

Growth, precocity, enzyme activity and chemical composition of juvenile Chinese mitten crab, *Eriocheir sinensis*, fed different dietary protein-to-energy ratio diets

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Abstract

A growth experiment was conducted to determine the optimum dietary protein-to-energy (P/E) ratio of juvenile Chinese mitten crab *Eriocheir sinensis*. Six practical diets were formulated with P/E ratios ranging from 15.94 to 21.25 g protein MJ⁻¹ gross energy (g MJ⁻¹). Results showed that specific growth rates related with molting were significantly affected by dietary P/E ratio ($P < 0.05$). The specific growth rates both in body weight (SGR_w) and carapace width (SGR_{cw}) for the entire experimental period were not significantly affected by dietary P/E ratio ($P > 0.05$). Molting frequency, survival rate and crab body composition were also significantly affected by dietary P/E ratio ($P < 0.05$). Rate of precocity displayed negative trends with growth rate, and the trend with SGR_{cw} was significant ($P < 0.01$). The activities of alkaline phosphatase, trypsinase and steapsin were significantly affected by dietary P/E ratio ($P < 0.05$). Molting frequency ($P < 0.001$), SGR_{w1-2} ($P < 0.05$) and SGR_{w0-2} ($P < 0.05$) were positively and significantly related to alkaline phosphatase activity. We recommend 18.13–19.20 g MJ⁻¹ as the optimum dietary P/E ratio for juvenile *E. sinensis*, based on growth performance, rate of precocity and digestive enzyme activities.

Keywords: protein-to-energy ratio, growth, precocity, enzyme activity, chemical composition, *Eriocheir sinensis*

Introduction

Protein plays an important role in organisms and is the most expensive ingredient in most aquaculture feeds. The utilization of dietary protein is closely related to dietary protein level and the availability of non-protein energy sources. It is well known that if the diet does not contain sufficient energy, protein will be used for energy depletion rather than for growth (NRC 1993; Ai, Mai, Li, Zhang, Zhang, Duan, Tan, Xu, Ma, Zhang & Liufu 2004; Ali, Al-Ogaily, Al-Asgah, Goddard & Ahmed 2008). Conversely, if excess dietary energy is provided, food intake may be limited and it may cause inefficient use of protein and nutrients (Ali & Jauncey 2005; Okorie, Kim, Lee, Bae, Yoo, Han, Bai, Park & Choi 2007). Growth and body composition of fish are significantly affected by dietary protein and energy levels (Lovell 1989). Providing optimum dietary protein-to-energy (P/E) ratio in aquatic diets is widely accepted as a means to spare protein for increased growth rates at a lower cost (Mu, Shim & Guo 1998; Ali & Jauncey 2005; Ali *et al.* 2008). Conversely, improper dietary P/E ratio may result in lower growth and poor protein and energy utilization (Shiau & Lan 1996). Improper dietary P/E ratios may cause more protein to be stored as fat, which leads to increased body lipid deposition (Van der Meer, Zamora & Verdegem 1997; Lee & Kim 2001). In crustaceans, the hepatopancreas is the main storage depot for

energy reserves. Excess lipid storage in the hepatopancreas of juvenile Chinese mitten crab (*Eriocheir sinensis*) can arouse precocity (Cheng & Wang 2000; Wang, Li, Li & Zhao 2001), which is considered as a main constraint for *E. sinensis* aquaculture (Zhang & Xu 2001; Li, Kang, Zhao, Wen & Han 2005).

Previous studies of dietary P/E ratios have focused on conversion efficiency for primary nutrients, such as protein conversion efficiency (e.g. Catacutan & Coloso 1995; Ai *et al.* 2004; Ali *et al.* 2008). In fact, the ability of organisms to hydrolyse, absorb and assimilate the principal nutrients can be effectively examined by analysing the activity of digestive enzymes (Lee, Smith & Lawrence 1984). Digestive enzyme activity is a good indicator of digestive capacity, and many recommendations for optimum requirements of nutrients were based on digestive enzyme activity (Lee *et al.* 1984; Lin, Luo, Ye, Zhou, Xue & Yang 2000). Among various digestive enzymes, the levels of secretion of pancreatic enzymes (e.g. amylase, trypsinase and lipase) are commonly used as indices of digestive system function (Ribeiro, Zamboni-Infante, Cahu & Dinis 1999; Shan, Xiao, Huang, Bellagamba & Carnevia 2008). Activity of digestive enzymes changes with ontogenetic development, molting and circadian rhythms (Le Moulac, Klein, Sellos & Van Wormhoudt 1996). Dietary composition has great effects on digestive enzyme activity (Kumulu & Jones 1995; Guzman, Gaxiola, Rosa & Torre-Blanco 2001), but the effect of dietary P/E ratio on digestive enzyme activities is little studied.

Eriocheir sinensis is a very popular table delicacy in many Asian countries, especially in China and Japan. The species has been widely cultured in ponds, reservoirs, and lakes in China for years, with an annual production of 518 400 tons in 2008 (FBMA 2009). Previous studies on P/E ratio in *E. sinensis* have used semi-purified or purified diets with a relatively high protein level (Xu & He 1998; Zhu & Qian 2000). In these studies, the recommended optimum dietary P/E ratios were just the designed value from a better growth group. Information on appropriate P/E ratio in *E. sinensis*, fed fishmeal and soybean meal-based practical diets is rare, except for Lin *et al.* (2000), who, however, tested only three different P/E ratio diets. The objective of the present study was to investigate the optimum dietary P/E ratio for *E. sinensis* with practical feeds, in terms of molting, growth, digestive enzyme

activities and survival rate with practical feeds. As precocity has strong negative effects on the aquaculture of the crab, and crabs with higher growth rate, survival rate and lower rate of precocity are welcomed by farmers, and therefore the effect of P/E ratio on precocity and production complex index of *E. sinensis* was also investigated.

Materials and methods

Experimental diets

Six experimental diets (designated D1–D6) were formulated with P/E ratio ranging from 15.94 to 21.25 g MJ⁻¹ based on normally used diet P/E ratios. Ingredients and chemical composition of the experimental diets are shown in Table 1. Oil and water were added to dry ingredients and thoroughly mixed to form a pelletable mixture. The diets were formed into 1.5 mm (diameter) pellets, oven-dried at 60°C and stored at –4°C until used.

Experimental animals and rearing conditions

Juvenile *E. sinensis* were obtained from Hannan Fish Farm, Wuhan, Hubei, China in July, 2008 and then reared in an indoor 27-m² rectangular concrete pool (water depth, 25 cm). To reduce aggressive behaviours of crabs, some live alligator weed *Alternanthera philoxeroides* covered about half the area of the pool. *E. sinensis* were fed mixed experimental feed of the same amount of D1–D6 to satiation twice a day at 0830 and 1700 for 4 weeks to acclimate them to the experimental conditions and diets.

At the beginning of the trial, 180 healthy crabs with initial body size of 9.92 ± 0.25 g (mean ± SE) were randomly distributed to 180 plastic tanks (one crab in each tank). Thirty replicates were set for each group. The tanks (length × width × height: 50 × 40 × 30 cm) were placed in three concrete pools which were the same as the acclimation pool. Five holes (diameter: 1.5 cm) were drilled previously on each tank to maintain cross-flow and similar water quality. One brick (24 × 6 × 5 cm) was placed in each tank to simulate the natural habitat. Water was aerated all the time except for feeding. Water depth in ponds was about 25 cm. Half of the water in the pools was replaced with aerated tap water each week. During the experiment, natural photoperiod and water temperature were maintained. Water

Table 1 Formulation and proximate composition of the experimental diets (% dry matter)

| Ingredients | Diets | | | | | |
|--|-------|-------|-------|-------|-------|-------|
| | D1 | D2 | D3 | D4 | D5 | D6 |
| Fish meal | 23.00 | 23.00 | 28.00 | 28.00 | 36.00 | 36.00 |
| Soybean meal | 24.00 | 24.00 | 24.00 | 24.00 | 24.00 | 24.00 |
| Fish oil | 1.10 | 0.90 | 0.70 | 0.80 | 0.45 | 0.25 |
| Soybean oil | 1.10 | 0.90 | 0.70 | 0.80 | 0.45 | 0.25 |
| Cornstarch | 26.00 | 15.00 | 10.00 | 21.00 | 13.00 | 2.00 |
| Rice chaff | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| Mineral & vitamin premix* | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Choline chloride | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| CaH ₂ PO ₄ | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Cholesterol | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Lecithin | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Swine liver powder† | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Sodium alginate | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Cellulose | 0.30 | 11.70 | 12.10 | 0.90 | 1.60 | 13.00 |
| Proximate composition (% dry matter) | | | | | | |
| Moisture | 14.34 | 14.26 | 11.85 | 7.62 | 8.68 | 12.48 |
| Crud protein | 32.19 | 33.43 | 35.61 | 35.03 | 39.72 | 40.72 |
| Crud lipid | 6.90 | 7.39 | 7.03 | 7.29 | 7.26 | 6.88 |
| Ash | 10.47 | 10.42 | 11.13 | 11.04 | 12.26 | 12.03 |
| Gross energy (kJ g ⁻¹) | 20.20 | 20.25 | 19.27 | 18.37 | 19.21 | 19.16 |
| P/E (g protein MJ gross energy ⁻¹) | 15.94 | 16.51 | 18.48 | 19.07 | 20.68 | 21.25 |

*Mineral & vitamin premix was bought from Haid Feeds Co., Ltd., Guangzhou, P. R. China.

†Swine liver powder was bought from Wuhan Coland Aquaculture Co., Ltd., Wuhan, P. R. China.

temperature fluctuated from 19.7 to 28.5°C, pH was 6.9–7.4; ammonia-N concentration was less than 0.31 mg L⁻¹; and dissolved oxygen concentration was more than 5 mg L⁻¹.

During the 76-day experiment (from August 16th to October 30th), crabs were fed experimental feeds to satiation twice a day at 0830 and 1700. Uneaten food and faeces were removed before feeding each morning. Newly molted shells and dead crabs were also removed daily. Newly molted crabs were weighed and measured 1 day later when the new shell had become hard to reduce stress. At the end of the experiment, each crab was weighed to 0.01 g, and carapace width was measured to 0.1 mm. Precocity of crabs was evaluated according to the methods of Wang *et al.* (2001), which were mainly by morphological characters, especially secondary sex characters are related to maturity. The characteristics typically were described in detail by Li, Li, Liu and De Silva (2011) as following: (1) *Body colour*. The dorsal carapace of normal crabs is slightly yellow, also known as 'yellow crab'; however, the dorsal carapace of sexually precocious crabs is dark-green or blue with obvious stripes, also known as the 'green crab'. (2) *Abdominal segment*. The abdominal

segment of mature female crabs reaches the pereopod base, and is surrounded by long and dense hair; the belly of mature males is protruded out of the segment breastplate. (3) *Appendages*. The pereopods have long and thick setae in mature crabs and the pincers have very dense and long hair in mature male crabs.

Sample collection

At the end of the experiment, haemolymph was collected with a syringe from each of six crabs in each group, and the crabs were sacrificed. The hepatopancreas was removed and weighed individually on an ice plate. Sampled hepatopancreases were frozen in liquid nitrogen and stored at -20°C for further tests. Another 6–8 crabs in each group were also sampled and stored at -20°C for chemical composition analysis.

Sample analysis

Collected haemolymph was centrifuged at 4°C with 20817 g for 10 min, and the supernatant serum was used for testing alkaline phosphatase (ALP) activity. ALP activity was determined by the method

of Chen (1995) with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Frozen hepatopancreas samples were homogenized with glass homogenizers on ice with ice-cold buffer. The homogenized suspension was centrifuged at 4°C with 664 g for 10 min, and the supernatant was used for enzyme activity determinations. Trypsinase and steapsin activity were tested with commercial kits (Nanjing Jiancheng Bioengineering Institute). Total protein concentration of tissue was measured using Bradford (1976) method with bovine serum albumin as the standard.

Moisture, crude protein, lipid, ash and energy contents of the experimental diets and crab bodies were analysed. Moisture content was determined by drying to constant weight at 105°C. Crude protein content was analysed using the Kjeldahl method with auto nitrogen analyzer (Kjeltec Auto Analyzer 2300; FOSS Company, Eden Prairie, MN, USA). Crude lipid was determined for crabs and diets by ether extraction using a Soxtec System (Soxtec System HT6; Tecator, Hoganas, Sweden). Ash content was measured by combustion at 550°C in a muffle furnace for more than 3 h. Energy content was tested by bomb calorimetry (Phillipson microbomb calorimeter; Gentry Instrument, Aiken, SC, USA).

Calculation of data

$$\text{Rate of precocity(\%)} = 100 \times \frac{\text{precocious crab number}}{\text{final crab number}}$$

$$\text{Specific growth rate in weight (SGRw)(\%d}^{-1}\text{)} = 100 \times \frac{(\ln W_f - \ln W_i)}{t}$$

$$\text{Specific growth rate in carapace width (SGRcw)(\%d}^{-1}\text{)} = 100 \times \frac{(\ln CW_f - \ln CW_i)}{t}$$

$$\text{Molt frequency} = \frac{\text{total molt times}}{((\text{initial crab number} + \text{final crab number})/2)}$$

$$\text{Survival rate(\%)} = 100 \times \frac{\text{final crab number}}{\text{initial crab number}}$$

$$\text{Production complex index(\%)} = 100 \times \frac{(\text{final body weight} - \text{initial body weight})}{\text{initial body weight}} \times \frac{\text{survival rate}}{100} \times (100 - \text{rate of precocity})/100$$

Where, W_f stands for final body weight of a certain period (e.g. from the beginning to the

first molting), W_i for initial body weight of a certain period, CW_f for final carapace width of a certain period, CW_i for initial carapace width of a certain period and t for the days of a certain period.

Statistical analysis

As the crabs were reared individually, specific growth rate was calculated individually and then pooled for group comparison. STATISTICA 6.0 (Stat-Soft Inc., Tulsa, OK, USA) was used for statistical tests. Duncan's multiple-range test was used to compare the differences between the mean values, followed by one-way analysis of variance (ANOVA). Growth rate related to the second molting was analysed by ANCOVA with body weight after first molting as covariance. Statistical significance was set at $P < 0.05$. Broken-line regression and polynomial regression analysis were used to determine the optimum dietary P/E ratio.

Result

Molting and growth

Specific growth rate in body weight from the beginning of the experiment to the first molting (SGRw0-1) increased with increasing dietary P/E ratio, reached the highest value at 18.48 g MJ⁻¹ and then decreased slightly with increasing dietary P/E ratio (Table 2). Based on SGRw0-1, broken-line regression analysis indicated that the optimum dietary P/E ratio for juvenile *E. sinensis* is 18.64 g MJ⁻¹ ($y = -0.3573x + 9.2527$, $R^2 = 0.7652$; $y = 2.5922$). Specific growth rate in weight from the first molting to second molting (SGRw1-2) in group D1 (15.94 g MJ⁻¹) was significantly lower than that of group D4 (19.07 g MJ⁻¹), D5 (20.68 g MJ⁻¹) and D6 (21.25 g MJ⁻¹) ($P < 0.05$). Based on SGRw1-2, broken-line regression analysis indicated that the optimum dietary P/E ratio for juvenile *E. sinensis* is 19.20 g MJ⁻¹ ($y = 0.3228x - 4.4696$, $R^2 = 0.8352$; $y = 1.7269$). Specific growth rate in weight from the beginning of the experiment to the second molting (SGRw0-2) in group D1 (15.94 g MJ⁻¹) was significantly lower than that in group D3 (18.48 g MJ⁻¹), D4 (19.07 g MJ⁻¹) and D5 (20.68 g MJ⁻¹) ($P < 0.05$) (Table 2). The result of quadratic curve regression analysis, based on SGRw0-2, indicated

that the optimum dietary P/E ratio for juvenile *E. sinensis* is 19.17 g MJ⁻¹ ($y = -0.0886x^2 + 3.3963x - 30.693$, $R^2 = 0.9707$).

Specific growth rate in body weight (SGR_w) for the entire experimental period increased with increasing dietary P/E ratio up to 18.48 g MJ⁻¹, and then decreased slightly with further increases in dietary P/E ratio (Fig. 1a). The results of quadratic curve regression analysis, based on SGR_w, indicated that the optimum dietary P/E ratio for juvenile *E. sinensis* is 18.93 g MJ⁻¹ (Fig. 1a). Specific growth rate in carapace width (SGR_{cw}) for the entire experimental period increased with increasing dietary P/E ratio up to the highest value for group D4 (19.07 g MJ⁻¹) and then decreased slightly with increasing dietary P/E ratio, but there were no significant difference in SGR_{cw} between groups ($P > 0.05$). According to quadratic curve analysis based on SGR_{cw}, the optimum dietary P/E ratio for juvenile *E. sinensis* is 18.71 g MJ⁻¹ (Fig. 1b).

Molting frequency (MF) increased with increasing dietary P/E ratio up to the highest value for group D5 (20.68 g MJ⁻¹) and then decreased (Table 2). The MF in group D1 (15.94 g MJ⁻¹) and D2 (16.58 g MJ⁻¹) were significantly lower than that in group D5 (20.68 g MJ⁻¹) ($P < 0.05$). According to broken-line regression analysis, based on MF, the optimum dietary P/E ratio for juvenile *E. sinensis* is 18.47 g MJ⁻¹ ($y = 0.2366x - 2.3289$, $R^2 = 0.937$; $y = 2.0401$).

Rate of precocity, survival rate and production complex index

Rate of precocity first decreased with increasing dietary P/E ratio and reached the lowest value for group D4 (19.07 g MJ⁻¹), and then increased with further increasing dietary P/E ratio (Table 2). Quadratic curve analysis indicated that the lowest rate of precocity appeared at 18.47 g MJ⁻¹ ($y = -0.0072x^2 + 0.2694x - 2.0858$, $R^2 = 0.6036$). A significant negative correlation was observed between rate of precocity and SGR_{cw} (Fig. 2).

Survival rates in this experiment for juvenile *E. sinensis* are shown in Table 2. The survival rate in group D1 (15.94 g MJ⁻¹) was significantly lower than that in group D2 (16.58 g MJ⁻¹) and D4 (19.07 g MJ⁻¹) ($P < 0.05$).

Production complex index was significantly affected by dietary P/E ratio, Quadratic curve analysis indicated that the lowest rate of precocity

appeared at 18.61 g MJ⁻¹ ($y = -4.0433x^2 + 150.49x - 1353.3$, $R^2 = 0.968$) (Fig. 3).

Enzyme activity

Alkaline phosphatase, trypsinase and steapsin activities are presented in Table 3. ALP activity increased with increase in dietary P/E ratio before reaching the highest value for group D5 (20.68 g MJ⁻¹) and then decreased slightly. The ALP activity in group D5 was significantly higher than those of groups D1, D2 and D3 ($P < 0.05$). Significant positive correlations were observed between ALP activity and molting frequency (Fig. 4a), SGR_{w1-2} (Fig. 4b) and SGR_{w0-2} (Fig. 4c). The crabs fed D3 (18.48 g MJ⁻¹) exhibited significantly higher trypsinase activity ($P < 0.05$). According to the quadratic curve regression analysis, the highest trypsinase activity appeared at 18.13 g MJ⁻¹ ($y = -297.8x^2 + 10797x - 92717$, $R^2 = 0.5274$). The steapsin activity in group D4 (19.07 g MJ⁻¹) was significantly higher than that of group D1 (15.94 g MJ⁻¹) ($P < 0.05$). The result of quadratic curve regression analysis showed that the highest steapsin activity appeared at 18.65 g MJ⁻¹ ($y = -6.6068x^2 + 246.45x - 2206.3$, $R^2 = 0.9469$).

Chemical composition

Chemical composition of juvenile *E. sinensis* in this experiment is presented in Table 4. Moisture content differed significantly between groups. Crabs fed D1 (15.94 g MJ⁻¹) had significantly higher moisture content and ash content, but had the significantly lower lipid content when compared with other groups ($P < 0.05$). The protein content in groups D3 (18.48 g MJ⁻¹) and D5 (20.68 g MJ⁻¹) was significantly higher than that in group D2 (16.58 g MJ⁻¹) ($P < 0.05$).

Discussion

Some authors believe that high rates of growth will cause precocity in juvenile *E. sinensis* (e.g. Gu, Zhang, Liu & Du 1997). In the present study, rate of precocity showed a negative relationship with growth rate. Somatic growth and reproduction have been known to be antagonistic (Charmantier, Charmantier-Daures & Van Herp 1997). It is commonly accepted that nutrients must be available first for metabolism, and second for growth and

Table 2 Body weight, growth rate-related to molting, molting frequency, rate of precocity and survival rate of juvenile *E. sinensis* fed different P/E ratio diets (means \pm SE)

| Diets (P/E) (g MJ ⁻¹) | Body weight (g) | | SGR [*] (% d ⁻¹) | | | | Molting frequency | Rate of precocity (%) | Survival (%) |
|-----------------------------------|------------------|------------------|---------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------|----------------------------------|--------------|
| | Initial | Final | SGRw0-1 | SGRw1-2 | SGRw0-2 | SGRw1-2 | | | |
| D1 (15.94) | 10.37 \pm 0.48 | 21.62 \pm 0.22 | 2.34 \pm 0.29 ^{ab} | 0.56 \pm 0.22 ^a | 0.87 \pm 0.14 ^a | 1.45 \pm 0.35 ^a | 40.00 \pm 0.00 | 33.33 \pm 0.00 ^a | |
| D2 (16.58) | 9.62 \pm 0.35 | 18.08 \pm 0.92 | 2.51 \pm 0.30 ^{ab} | 1.08 \pm 0.17 ^{ab} | 1.32 \pm 0.10 ^{ab} | 1.70 \pm 0.13 ^a | 37.50 \pm 12.50 | 53.33 \pm 0.00 ^{bc} | |
| D3 (18.48) | 10.15 \pm 0.36 | 21.83 \pm 1.57 | 2.92 \pm 0.35 ^b | 1.25 \pm 0.28 ^{ab} | 1.86 \pm 0.26 ^b | 1.87 \pm 0.04 ^{ab} | 33.33 \pm 0.00 | 50.00 \pm 10.00 ^{abc} | |
| D4 (19.07) | 9.58 \pm 0.33 | 18.46 \pm 0.93 | 2.09 \pm 0.21 ^a | 1.85 \pm 0.29 ^b | 1.75 \pm 0.23 ^b | 2.13 \pm 0.21 ^{ab} | 22.50 \pm 2.50 | 60.00 \pm 6.67 ^c | |
| D5 (20.68) | 9.45 \pm 0.38 | 18.10 \pm 1.60 | 1.88 \pm 0.17 ^a | 1.65 \pm 0.15 ^b | 1.66 \pm 0.11 ^b | 2.67 \pm 0.37 ^b | 28.33 \pm 11.67 | 36.67 \pm 3.33 ^{ab} | |
| D6 (21.25) | 10.16 \pm 0.41 | 20.76 \pm 1.74 | 1.72 \pm 0.16 ^a | 1.69 \pm 0.38 ^b | 1.45 \pm 0.29 ^{ab} | 1.83 \pm 0.26 ^{ab} | 54.46 \pm 16.96 | 50.00 \pm 3.33 ^{abc} | |

Means in the same column with different superscripts are significantly different ($P < 0.05$).

*0–1 stands for the period from the beginning of the experiment to the first molting, 1–2 stands for the period from the first molting to second molting, 0–2 stands for the period from the beginning of the experiment to the second molting.

third for reproduction in nature (Bray & Lawrence 1992). In other words, once the nutrient or energy needed for metabolism and growth is met, the surplus nutrient or energy will be stored or used up for reproduction. In the present study, rate of precocity was observed negatively correlated to SGR_{cw} (Fig. 2). Therefore, precocity could not be simply attributed to rapid growth. It may also be due to the effects of dietary P/E ratio on nutrient or energy utilization. It is well known that dietary P/E ratio has significant effects on nutrient utilization (Van der Meer *et al.* 1997; Lee & Kim 2001). In crustaceans, the hepatopancreas is not only the main digestive gland but also the main depot for energy reserves (Mikami & Greenwood 1994; Cheng, Du & Lai 1998). Excess lipid stored in the hepatopancreas of juvenile *E. sinensis* has been thought to arouse a higher proportion of precocity (Cheng & Wang 2000; Wang *et al.* 2001). In our study, rate of precocity decreased with increasing dietary P/E ratio, reached the lowest value at 19.07 g MJ⁻¹ and then increased with increasing dietary P/E ratio. The relationship between precocity and dietary P/E ratio is poorly understood. It may be that a relatively higher proportion of nutrients or energy was used for growth in groups fed optimal dietary P/E ratio. In contrast, a higher proportion of energy was stored in the hepatopancreas in our experimental groups fed improper dietary P/E ratio diets, which then aroused a higher proportion of precocity.

Our results demonstrate that dietary P/E ratio has a great effect on growth of juvenile *E. sinensis*, which are in line with most of the studies related to dietary P/E ratio (Catacutan & Coloso 1995; Lee & Kim 2001; Ali *et al.* 2008). In our study, molting frequency (MF) was significantly affected by dietary P/E ratio ($P < 0.05$). Also, MF was significantly and positively related with alkaline phosphatase (ALP) activity (Fig. 4a). ALP is important for crustaceans, as it influences the absorption of phosphate and calcium from water during the biomineralization process that leads to the molting and replacement of the shell (Zhou, Chen, Chen, He & Zhou 2000; Xiao, Xie, Lin, Li, Chen, Zhou & Zhang 2002). As calcium is one of the basic elements of the shell, crab shell growth must rely on calcium uptake and metabolism. Therefore, ALP activity theoretically has a great effect on crab molting and growth. Besides MF, the SGR_{w1-2} (Fig. 4b) and SGR_{w0-2} (Fig. 4c)

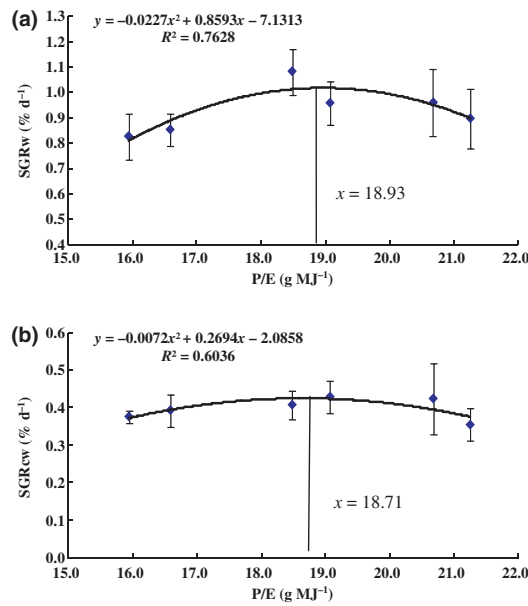


Figure 1 Relationship between the entire-experiment specific growth rate in body weight (SGRw, a), carapace width (SGRcw, b) and dietary P/E ratio for juvenile *E. sinensis* fed different P/E ratio diets.

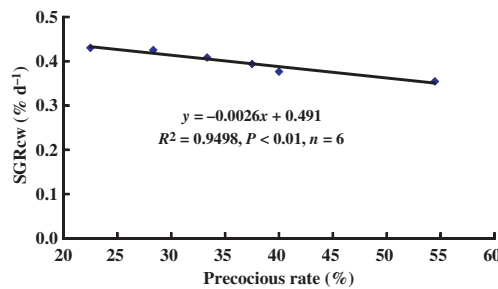


Figure 2 Relationship between the entire-experiment specific growth rate in carapace width (SGRcw) and rate of precocity for juvenile *E. sinensis* fed different P/E ratio diets.

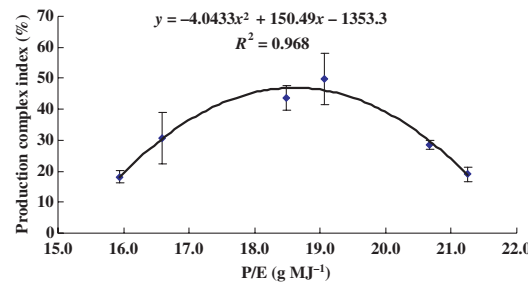


Figure 3 Relationship between production complex index and dietary P/E ratio for juvenile *E. sinensis* fed different P/E ratio diets.

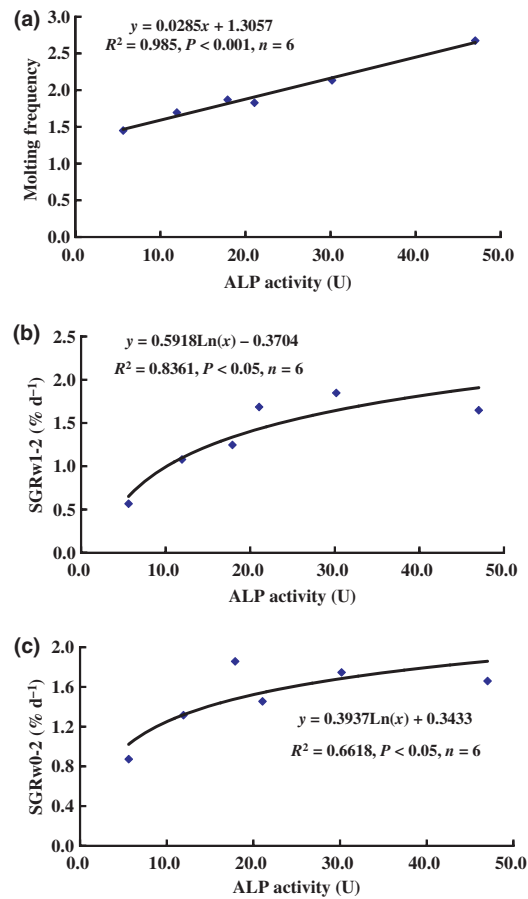


Figure 4 Relationship between molting frequency (a), specific growth rate in body weight during the first and second molting (SGRw1-2, b), specific growth rate in body weight from the beginning to the second molting (SGRw0-2, c) and alkaline phosphatase (ALP) activity for juvenile *E. sinensis* fed different P/E ratio diets.

were also significantly and positively correlated with ALP activity in the present study. Immediately following molting, crustacean body size and biomass can increase remarkably (Charmantier *et al.* 1997), but conversely, it has been documented in some species that intermolt growth is negligible (Hartnoll 1982). Therefore, higher MF means that higher growth rates and body weight occur at certain times. In the present study, growth rate (SGRw0-1, SGRw1-2 and SGRw0-2) of juvenile *E. sinensis* was significantly affected by dietary P/E ratio ($P < 0.05$).

For crustaceans, molting requires an adequate supply of nutrients and energy (Charmantier *et al.* 1997). In this regard, higher growth rate may also

Table 3 Alkaline phosphatase (ALP), trypsinase and steapsin activities of juvenile *E. sinensis* fed different P/E ratio diets (means \pm SE)

| Diets (P/E) (g MJ ⁻¹) | ALP activity (U) | Trypsinase activity (U mg protein ⁻¹) | Steapsin activity (U g protein ⁻¹) |
|-----------------------------------|---------------------------------|---|--|
| D1 (15.94) | 5.61 \pm 1.86 ^a | 3145.7 \pm 1004.5 ^{ab} | 41.13 \pm 13.33 ^a |
| D2 (16.58) | 11.93 \pm 0.70 ^a | 5157.7 \pm 758.1 ^{bc} | 67.34 \pm 8.33 ^{ab} |
| D3 (18.48) | 12.63 \pm 5.30 ^a | 6130.3 \pm 1194.6 ^c | 84.78 \pm 16.72 ^{ab} |
| D4 (19.07) | 30.18 \pm 12.77 ^{ab} | 3198.1 \pm 842.0 ^{ab} | 97.39 \pm 15.65 ^b |
| D5 (20.68) | 47.02 \pm 11.68 ^b | 3961.2 \pm 835.1 ^{abc} | 61.90 \pm 7.36 ^{ab} |
| D6 (21.25) | 21.05 \pm 9.18 ^{ab} | 1972.1 \pm 382.2 ^a | 48.68 \pm 24.71 ^{ab} |

Means in the same column with different superscripts are significantly different ($P < 0.05$).

Table 4 Body composition of juvenile *E. sinensis* fed different P/E ratio diets (means \pm SE)

| Diets (P/E) (g MJ ⁻¹) | Moisture (%) | Protein (% dry matter) | Lipid (% dry matter) | Ash (% dry matter) |
|-----------------------------------|-------------------------------|--------------------------------|------------------------------|-------------------------------|
| D1 (15.94) | 82.36 \pm 0.00 ^a | 38.79 \pm 0.17 ^{ab} | 3.24 \pm 0.20 ^a | 47.56 \pm 0.15 ^a |
| D2 (16.58) | 72.00 \pm 0.00 ^f | 37.20 \pm 0.61 ^a | 7.13 \pm 0.02 ^c | 41.55 \pm 0.01 ^c |
| D3 (18.48) | 77.53 \pm 0.00 ^c | 39.85 \pm 0.70 ^b | 5.48 \pm 0.11 ^b | 42.70 \pm 0.08 ^b |
| D4 (19.07) | 77.45 \pm 0.01 ^d | 38.88 \pm 0.53 ^{ab} | 7.73 \pm 0.05 ^d | 41.17 \pm 0.03 ^d |
| D5 (20.68) | 78.09 \pm 0.01 ^b | 39.52 \pm 0.17 ^b | 6.81 \pm 0.02 ^c | 39.50 \pm 0.20 ^e |
| D6 (21.25) | 77.25 \pm 0.00 ^e | 39.12 \pm 0.69 ^{ab} | 6.84 \pm 0.08 ^c | 42.96 \pm 0.03 ^d |

Means in the same column with different superscripts are significantly different ($P < 0.05$).

be related with higher food intake, digestive ability and feed efficiency. In the present study, trypsinase and steapsin activities were significantly affected by dietary P/E ratio ($P < 0.05$), which is in line with the findings of Guzman *et al.* (2001). Conversely, Ali and Jauncey (2005) found that digestive enzyme activities (protease and lipase) did not significantly change in response to dietary P/E ratio, but the lack of enzyme activity data presented for that study makes interpretation difficult. In our study, the activity of trypsinase and steapsin peaked in group D3 (18.48 g MJ⁻¹) and D4 (19.07 g MJ⁻¹) respectively. Higher digestive enzyme activity means higher digestive ability, which should translate to higher animal growth rate (Lin *et al.* 2000). Our results indicated that the highest growth rate of each period peaked in groups D3 or D4. Therefore, we conclude that higher digestive enzyme activities correspond to higher growth rates in crabs, as was found by Lin *et al.* (2000).

Optimizing P/E ratios in aquatic diets is widely accepted as a means to spare protein for higher growth at a lower cost (Mu *et al.* 1998; Ali & Jauncey 2005; Ali *et al.* 2008). Previous studies on *E. sinensis* protein requirements were simple dose-response experiments, where experimental

diets contained different levels of protein without consideration of the effect of dietary P/E ratio. Chen, Du and Lai (1994) found that SGRw of *E. sinensis* was positively related to dietary protein levels between 16.5% and 53.6%. The particular dose-response that they observed may be due mainly to low dietary energy levels used in their study. In our study, growth rate did not increase with increasing dietary protein level. Groups D5 and D6 had relatively lower growth rates compared with group D4, even though the protein level of those diets was closer to the optimum protein levels reported in previous studies (Mu *et al.* 1998; Qian & Zhu 1999) than was that of group D4. Our results document that dietary P/E ratio has a great effect on growth performance and protein utilization. The optimum dietary P/E ratios for juvenile *E. sinensis* based on SGRw0-1, SGRw1-2, SGRw0-2, SGRw and SGRcw are 18.64, 19.20, 19.17, 18.93 and 18.71 g MJ⁻¹ respectively. These results are similar to others. Based on survival, Xu and He (1998) recommended 19.95 g MJ⁻¹ as optimal P/E ratio for juvenile *E. sinensis*. Similarly, the optimal P/E ratio for juvenile white shrimp recommended by Hu, Tan, Mai, Ai, Zheng and Cheng (2008) was 21.1 g MJ⁻¹.

Dietary protein and energy levels are known to have effects on growth and body composition of fish (Lovell 1989). In our study, significant differences were observed in proximate body composition of juvenile *E. sinensis* fed different dietary P/E ratios. However, the changes in body composition were inconsistent. Ali *et al.* (2008) also reported that body composition of juvenile *Oreochromis niloticus* was not consistently affected by dietary P/E ratio. It is well documented that body lipid content is conversely related with moisture content (Lee & Kim 2001; Ali & Jauncey 2005). In the present study, crabs in group D1 had the highest moisture content and had the lowest lipid content.

In conclusion, the present study documents that dietary P/E ratio has significant effects on molting, growth and digestive enzymes activity in juvenile *E. sinensis*. Molting frequency, growth rate and digestive enzyme activity, all increase with increasing dietary P/E ratio until an optimal P/E ratio is reached and then decrease. Rate of precocity has an inverse relationship with specific growth rate of the carapace. Crabs show better growth performance and a reduced rate of precocity at a relatively low protein level when fed optimal dietary P/E ratios. In accordance with our results, we recommend 18.13–19.20 g MJ⁻¹ as the optimum dietary P/E ratio for juvenile *E. sinensis*.

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