Growth and Diet Utilization Efficiencies of Overwinter Juvenile Mud Crabs Fed with Different Diets

Shengping ZHONG[1], Yan JIANG[2], Changzi GE[3], Xiaohui CAI[4], Jianda SONG[3], Yonghong LIU[1], Lianghua HUANG[1], Yinhui PENG[4], Guoqiang HUANG[1*]

1. Guangxi Key Laboratory of Marine Medicine, Guangxi University of Chinese Medicine, Nanning 530200. China; 2. Guangxi Institute of Oceanology Co., Ltd., Beihai 536000, China; 3. Marine College, Shandong University at Weihai, Weihai 264209, China; 4. Guangxi Key Laboratory of Marine Biodiversity Conservation, Oceanography Institute, Beibu Gulf University, Qinzhou 535011, China

Abstract [**Objectives**] To study the utilization efficiency of different diets for juvenile *Scylla paramamosain*. [**Methods**] Low-value fish, shrimp, clam and clamworm were fed to overwintering young mud crabs, and the performance of the feed was evaluated by growth, feed utilization and energy allocation. [**Results**] Shrimp-fed mud crabs showed the best growth performance, while fish-fed mud crabs showed the worst growth performance and converted the least protein and energy for growth. Shrimp-fed mud crabs molted more frequently, and the dry matter mass and energy of Exuviation were significantly higher than those of fish-fed mud crabs. The shrimp-fed crabs also had significantly higher food intake than those fish-fed crabs. The order of feed conversion efficiency (FCE) of fish-fed mud crabs was Polychaete > clam > shrimp > fish, while the order of FCE calculated with FCE-P and FCE-E was Polychaete > clam > shrimp > fish. Fish-fed mud crabs had the least energy intake and the least energy for growth, molting, excretion, metabolism, and feces. Among the four treatments, the mud crabs fed on Polychaete had the largest proportion of energy used for growth, while the mud crabs fed on fish had the largest proportion of energy used for molting. The proportion of energy consumed by mud crabs fed on Polychaete was the smallest, which may be the main reason for the higher FCE-P and FCE-E of mud crabs fed on Polychaete. [**Conclusions**] The results of this experiment showed that the utilization of low-value fish by mud crabs was the worst, suggesting that fish should not be used as control or reference food when studying the formula feed of mud crabs.

Key words Mud crab (*Scylla paramamosain*), Natural feed, Growth, Feed conversion efficiency (FCE), Energy allocation

1 Introduction

Scylla serrata, *Scylla tranquebarica*, *Scylla paramamosain* and *Scylla olivacea* are the four most valuable mud crabs in the world. In their distribution areas, they are highly valued by fishing and aquaculture production[1]. *S. paramamosain* is the main mud crab distributed in the southeast coast of China, and it is also the most important cultured mud crab in China, and its cultured output accounts for more than 90% of the cultured output of mud crab in China[2]. *S. paramamosain* is also important predator in estuaries and mangrove areas. The diet of wild mud crabs is mainly composed of marine debris, bivalves, crustaceans, fish, *etc.*, and the importance of different food varies greatly in different places[1,3]. In aquaculture production, mud crabs are often fed with low-value fish leftovers (fish head, fish skin, broken fish, fish viscera, squid head, *etc.*), snails, bivalves, slaughter leftovers, shrimp head, shrimp feed, and so on[1,4–6].

Received: July 12, 2024 Accepted: October 20, 2024

Supported by Guangxi Natural Science Foundation (2017GXNSFAA198022); Guangxi Key R & D Program (Guike AB16380105); Open Fund of Guangxi Key Laboratory of Marine Biodiversity Conservation (2023KA01); Doctoral Initiation Funding Project of Guangxi University of Chinese Medicine (2018BS034).

Shengping ZHONG, associate researcher, research fields: marine animal germplasm resources.

* Corresponding author. Guoqiang HUANG, researcher, research fields: aquaculture ecology.

The southeast coast of China extensively carries out *S. paramamosain* cultivation, and crab seedlings can be provided all year round in the south of China. In Guangxi at the southern tip of China, the breeding of *S. paramamosain* can continue until winter, and then the crab seedlings are put into ponds for overwintering. Because of the warm winter along the coast of Guangxi, the water temperature in ponds is generally maintained above 10 ℃, but it may be reduced to the lowest 5 ℃ in case of cold wave. Overwintering mud crabs usually grow slowly and are uneven in size. Low-value fish, shrimp, bivalve and snails are usually used as diet for overwintering young crabs, but there is little research on the utilization of these fdiets by mud crabs, which may lead to slow growth of mud crabs and waste of diets, and consequently leading to the deterioration of pond environment. Therefore, it is necessary to investigate the growth, food utilization and energy allocation of overwintering mud crabs after feeding on different diets.

The parameters of energy metabolism are the basic parameters to evaluate the utilization of feeds for cultured crabs. Many experimental studies on bioenergetics have been carried out on *Portunus trituberculatus*[7–10], the most important maricultural crab in China, and *Eriocheir sinensis*[11–15], the most important freshwater cultured crab. The energy budget model of mud crab larvae has also been established[16–18], and Liu *et al.*[19–20] studied the response of energy metabolism of *S. paramamosain* crab larvae to temperature fluctuations. However, only Nguyen *et al.*[21] established the energy allocation model of juvenile *S. serrata* in the study on the effect of different fish meal and soybean protein ratios on the energy allocation of mud crabs. The energy budget of juvenile mud crab has rarely been reported, which may be due to the long molting cycle of mud crab and the easy fragmentation of feces, resulting in difficulties in collecting enough feces and molting samples, so it is necessary to have a longer period of experiments to establish the energy budget model of juvenile mud crabs.

In order to establish the energy budget model of the overwintering mud crab, *S. paramamosain*, and evaluate the utilization of different diets during the overwintering period, four different diets were used to feed the overwintering mud crab for 90 d, and the results will provide a reference for the selection of high-quality diets and the improvement of the breeding technology of overwintering mud crabs.

2 Materials and methods

2.1 Experimental facilities Sixty 50 cm × 40 cm × 40 cm plastic boxes were used as culture containers in the experiment. Before use, the plastic boxes were soaked in potassium permanganate for 6 h, then rinsed, and then 20 cm sand filtration seawater was added. An air stone was placed in each culture box, and air was continuously filled to maintain the dissolved oxygen content not less than 5.0 mg/L.

2.2 Seawater for experiment The seawater used in the experiment was filtered by three grades of sand and then disinfected by ultraviolet irradiation. The seawater salinity ranges from 26 to 30, the pH ranges from 7.9 to 8.3, and the dissolved oxygen content is not less than 5.0 mg/L.

2.3 Source and cultivation of juvenile crabs Juvenile crabs were bred from the 5^{th} stage of juvenile crabs provided by Guangxi Institute of Oceanography Co. Ltd. through standard rough culture. Two hundred round plastic buckets (with a volume of about 2 L) were used, 2 cm thick sand was laid at the bottom of the bucket, and then 1 L of water was added, and one juvenile crab of stage 5 was put into each bucket. The juvenile crabs were fed with sufficient prawn formula feed once a day, and the juvenile crabs with a body mass of about 1.5 g were cultured for 30 d, and healthy individuals were selected as experimental materials.

2.4 Diet preparation In recent years, low-value fish, shrimp, clam and Polychaete with large local production have been used as the main diet for mud crab farming. *Callionymus richardsoni*, *Penaeus latisulcatus*, *Ruditapes philippinarum*, Polychaete (*Perinereis aibuhitensis*) was used as the representative diet. Before the experiment, *R. philippinarum* and Polychaete were temporarily cultured in sand filtered seawater for 24 h, and then *C. richardsoni* was treated to remove fish heads, bones, scales and viscera, and shells were removed *R. philippinarum*. The shells and heads of the prawns of *P. latisulcatus* were removed. For Polychaete, took out and drained until no more water drips. And then subpackaged each diet into a sealed bag according to that daily feed requirement, and stored in a refrigerator at the temperature of −20 ℃. Before feeding, that diet which was fed on the same day was taken out from a refrigerator, and after the diet was thaw, the diet was cut into a proper size with scissors according to the require feeding amount and is fed after being weighed.

2.5 Experimental design Sixty culture boxes were randomly (by drawing lots) assigned to four diet treatments, with 15 replicates of each diet. After fasting for 24 h, 60 healthy and uniform individuals were selected from 200 juvenile crabs. After weighing and recording, one juvenile crab was put into each culture box for culture. During the experiment, the experiment of diet dissolution was designed. Six portions of each prepared diet were weighed directly (about 100 g each) and dried in an oven at 60 ℃ to constant weight. Then another 6 portions of diet were weighed and soaked in a sorting box with seawater for 22 h, then collected, soaked in distilled water for 15 min, washed three times with distilled water, dried in an oven at 60 ℃ to constant weight, and then stored in a dryer.

2.6 Mud crab farming The mud crabs are fed once a day at 16:00 in the afternoon, and the diets were thawed in advance and weighed before feeding. Changed 100% of the water 2 h before feeding every day. The body mass of mud crab was weighed once every 30 d.

2.7 Sample collection After each diet was prepared, 6 samples (about 100 g for each sample) were randomly selected, dried in an oven at 60 ℃ to constant weight, and then stored in a dryer. The residual bait in each culture box was collected 2 h before feeding every day, washed with purified water, drained and put into sealed bags, and stored in a refrigerator at −20 ℃. The residual diet samples were taken out every 30 d, dried in an oven at 60 ℃ to constant weight, and then stored in a dryer. Before changing the water every day, siphoned the feces of each breeding box onto a 80-mesh sieve, transferred the filtered feces to a beaker, and then stored them in a refrigerator at −20 ℃. After the experiment, all fecal samples were taken out and dried to constant weight in an oven at 60 ℃, and then stored in a dryer. At 8:00 am and 18:00 pm every day, checked the molting condition of each culture box. Once the molting was found, immediately took out the molted shell, washed it with purified water, sealed in a sealed bag for each culture box, made a record and stored in a refrigerator at −20 ℃. After the experiment, all the molting samples were taken out, counted and weighed, dried in an oven at 60 ℃ to constant weight, and then stored in a dryer.

Six samples were randomly collected from the remaining crude young mud crabs after the experimental objects were selected, each sample consisted of 15 juvenile mud crabs. After counting and weighing, they were stored in a refrigerator at −20 ℃, then taken out and dried in an oven at 60 ℃ to constant weight, and then stored in a dryer. After the experiment, after 24 h of fasting, each mud crab was taken as a sample, weighed and put into a sealed bag, and stored in a refrigerator at −20 ℃, then taken out and dried in an oven at 60 ℃ to constant weight, and then put into a dryer for storage.

2.8 Sample processing After the culture experiment, all the samples were dried to constant weight, and then the samples were crushed by a small electric crusher, and then sieved by a 80-mesh sieve, and stored in a dryer as samples to be tested.

2.9 Sample analysis The nitrogen content of the sample was determined by the Micro-Kjeldahl method and converted to the crude protein content of the sample by multiplying the nitrogen content by 6.25. The energy content of the samples was determined by PARR 6400 Bomb Calorimeter (PARR Instruments, USA), and the acid insoluble ash (AIA) content of the feed and feces was determined by the method of the literature[22]. Each sample was measured for 3 times, and the average value was taken as the measured value.

2.10 Data calculation The formulas for calculating the mass growth rate (*MGR*, %), the specific growth rate (*SGR*, %) and the food conversion efficiency (*FCE*, %) of the mud crabs were as follows:

$$MGR = 100 \times (M_{t2} - M_{t1}) / M_{t1}$$

$$SGR = 100 \times ln\ (M_{t2}/M_{t1}) / (t_2 - t_1)$$

$$FCE = 100 \times (M_{t2} - M_{t1}) / I$$

where t_1 and t_2 represent different experimental time points, M_{t1} and M_{t2} represent the body mass of mud crabs at the corresponding time points, and I represents the food intake during the period from t_1 to t_2. In this experiment, *MGR*, *SGR* and *FCE* expressed by dry matter, protein and energy were calculated according to the crude protein content and energy content of the samples, which were expressed as *MGR-D*, *MGR-P*, *MGR-E*, *SGR-D*, *SGR-P*, *SGR-E*, *FCE-D*, *FCE-P* and *FCE-E*, respectively. The relevant parameters in the energy budget formula were calculated according to the method of the literature[23]:

$$IE = Id \times GEd$$

$$GRE = FCe - ICe$$

$$FE = Fd \times GEf$$

$$EE = Ed \times GEe$$

$$UE + ZE = (UN + ZN) \times 24.83$$

$$ME = IE - GRE - FE - EE - (UE + ZE)$$

where *IE*, *GRE*, *FE*, *EE*, *UE + ZE* and *ME* denote ingestion energy, growth energy, fecal energy, molting energy, excretion energy and metabolic energy, respectively. *Id*, *Fd*, *Ed*, *GEd*, *GEf* and *GEe* represent the amount of dry matter ingested, the amount of dry matter in feces, the amount of dry matter in molting, the energy content of food, the energy content of feces, and the energy content of molting, respectively. *FCe* and *ICe* represent the energy of the mud crabs at the end of the experiment and the energy of the mud crabs at the beginning of the experiment. *UN + ZN* represents the nitrogen excreted through urine and gills, while *UE + ZE* represents the energy loss of nitrogen excretion. The energy loss of 1 g nitrogen excretion was calculated according to the parameter 24.83 KJ given by Elliott[24]. The calculation method of *UN + ZN* was based on the literature[16] and[25]:

$$UN + ZN = I_N - G_N - F_N - E_N$$

where *IN*, *GN*, and *EN* separately denote ingestion nitrogen, growth nitrogen, excretion nitrogen and molting nitrogen. The calculation method of *FN* and *FE* was as follows:

$$F_N = I_N \times (100 - DR_N) / 100$$

$$F_E = I_E \times (100 - DR_E) / 100$$

where DR_N and DR_E represent the apparent digestibility of nitrogen and energy of mud crabs to diets, and they can be determined by acid-insoluble ash method[22]:

$$DR_N = 100 \times [1 - (N_{feces}/N_{diet}) \times (AIA_{diet}/AIA_{feces})]$$

$$DR_E = 100 \times [1 - (GE_{feces}/GE_{diet}) \times (AIA_{diet}/AIA_{feces})]$$

where N_{feces} and N_{diet} represent the nitrogen content of the feces and diet, GE_{feces} and GE_{diet} represent the energy content of the feces and diet, and AIA_{diet} and AIA_{feces} represent the acid-insoluble ash content of the diet and feces.

2.11 Statistical analysis The statistical analysis of the data was completed by SPSS 11.5 statistical analysis software. The body mass, molting related indicators, energy consumption, energy for growth, energy for molting, energy for feces, and metabolic energy of mud crabs fed with different diets were compared by one-way ANOVA, and the differences among treatments were analyzed by Duncan's multiple comparisons. For the percentage data of body mass growth rate, specific growth rate, feed conversion efficiency, and the distribution ratio of feeding energy in growth, molting, feces, excretion, and metabolism expressed in percentage, arcsine ($\sin^{-1}\sqrt{x}$) transformation was performed before variance analysis. When the data were in line with normal distribution and the variance of each group was the same, one-way analysis of variance was used, and $P < 0.05$ was taken as the standard of significant difference. Kruskal – Wallis test was carried out for non-parametric statistical analysis if the variance of the data was not homogeneous, and $P < 0.05$ was used as the criterion for significant difference.

3 Results and analysis

3.1 Water temperature and salinity The experiment was conducted from October 2019 to April 2020, with water temperature ranging from 13.8 ℃ to 28.1 ℃ and salinity ranging from 25.5 to 29.0 (Fig. 1).

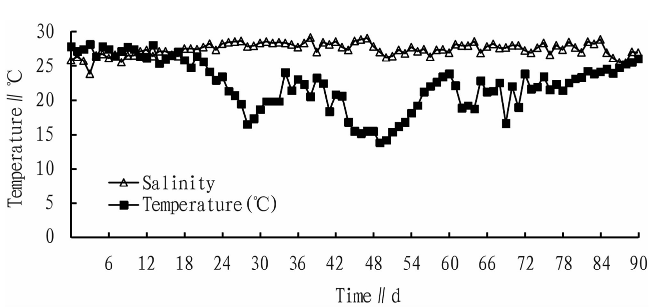

Fig. 1 Water temperature and salinity

3.2 Growth of juvenile mud crabs The changes in body mass of mud crabs fed with different diets were shown in Table 1. At the end of the experiment, the body mass of the mud crabs fed with fish was significantly smaller than that of the mud crabs fed with the other three diets, and was also smaller than that of the other mud crabs during the whole experiment. The growth performance of the mud crabs fed with shrimp was the best. During the 61 – 90 d of the experiment, the body mass of the mud crabs fed with clam decreased. When the body mass of mud crabs was converted into dry matter, protein and energy, the value of mud crabs fed with fish was much lower than that of mud crabs fed with shrimp, clam and polychaete at the end of the experiment (Table 2).

Table 1 Changes in body mass (g) of crabs fed with different diets

Diet	0 d	30 d	60 d	90 d
Fish	1.40 ± 0.10^{a}	2.88 ± 0.22^{a}	3.71 ± 0.26^{a}	3.95 ± 0.23^{a}
Shrimp	1.43 ± 0.08^{a}	4.37 ± 0.51^{b}	6.75 ± 0.89^{b}	7.85 ± 0.96^{b}
Clam	1.54 ± 0.09^{a}	4.30 ± 0.46^{b}	6.47 ± 0.44^{b}	6.19 ± 0.44^{b}
Polychaete	1.44 ± 0.09^{a}	4.09 ± 0.45^{b}	5.88 ± 0.35^{b}	6.10 ± 0.25^{b}

NOTE Values without same letter in the same cluster were significantly different from each other ($P < 0.05$). The error bars mean SE ($n = 14$). The same in Table 2.

The differences in the body mass growth rate (*MGR*) and specific growth rate (*SGR*) of mud crabs fed with different diets showed similar trends with body mass (Table 3 and Table 4). During the period of 61 – 90 d, the *MGR* and *SGR* of mud crabs fed with clam decreased to below 0. *MGR* and *SGR* of mud crabs fed with shrimp were the highest, and *MGR* and *SGR* of mud crabs fed with fish were the lowest. When *MGR* and *SGR* were converted into dry matter, protein and energy, the order of *MGR* and *SGR* of mud crabs fed with different diets was consistent with that expressed by body mass.

Table 2 Wet matter, dry matter, protein and energy content of individual crab at the end of experiment

Diet	Wet mass//g	Dry mass//g	Protein//g	Energy//KJ
Fish	3.95 ± 0.23^{a}	0.85 ± 0.07^{a}	0.30 ± 0.02^{a}	7.13 ± 0.56^{a}
Shrimp	7.87 ± 0.96^{b}	2.53 ± 0.34^{b}	0.89 ± 0.12c	24.14 ± 3.12^{b}
Clam	6.19 ± 0.44^{b}	1.84 ± 0.18^{b}	0.59 ± 0.07^{b}	19.00 ± 2.05^{b}
Polychaete	6.10 ± 0.25^{b}	1.94 ± 0.09^{b}	0.72 ± 0.03^{bc}	22.06 ± 1.03^{b}

Table 3 Body mass gain rate of wet mass of mud crabs fed with different diets in different periods (*MGR*, %)

Diet	Fish	Shrimp	Clam	Polychaete
MGR1-30	116.68 ± 10.80^{a}	209.94 ± 23.84^{b}	184.39 ± 18.42^{b}	191.49 ± 20.71^{b}
MGR31-60	31.49 ± 8.23^{a}	62.28 ± 13.40^{b}	62.88 ± 11.36^{b}	56.72 ± 12.28^{b}
MGR61-90	9.67 ± 6.37^{ab}	20.52 ± 10.04^{b}	–4.39 ± –1.06^{a}	7.32 ± 6.14^{ab}
MGR1-90	194.49 ± 19.93^{a}	458.79 ± 64.73c	311.06 ± 28.87^{ab}	337.56 ± 20.78^{bc}
MGRd1-90	200.83 ± 22.24^{a}	773.17 ± 108.68c	498.85 ± 62.97^{b}	578.27 ± 38.25^{bc}
MGRp1-90	246.63 ± 26.32^{a}	889.72 ± 126.85c	534.68 ± 83.26^{b}	715.54 ± 47.19^{b}
MGRe1-90	160.43 ± 20.63^{a}	752.87 ± 103.70^{b}	538.27 ± 73.70^{b}	689.11 ± 43.72^{b}

NOTE The body mass gain rate (*MGR*, %) of mud crabs fed with different diets (MGR-W, MGR-D, MGR-P, MGR-E mean MGR was calculated in terms of wet mass, dry mass, protein, and energy respectively). Values without same letter in the same column were significantly different from each other ($P < 0.05$). The error bars mean SE ($n = 14$). The same below.

Table 4 Specific growth rates of mud crabs at different experimental stages (*SGR*, %)

Diet	Fish	Shrimp	Clam	Polychaete
SGR1-30	2.40 ± 0.21^{a}	3.53 ± 0.27^{b}	3.27 ± 0.32^{b}	3.34 ± 0.33^{b}
SGR31-60	0.84 ± 0.20^{a}	1.46 ± 0.30^{b}	1.51 ± 0.25^{b}	1.35 ± 0.28^{b}
SGR61-90	0.25 ± 0.18^{ab}	0.50 ± 0.25^{b}	–0.15 ± 0.04^{a}	0.18 ± 0.16^{ab}
SGR1-90	1.16 ± 0.09^{a}	1.83 ± 0.12^{b}	1.54 ± 0.06^{b}	1.63 ± 0.05^{b}
SGRd1-90	1.19 ± 0.08^{a}	2.29 ± 0.15c	1.92 ± 0.11^{b}	2.11 ± 0.06^{bc}
SGRp1-90	1.34 ± 0.08^{a}	2.43 ± 0.15c	1.94 ± 0.14^{b}	2.31 ± 0.06^{bc}
SGRe1-90	1.02 ± 0.09^{a}	2.28 ± 0.14^{b}	1.97 ± 0.12^{b}	2.27 ± 0.06^{b}

NOTE The specific growth rate (*SGR*, %) of mud crabs fed with different diets (SGR-W, SGR-D, SGR-P, SGR-E mean SGR was calculated in terms of wet mass, dry mass, protein, and energy, respectively).

3.3 Effects of different diets on molting The average molting frequency of mud crabs fed with shrimp was significantly higher than that of mud crabs fed with fish, but the average molting frequency of mud crabs fed with fish was not significantly lower than that of mud crabs fed with clam and polychaete. The amount of dry matter and energy of mud crabs fed with shrimp was significantly more than that of mud crabs fed with fish, while the amount of dry matter and energy of mud crabs fed with polychaete was only slightly higher than that of mud crabs fed with fish.

Table 5 Molting of mud crabs fed with different diets

Diet	Molting frequency	Dry matter of exuviations//g	Energy of exuviations//KJ
Fish	1.85 ± 0.10^{a}	0.37 ± 0.04^{a}	1.09 ± 0.12^{a}
Shrimp	2.38 ± 0.14^{b}	0.94 ± 0.13c	2.28 ± 0.30^{b}
Clam	2.07 ± 0.07^{ab}	0.65 ± 0.07^{b}	1.68 ± 0.17^{ab}
Polychaete	2.07 ± 0.07^{ab}	0.61 ± 0.02^{ab}	1.38 ± 0.05^{a}

3.4 Food intake of mud crabs The crabs fed with shrimp had the largest food intake, followed by the crabs fed with Polychaete, and the crabs fed with fish had the smallest food intake. The order was the same at different experimental stages, and the order of food intake did not change after converting food intake into dry matter, protein and energy.

Table 6 Food intake of mud crabs at different experimental stages

Diet	Fish	Shrimp	Clam	Polychaete
FI1-30	3.65 ± 0.33^{a}	12.98 ± 1.19^{c}	8.88 ± 0.51^{b}	8.42 ± 0.66^{b}
FI31-60	1.86 ± 0.30^{a}	7.47 ± 0.95^{c}	6.48 ± 0.72^{b}	4.63 ± 0.55^{b}
FI61-90	0.48 ± 0.06^{a}	5.75 ± 1.10^{c}	2.12 ± 0.56^{b}	2.19 ± 0.33^{b}
FI1-90	5.99 ± 0.52^{a}	26.19 ± 2.72^{c}	17.48 ± 1.51^{b}	15.24 ± 0.68^{b}
FId	1.14 ± 0.10^{a}	4.78 ± 0.49^{c}	3.38 ± 0.29^{b}	3.21 ± 0.14^{b}
FIp	0.79 ± 0.07^{a}	3.33 ± 0.35^{c}	2.08 ± 0.18^{b}	2.08 ± 0.09^{b}
FIe	21.63 ± 1.89^{a}	88.60 ± 9.16^{c}	66.53 ± 5.71^{b}	61.24 ± 2.72^{b}

3.5 Feed conversion efficiency Except at the stage of 31 – 60 d, the highest feed conversion efficiency (FCE) of fresh weight was obtained by feeding mud crabs with fish, and the greatest difference of FCE occurred at the stage of 61 – 90 d among mud crabs fed with different diets. When converted into dry matter, protein and energy, the dry matter conversion efficiency (FCE-D) of crab fed polychaete was slightly higher than that of other diets. The order of protein conversion efficiency (FCE-P) and energy conversion efficiency (FCE-E) was polychaete > clam > shrimp > fish.

Table 7 Feed conversion efficiency of mud crab to different diets at different experimental stages

Diet	Fish	Shrimp	Clam	Polychaete
FCE1-30	40.45 ± 4.83^{b}	21.35 ± 8.24^{a}	30.40 ± 7.26^{ab}	29.37 ± 7.80^{ab}
FCE31-60	36.51 ± 2.10^{a}	30.89 ± 6.39^{a}	39.08 ± 10.12^{a}	36.83 ± 5.81^{a}
FCE61-90	24.48 ± 3.87^{b}	8.39 ± 7.39^{ab}	–7.36 ± –5.39^{a}	4.30 ± 1.35a^{b}
FCE1-90	46.36 ± 2.65^{b}	24.13 ± 6.56^{a}	27.18 ± 7.39^{a}	30.80 ± 5.23^{a}
FCEd	49.86 ± 3.85^{a}	45.44 ± 3.71^{a}	44.75 ± 3.00^{a}	51.95 ± 2.18^{a}
FCEp	21.45 ± 1.70^{a}	23.21 ± 2.08^{a}	26.77 ± 1.88^{ab}	29.68 ± 1.31^{b}
FCEe	20.04 ± 1.61^{a}	23.64 ± 2.15^{a}	23.70 ± 1.84^{a}	31.69 ± 1.37^{b}

3.6 Moisture, crude protein and energy content of mud crabs Compared with crabs fed with shrimp, clam and polychaete, crabs fed with fish had the highest moisture and the lowest protein and energy content at the end of the experiment, indicating that crabs fed with fish had less protein and energy stored in their bodies.

Table 8 Contents of body water, crude protein and energy of mud crabs fed with different diets

Diet	Fish	Shrimp	Clam	Polychaete
Moisture//%	78.41 ± 1.36^{b}	67.80 ± 2.52^{a}	70.35 ± 2.04^{a}	67.93 ± 1.22^{a}
Protein//%	7.71 ± 0.49^{a}	11.28 ± 0.89^{b}	9.69 ± 0.98^{ab}	11.95 ± 0.48^{b}
Energy//KJ/g	1.82 ± 0.12^{a}	3.10 ± 0.28^{b}	3.07 ± 0.25^{b}	3.64 ± 0.14^{b}

3.7 Energy budget of mud crabs and its distribution ratio During the whole experimental period, the energy intake for growth, molting, excretion, metabolism and feces of mud crabs fed with fish was the lowest, and the corresponding indicators of mud crabs fed with shrimp were the highest, while the corresponding indicators of mud crabs fed with clam and polychaete were between them, and there was no significant difference between them. The distribution of feeding energy among different activities is shown in Table 9. The proportion of energy used for growth ranged from 20.04% to 30.69%, and the proportion of energy used for growth of mud crabs fed with polychaete was significantly higher than that of other diets. The proportion of energy loss in exuviation of mud crabs fed with fish was significantly higher than that of the other three diets. The energy of metabolism in mud crabs fed with polychaete was significantly lower than that of the other three diets.

Table 9 Energy budget of mud crabs

Item	Diet	Ingestion	Growth	Exuviation	Feces	Excretion	Metabolism
Energy budget//KJ	Fish	21.63 ± 1.89^{a}	4.32 ± 0.50^{a}	1.09 ± 0.12^{a}	1.93 ± 0.17^{a}	1.22 ± 0.11^{a}	13.07 ± 1.21^{a}
	Shrimp	88.60 ± 9.16c	21.27 ± 3.09^{b}	2.28 ± 0.30c	12.44 ± 1.27c	4.71 ± 0.45c	47.91 ± 4.98^{c}
	Clam	66.53 ± 5.71^{b}	15.91 ± 2.03^{b}	1.68 ± 0.17^{b}	6.62 ± 0.71^{b}	3.00 ± 0.27^{b}	39.32 ± 3.21^{bc}
	Polychaete	61.24 ± 2.72^{b}	19.18 ± 0.93^{b}	1.38 ± 0.05^{b}	8.69 ± 0.45^{b}	2.66 ± 0.16^{b}	29.34 ± 1.86^{b}
Energy allocation//%	Fish	100	20.04 ± 1.61^{a}	5.37 ± 0.64^{b}	8.99 ± 0.22^{a}	5.63 ± 0.16^{b}	59.98 ± 1.74^{b}
	Shrimp	100	23.64 ± 2.15^{a}	2.66 ± 0.27^{a}	13.87 ± 0.38^{b}	5.41 ± 0.19^{b}	54.43 ± 2.02^{b}
	Clam	100	23.70 ± 1.84^{a}	2.56 ± 0.13^{a}	9.83 ± 0.45^{a}	4.51 ± 0.15^{a}	59.40 ± 1.40^{b}
	Polychaete	100	31.69 ± 1.37^{b}	2.30 ± 0.12^{a}	14.13 ± 0.21^{b}	4.33 ± 0.11^{a}	47.55 ± 1.31^{a}

4 Discussion

4.1 Effects of different diets on the growth of overwintering mud crabs A variety of different diets are used in the production of mud crabs[2,4–6]. In the mud crab culture ponds near the mangrove area, low-value fish and bivalve are used as the main food[6], while for young mud crabs, live brine shrimp, minced fresh shrimp or tilapia can be used as better diets[27], leading to significant differences in growth performance in different studies. The growth of *S. paramamosain* fed with razor clam (*Sinonovacula constricta*) was significantly faster than that of silver carp (*Hypophthalmichthys molitrix*)[28–29]. Mud crabs fed with formula feed also grew faster than those fed with low-value fish[28–30]. In studies of other crabs, the growth rate of *Portunus trituberculatus* fed with *Ruditapes philippinarum* was significantly faster than that of the crabs fed with a goby (*Acanthogobius flavimanu*)[31], and the growth rate of *Eriocheir sinensis* fed with formula feed was also faster than that of the crabs fed with fresh trash fish[12,32]. In this experiment, the mud crabs fed with fish showed the worst growth performance. These results indicate that low-value fish is not a good diet for mud crabs, and similar results are found in prawns[33–34]. Therefore, low-value fish should not be used as the main bait for mud crab culture.

The dietary protein and lipid requirements of cultured crabs ranged from 35% – 50% and 5% – 10%, respectively[34]. In the study of nutritional requirements and feed of mud crabs, it is found that mud crabs have high protein and lipid requirements, and properly increasing the lipid content of feed can promote the growth of mud crabs[2]. In a series of studies on the nutritional requirements of juvenile *S. paramamosain*, it was found that the optimum protein content in the diet was 49.03%[36], and the optimum lipid content was 8.14%[7]. Another study considered that the suitable lipid content of *S. paramamosain* diet was 8.52% – 11.63% (the best was 9.5%), which could promote the good growth of mud crabs[37]. For *S. paramamosain*, increasing the lipid content above 10% does not further promote growth[2]. Natural diets usually have a high protein content (generally more than 60%), but fish meat generally has a low lipid content (usually less than 3%)[28-29,34]. Clearly, the lower lipid content relative to the nutritional requirements of *S. paramamosain* may be a major disadvantage of low value fish. In this experiment, in addition to polychaete containing more lipid (8.71 - 12.49%)[34,38-40], the lipid content of shrimp and clam was also lower (less than 5%)[34], but the growth of mud crabs fed with shrimp and clam was also better than that of mud crabs fed with fish and polychaete. The higher optimal lipid content in the formula feed may be due to the fact that the protein in the formula feed is lower than that in the natural animal diet, generally not more than 55%[2,36,41], in which case the increase in lipid content may have a protein-saving effect.

Natural diets such as low-value fish, clams and shrimps are usually used as comparative baits when evaluating the formula feed of crabs. The results of these studies show that low-value fish is not a good natural diet, and other studies have found that clams, shrimps and polychaetes are better natural diets[7,28-29,34]. Therefore, the breeding effect of some formula feeds is better than that of low-value fish, but it is not enough to prove that these formula feeds are good formula feeds. Thus, a better reference natural diet should be used in the evaluation of formula feeds. *Fenneropenaeus chinensis* will show differences in molting related indicators when ingesting different diets. Huang *et al.* [42] believed that natural diets such as shrimp, clam and polychaete are rich in sterols, which can promote the synthesis of ecdysone. Therefore, the molting frequency of *F. chinensis* fed with natural diet was higher than that of shrimp fed with formulated diet. Similar to *F. chinensis*, the molting frequency of *S. paramamosain* fed with shrimp, clam and worm was also higher than that of mud crabs fed with fish.

4.2 Utilization of different diets by mud crab The diets of wild mud crabs mainly include marine debris, bivalves, crustaceans, fish, *etc.* The importance of different diets is related to the location[2]. In this experiment, under the condition of no food selection and sufficient supply of each food, the intake of mud crabs fed with fish was significantly less, while the intake of shrimp was the largest. However, in the study of *F. chinensis*[35] and *Portunus trituberculatus*[7], the feeding rate of the treatment fed with low-value fish was higher, and the difference in feeding of different shrimps and crabs on natural food may reflect the difference in food preference among species. In the pond culture of green crabs, low-value fish are scattered into the pond without corresponding feeding inspection, which may lead to excessive diet and deterioration of pond water quality.

Compared with prawns, mud crabs eat food by holding the diet[1] with their feet, which leads to a lot of debris and waste of feed. This may lead to the difficulty of collecting residual food in the experiment, so there are few studies on the food utilization efficiency of mud crab. The feed conversion factor of juvenile *S. serrata* ranges from 1.2 to 2.1[2], but the feed conversion factor is not a parameter suitable for expressing the utilization efficiency of ingested feed for *S. serrata*, but an index for evaluating feed input and output of *S. serrata*, and the feed conversion efficiency is more suitable for evaluating the utilization efficiency of different feeds. Different diets cause significant changes in feed conversion efficiency of shrimps and crabs[11-12,31,34-35]. In this experiment, the *S. paramamosain* fed fish obtained the best feed conversion efficiency in terms of fresh matter, which is different from the *P. trituberculatus* fed fish which obtained the worst feed conversion efficiency[31]. In *Eriocheir sinensis*, better feed conversion efficiency can be obtained by feeding with fish than with formula feed[12].

When the feed conversion efficiency was converted into dry matter, protein and energy, the order was obviously different from that calculated by fresh matter mass, which was obviously different from the previous research results of prawns and other crabs[11-12, 31, 33, 35]. Body composition data showed that *S. paramamosain* fed to fish had a high moisture content and a low dry matter, protein, and energy content, resulting in the lowest feed conversion efficiency when converted to dry matter, protein, and energy, although the mud crab fed to fish had the highest feed conversion efficiency in terms of fresh matter. These results indicate that fish as food can maintain the survival of mud crabs, but it can't support the growth and nutrient accumulation of mud crabs.

4.3 Energy allocation Bioenergetics research plays an important role in aquaculture[17], but the energy budget of mud crab and its distribution among different physiological activities are still rare. Previous studies have established the energy budget model of *S. paramamosain* larvae[16,18] and studied the effects of temperature on oxygen consumption rate and ammonia excretion rate of *S. serrata* zoea larvae[43]. Bioenergetics research on some crustaceans with important economic value, such as *F. chinensis*, *E. sinensis* and *P. trituberculatus*, shows that different diets can change the distribution mode of ingestion energy[12,31,33]. The results of this study show that when the *S. paramamosain* was fed with fish, the ingestion energy was the least, and the energy allocated to growth, Exuviation, feces, excretion and metabolism was also the least, while when the *P. trituberculatus* was fed with fish, the ingestion energy was the largest, and more energy was allocated to exuviation and metabolism[31]. Different diets can also change the proportion of energy allocated to crabs in different activities[11-12, 31]. When crabs were fed with fish, 25.00% of the energy was used for growth, while only 8.53% of the energy is used for growth when crabs are fed with formula feed[12]; the proportion of energy devoted to growth is significantly lower when *P. trituberculatus* was fed with fish than when feeding with shrimp and clams[31]. In this experiment, the proportion of growth energy of *S. paramamosain* fed with fish was lower than that fed with shrimp, clam and polychaete, while the proportion of exuviation energy loss was higher than that fed with other diets.

The results of bioenergetics in this experiment indicated that low value fish was not a good diet for *S. paramamosain*, which would lead to low diet consumption of mud crab and a high proportion of energy loss during molting and metabolism. Feed quality has a significant impact on the long-term energy accumulation of *Callinectes sapidus*[44], so the proportion of energy used for growth of mud crabs feeding on fish is smaller, and the feed conversion efficiency is also lower. In this study, polychaete was found to be a very good diet. Although the intake of polychaete was only moderate among the four diets, the proportion of energy lost in metabolism was small, so there was more energy to support the growth performance of mud crabs.

Digestive enzyme gene analysis of crabs showed that herbivorous crabs enhanced their ability to digest carbohydrates, while carnivorous crabs acquired a stronger ability to digest protein and lipid[45]. Previous studies have shown that *S. paramamosain*, *P. trituberculatus* and *E. sinensis* support this conclusion. In the juvenile stage of *S. paramamosain*, the activity of protease increased with the increase of the protein content of the compound diet[36], and the activity of protease and lipase reached the peak when the lipid content of the compound diet was 8.14%[7]. The protease and lipase activities of *S. paramamosain* fed with low-value fish and *S. constricta* were significantly higher than those fed with formula feed[29]. In this experiment, the ratio of energy lost by feces to ingested energy by *S. paramamosain* ranged from 8.99% to 14.13%, which indicated that the digestion and absorption rates of the four natural foods were relatively high. Fecal energy loss of *P. trituberculatus* ranged from 3.26% to 4.73% when feeding on fish, shrimp and clam[31], which means higher digestibility. The apparent digestibility of *E. sinensis* was also higher when fed fish than when fed formula feed, and the proportion of energy lost in feces was significantly lower[12]. These results indicate that the cultured crabs such as *S. paramamosain*, *P. trituberculatus* and *E. sinensis* are typical carnivores, and therefore, the diets suitable for crab culture should be high in protein and lipid.

5 Conclusions

Through establishing the energy allocation model of four kinds of food taken by juvenile *S. paramamosain*, we analyzed the effects of different food on the growth, molting, feeding, conversion efficiency and bioenergetics indexes of juvenile *S. paramamosain*. It was found that less consumption of low-value fish by mud crabs may lead to more food wastage, and mud crabs had lower conversion efficiency of dry matter, protein and energy after consuming low-value fish, while shrimp, clam and polychaete had higher consumption and better performance in growth and energy indicators. Low-value fish can not support the growth and nutrient storage of *S. paramamosain*, so it should not be used as the main food of mud crab culture. Low-value fish should not be used as reference feed in the research and development of formula feed for mud crabs.

References

[1] SHELLEY C, LOVATELLI A. Mud crab aquaculture: A practical manual [M]. FAO Fisheries and Aquaculture Technical Paper. 2011, No. 567. Rome, FAO.

[2] WANG GZ, LI SJ, CHEN ZG. Status of mud crabs (*Scylla* spp.) Farming and studies on the population biology of *S. paramamosain*[J]. Journal of Xiamen University, 2016, 55(5): 617-623. (in Chinese).

[3] VISWANATHAN C, RAFFI SM. The natural diet of the mud crab *Scylla olivacea* (Herbst, 1896) in Pichavaram mangroves, India[J]. Saudi Journal of Biological Sciences, 2015, 22: 698-705.

[4] MWALUMA J. Pen culture of the mud crab *Scylla serrata* in Mtwapa Mangrove System, Kenya[J]. Western Indian Ocean Journal of marine Sciences, 2002, 1(2): 127-133.

[5] SHEN BQ, CHEN JM, YE JY, *et al.* Effects of various substrates, stocking density and diets on cage nursing of instar *Scylla paramamosain*[J]. Journal of Zhejiang Ocean University (Natural Science), 2009, 28(4): 461-464. (in Chinese).

[6] GUO JM, LIN GH. Stable isotope compositions of wild and farmed *S. saramoamosion* and its implication for tracebility[J]. Journal of Isotopes, 2014, 27(1): 1-7.

[7] XU MZ, ZHANG Q, DONG LF, *et al.* Effects of dietary lipid level on growth performance, body composition and digestive enzyme activities of juvenile *Scylla paramamosain*[J]. Chinese Journal of Animal Nutrition, 2017, 29(3): 987-994. (in Chinese).

[8] WANG F, WANG X, LIU H, *et al.* Effects of light color on growth and energy budget of swimming crab[J]. Periodical of Ocean University of China, 2014, 44(11): 25-29. (in Chinese).

[9] LI YS, LIU JJ, SU XP, *et al.* Effect of dissolved oxygen on agonistic behavior and energy metabolism of the swimming crab (*Portunus trituberculatus*)[J]. Journal of Fishery Sciences of China, 2021, 28(10): 1311-1318. (in Chinese).

[10] ZHAO MM, YUAN Y, JIN M, *et al.* 2021. Influence of dietary calcium to phosphorus ratio on growth, minerals bioaccumulation and energy metabolism in juvenile swimming crab (*Portunus trituberculatus*)[J]. Journal of Fisheries of China, 2021, 45(10): 1677-1691. (in Chinese).

[11] ZHOU HX, LIU CQ, WANG JX, *et al.* Effects of dietary fat on the energy budget and growth of *Eriocheir sinensis*[J]. Hebei Fisheries, 2009, 9: 4-7. (in Chinese).

[12] LI HY, WU LX, CHEN W, *et al.* Effects of feeding frequency and food type on growth and energy budget of juvenile Chinese mitten crab *Eriocheir sinensis*[J]. Journal of Dalian Ocean University, 2013, 28(5): 475-480. (in Chinese).

[13] MAGDALENA J, MONIKA N. Effect of temperature on the physiology and bioenergetics of adults of the Chinese mitten crab *Eriocheir sinensis*: Considerations for a species invading cooler waters[J]. Marine and Freshwater Behaviour and Physiology, 2014, 44(3): 171-183.

[14] BAO J, LI XD, YU H, *et al.* Respiratory metabolism responses of Chinese mitten crab, *Eriocheir sinensis* and Chinese grass shrimp, Palaemonetes sinensis, subjected to environmental hypoxia stress[J]. Frontiers in Physiology, 2018(9): 1-8.

[15] LI CB, SHEN CC, FENG GP, *et al.* Effects of different water velocities on the energy metabolism of parent *Eriocheir sinensis* under starvation [J]. Freshwater Fisheries, 2023, 53(6): 52-57. (in Chinese).

[16] ZHU XM, LI SJ. Studies on the energy budget of the larvaes of *Scylla serrata* (Forskal). In: Abstracts of 2000 Symposium of the Branch of Crustacean in Oceanographic and Limnological Society of China, Chinese Zoological Society, Ecology Branch of Oceanographic and Limnological Society of China. 2000, Chengdu, Sichuan, China. Pp: 69. (in Chinese).

[17] ZHU XM, YE L, LI SJ. Bioernegetics and some recent progress in studies of decapod crustaceans[J]. Journal of Xiamen University (Natural Sciences), 2006, 45(Sup. 2): 162-169. (in Chinese).

[18] ZHANG SF, JIANG KJ, GU XL, *et al.* Effects of light density on growth and energy budget of the larvae of *Scylla paramamosain*[J]. Marine Fishery, 2011, 33(2): 187-194. (in Chinese).

[19] LIU JH, CHEN SJ, REN ZM, *et al.* Effects of diurnal temperature fluctuations on growth performance, energy metabolism, stress response, and gut microbes of juvenile mud crab *Scylla paramamosain*[J]. Frontiers in Marine Science, 2022, 9: 1076929.

[20] LIU JH, SHI C, YE YF, *et al.* Effects of temperature on growth, molting, feed intake, and energy metabolism of individually cultured juvenile mud crab *Scylla paramamosain* in the recirculating aquaculture system [J]. Water, 2022, 14: 2988.

[21] NGUYEN NTB, WANTIEZ L, LEMAIRE P, *et al.* Feed efficiency, tissue growth and energy budget changes during the molting cycle of juvenile mud crab, *Scylla serrata*: Effects of dietary proteins, fishmeal versus

soy protein concentrate[J]. Fishes, 2022, 7: 334.

[22] ATKINSON JL, HILTON JW, SLINGER SJ. Evaluation of acid-insoluble ash as an indicator of feed digestibility in Rainbow Trout (*Salmo gairdneri*)[J]. Canadian Journal of Fisheries and Aquatic Sciences, 1984, 41(9): 1384–1386.

[23] KLEIN BRETELER WCM. Food consumption, growth and energy metabolism of juvenile shore crabs, Carcinus maenas[J]. Netherlands Journal of Sea Research, 1975, 9: 255–272.

[24] ELLIOTT JM. Energy loss in the waste products of brown trout (Salmo trutta L.)[J]. Journal of Animal Ecology, 1976, 45(2): 561–580.

[25] LEVINE DM, SULKIN SD. Partitioning and utilization of energy during the larval development of the xanthid crab, *Rithropanopeus harrisii* (Gould)[J]. Journal of Experimental Marine Biology and Ecology, 1979, 40: 247–257.

[26] LEMOS D, PHAN VN. Energy partitioning into growth, respiration, excretion and exuviae during larval development of the shrimp *Farfantepenaeus paulensis*[J]. Aquaculture, 2001, 199: 131–143.

[27] VU NU LEWIS LV, TRUONG TN, *et al.* Development of nursery culture techniques for the mud crab *Scylla paramamosain*[J]. Aquaculture Research, 2007, 38: 1563–1568.

[28] CAI YL, ZHANG LB, HU GY, *et al.* Effects of different diets on growth performance and environmental water quality of *Scylla paramamosain* cultured in carb apartment[J]. Acta Agriculturae Zhejiangensis, 34(11): 2404–2415. (in Chinese).

[29] SU GS, XIAO GQ, CAI JB, *et al.* Effects of different diets on growth performance, enzyme activities and biochemical indexes of *Scylla paramamosain*[J]. Transactions of Oceanology and Limnology, 2022, 44(2): 41–48.

[30] ZHOU F, PENG J, CHEN LZ, *et al.* Evaluation of the effects of formulated diet and trash fish diet on the aquaculture of mud crab, *Scylla paramamosain*[J]. Journal of Fisheries Research, 2020, 42(4): 356–365. (in Chinese).

[31] XU Y, MA S. Effects of Different Diets on the Growth and Bioenergetics of Swimming Crab *Portunus Trituberculatus*[J]. Periodical of Ocean Universityof China, 2009, 39(Sup.): 353–358. (in Chinese).

[33] HUANG GQ, DONG SL, WANG F. Effects of different combinations of diets on the growth and food conversion efficiency of Chinese shrimp, *Fenneropenaeus chinensis*[J]. South China Fisheries Science, 2005, 5(1): 26–30. (in Chinese).

[34] HUANG GQ, DONG SL, WANG F. Growth, energy allocation, and biochemical composition of the body of Chinese shrimp, *Fenneropenaeus chinensis*, fed different diets[J]. Crustaceana, 2005, 78 (2): 141–162.

[35] ESMAEILI N, MA HY, KADRI S, *et al.* Protein and lipid nutrition in crabs[J]. Reviews in Aquaculture, 2024. DOI: 10.1111/raq.12908.

[36] DONG LF, TONG T, ZHANG Q, *et al.* Effect of dietary protein level on growth performance, body composition, and digestive enzyme activities in green mud crab (*Scylla paramamosain*) juveniles[J]. Journal of Fishery Sciences of China, 2017, 24(3): 524532. (in Chinese).

[37] ZHAO J, WEN XB, LI SK, *et al.* Effects of dietary lipid levels on growth, feed utilization, body composition and antioxidants of juvenile mud crab *Scylla paramamosain* (Estampador)[J]. Aquaculture, 2015, 435: 200–206.

[38] CAO QM, LIU QB, YU YQ, *et al.* Nutritional composition and safety of cultured and wild sandworm (*Perinereis aibuhitensis*) from a coastal area of Shandong Province[J]. Journal of Fishery Sciences of China, 2016, 23(5): 1164–1172. (in Chinese).

[39] LIN T, YANG Y, WANG SM, *et al.* Nutritional analysis and food safety study of *Perinereis aibuhitensis*[J]. Journal of Applied Oceanography, 2016, 35(3): 412–417. (in Chinese).

[40] HU Y, CHEN R, HU LH, *et al.* Comparative analysis of nutrient composition between wild and cultured clamworm, Perinereis aibuhitensis from different areas[J]. Fisheries Science, 2018, 37(1): 93–100. (in Chinese).

[41] AI CX, LIU JG, LIN QW, *et al.* Nutrient requirements and development of formulated diets of mud crab, *Scylla* sp.[J]. Journal of Fisheries of China, 2007, 31(suppl): 122–128. (in Chinese).

[42] HUANG GQ, DONG SL, WANG F, *et al.* Effects of diet differences and ration levels on the molting of Chinese shrimp, *Fenneropenaeus chinensis*[J]. Periodical of Ocean University of China, 2004, 34(6): 942–948. (in Chinese).

[43] ZHU XM, LI SJ, SONG XY. Effects of temperature on oxygen consumption and ammonia excretion of mud crab Zoea, *Scylla serrata*[J]. Journal of Xiamen University (Natural Sciences), 2003, 42(1): 92–96. (in Chinese).

[44] BELGRAD BA, GRIFFEN BD. The influence of diet composition on fitness of the blue crab, *Callinectes sapidus*[J]. PLoS ONE, 2016, 11(1): e0145481.

[45] WANG ZF, TANG D, GUO HY, *et al.* Evolution of digestive enzyme genes associated with dietary diversity of crabs[J]. Genetica, 2020, 148: 87–99.

(From page 35)

[15] JIN JB, ZHU ZL, LIN QH. Rooting characteristics and anatomical structure of Carpinus betulus cuttings[J]. Journal of Northwest A&F University (Natural Science Edition), 2015, 43(2): 92–98. (in Chinese).

[16] TANG Z, DU W, DU XL, *et al.* iTRAQ protein profiling of adventitious root formation in mulberry hardwood cuttings[J]. Journal of Plant Growth Regulation, 2016, 35(3): 618–631.

[17] HUANG XX. Study on cutting propagation technology and rooting mechanism of Dendrocalamus latiflorus[D]. Nanning: Guangxi University, 2022. (in Chinese).

[18] ZHANG JC, LI JW, WANG FM, *et al.* Effect of developmental stage on rooting of 7 peach cultivars by hardwood cutting[J]. South China Fruits, 2024, 53(4): 188–194. (in Chinese).

[19] ZHAO X, LI QY, JIANG QB, *et al.* Effects of substrates and rooting regulators on rooting of cuttings in *Manglietia conifera* Dandy[J]. Journal of Nanjing Forestry University: Natural Sciences Edition, 2019, 43(2): 23–30. (in Chinese).

[20] JIA ZY, GE XM, TANG LZ. Research progress in cutting propagation technology for woody plants and its affecting factors[J]. World Forestry Research, 2015, 28(2): 36–41. (in Chinese).

[21] WANG FR, WANG HL, GONG LZ, *et al.* Hardwood cuttage rooting of four varieties of peach rootstocks[J]. Journal of Anhui Agricultural Sciences, 2016, 44(17): 39–40, 60. (in Chinese).

[22] ZHU TF, LI X, SU XL, *et al.* Analysis of changes of substances in the cuttings of four Rosaceae fruit trees during cutting[J]. South China Fruits, 2023, 52(2): 139–146. (in Chinese).