



The Effects of Dietary Protein Sources on the Growth and Survival of *Scylla tranquebarica* (Fabricius, 1798) Megalopa to Early Crab Instar

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Abstract

The effects of different dietary protein sources on the growth and survival of *Scylla tranquebarica* (Fabricius, 1798) megalopa to early crab instar were elucidated. Four isoproteic (51 %) and isolipidic (10 %) diets were formulated using fish meal (FM) as the main protein source, combined with squid meal (FM:SQ), scallop meal (FM:SC), or bloodworm meal (FM:BW) at a ratio of 3:2. The 2-day-old megalopa were reared in triplicate in 35 L black rectangular tanks and fed the experimental diets for 14 days. The larval stage composition, survival, and growth performance were evaluated on days 7 and 14. On day 7, the production of crab instar 1 was significantly higher in the FM, FM:SQ, and FM:SC treatments compared to FM:BW ($P < 0.05$). On day 14, the proportion of crab instar 2 and the mean total biomass were significantly higher in the FM:SQ and FM:SC treatments ($P < 0.05$). These two treatments also exhibited significantly lower feed intake and feed conversion ratios than the others, with the highest protein and lipid efficiency ratios ($P < 0.05$). In conclusion, combining fish meal with squid or scallop meal improves megalopa growth and survival during the transition to the early instar stage.

Keywords: crablet, formulated diets, larval development, mud crab, protein combination

Introduction

Feed is the key factor in successful crab seed production in mud crabs (*Scylla* spp.) (Sanudin et al., 2024). Mud crab larvae hatch as zoeae and pass through five zoeal stages before metamorphosing into the megalopa. The megalopa is the transitional stage following the last crab larvae instar. The megalopa further metamorphoses into the benthic crab instar stage, which exhibits the body structure of adult mud crabs (Thien et al., 2025). During larval rearing, live feeds such as rotifers and *Artemia* are commonly provided from zoea I to zoea V (Sanudin et al., 2022). Owing to the incomplete development of the digestive system in zoeal larvae, formulated diets are generally introduced only at the juvenile and adult stages (Serrano, 2012). Weaning from live feeds to formulated diets has been reported to occur during the megalopa stage, during which megalopae are typically fed adult *Artemia*, fresh feeds such as shredded fish, shrimp,

and mussel meat, or commercial diets (Holme et al., 2006; Quintio & Parado-Estapa, 2003). In addition, no significant difference in survival was observed between *Scylla serrata* megalopae fed a 100 % micro-bound diet and those fed 100 % *Artemia* (Genodepa et al., 2004).

Among protein sources, fish meal has conventionally been used as the primary protein in commercial diets due to its high palatability, digestibility, and well-balanced amino acid composition (Hua et al., 2019). Despite these advantages, acceptance of formulated diets remains a challenge in some crustacean species, such as the mud crab *Scylla tranquebarica* (Fabricius, 1798), which rejected formulated feed under culture conditions (Liew et al., 2022). To improve feed acceptance, natural ingredients such as squid and shellfish are incorporated as feed attractants to enhance feed intake in cultured animals (Ndobe et al., 2022; Rasidi, 2022). The inclusion of squid meal and

squid extract in the diet of the freshwater crayfish *Procambarus clarkii* is an effective feed stimulant, thereby improving feed intake (Hua et al., 2014). These natural ingredients are also rich in carotenoids and cholesterol, two essential nutrients that crustaceans have a limited capacity to synthesise *de novo* (Kumar et al., 2018; Matsuno, 2001).

The combination of various protein sources with fish meal in formulated diets has been shown to improve feed acceptance in crustaceans, thereby supporting better growth and survival (Marchese et al., 2019; Simon et al., 2019). In this context, squid meal, fish meal, and *Artemia* meal have been tested as alternatives to rotifer meal in the diet of *S. serrata* megalopa (Holme et al., 2006). This study demonstrated that megalopae fed solely on squid meal, as well as those fed a mixture of squid meal and fish meal, showed higher survival rates and shorter times to reach the first crab instar compared with megalopae fed diets combining squid meal with either rotifer meal or *Artemia* meal (Holme et al., 2006).

The present study was conducted to assess the effects of combining fish meal with other protein sources on the growth and survival of *S. tranquebarica* megalopa from the early instar stage onward. The purple mud crab, *S. tranquebarica*, was selected as the target species because it is one of the four highly sought-after mud crab species in both domestic and international markets (Mohd Sharif et al., 2019). This species is widespread across the Indo-Pacific region, from Southeast and East Africa to Southeast Asia (Alberts-Hubatsch et al., 2016). The data generated from this study are expected to provide valuable information to assist the expansion of mud crab aquaculture.

Materials and Methods

Ethical approval

The present study received ethical approval from the Universiti Malaysia Sabah Code of Practice and Guidelines for the Care and Use of Animals for Scientific Purposes (AEC006/2022).

Source of mud crab megalopa

The feeding trial was conducted at the Crustacean Hatchery, Borneo Marine Institute, Universiti Malaysia Sabah, Malaysia. Megalopa of *S. tranquebarica* were

obtained from a single broodstock spawning at the hatchery. Broodstock management and larval rearing from zoea I to the megalopa stage were carried out according to the protocols described by Thien and Yong (2017) and Sanudin et al. (2022), respectively. The selection of specimens from a single spawning batch was designed to minimize variability arising from differences in parental genetics, maternal nutrient allocation, and early rearing history. This approach allowed the effects of dietary treatments to be evaluated under a more controlled experimental setting.

Experimental feed

The experimental diets were formulated using fish meal (FM), squid meal (SQ), scallop meal (SC), and bloodworm meal (BW) as the primary protein sources. The crude protein and crude lipid contents, and the amino acid profiles of the protein sources used in the present study are presented in Tables 1 and 2, respectively. The crude protein content of the FM, SQ, SC, and BW meals was 78.41 %, 85.04 %, 79.41 %, and 61.8 %, respectively, and their crude lipid content was 7.31 %, 5.22 %, 5.43 %, and 9.74 %, respectively (Table 1). The amino acid analysis was conducted for FM, SQ, and SC. However, due to an insufficient BW sample, the amino acid profile was not determined for this diet. Differences among ingredients were observed for several essential amino acids (Table 2). Lysine concentrations were higher in FM and SQ, and lower in SC. Methionine content was higher in SC compared with FM and SQ. Arginine levels were higher in SQ relative to the other ingredients. Variations were also present with leucine and isoleucine occurring at higher concentrations in FM and SQ than in SC. In contrast, valine levels were similar in FM and SC but lower in SQ. Among the non-essential amino acids, alanine content was higher in SC, while glutamic acid concentrations were higher in FM and SQ.

The experimental diets consisted of four isoproteic (51 %) and isolipidic (10 %) diets (Sanudin et al., 2024) (Table 3). The control diet used fish meal (FM) as the sole protein source. The remaining three diets were formulated by partially replacing fish meal with squid meal (FM:SQ), scallop meal (FM:SC), or bloodworm meal (FM:BW) at a fixed ratio of 3:2 (Thien & Yong, 2017). Squid meal and bloodworm meal were prepared by oven-drying squid (*Loligo* sp.) and bloodworm (*Chironomus* sp.), respectively, and ground into a fine powder. Scallop meal was a commercial product obtained from a local supplier.

Table 1. The crude protein and lipid content (mean $\pm$ SD) of the different protein ingredient sources (g.100 g⁻¹ dry weight basis) fed to purple mud crab *Scylla tranquebarica*.

Proximate analysis(%)	Protein sources			
	FM	SQ	SC	BW
Crude protein	78.41 $\pm$ 0.41	79.64 $\pm$ 0.58	69.41 $\pm$ 0.75	61.80 $\pm$ 0.21
Crude lipid	7.31 $\pm$ 0.22	5.22 $\pm$ 0.22	5.43 $\pm$ 0.20	9.74 $\pm$ 0.27

FM, fish meal; SQ, squid meal; SC, scallop meal; BW, bloodworm meal.

Table 2. Compound weight (ng, dry weight basis) of amino acid compositions in of ingredients fish meal (FM), squid meal (SQ), and scallop meal (SC) fed to purple mud crab *Scylla tranquebarica*.

Amino acid compounds	Protein sources		
	FM	SQ	SC
Essential amino acids			
Threonine	34.0	38.0	31.9
Methionine	24.5	25.8	33.6
Isoleucine	38.3	41.3	36.6
Leucine	62.2	66.3	56.5
Phenylalanine	27.4	29.7	26.2
Lysine	81.8	76.0	52.0
Valine	45.0	37.8	45.5
Histidine	17.8	18.7	15.4
Arginine	51.3	67.4	43.1
Total essential amino acids	382.2	401.0	340.8
Non-essential amino acids			
Aspartic acid	82.4	80.5	74.3
Serine	33.7	36.2	29.8
Glutamic acid	117.7	123.7	104.2
Glycine	43.6	39.7	37.7
Alanine	54.4	46.0	61.8
Cysteine	3.1	3.9	1.6
Tyrosine	20.1	21.9	16.4
Proline	35.5	31.0	29.6
Hydroxyproline	5.4	5.6	3.2
Total non-essential amino acids	395.9	388.7	358.6
Total amino acids	778.0	789.7	699.4

Table 3. Formulation and proximate analysis of experimental diets (g.100 g⁻¹ dry weight basis) fed to purple mud crab *Scylla tranquebarica*.

Ingredients (g.100 g ⁻¹)	Diets			
	FM	FM:SQ	FM:SC	FM:BW
Fish meal	67.37	40.00	40.00	40.00
Squid meal	-	25.11	-	-
Scallop meal	-	-	25.93	-
Bloodworm meal	-	-	-	34.34
Fish oil	6.00	5.06	4.86	3.46
Cholesterol	0.60	0.60	0.60	0.60
Alpha starch	11.03	14.23	13.61	6.60
Soy lecithin	3.00	3.00	3.00	3.00
Calcium phosphate	2.00	2.00	2.00	2.00
¹ Mineral premix	3.00	3.00	3.00	3.00
² Vitamin premix	3.00	3.00	3.00	3.00
Carboxymethyl-cellulose	4.00	4.00	4.00	4.00
Proximate analysis (%)				
Crude protein	51.39 ± 0.20	51.22 ± 0.36	51.73 ± 0.52	51.48 ± 0.24
Crude lipid	10.61 ± 0.21	10.24 ± 0.38	10.11 ± 0.61	10.49 ± 0.33
Moisture	8.65 ± 0.39	8.79 ± 0.07	8.44 ± 0.29	8.12 ± 0.18
Ash	21.56 ± 1.53	16.05 ± 2.30	21.11 ± 0.76	19.87 ± 1.68
³ Estimated gross energy (kcal.100g ⁻¹)	422.6 ± 2.41	444.2 ± 3.49	426.2 ± 6.93	433.0 ± 2.34
⁴ Gross energy-to-protein ratio (kcal.g ⁻¹ crude protein)	8.22 ± 0.03	8.67 ± 0.07	8.24 ± 0.10	8.41 ± 0.06

Data are presented as mean ± standard deviation (n = 3).

FM, fish meal; FM:SQ, fish meal and squid meal; FM:SC, fish meal and scallop meal; FM:BW, fish meal and bloodworm meal.

¹Mineral mixture (g.kg⁻¹ mixture): Calcium phosphate monobasic, 270.98; Calcium lactate, 327.0; Ferrous sulphate, 25.0; Magnesium sulphate, 132.0; Potassium chloride, 50.0; Sodium chloride, 60.0; Potassium iodide, 0.15; Copper sulphate, 0.785; Manganese oxide, 0.8; Cobalt carbonate, 1.0; Zinc oxide, 3.0; Sodium selenite, 0.011; Calcium carbonate, 122.274.

²Vitamin mixture (g.kg⁻¹ mixture): Ascorbic acid, 45.0; Inositol, 5.0; Choline chloride, 75.0; Niacin, 4.5; Riboflavin, 1.0; Pyridoxine HCl, 1.0; Thiamine HCl, 0.92; D-calcium pantothenate, 3.0; Retinyl acetate, 0.60; Vitamin D3, 0.083; Menadione, 1.67; DL alpha-tocopherol acetate, 8.0; D-biotin, 0.02; Folic acid, 0.09; Vitamin B12, 0.00135. All ingredients were diluted with alpha-cellulose to a total of 1 kg.

³Gross energy (kcal.100g⁻¹) = 5.65 kcal g⁻¹ protein, 9.45 kcal g⁻¹ lipid, and 4.10 kcal g⁻¹ NFE, ⁴Gross Energy to protein ratios (kcal.g⁻¹) = Gross energy (kcal.100g⁻¹) / Crude protein (g.100g⁻¹) (Taj et al., 2023).

The experimental diets were prepared by thoroughly mixing all ingredients with water to form a dough. Pre-gelatinised α -starch and carboxymethyl cellulose (CMC) were incorporated into all diets as binders to improve pellet integrity. The dough was pelletised using a mincer (TBS-200 Orimas, Taiwan). The noodle-like strands produced were oven-dried at 40 °C for 8 h. After drying, the pellets were crushed into smaller particles and sieved sequentially through 710 μ m and 500 μ m mesh strainers to obtain pellet sizes ranging from 500 to 710 μ m (Genodepa et al., 2004). The pellets were then stored at -20 °C until use. Proximate composition analysis of the experimental diets was conducted before the commencement of the feeding trial. The crude protein and lipid in the diets were in the ranges of 51.22–51.73 % and 10.11–10.61 %, respectively. The gross energy (GE) of the diets was estimated based on physiological fuel values of 5.65 kcal g⁻¹ protein, 9.45 kcal g⁻¹ lipid, and 4.10 kcal g⁻¹ carbohydrate (NRC, 2011). The diets showed comparable GE (422.6 to 444 kcal 100 g⁻¹) and GE:P ratios (8.22 to 8.67 kcal g⁻¹ protein), indicating that the experimental diets were approximately iso-energetic.

Feeding trial

In this experiment, two-day-old megalopa larvae were used (initial body weight [mean $\pm$ SD]: 4.18 $\pm$ 0.20 mg; initial carapace width: 1.73 $\pm$ 0.09 mm). A total of 30 megalopae were randomly stocked into 35-L black rectangular tanks (58.5 cm length $\times$ 38.5 cm width $\times$ 24.5 cm height) and reared in triplicate (n = 3), in a stocking density of 2 megalopae L⁻¹ (Xu et al., 2005). Each tank was filled with 15 L of seawater, and gentle aeration was provided. Shelters consisting of PVC pipes (2.5 cm diameter $\times$ 4.0 cm length) and black netting were placed in the tanks to reduce cannibalism among the megalopae (Thien et al., 2022). The natural photoperiod consisted of approximately 12 h of light and 12 h of darkness. Water quality parameters were maintained at 28.8 $\pm$ 0.8 °C for temperature, 28.62 $\pm$ 1.0 ppt for salinity, 5.5 $\pm$ 0.5 mg L⁻¹ for dissolved oxygen, and 7.82 $\pm$ 0.4 for pH.

The larvae were fed twice daily, in the morning (09:00) and afternoon (16:00), at a rate of 2 mg of feed per megalopa (Holme et al., 2006). To calculate feed intake during the trial, the amount of feed provided was recorded, and any uneaten feed was removed before

the next feeding. The leftover feed was then dried in an oven at 55 °C (Unnikrishnan & Paulraj, 2010). A 50 % water exchange was performed each morning before feeding (Cabacaba et al., 2020), and any dead larvae were removed and recorded. The feeding trial lasted 14 days, during which growth, survival, and larval stage composition were monitored on days 7 and 14.

Data collection

Throughout the feeding trials, the megalopa metamorphosed into the first two crab instars 1 and 2 (C1 and C2). Body weight (BW) and carapace width (CW) of the C1 and C2 were measured on days 7 and 14. Survival rate, larval stage composition, body weight gain (BWG), carapace width gain (CWG), specific growth rate of body weight (SGR_{BW}), specific growth rate of carapace width (SGR_{CW}), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and lipid efficiency ratio (LER) were determined at the end of the experiment as percentages based on the following formulae:

$$\text{Survival rate (\%)} = \frac{\text{Final number of survived crabs}}{\text{Initial number of crabs}} \times 100$$

Larval stage composition (%)

$$= \frac{\text{Number of surviving crabs at certain stage}}{\text{Total surviving crabs}} \times 100$$

$$\text{BWG (\%)} = \frac{(\text{Final} - \text{Initial}) \text{ body weight (mg)}}{\text{Initial body weight (mg)}} \times 100$$

$$\text{CWG (\%)} = \frac{(\text{Final} - \text{Initial}) \text{ carapace width (mm)}}{\text{Initial carapace width (mm)}} \times 100$$

SGR_{BW} (%)

$$= \frac{\ln(\text{final body weight (mg)}) - \ln(\text{initial body weight (mg)})}{\text{Duration (days)}} \times 100$$

SGR_{CW} (%) =

$$\frac{\ln(\text{final carapace width (mm)}) - \ln(\text{initial carapace width (mm)})}{\text{Duration (days)}} \times 100$$

$$\text{FI (\% . day}^{-1}\text{)} = \frac{100 \times \text{Feed consumption (g)}}{\text{Average biomass (g)} \times \text{days}}$$

$$\text{FCR} = \frac{\text{Dry feed intake (mg)}}{\text{Wet weight gain (mg)}}$$

$$PER = \frac{\text{Wet weight gain (mg)}}{\text{Total protein intake (mg)}}$$

$$LER = \frac{\text{Wet weight gain (mg)}}{\text{Total lipid intake (mg)}}$$

Proximate and amino acids analyses

The proximate composition of the experimental diets, including moisture, dry matter, ash, crude lipid, and protein content, was determined following AOAC (1999) methods. Moisture content was measured by oven-drying the samples at 105 °C for 24 h, while crude ash was obtained by incineration in a muffle furnace at 550 °C for 5 h. Crude lipid was analysed using a Soxtec Lipid Analyser (Soxtec™ 2043, Foss, Sweden), and protein content was determined via Kjeldahl distillation and titration using a Vapodest 500 (Gerhardt, Germany). The amino acid analysis was performed by the Laboratory of Halal Services, Universiti Putra Malaysia, Malaysia. The amino acid solution was derivatised using the AccQ-Fluor™ reagent, with aminobutyric acid used as the internal standard. The resulting amino acid derivatives were analysed by high-performance liquid chromatography (Waters Alliance 2695) coupled with a fluorescence detector (Waters 2475). The amino acid content (mg) was determined through external standard calibration.

Statistical analyses

The proportion data were arcsine transformed before analysis. The Shapiro-Wilk and Levene's tests were used to assess data normality and homogeneity of variance, respectively. A one-way ANOVA was performed to evaluate statistical differences at $P < 0.05$ between diets at each time of measurement (7d and 14d), followed by Tukey's post hoc test for pairwise comparisons. All statistical analyses were conducted using IBM SPSS Statistics version 20. Data are presented as mean $\pm$ standard deviation (SD).

Results

Larval stage composition and survival

On day 7, the composition of megalopa in the FM, FM:SQ, and FM:SC treatments was significantly lower (51.67–

56.96 %) than in the FM:BW treatment (67.81 %)($P < 0.05$) (Table 4). The highest C1 instar composition was observed in the FM:SC treatment (48.34 %); however, this value did not differ significantly from those of the other treatments, except for FM:BW, which had the lowest C1 instar proportion (32.19 %)($P < 0.05$).

On day 14, megalopae were absent in all treatments except FM:BW, in which a small proportion (1.96 %) remained. In the other treatments, larval composition consisted predominantly of C1 and C2 instars (Table 4). The C1 instar composition was significantly lower in the FM:SQ and FM:SC treatments than in the other treatments ($P < 0.05$). Consequently, these treatments exhibited significantly higher C2 instar composition, reaching 57.00 % and 51.86 % in FM:SQ and FM:SC, respectively, compared with FM and FM:BW (33.3 and 21.6 %, respectively $P < 0.05$).

Survival was highest in the FM:BW treatment day 7 (65.56 %), but there were no significant differences among treatments (range = 61.1–64.4 %, $P > 0.05$) (Table 4). By day 14, the highest survival was observed in the FM:SQ treatment (58.89 %); however, survival did not differ significantly among treatments (range = 50.0–57.8 %, $P > 0.05$).

Body weight and carapace growth performance

The BW of C1, BWG, and SGR_{BW} from megalopa to C1 (M–C1), as well as the CW of C1, CWG, and SGR_{CW} from M–C1, did not differ significantly among treatments on day 7 ($P > 0.05$, Table 5).

On day 14, the BW of C2 instar crabs was significantly higher in the FM:SQ treatment compared with FM:BW ($P < 0.05$), although no significant differences were observed relative to the other treatments (Table 6). On day 14, no significant differences in BWG or SGR_{BW} were observed among dietary treatments for the M–C1, C1–C2, or M–C2 stages ($P > 0.05$).

The total biomass was significantly higher in the FM:SQ and FM:SC groups compared with the other treatments ($P < 0.05$). In addition, C1 instar crabs on day 14 exhibited lower BW, BWG, and SGR_{BW} of M–C1 than

Table 4. Larval stage composition and survival of megalopa and crab instars of purple mud crab *Scylla tranquebarica* fed diets containing different protein sources.

Diets	Day 7			Day 14			
	Larval stage composition (%)		Survival (%)	Larval stage composition (%)			Survival (%)
	M	C1		M	C1	C2	
FM	56.96 $\pm$ 4.24 ^a	43.04 $\pm$ 4.24 ^b	64.44 $\pm$ 3.85 ^a	0	66.71 $\pm$ 5.81 ^b	33.29 $\pm$ 5.81 ^a	50.00 $\pm$ 3.33 ^a
FM:SQ	52.65 $\pm$ 2.51 ^a	47.35 $\pm$ 2.52 ^b	61.11 $\pm$ 5.09 ^a	0	42.96 $\pm$ 6.12 ^a	57.04 $\pm$ 6.12 ^b	58.89 $\pm$ 8.39 ^a
FM:SC	51.67 $\pm$ 4.29 ^a	48.34 $\pm$ 4.29 ^b	62.22 $\pm$ 6.94 ^a	0	48.14 $\pm$ 1.62 ^a	51.86 $\pm$ 1.62 ^b	57.78 $\pm$ 5.09 ^a
FM:BW	67.81 $\pm$ 2.56 ^b	32.19 $\pm$ 2.56 ^a	65.56 $\pm$ 1.92 ^a	1.96 $\pm$ 3.40	76.43 $\pm$ 10.21 ^b	21.61 $\pm$ 6.84 ^a	50.00 $\pm$ 8.82 ^a
P value	0.002	0.001	0.697	0.441	0.001	0.000	0.292

Data are presented as mean $\pm$ standard deviation (n = 3). Different superscripted letters within a column indicate significant differences ($P < 0.05$).

M, megalopa; C1, crab instar 1; C2, crab instar 2; FM, fish meal; FM:SQ, fish meal and squid meal; FM:SC, fish meal and scallop meal; FM:BW, fish meal and bloodworm meal.

Table 5. Body weight, body weight gain, specific growth rate of body weight, carapace width, carapace width gain, and specific growth rate of carapace width were measured on day 7 for C1 and C2 instars of purple mud crab *Scylla tranquebarica*.

Diets	Initial BW megalopa (mg)	BW C1 (mg)	M-C1		Initial CW megalopa (mg)	CW C1 (mm)	M-C1	
			BWG (%)	SGR _{BW} (%.day ⁻¹)			CWG (%)	SGR _{CW} (%.day ⁻¹)
FM	4.19 ± 0.26 ^a	7.49 ± 0.21 ^a	79.08 ± 5.71 ^a	8.31 ± 0.11 ^a	1.73 ± 0.14 ^a	3.03 ± 0.04 ^a	75.15 ± 4.68 ^a	7.99 ± 0.17 ^a
FM:SQ	4.20 ± 0.20 ^a	7.82 ± 0.26 ^a	86.30 ± 8.56 ^a	8.87 ± 0.38 ^a	1.73 ± 0.11 ^a	3.04 ± 0.05 ^a	76.21 ± 5.12 ^a	8.08 ± 0.08 ^a
FM:SC	4.19 ± 0.13 ^a	7.85 ± 0.34 ^a	87.07 ± 6.00 ^a	8.95 ± 0.63 ^a	1.72 ± 0.10 ^a	3.03 ± 0.05 ^a	76.08 ± 4.66 ^a	8.07 ± 0.04 ^a
FM:BW	4.15 ± 0.25 ^a	7.47 ± 0.34 ^a	79.97 ± 3.54 ^a	8.39 ± 0.35 ^a	1.72 ± 0.06 ^a	2.99 ± 0.06 ^a	74.09 ± 2.14 ^a	7.92 ± 0.00 ^a
P value	0.99	0.124	0.324	0.212	1.00	0.217	0.925	0.253

Data are presented as mean ± standard deviation (n = 3). Different superscripted letters within the same column indicate a significant difference ($P < 0.05$).

M, megalopa; C1, crab instar 1; C2, crab instar 2; BW, body weight; BWG, body weight gain; SGR_{BW}, specific growth rate of body weight; CW, carapace width; CWG, carapace width gain; SGR_{CW}, specific growth rate of carapace width; FM, fish meal; FM:SQ, fish meal and squid meal; FM:SC, fish meal and scallop meal; FM:BW, fish meal and blood worm meal.

Table 6. Body weight, body weight gain, specific growth rate of body weight, and total biomass were measured on day 14 for C1 and C2 instars of purple mud crab *Scylla tranquebarica*.

Diets	BW C1 (mg)	BW C2 (mg)	M-C1		C1-C2		M-C2		Mean total biomass (mg)
			BWG (%)	SGR _{BW} (%.day ⁻¹)	BWG (%)	SGR _{BW} (%.day ⁻¹)	BWG (%)	SGR _{BW} (%.day ⁻¹)	
FM	6.92 ± 0.32 ^a	17.32 ± 0.37 ^{ab}	65.48 ± 6.36 ^a	3.59 ± 0.13 ^a	131.34 ± 4.18 ^a	11.98 ± 0.31 ^a	314.29 ± 13.52 ^a	10.14 ± 0.15 ^a	155.90 ± 10.11 ^a
FM:SQ	7.24 ± 0.36 ^a	17.94 ± 0.35 ^b	72.35 ± 7.26 ^a	3.88 ± 0.29 ^a	129.51 ± 7.63 ^a	11.86 ± 0.28 ^a	327.30 ± 15.40 ^a	10.37 ± 0.14 ^a	235.00 ± 13.36 ^b
FM:SC	7.15 ± 0.43 ^a	17.51 ± 0.45 ^{ab}	70.68 ± 6.95 ^a	3.81 ± 0.26 ^a	123.09 ± 4.21 ^a	11.46 ± 0.36 ^a	317.59 ± 11.54 ^a	10.21 ± 0.18 ^a	216.88 ± 5.73 ^b
FM:BW	6.83 ± 0.49 ^a	16.26 ± 0.89 ^a	64.82 ± 5.38 ^a	3.56 ± 0.18 ^a	117.90 ± 12.30 ^a	11.11 ± 0.77 ^a	292.04 ± 16.51 ^a	9.75 ± 0.39 ^a	132.53 ± 9.31 ^a
P value	0.162	0.033	0.454	0.279	0.214	0.177	0.082	0.064	0.000

Data are presented as mean ± standard deviation (n = 3). Different superscripted letters within the same column indicate a significant difference ($P < 0.05$).

FM, fish meal; FM:SQ, fish meal and squid meal; FM:SC, fish meal and scallop meal; FM:BW, fish meal and bloodworm meal; BW, body weight; BWG, body weight gain; SGR_{BW}, specific growth rate of body weight; M, megalopa; C1, crab instar 1; C2, crab instar 2.

those that had undergone earlier metamorphosis on day 7. From day 7 to day 14, BW, BWG, and SGR_{BW} in the M-C1 stage decreased across treatments, with no significant differences among diets, including the FM:SQ and FM:SC treatments ($P > 0.05$).

On day 14, CW of C2 instars and the CWG and SGR_{CW} for M-C1, C1-C2, and M-C2 did not differ significantly among dietary treatments ($P > 0.05$) (Table 7). In contrast, the FM:BW treatment consistently recorded the lowest CW of C2, CWG, and SGR_{CW} across M-C1, C1-C2, and M-C2 ($P > 0.05$). Crabs that remained in the C1 instar stage on day 14 exhibited lower CW, CWG, and SGR_{CW} of M-C1 than those that had undergone metamorphosis on day 7.

Feed utilisation

The treatment fed with FM:SQ and FM:SC showed a

significantly lower FI and FCR compared to the FM and FM:BW treatments ($P < 0.05$) (Table 8). The FM:BW group had the highest FI and FCR among all treatments ($P < 0.05$). The PER and LER were significantly higher in the FM:SQ and FM:SC treatments than in the FM and FM:BW treatments ($P < 0.05$).

Discussion

Proteins play a fundamental role in supporting the growth and physiological maintenance of animals. The selection of nutritionally valuable protein sources is a critical consideration in feed development, particularly during key transitional phases such as metamorphosis and the weaning period from live feed to formulated diets. In mud crabs, this transition is especially pronounced, as feeding habits change during the megalopa stage before metamorphosis into the crab instar stage. The present findings demonstrate that

Table 7. The carapace width, carapace width gain, and specific growth rate of carapace width were measured on day 14 for C1 and C2 instars of purple mud crab *Scylla tranquebarica*.

Diets	CWC1 (mg)	CWC2 (mg)	M-C1		C1-C2		M-C2	
			CWG (%)	SGR _{CW} (%.day ⁻¹)	CWG (%)	SGR _{CW} (%.day ⁻¹)	CWG (%)	SGR _{CW} (%.day ⁻¹)
FM	2.84 ± 0.21 ^a	4.27 ± 0.20 ^a	64.28 ± 6.78 ^a	7.09 ± 0.88 ^a	41.04 ± 5.44 ^a	4.91 ± 0.56 ^a	146.89 ± 6.07 ^a	12.89 ± 0.56 ^a
FM:SQ	2.92 ± 0.12 ^a	4.34 ± 0.13 ^a	69.53 ± 6.80 ^a	7.52 ± 0.37 ^a	42.72 ± 1.57 ^a	5.08 ± 0.15 ^a	151.47 ± 6.93 ^a	13.16 ± 0.15 ^a
FM:SC	2.89 ± 0.18 ^a	4.31 ± 0.13 ^a	68.34 ± 7.29 ^a	7.41 ± 0.29 ^a	42.19 ± 1.19 ^a	5.03 ± 0.11 ^a	150.46 ± 9.31 ^a	13.10 ± 0.11 ^a
FM:BW	2.79 ± 0.22 ^a	4.21 ± 0.14 ^a	61.73 ± 7.30 ^a	6.85 ± 0.59 ^a	40.22 ± 1.18 ^a	4.83 ± 0.12 ^a	144.25 ± 6.22 ^a	12.75 ± 0.12 ^a
P value	0.522	0.367	0.536	0.501	0.733	0.732	0.617	0.378

Data are presented as mean ± standard deviation (n = 3). Different superscripted letters within the same column indicate a significant difference ($P < 0.05$).

M, megalopa; C1, crab instar 1; C2, crab instar 2; CW, carapace width; CWG, carapace width gain; SGR_{CW}, specific growth rate of carapace width; FM, fish meal; FM:SQ, fish meal and squid meal; FM:SC, fish meal and scallop meal; FM:BW, fish meal and bloodworm meal.

Table 8. Feed utilisation of the purple mud crab, *Scylla tranquebarica*, fed experimental diets with different protein sources.

Diets	FI (%.day ⁻¹)	FCR	PER	LER
FM	3.50 ± 0.13 ^b	12.39 ± 1.23 ^b	0.16 ± 0.01 ^a	0.77 ± 0.07 ^a
FM:SQ	2.25 ± 0.08 ^a	8.16 ± 0.63 ^a	0.24 ± 0.02 ^b	1.20 ± 0.11 ^b
FM:SC	2.43 ± 0.09 ^a	8.90 ± 0.81 ^a	0.22 ± 0.02 ^b	1.12 ± 0.11 ^b
FM:BW	3.95 ± 0.08 ^c	15.05 ± 1.12 ^c	0.13 ± 0.01 ^a	0.64 ± 0.05 ^a
P value	0.000	0.000	0.000	0.000

Data are presented as mean ± standard deviation (n = 3). Different superscripted letters within the same column indicate a significant difference ($P < 0.05$).

FI, feed intake; FCR, feed conversion ratio; PER, protein efficiency ratio; LER, lipid efficiency ratio; FM, fish meal; FM:SQ, fish meal and squid meal; FM:SC, fish meal and scallop meal; FM:BW, fish meal and bloodworm meal.

the inclusion of different dietary protein sources in *S. tranquebarica* diets resulted in variable growth performance of megalopa during their transition to the early instar stage. Such variability may be attributed to differences in protein quality, which are commonly associated with the amino acid composition of each protein source and its ability to meet the specific nutritional requirements of megalopa (Kusnadi et al., 2023). In addition, these differences may also reflect variation in diet acceptance, highlighting the importance of protein source palatability and feed attractiveness in formulated diets (Hua et al., 2019). Consistent with these results, the use of squid meal and squid extract has been reported to act as effective feeding stimulants, enhancing feed intake in freshwater crayfish (*P. clarkii*) (Hua et al., 2014).

In the current study, incorporating squid and scallop meals into megalopa diets improved growth performance compared with a diet based solely on fish meal. This suggests that combining multiple protein sources, such as squid and scallop meals, enhances the nutritional quality of formulated diets, thus improving crab growth and survival. The benefits of using blended protein sources are often attributed to their complementary nutritional profiles, which meet the crustacean's physiological requirements (Hua et al., 2019).

Similar positive effects have been reported in crustaceans, where supplementation of fish meal with bivalve meal significantly enhanced reproductive performance in giant freshwater prawn (*Macrobrachium rosenbergii*) broodstock, while dietary inclusion of squid meal improved larval growth in white-leg shrimp (*Penaeus vannamei*) (Cordova-Murueta & García-Carreño, 2002; Sin et al., 2016). Although FM alone provided a high total essential amino acid (EAA) content, its profile was relatively limited in amino acids such as methionine, which are critical for crustacean protein synthesis, moulting, and overall growth (Nunes & Masagounder, 2023). In the present study, the methionine level was 37 % higher in the SC than in the fish meal. The inclusion of SC has been reported to compensate for this limitation by increasing methionine levels and diversifying the EAA profile, thereby reducing potential amino acid antagonism and enhancing nutritional efficiency (Teoh, 2025).

Similar improvements in growth performance resulting from optimised dietary protein profiles have been reported in mud crab feeding studies where balanced amino acid supply enhanced SGR, PER, and FCR (Guo et al., 2026). This is also consistent with the behavioural evidence reported by Teoh et al. (2025), in which diets richer in marine protein sources, such as

squid meal, elicited stronger feeding responses in the slipper lobster *Thenus orientalis*, indicating that amino acid composition influences both palatability and feed intake in decapod crustaceans. Compared with fishmeal-only diets, protein sources enhanced with functional amino acids, such as glutamic acid and glycine, have been shown to improve feeding behaviour and intake, which directly affects growth performance (Teoh et al., 2023). Collectively, these findings indicate that FM alone, while nutritionally rich, may not provide the most effective amino acid pattern for maximal growth in mud crabs.

In the present study, the squid meal (FM:SQ) and scallop meal (FM:SC) treatments exhibited significantly lower feed intake (FI) than the FM and FM:BW groups. These results suggest that, despite lower feed intake, crabs fed the FM:SQ and FM:SC diets achieved significantly higher total biomass than groups with higher FI, demonstrating superior overall feed and protein utilisation efficiency. The enhanced growth performance observed in the FM:SQ and FM:SC groups was supported by significantly higher PER values than in the other treatments. The protein efficiency ratio (PER) is a widely used index for assessing dietary protein utilisation efficiency (NRC, 2011). The present results indicate that crabs fed the FM:SQ and FM:SC diets utilised dietary protein effectively for tissue synthesis. In contrast, protein utilisation for growth was comparatively less efficient in the FM:BW and FM only treatment groups.

In the present study, the FM:SQ and FM:SC treatments also exhibited significantly lower FCR than the FM and FM:BW groups. Furthermore, the FCR was 34 % lower than the control diet, from 12.39 in FM to 8.16 in FM:SC. The FCR is a key indicator of feed utilisation efficiency, with lower values reflecting more efficient conversion of consumed feed into body biomass over a given period (Fry et al., 2018). The FCR values in crustacean post-larvae are high due to feed loss, incomplete ingestion, and leaching (Barreto et al., 2023). Therefore, a 34 % FCR reduction indicates greatly improved feeding efficiency, which is biologically meaningful at the megalopa stage and suggests enhanced feed utilisation and reduced wastage. From a practical perspective, such an improvement would substantially reduce feed inputs and associated costs in commercial hatcheries, where feed constitutes a major operational expense (Junior et al., 2025). A lower FCR indicates better animal growth than a higher FCR value (Shi et al., 2024). Animals cultured with feeds exhibiting higher FCR require more feed to achieve the desired total yield, resulting in increased farm operating costs and additional environmental impacts associated with feed production, processing, transportation, and waste generation (Eissa et al., 2022).

In this study, feeding FM as the sole protein source resulted in lower growth and survival than diets combining FM with SQ or SC. Although crabs fed FM

exhibited higher FI than those fed FM:SQ and FM:SC, they showed significantly poorer FCR, lipid efficiency ratio (LER), and PER. These findings suggest that reliance on fish meal alone is suboptimal for mud crab rearing during the transition from megalopa to the early instar stage. Similar trends have been reported in other aquatic species. For example, juvenile tiger prawn (*Penaeus monodon*) fed diets containing only fish meal resulted in significantly lower weight gain than a combination of fish meal, krill meal or squid meal (Simon et al., 2019). Likewise, juvenile spiny lobster (*Panulirus ornatus*) fed a diet primarily based on fish meal exhibited lower survival and biomass than those receiving a combination of fish meal and krill meal as the main protein sources (Marchese et al., 2019).

Bloodworm is recognised as an excellent protein source in aquaculture (Sulistiyarto & Restu, 2018). In shrimp culture, incorporating marine bloodworms (*Dendronereis* sp.) into the diet of *P. monodon* enhanced nutrient utilisation in faster biomass growth compared to treatments without marine bloodworms (Yong et al., 2004). Furthermore, introducing bloodworms into the diet of white-leg shrimp, *Penaeus vannamei*, has been associated with increased fecundity, higher hatching rate, and improved ovarian development in other crustacean species (Laufer et al., 1998). Despite the reported benefits of bloodworms in the diets of various crustacean species, their inclusion in megalopa diets did not enhance growth, development, or survival compared with other marine protein sources such as squid meal and scallop meal. In the present study, crabs fed a bloodworm meal diet (FM:BW) exhibited lower growth than the fishmeal-alone diet (FM). Although FI was the highest in the FM:BW group, the FCR was also the highest, indicating inefficient conversion of consumed feed into body weight gain. Moreover, despite having a gross energy content comparable to the other formulated diets, the FM:BW diet did not improve larval growth performance, suggesting that combining fish meal with bloodworm meal may not be optimal for promoting megalopa development.

The crab megalopa used in the present study were obtained from a single broodstock and reared under controlled laboratory conditions. Therefore, genetic and maternal factors such as egg quality, maternal nutrition, and broodstock health may have influenced the outcomes (Thien & Yong, 2017). Nevertheless, this study provides a foundational understanding of how different protein sources can be manipulated to enhance the growth and survival of megalopa. Optimising diet at this stage is a crucial aspect of megalopa rearing, as it directly contributes to their well-being under captivity. Maintaining consistent survival rates is essential for reliable crab seed production and the sustainability of the mud crab aquaculture industry. Future studies are recommended to evaluate these protein sources using multiple spawns of broodstock under farm-scale

conditions to validate their broader applicability in commercial settings.

Conclusion

In conclusion, it is preferable to combine fish meal with other marine protein sources, such as squid meal and scallop meal, into the diet formulation for the megalopa of *S. tranquebarica* rather than using fish meal as the primary protein source. These benefits are likely attributable to the high-quality protein provided by combining different protein sources.

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