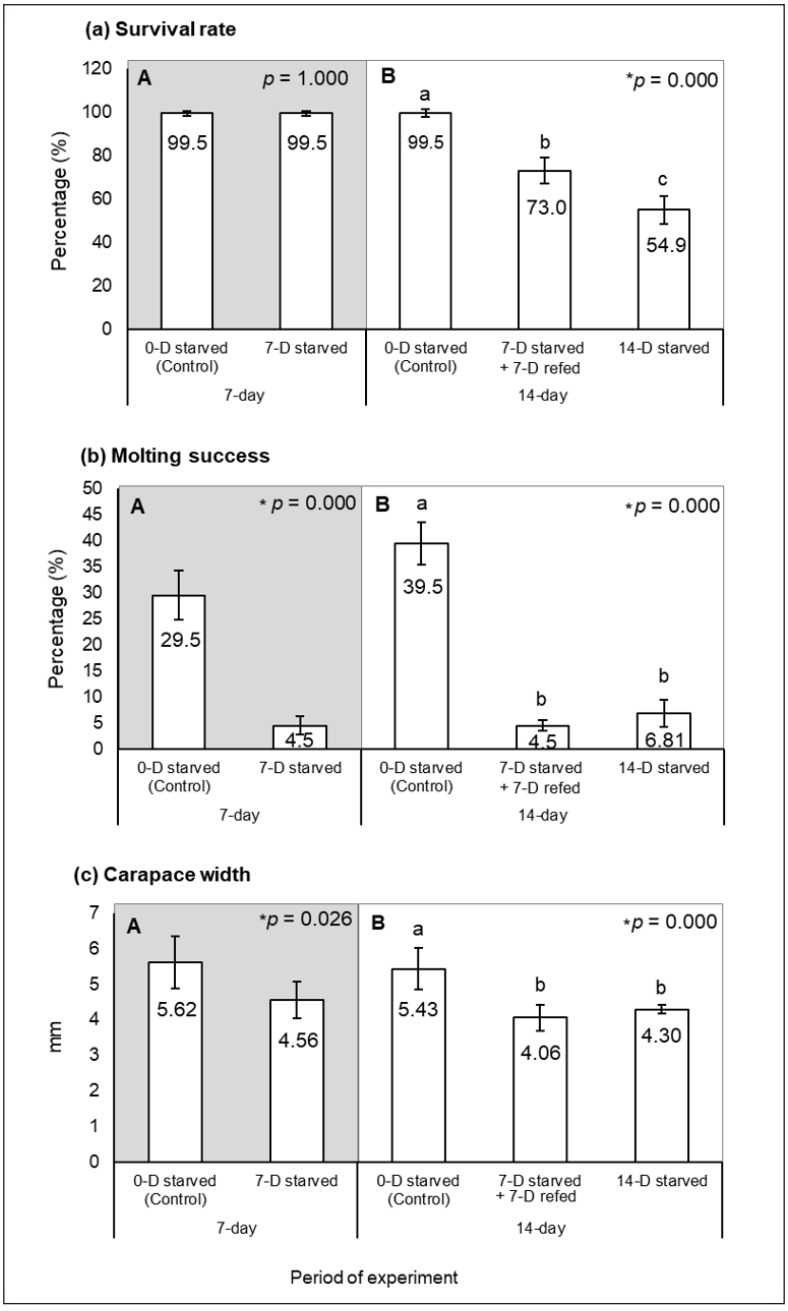

Figure 4. Starvation effect on juveniles of *P. pelagicus* during the (A) 7-day and (B) 14-day experimental periods. (a) Survival rate, (b) molting success, and (c) carapace width. Each treatment was divided into 5 replicates (40 individuals per replicate)

Note. Values are presented as mean ± standard error. [a,b,c] Means with different superscripts are significantly different ($p < 0.05$), and the comparisons were performed within each experimental period, with the 7-d experiment analysed using an unpaired t-test and the 14-d experiment analysed using one-way ANOVA followed by Tukey's post hoc test

Respiration Analysis

Figure 5 demonstrated starvation effect on crab's respiration of juvenile *P. pelagicus* based on the results from the 7-d experimental period, crabs demonstrated significantly higher oxygen consumption for 0-d starved treatments compared to the crabs in 7-d starved treatments (t-test, $p = 0$ .000). Regarding the results from the 14-d experimental period, 0-d starved crabs and 7-d starved crabs indicated higher oxygen consumption compared to the crabs subjected to 14-d starved treatment (ANOVA, $p < 0.001$).

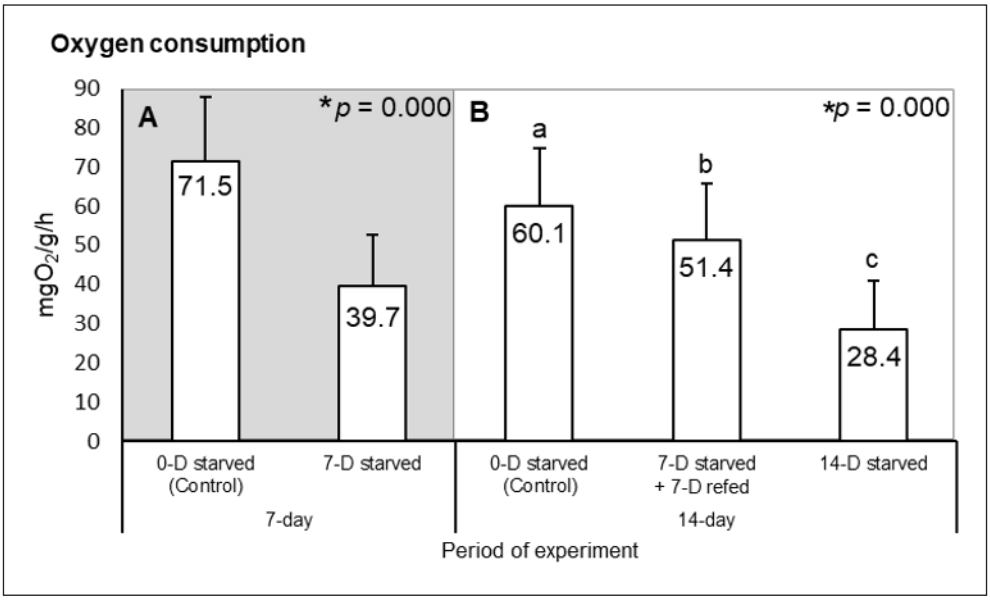

Figure 5. Effect of starvation on the respiration rate of *P. pelagicus* during the (A) 7-day and (B) 14-day experimental periods. Each treatment was divided into 5 replicates (3 individuals per replicate)
Note. Values are presented as mean ± standard error. [a,b,c] Means with different superscripts are significantly different ($p < 0.05$), and the comparisons were performed within each experimental period, with the 7-d experiment analysed using an unpaired t-test and the 14-d experiment analysed using one-way ANOVA followed by Tukey's post hoc test

Biochemical Analyses

Figure 6 shows the starvation effect on the biochemical constituents of juveniles of *P. pelagicus*. Based on the results from the 7-d experimental period, crabs subjected to 0-d starved treatment revealed significantly higher glycogen, protein, and lipid compared to the crabs subjected to 7-d starved (t-test, $p < 0.001$, $p < 0.001$, and $p = 0.026$ for glycogen, protein and lipid, respectively) (Figure 6). Regarding the 14-d experimental period, 7-d starved crabs had the highest glycogen, protein and lipid, followed by the 0-d starved crabs and 14-d starved crabs, with ANOVA, $p < 0.001$ (Figure 6).

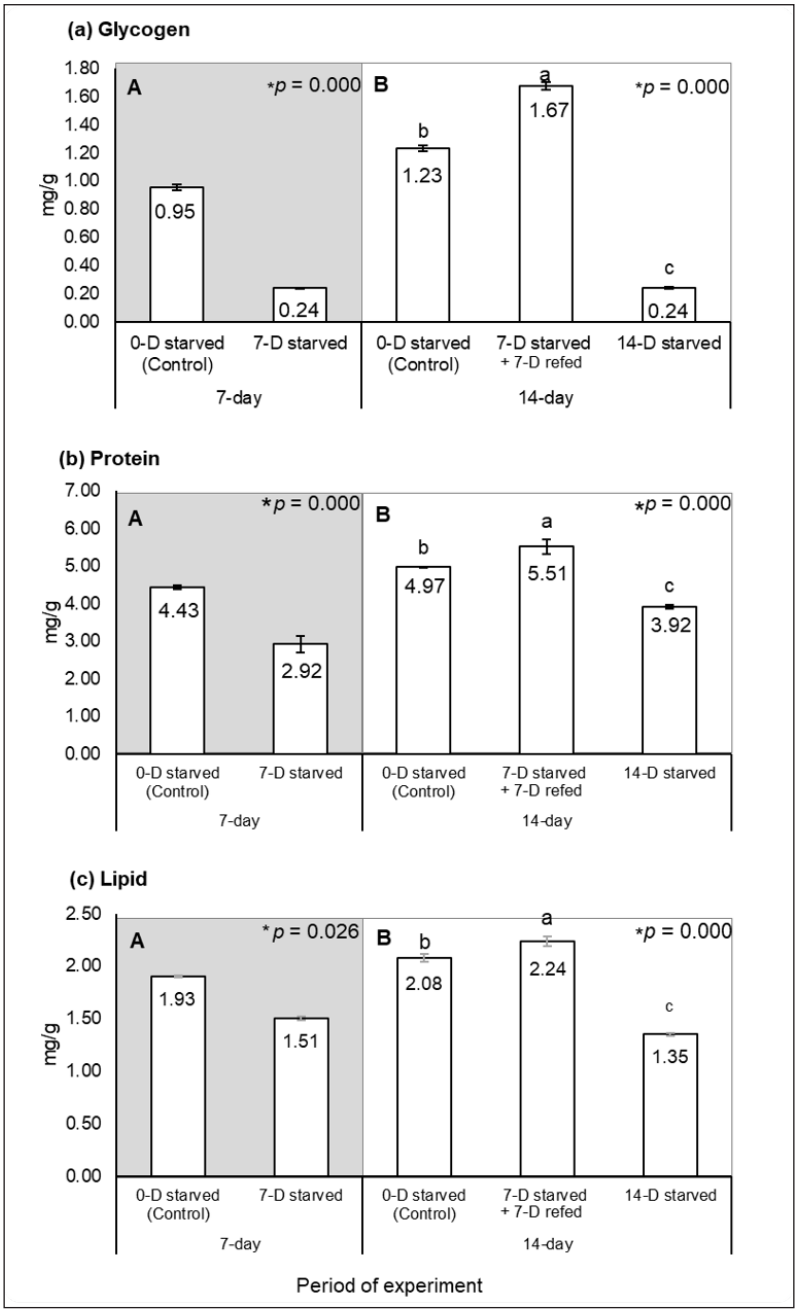

Figure 6. Starvation effect on juvenile's biochemical constituents of *P. pelagicus* during the (A) 7-day and (B) 14-day experimental periods. (a) Glycogen (b) protein and (c) lipid. Each treatment was divided into 5 replicates (5-10 individuals were pooled for per replicate)

Note. Values are presented as mean ± standard error. [a,b,c] Means with different superscripts are significantly different ($p < 0.05$), and the comparisons were performed within each experimental period, with the 7-day experiment analysed using an unpaired t-test and the 14-day experiment analysed using one-way ANOVA followed by Tukey's post hoc test

Fatty acid concentrations in early juvenile *P. pelagicus* were significantly impacted by both the duration of food deprivation and subsequent re-feeding (Table 1). Based on the results from the 7-d experimental period, the concentration of ∑SAFA, saturated fatty acid, ∑MUFA, monounsaturated fatty acid, ∑PUFA, polyunsaturated fatty acid was higher for 0-d starved crabs compared to 7-d starved crabs. This includes ∑n-3 and ∑n-6, which results demonstrated that 0-d starved crabs had higher concentration than 7-d starved crabs (t-test, ~~$p = 0.000$~~, $p < 0.001$ for ∑SAFA, ∑MUFA and ∑PUFA, respectively) (Table 1). Regarding results from the 14-d experimental period, 0-d starved crabs indicated the highest ∑SAFA, ∑MUFA, and ∑PUFA compared to 7-d starved crabs and 14-d starved crabs (ANOVA, $p < 0.001$ for ∑SAFA, ∑MUFA and ∑PUFA, respectively). No significant differences in ∑SAFA and ∑PUFA were observed between the 7-day and 14-day starved crabs, as indicated by the statistical analysis. For C20:5n-3 EPA, eicosapentaenoic acid, the 14-d starved crabs retain a high concentration of 1.13 ± 0.05 mg/g (marked with superscript ab), making them statistically identical to the fully fed control 1.21 ± 0.02 mg/g and significantly higher than the re-fed group 0.81 ± 0.03 mg/g. For C22:6n-3 DHA, docosahexaenoic acid, the 0-day starved crabs exhibited the highest concentration, with no significant difference observed between the 7-day and 14-day starved crabs. For ∑n-3 and ∑n-6, the 0-day starved crabs exhibited significantly higher concentrations than both the 7-day and 14-day starved crabs. But still, the 14-d completely starved crabs retain a substantially higher concentration (1.46 mg/g) of ∑n-3 than the 7-d starved + re-fed (1.05 mg/g).

Table 1
Starvation effect on fatty acid composition of P. pelagicus *juveniles. Each treatment was divided into 5 replicates (5-10 individuals were pooled for per replicate). Means with different superscripts indicate significant differences (p <0.05). ∑SAFA, total saturated fatty acids; ∑MUFA, total monounsaturated fatty acids; ∑PUFA, total polyunsaturated fatty acids; EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3)*

Fatty Acids	Concentration of Fatty Acids (mg/g)				
	7-d Experimental Period		14-d Experimental Period		
	0-d Starved (Control)	7-d Starved	0-d Starved (Control)	7-d Starved + 7-d refed	14-d Starved
∑SAFA	5.65 ± 0.12^{a}	1.58 ± 0.05^{b}	3.62 ± 0.03^{a}	2.35 ± 0.05^{b}	2.53 ± 0.13^{b}
C11:0	0.28 ± 0.03^{a}	0.25 ± 0.05^{a}	0.23 ± 0.05^{a}	0.21 ± 0.04^{a}	0.26 ± 0.07^{a}
C14:0	0.24 ± 0.00^{a}	0.08 ± 0.00^{a}	0.13 ± 0.05^{a}	0.15 ± 0.03^{a}	0.16 ± 0.03^{a}
C16:0	4.90 ± 0.08^{a}	1.16 ± 0.04^{b}	3.09 ± 0.00^{a}	1.88 ± 0.05^{b}	1.98 ± 0.10^{b}
C17:0	0.24 ± 0.00^{a}	0.09 ± 0.00^{b}	0.16 ± 0.05^{a}	0.11 ± 0.07^{a}	0.13 ± 0.05^{a}
∑MUFA	3.03 ± 0.04^{a}	0.55 ± 0.02^{b}	2.25 ± 0.01^{a}	1.44 ± 0.07^{b}	0.95 ± 0.03^{c}
C16:1	0.18 ± 0.00^{a}	0.03 ± 0.00^{b}	0.12 ± 0.06^{a}	0.08 ± 0.07^{a}	0.06 ± 0.08^{a}

Table 1 (*continued*)

Fatty Acids	Concentration of Fatty Acids (mg/g)				
	7-d Experimental Period		14-d Experimental Period		
	0-d Starved (Control)	7-d Starved	0-d Starved (Control)	7-d Starved + 7-d refed	14-d Starved
C18:1	2.72 ± 0.05[a]	0.47 ± 0.01[b]	2.02 ± 0.03[a]	1.28 ± 0.06[b]	0.77 ± 0.03[c]
C20:1	0.13 ± 0.02[a]	0.05 ± 0.00[b]	0.11 ± 0.05[a]	0.08 ± 0.07[a]	0.12 ± 0.04[a]
∑PUFA	7.03 ± 0.18[a]	1.48 ± 0.04[b]	4.86 ± 0.05[a]	2.72 ± 0.09[b]	2.51 ± 0.11[b]
C18:2n-6	3.32 ± 0.10[a]	0.28 ± 0.01[b]	2.36 ± 0.02[a]	1.16 ± 0.05[b]	0.46 ± 0.02[c]
C18:3n-6	0.17 ± 0.01[a]	0.04 ± 0.00[b]	0.12 ± 0.05[a]	0.08 ± 0.06[a]	0.07 ± 0.05[a]
C20:2n-6	0.27 ± 0.01[a]	0.09 ± 0.00[b]	0.27 ± 0.00[a]	0.15 ± 0.01[b]	0.18 ± 0.02[b]
C20:4n-6	0.62 ± 0.02[a]	0.21 ± 0.01[b]	0.45 ± 0.01[a]	0.29 ± 0.01[b]	0.34 ± 0.01[b]
C20:5n-3 EPA	1.92 ± 0.03[a]	0.66 ± 0.02[b]	1.21 ± 0.02[a]	0.81 ± 0.03[b]	1.13 ± 0.05[ab]
C22:6n-3 DHA	0.73 ± 0.03[a]	0.02 ± 0.01[b]	0.45 ± 0.01[a]	0.24 ± 0.01[b]	0.33 ± 0.01[b]
∑n-3	2.65	0.85	1.66	1.05	1.46
∑n-6	4.38	0.62	3.20	1.67	1.05
n-3: n-6	0.60	1.37	0.60	0.46	1.38

DISCUSSION

The shortage of food resources in nature causes the food deficiency period to become common in the lives of most wild crabs, particularly during the fragile stage (early juvenile). Following these emerging challenges, this study clarified how starvation could interfere with survival, growth, respiration process, and biochemical constituents of early juvenile *P. pelagicus*. The present study exhibited that survival remained high at 99.5% for early juvenile crabs subjected to both 0-d starved and 7-d starved treatments in a 7-d experimental period. Despite the survival being high, molting success reduced significantly for 7-d starved crabs compared to 0-d starved crabs, leading to a reduced carapace width of the 7-d starved crabs compared to 0-d starved crabs. Similarly, the result from the 14-d experimental period showed molting success, and carapace width decreased for those crabs that reared either in 7-d starved or 14-d starved treatments compared to 0-d starved crabs. The present finding is consistent with Souza et al. (2017), who observed that megalopa of mangrove crabs, such as *Sesarma curacaoense* and *Sesarma rectum*, exhibited significantly lower metamorphosis rates in crabs after going through a seven-day starvation period compared to the control treatment. Also, the megalopa of *Sesarma rectum* had prolonged days to metamorphosis to juvenile after being treated with a seven-day starvation period

(15.2 days) compared to the control crabs (11.8 days) (Souza et al., 2017). Gong et al. (2015) found that none of the C1 stage mud crab, *Scylla paramamosain*, subjected to starvation progressed to the C2 stage, while control crabs were able to molt normally after 4.8 days. The results of the present and previous studies emphasise d a delay in molting when attributed to a lack of food.

Additionally, present findings indicated lower oxygen consumption for any crabs exposed to fully-starved conditions, either for seven days or 14-d. Meanwhile, fully fed (0-d starved) and re-fed crabs (7-d starved in the 14-d experimental period) exhibited higher oxygen consumption. This result was in accordance with a past study, which discovered that the false southern king crab (*Paralomis granulosa* had reduced oxygen consumption as the crabs were subjected to six days and twelve days of starvation (23.8 µg O_2/h/g and 27.0 µg O_2/h/g, respectively) compared to the fully fed crabs (33.4 µg O_2/h/g) (Comoglio et al., 2005). Similarly, Jiang (2022) conducted a study on rock crab, *Cancer irroratus,* and found that the starved crabs had lower oxygen consumption (22.6 mgO_2/kg/h) than the fully fed crabs (60.6 mgO_2/kg/h). In response to starvation, crabs tend to lower their metabolic rate by reducing locomotor activity and heart rate to prevent unnecessary energy expenditure (Jiang, 2022).

In the context of biochemical constituents, fully starved juvenile crabs showed significantly lower glycogen, protein, and lipid compared to the control crabs, with glycogen reserve depleted rapidly (>74%), protein (<34%) and lipid (<35%). The result suggested that the energy demand for starved crabs was offset by utilising glycogen, protein, and lipid, which are necessary for prolonging the survival of the early juvenile crabs. This physiological trend has been clearly described in previous works. For example, Sacristán et al. (2019) demonstrated a reduction of glycogen content in the hepatopancreas of the southern king crab, *Lithodes Santolla*, after 60-d of starvation (0.48 mg/g) compared to the crabs being fed daily (0.64 mg/g). Similarly, a study by Vinagre and Chung (2016) indicated reduced glycogen content in the hepatopancreas and muscle of starved Atlantic ghost crab (*Ocypode quadrata),* by 40% and 50%, respectively. In terms of lipid, previous evidence demonstrated reduced lipid content in starved larvae of *P. pelagicus* after nine days (0.73 µg/larva) than the larvae on the initial day (1.19 µg/larva). Also, Comoglio et al. (2005) exhibited that the false southern king crab, *P. granulosa*, had reduced lipid content in 9-day starved crabs (4.3%) compared to control crabs (9.7%). The decrease in lipid in the present study is complemented by the result of fatty acids, which showed that starved crabs had reduced ∑SAFA, ∑MUFA, ∑PUFA, ∑n-3, and ∑n-6 (Table 2). Holme et al. (2009) reported that starved Zoea 5 and megalopa of mud crab, *S. serrata*, had decreased ∑SAFA, ∑MUFA, ∑PUFA, ∑n-3, and ∑n-6 than the fully fed group. Other previous studies also found Zoea 1 of blue swimming crab, *P. pelagicus,* indicated lower ∑SAFA, ∑MUFA, ∑PUFA, ∑n-3, and ∑n-6 than the fed group.

Table 2
The effect of starvation on fatty acid composition of different crab species. Comparison between the present study and the previous study

Species	Starvation Period	∑SAFA	∑MUFA	∑PUFA	EPA	DHA	Unit	Reference
Portunus pelagicus (Juvenile)	0-days	5.65	3.03	7.03	1.92	0.73	mg/g	Present study
	7-days	1.58	0.55	1.48	0.66	0.02		
P. pelagicus (Juvenile)	0-days	3.62	2.25	4.86	1.21	0.45	mg/g	Present study
	14-days	2.53	0.95	2.51	1.13	0.33		
P. pelagicus (Zoea 1)	0-days	0.13	0.10	0.26	0.10	0.10	%	Wu et al., 2017
	3-days	0.08	0.08	0.10	0.04	0.03		
P. trituberculatus (Adult)	0-days	29.7	37.4	32.7	8.08	12.3	%	Ding et al., 2017
	30-days	26.7	36.9	36.3	8.92	14.9		
P. trituberculatus (Adult)	0-days	36.6	52.3	10.3	1.1	1.9	%	Ding et al., 2017
	30-days	31.3	53.5	12.3	1.73	2.43		
Scylla serrata (Zoea 5)	0-days	11.0	11.4	10.3	4.4	1.47	µg/mg	Holme et al., 2009
	4-days	4.94	3.08	5.43	2.78	0.79		
S. serrata (Megalopa)	0-days	8.19	10.6	8.93	2.86	2.23	µg/mg	Holme et al., 2009
	6-days	4.20	4.20	3.90	1.55	0.77		

Note. The highlighted number indicated a higher fatty acid reading

However, looking at the fatty acid trend in adult blue crab, *Portunus trituberculatus* (Table 2), indicated no reduction of ∑PUFA, DHA and EPA in starved crabs, probably to ensure the functioning of the nervous system, membrane integrity, stress resistance, and immunity at an optimum state rather than utilising these fatty acids as a source of energy (Wu et al., 2017). This observation demonstrated differences in fatty acid utilisation between the early growth stage and the adult stage in crabs. Perhaps, owing to incomplete growth development, early-stage crabs would use all the fatty acids to prolong their survival as long as possible, regardless of the importance of specific fatty acids to carry out certain vital functions. Through this process, the larvae or early crabs expend considerable energy, which ultimately causes higher mortality among the early-stage crabs. Interestingly, regarding to 14-day experimental period, 7-d starved crablets stated higher glycogen, protein and lipid compared to the 0-d starved crablets. The result highlighted that the crablet subjected to 7-d of starvation, then re-fed for 7-d, had the highest glycogen, lipid, and protein content compared to the fully fed crablet. Perhaps, as the crablet experienced a 7-day starvation, it responded to this starvation stress by storing as much energy as possible during the re-fed state. The stored glycogen, lipids, and proteins could subsequently be utilised as energy reserves to support survival during prolonged starvation. This event is considered part of the acclimation process.

Additionally, the higher fatty acid concentrations observed in the 14-day starvation experiment compared with the 7-day starvation experiment should be interpreted with caution. Although prolonged starvation is generally expected to further deplete lipid reserves, the initial fatty acid composition of the control groups differed between the two independent experiments. Specifically, the 7-day experiment began with a higher total fatty acid content than the 14-day experiment, reflecting variation between experimental batches of crablets. This variation may be attributed to differences in the physiological condition, nutritional history, broodstock quality, or natural biological variability of juvenile *P. pelagicus* obtained from different spawning batches. Consequently, the fatty acid concentrations between the 7- and 14-day experiments are not directly comparable. Instead, each starvation period should be interpreted relative to its respective control, allowing the effects of starvation to be evaluated within each independent experimental batch. This batch-to-batch variation represents a limitation of the present study and should be considered when comparing the magnitude of lipid depletion between the two starvation durations.

Many crustaceans likely use glycogen reserves as a primary energy source rather than using lipids and proteins during starvation (Sacristán et al., 2019). The rapid depletion of glycogen observed during starvation is likely attributable to its role as the primary and readily accessible energy reserve in crustaceans. The hepatopancreas is the main site for crustacean energy reserves, which are stored as glycogen (Vinagre et al., 2020). As the

glucose level drops in the haemolymph due to starvation, the X-organ secretes CHH to stimulate glycogenolysis in the hepatopancreas, breaking down glycogen into sugar to maintain glucose levels in the haemolymph for sustaining muscular energy requirements (Sanchez-Paz et al., 2006). Glycogen also plays a role in crab moulting, which is imperative for chitin synthesis (Sugumar et al., 2013; Sanchez-Paz et al., 2006). Since the glycogen reserve is used to provide energy during the starvation period, it reduces the amount of glycogen available for crustacean molting, leading to molt delay for starved crabs, as shown in the present study. Additionally, lipids serve as an additional source of energy during prolonged periods of starvation. The hepatopancreas is also known as the main lipid storage tissue, and lipolysis occurs to break down lipids into glycerol and fatty acids (Sanchez-Paz et al., 2006). Glycerol is then converted into glucose through gluconeogenesis, whereas fatty acids proceed through the β-oxidation pathway, resulting in acetylCoA that can be oxidised by the tricarboxylic acid cycle, producing ATP or energy (Vinagre et al., 2020). The present study also found that while glycogen was rapidly utilised in starved crabs, protein concentration decreased slightly because some protein breakdown is necessary to support glycogenolysis (glycogen to glucose) during the initial state of starvation. When energy sources from glycogen and lipids are limited, then the gluconeogenesis (protein to glucose) process is initiated, whereby proteolysis in crab muscle is stimulated to degrade proteins into amino acids and mobilise them into tissues for glucose synthesis and ATP production (Pellegrino et al., 2013). However, prolonged starvation may cause harmful effects when proteins are used as an energy source due to insufficient nutrient availability.

CONCLUSION

Overall, this study provides insight into the starvation effect on survival, growth, respiration process, and biochemical constituents of early juvenile crabs by using *P. pelagicus as a model.* It was observed that starvation negatively affected survival, molting, and growth of early juvenile crabs after seven days of being subjected to starvation, while fully-fed crabs increased in growth and molting frequency. Likewise, respiration rate is reduced with the prolonged starvation period as one of the ways to lower crab metabolic rate in grappling with such adverse conditions. In the context of biochemical constituents, starved crabs demonstrated a reduction in glycogen, protein, and lipids, including all fatty acid elements such as ∑SAFA. ∑PUFA, ∑MUFA, DHA, and EPA in their body tissues. Therefore, we confirmed that the internal body of *P. pelagicus* lined up several strategies (such as delayed molting, reduced oxygen consumption, used reserve glycogen, lipid and protein) to overcome feed deprivation. This is until the crab's internal body mechanism exhausts all avenues to compensate for energy demand since all the storage energy is already being utilised, hence causing the mortality of the crabs. Overall, the present finding demonstrated the relation between the nutritional condition and starvation on *the early growth of crabs*.

Such knowledge should be valuable for a better understanding of early juveniles' resilience in enduring starvation. Likewise, studying the effects of starvation on crablets provides important insights for optimising feeding strategies and culture protocols in *P. pelagicus* aquaculture by identifying the species' tolerance to temporary feed deprivation and improving feed management practices.

ACKNOWLEDGEMENT

The authors are grateful to all the staff at the Faculty of Fisheries and Food Science and the Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu**.**

ETHICAL APPROVAL

The authors followed research ethics guidelines from the Office of Research Management (RMO), Universiti Malaysia Terengganu (UMT), Kuala Terengganu, for the care and use of animals in this whole experiment as outlined in the Research Ethics Guidelines for the Ethics of Research on Animals 2014 (UMT; https://studylib.net/doc/8738550/research-ethics-guidelines—pusat-pengurusan-penyelidikan).

DATA AVAILABILITY

This manuscript has no associated data.

COMPETING INTERESTS

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

FUNDING

The study was fully funded by the Fundamental Grant Research Scheme (FRGS), Universiti Malaysia Terengganu (Vot. No. 59460). The study is also part of the research program under the Higher Institution Center of Excellence (HICoE) Grant for the Development of Future Food Through Sustainable Shellfish Aquaculture (HICoE AKUATROP Trust Account No. 66955).

ABBREVIATION

D - day

SAFA - saturated fatty acid

MUFA - monounsaturated fatty acid

PUFA - polyunsaturated fatty acid

DHA - docosahexaenoic acid

EPA - eicosapentaenoic acid

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