Aquaculture Research

Aquaculture Research, 2010, **41**, 278–294 doi: 10.1111/j.1365-2109.2009.02330.x

Dietary protein requirement of giant mud crab *Scylla serrata* juveniles fed iso-energetic formulated diets having graded protein levels

Unniyampurath Unnikrishnan & Rajain Paulraj

Fish & Shellfish Nutrition Research Group, Central Marine Fisheries Research Institute, Cochin, India

Correspondence: Present address: U Unnikrishnan, Departamento de Biologia, CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal. E-mail: unnikrishnanu@gmail.com

Present address: R Paulraj, Coastal Aquaculture Authority (Government of India), Chennai 600 006, India.

Abstract

The protein requirement of juvenile mud crab *Scylla serrata* (body weight = 0.25 ± 0.051 g, carapace width = 9.3 ± 0.04 mm) fed with different iso-energetic, iso-lipidic diets with graded protein levels (15–55% crude protein at 5% intervals) was determined. The feeding trial was conducted for a period of 63 days to determine the minimum and optimum protein requirement of juvenile *S. serrata*. The crabs fed with 15% and 20% dietary protein levels showed 100% and 12.5% of mortalities respectively. The mortalities observed in the above treatments were associated with the prolonged intermoult duration (46 and 32 days respectively). All other treatments recorded 100% survival. The best growth performance as well as the nutrient turn-over was recorded in crabs fed with 45% crude protein in the diet. Second-order polynomial regression of specific growth rate (SGR) as well as body protein gain vs. dietary protein levels suggested that 46.9–47.03% dietary protein is required for the best growth response and protein deposition in juvenile *S. serrata*. An extrapolation of 'SGR' and 'daily protein gain' upon the 'dietary protein level' axis ($Y = 0$) showed that 14.7–16.2% dietary protein is necessary for the minimum maintenance metabolism.

Keywords: *Scylla serrata*, mud crab, protein, amino acid, growth, formulated diet

Introduction

The mud crabs of the genus *Scylla* are strongly associated with mangrove areas throughout the Pacific and Indian oceans (Keenan 1999). They are an important source of income for small-scale fishers throughout the Asia-Pacific region (Gillespie & Burke 1992). The history of mud crab aquaculture is of 100 years in China (Yalin & Qingsheng 1994) and for at least the past 30 years throughout Asia. Traditionally, mud crabs have been considered to be single species, *Scylla serrata* (Stephen & Campbell 1960). But based on the morphologic and genetic characteristics, recent taxonomic revision of the genus (Keenan, Davie & Mann 1998) has shown that four distinct *Scylla* species [*S. serrata* (Forskål), *S. tranquebarica* (Fabricius), *S. olivacea* (Herbst) and *S. parmamosain* (Estampador)] exist. Among these, giant mud crab *S. serrata* is a suitable candidate for aquaculture, owing to its faster growth and larger size. Hence, they are being widely used for mud crab farming.

The outbreak of the white spot syndrome virus and collapse of shrimp aquaculture throughout Asia in the 1990s (Boyd & Clay 1998) resulted in a growing demand for mud crab as a potential alternative crustacean species for brackish water aquaculture (Marichamy & Rajapackiam 2001). However, the present mud crab-farming systems are limited due to the dependence on wild-caught seed supply (Keenan 1999) and the use of conventional diets such as trash fish, molluscan meat, meat entrails, etc. These diets are highly polluting, causing the spoilage of water quality, thereby affecting the health of the farmed crabs. Also, such diets may not be reliable in terms of their quality and continuous supply.

Information on the nutrient requirement of a species is crucial for the development of scientifically designed, nutritionally balanced, cost-effective commercially formulated diets (Tacon 2000). Compared

with shrimps, very few attempts were made to investigate the nutritional requirements in Portunids. Preliminary attempts using formulated diets were made in species such as *Callinectes sapidus* (Millikin, Fortner, Fair & Sick 1980), *Eriocheir sinensis* (Chen, Du & Lai 1994; Mu, Shim & Guo 1998) and in mud crabs of the genus *Scylla*.

Feeding trials with formulated diets containing 35% and 40% crude protein were first reported in mud crabs (~ 600 g size) by Chin, Gunasekera and Amandokoon (1992). However, no significant difference in growth was recorded between the two treatments. Sheen and Wu (1999) reported that a dietary lipid level ranging from 5.3% to 13.8% in a formulated diet supported the best growth response in post-larval *S. serrata*. Sheen (2000) reported that a 0.50–0.79% dietary cholesterol level is adequate for a significantly higher weight gain in *S. serrata*, whereas 0.14–1% dietary cholesterol is required for megalopae (Genodepa, Zeng & Southgate 2004; Holme, Zeng & Southgate 2006). Reports are available on the essentiality of highly unsaturated fatty acids for juvenile mud crabs (Sheen & Wu 2003), eicosapentaenoic acid/docosahexaenoic acid interactions in larval survival (Hamasaki, Suprayudi & Takeuchi 2002; Suprayudi, Takeuchi & Hamasaki 2004) and larval phospholipid requirement (3–4%, Genodepa *et al.* 2004; Holme, Southgate & Zeng 2007). Apparent digestibility coefficients of various feed stuffs for *S. serrata* were reported by Catacutan, Eusebio and Teshima (2003),Tuan, Anderson, Luong-van, Shelley and Allan (2006) and Truong, Anderson, Mather, Paterson and Richardson (2008), which provide useful information regarding different ingredients suitable for the formulation of practical diets for the species.

The experimental diets used for estimating lipid and cholesterol requirement by Sheen and Wu (1999) and Sheen (2000) contained a constant basal dietary protein level of 48%. A more systematic study using varying protein levels (32–48%) along with 6–12% lipid levels was carried out by Catacutan (2002) on the same species. The results of the study showed that a dietary protein level ranging from 32% to 40% with a 6–12% lipid level promoted the best growth performance. However, the range of protein level under investigation was limited to provide any information regarding the maintenance metabolic requirement and protein turnover.

Hence, in the present investigation, a wide range of dietary protein levels (15–55%) was tested in the feeding experiment with *S. serrata*. Parameters of protein utilization [nutrient turnover, *A*/*E* ratio, essential amino acid index (EAAI), etc.], besides the general growth response parameters [specific growth rate (SGR), feed conversion, carapace width gain (CWG), etc.], were evaluated. Compared with the pioneering study on protein requirement in *S. serrata* by Catacutan (2002), the present investigation used smaller crabs, which allow for a better estimation of the dietary protein requirement owing to their faster growth and moulting frequency. In addition, a higher number of juvenile crabs were used per treatment (six replicates × four crabs). Hence, a more precise estimation of the minimum as well as the optimum protein requirement of the species, for maintenance metabolism and better growth, respectively, could be achieved.

Materials and methods

Experimental crabs

Two hundred and fifty individuals of *S. serrata* juveniles (body weight = 0.25 ± 0.051 g, carapace width = 9.3 ± 0.04 mm) collected from Cochin backwaters were acclimated to the laboratory conditions (salinity = 28 ± 1 g L^{-1}, temperature = 27 ± 4 °C, dissolved oxygen ≥ 5.5 mg L^{-1}, total NH_3 ≤ 0.01 mg L^{-1}, nitrite < 4.0 mg L^{-1}, nitrate < 3.0 mg L^{-1} and pH = 8.0 ± 0.5) for a period of 1 week. The juvenile crabs were stocked individually in plastic cages with 10 individual compartments of 0.5 L volume. Twenty-five cages, each holding 10 juvenile crabs, were floated in 2000 L of de-chlorinated and aerated brackish water, held in an FRP tank. The crabs were weaned to a formulated pellet feed containing 40% crude protein by starving them for a day and offering the pellet feed the following day.

Experimental set-up

Two hundred and sixteen newly moulted juvenile mud crabs were randomly selected over a period of 7 days for the trial. A completely randomized design was followed for the entire experiment. Nine different feed treatments were used as detailed in the following section. Each treatment had six replicate troughs of 50 L capacity, with each trough having four equal partitions using a netlon screen (mesh size = 3 mm, Netlon™, Chennai, India). Each replicate trough had four crabs stocked individually in each partition. The troughs were supplied with de-chlorinated, aerated brackish water to a volume of 40 L (salinity =

$28 \pm 1\,g\,L^{-1}$, temperature = $27 \pm 4\,^{\circ}C$, dissolved oxygen $\geq 5.5\,mg\,L^{-1}$, total $NH_3 \leq 0.01\,mg\,L^{-1}$, nitrite $< 4.0\,mg\,L^{-1}$, nitrate $< 3.0\,mg\,L^{-1}$ and pH = 8.0 ± 0.5). Each partition in the trough was provided with a piece of PVC pipe (5 cm length and 2.5 cm diameter) as a hide-out for the juvenile crab. The troughs were covered over by black netlon mesh to reduce the light intensity. Continuous water exchange was provided to the rearing system, ensuring 200% water volume exchange over a period of 24 h. Moults and deaths of individual crabs were observed and recorded on a day-to-day basis.

Uneaten feed accumulated at the bottom of each rearing troughs was siphoned into separate filter cones made of bolting cloth (< 100 μm mesh), after 3 h of feeding. These were further washed with double-distilled water to remove any adhering salts and were oven-dried at $55 \pm 2\,^{\circ}C$. The left-over feed samples thus collected for the entire experimental duration were stored for estimating the feed intake per crab at the termination of the growth trial. Similarly, the faecal matter accumulated at the bottom of each trough was siphoned out periodically into separate filter cones, washed, dried and stored for estimating the digestibility of diets.

Experimental diets and rationing

Nine iso-energetic ($\sim 17\,MJ\,kg^{-1}$) and iso-lipidic ($\sim 8\%$) formulated pellet diets containing a dietary crude protein level ranging from 15% to 55% at 5% intervals were prepared by mixing various ingredients. All the ingredients were purchased locally, except vitamins, minerals, cholesterol, lecithin, dextrin, cellulose, guar gum (Merck Che, Merck India, Mumbai, India) and cod liver oil (Seven Seas, Hull, UK). Proximate compositions of the locally collected ingredients are presented in Table 1. Vitamin and mineral mixes were prepared according to Kanazawa, Shimaya, Kawasaki and Kashiwada (1970) and Kanazawa, Teshima, Matsumoto and Nomura (1981). Ingredients, except vitamins, minerals and cod liver oil, were blended and moistened with water (15% volume/weight) and steamed for 20 min. After cooling, vitamins, minerals and cod liver oil were added and subsequently kneaded into a homogenous dough using a food processor (Whiteline, Maharaja Whiteline, New Delhi, India). After thorough kneading and homogenization, the dough was further pelletized using a manually operated rotary pelletizer fitted with a stainless-steel die-disc (6.2 mm thick, 62.5 mm disc diameter with 25 numbers of holes having a diameter of 1.75 mm). The pellet strands, spread over enamelled aluminium plates, were dried in a high-flux hot-air oven (Labline, Labline Scientific Instruments, Mumbai, India) at 45 °C for 8 h to reduce the moisture level below 7.5%. After drying, the pellet strands were broken into a uniform pellet size ($\sim$ 1.2 mm diameter, 4.0 mm length). The diets thus prepared were stored in airtight food grade and freezer-safe plastic containers at $-20\,^{\circ}C$ until used. The formulation, proximate composition, amino acid profile, water stability (Obaldo, Divakaran & Tacon 2002) and the apparent protein and gross energy digestibility (Carter *et al.* 1960; Spyridakis, Metailler & Gabaudan 1989; Jones & De Silva 1997) of the diets are given in Tables 2a, 2b and 3. The experimental diets were offered to the juvenile crabs twice daily at 07:00 and 17:00 hours to apparent satiation ($\simeq$ 6% of body weight on a daily basis). The feeding trial ensured a 63-day feeding period to individual crab in each dietary treatment. This was done to avoid any errors associated with asynchrony in the first moulting of juvenile crabs, from which the feeding trial commenced.

Table 1 Proximate composition (% dry matter basis) of locally available ingredients used for the formulation of experimental diets

Ingredient	Dry matter	Crude protein	Crude lipid	Crude ash	NFE*	Gross energy ($MJ\,kg^{-1}$)†
Fish meal	92.20	67.27	5.15	16.27	3.40	16.98
Squid meal	91.71	77.36	5.01	4.61	4.62	19.28
Shrimp meal	92.21	70.99	3.01	11.54	1.36	16.57
Clam meal	91.35	52.12	5.04	5.06	28.10	17.94
Soya flour (de-oiled)	91.89	51.22	1.30	9.24	28.59	16.36
Wheat flour	92.85	11.79	1.65	1.32	77.63	16.52

*Nitrogen-free extract ≡ carbohydrate content of ingredient, NFE (%) = 100 − (moisture+crude protein+crude lipid+crude fibre+crude ash), all in percentage of feed. (Same is applicable for the succeeding tables wherever given.)

†Calculated based on Cuzon & Guillaume (1997): 21.3, 17.2 and 39.5 $MJ\,kg^{-1}$ of protein, carbohydrate and lipid, respectively. (The same is applicable for the succeeding tables wherever given.)

Table 2a Formulation of experimental diets

	Dietary protein levels								
Ingredients (%)	**CP-15**	**CP-20**	**CP-25**	**CP-30**	**CP-35**	**CP-40**	**CP-45**	**CP-50**	**CP-55**
Fish meal	11.54	15.40	19.11	23.07	26.95	30.58	34.54	38.78	42.38
Squid meal	0.63	0.84	1.04	1.26	1.47	1.67	1.88	2.12	2.31
Shrimp meal	1.47	1.96	2.43	2.94	3.43	3.89	4.40	4.94	5.39
Clam meal	4.20	5.60	6.95	8.39	9.80	11.12	12.56	14.10	15.41
Soya flour (de-oiled)	3.15	4.20	5.21	6.29	7.35	8.34	9.42	10.58	11.56
Wheat flour	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Dextrin	51.81	47.00	40.00	33.00	26.00	19.20	12.30	4.80	0.00
Cellulose	3.28	1.33	1.91	2.00	2.25	2.75	2.80	2.88	1.96
Cod liver oil	5.42	5.17	4.85	4.55	4.25	3.95	3.60	3.30	2.49
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin mix*	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Mineral mix†	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Guar gum‡	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cr_2O_3§	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

CP-15, CP-20, CP-25, CP-30, CP-35, CP-40, CP-45, CP-50 and CP-55 are feeds containing 15–55% protein at 5% graded levels. The same is followed in the succeeding tables.

*Vitamin mix (in kg dry weight of feed): retinone, 45 000 IU kg^{-1}; β-carotene, 90 mg kg^{-1}; cholecalciferol, 10 mg kg^{-1}; α-tocopherol, 600 IU kg^{-1}; menadione, 25 mg kg^{-1}; P-amino benzoic acid, 100 mg kg^{-1}; D-biotin, 4 mg kg^{-1}; nicotinic acid, 400 mg kg^{-1}; calcium pantothenate, 600 mg kg^{-1}; pyrodoxine hydrochloride, 120 mg kg^{-1}; riboflavin, 80 mg kg^{-1}; cyanocobalamine, 750 mcg kg^{-1}; folic acid, 7.95 mg kg^{-1}; inositol, 4 g kg^{-1}; choline chloride, 6 g kg^{-1}; thiamine hydrochloride, 39 mg kg^{-1}; ascorbic acid, 200 mg kg^{-1}; cellulose (as filler), 3 g kg^{-1}.

†Mineral mix (in kg dry weight of feed): $CaCO_3$, 14.5 g kg^{-1}; K_2HPO_4, 8.4 g kg^{-1}; $MgSO_4 \cdot 7H_2O$, 13 g kg^{-1}; $Ca_3(PO_4)_2$, 11.55 g kg^{-1}; $MnSO_4 \cdot H_2O$, 65 mg kg^{-1}; KI, 15 mg kg^{-1}; $NaHSeO_3$, 0.5 mg kg^{-1}; $CoCl_2 \cdot 6H_2O$, 40 mg kg^{-1}; $ZnSO_4 \cdot 7H_2O$, 0.15 g kg^{-1}; NaCl, 1.25 g kg^{-1}; $CuSO_4 \cdot 5H_2O$, 25 mg kg^{-1}; $FeSO_4 \cdot 7H_2O$, 0.63 g kg^{-1}.

‡Binder.

§Inert marker.

Table 2b Proximate composition, water stability and apparent digestibility of experimental diets

	Dietary protein levels								
	CP-15	**CP-20**	**CP-25**	**CP-30**	**CP-35**	**CP-40**	**CP-45**	**CP-50**	**CP-55**
Proximate composition (% as fed)									
Dry matter	92.93	92.84	92.38	92.36	92.34	92.50	92.30	92.23	91.81
Crude protein	15.53	20.41	25.1	30.11	35.01	39.60	44.61	49.96	54.51
Crude lipids	7.97	8.00	8.01	8.03	8.04	8.03	7.99	8.03	7.51
Crude ash	6.96	7.65	8.32	9.03	9.72	10.37	11.07	11.83	12.47
Nitrogen-free extract	57.90	54.03	48.10	42.20	36.30	30.54	24.74	18.42	14.52
Gross energy (MJ kg^{-1})	17.01	16.82	16.79	16.85	16.89	16.87	16.93	17.00	17.09
Protein/energy (mg kJ^{-1})	9.13	12.14	14.95	17.86	20.73	23.47	26.35	29.39	31.90
Water stability (% 4 h^{-1})	95.9	96.5	95.8	96.1	96.7	95.6	96.5	96.3	96.0
*Apparent digestibility (% as fed)**									
Protein	79.0	80.9^a	85.4^b	85.7^b	86.1^b	86.0^b	86.1^b	85.9^b	86.0^b
Gross energy	82.3	84.1^a	88.6^b	89.1^b	88.4^b	89.3^b	89.2^b	89.8^b	89.2^b

Means ($n = 21$, CP-15 excluded due to 100% mortality) in the same row with different superscripts are significantly different ($P < 0.05$); $PSEM_{protein} = 0.24$ and $PSEM_{gross\ energy} = 0.31$.

Pooled standard error of mean (PSEM) = $SD/\sqrt{n}$. (The same is applicable for the succeeding tables wherever given.)

*Apparent nutrient digestibility (% as fed) = 100 − 100 × (% Cr_2O_3 in feed/% Cr_2O_3 in faeces) × (% protein or MJ kg^{-1} gross energy in faeces/ % protein or MJ kg^{-1} gross energy in feed).

Table 3 Amino acid composition (% as fed) of nine formulated diets containing graded levels of protein

	Dietary protein levels									
	CP-15	CP-20	CP-25	CP-30	CP-35	CP-40	CP-45	CP-50	CP-55	*A*/*E* ratio*
EAA										
Arg	1.07	1.41	1.74	2.08	2.42	2.74	3.09	3.46	3.77	0.140
His	0.39	0.51	0.63	0.76	0.88	1.00	1.12	1.26	1.37	0.051
Ile	0.65	0.85	1.05	1.26	1.46	1.66	1.87	2.09	2.28	0.085
Leu	1.16	1.53	1.88	2.25	2.62	2.96	3.34	3.74	4.08	0.151
Lys	1.21	1.59	1.96	2.35	2.73	3.09	3.48	3.89	4.25	0.157
Met	0.36	0.48	0.59	0.71	0.82	0.93	1.05	1.17	1.28	0.047
Thr	0.69	0.91	1.12	1.34	1.56	1.77	1.99	2.23	2.43	0.090
Trp	0.68	0.89	1.09	1.31	1.53	1.73	1.94	2.18	2.37	0.088
Phe	0.63	0.83	1.03	1.23	1.43	1.62	1.82	2.04	2.23	0.083
Val	0.83	1.09	1.35	1.61	1.88	2.12	2.39	2.68	2.92	0.108
Σ EAA	7.69	10.10	12.43	14.91	17.33	19.61	22.09	24.73	26.99	
NEAA										
Ala	1.00	1.32	1.62	1.95	2.26	2.56	2.88	3.23	3.52	
Asp	0.95	1.25	1.53	1.84	2.14	2.42	2.72	3.05	3.33	
Cys	0.23	0.31	0.38	0.45	0.53	0.60	0.67	0.75	0.82	
Glu	2.14	2.81	3.46	4.15	4.82	5.45	6.14	6.88	7.51	
Gly	1.46	1.92	2.36	2.84	3.30	3.73	4.20	4.71	5.13	
Pro	0.81	1.07	1.31	1.57	1.83	2.07	2.33	2.61	2.85	
Ser	0.74	0.97	1.19	1.43	1.66	1.87	2.11	2.37	2.58	
Tyr	0.51	0.67	0.82	0.99	1.15	1.30	1.46	1.64	1.79	
Σ NEAA	7.84	10.31	12.67	15.20	17.68	20.00	22.53	25.23	27.53	
ΣEAA/ΣNEAA	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	

*$A/E = 100 \times$ (individual EAA/total EAAs).

EAA, essential amino acid; NEAA, nonessential amino acid.

Estimation of feed intake, growth response and biochemical composition

At the end of the feeding trial, survival percentage (%S), live weight and carapace width of the juvenile crabs were noted. Specific growth rate, live weight gain (LWG), CWG and average intermoult duration (av.IMD) were determined. Voluntary feed intake (VFI), feed conversion ratio (FCR), protein efficiency ratio (PER) and nutrient intake (protein, lipid and energy) were calculated from the total feed intake and body weight gain.

The crabs were then subjected to euthanasia and oven-dried at 55 ± 2 °C. The moisture and dry matter contents were determined. Proximate composition (AOAC 1990) and amino acid analysis of feed samples as well as dried crab samples (pooled per treatment) were determined in triplicate sets. For amino acid analysis, excluding tryptophan, 0.1 g of feed or crab sample with 10 mL of 6 N HCl was digested at 110 °C in glass tubes sealed with a nitrogen atmosphere, for 24 h. The solution was filtered and flash evaporated to remove any acid. The acid-free sample was further made up with 0.05 N HCl, and filtered using a 0.2 μm nylon membrane filter (Whatman, Whatman plc, Kent, UK) to remove any residue. The pre-column derivatization of amino acids was performed with phenyl isothiocyanite to form phenylthiocarbamyl amino acids (Harris 1988), which can be detected with high sensitivity in a reversed-phase PICO-TAG HPLC Amino Acid Analytical System (Waters, MA, USA). Tryptophan was estimated using the spectrophotometric method ($\lambda = 500$ nm, Genesys^{TM} 5, Thermo Fisher Scientific, MA, USA) developed by Sastry and Tummuru (1985), after alkali hydrolysis of the sample in 5% sodium hydroxide solution at 110 °C for 24 h. The feed and crab samples were analysed in triplicate.

Statistical analysis

Duncan's multiple range 'T' (DMRT, 95% confidence level) test (Duncan 1955) was performed as the *post hoc* test of ANOVA on the raw data of various parameters (Snedecor and Cochran 1967), to estimate the level of significance, using SPSS-13 processor (SPSS, Chicago, IL, USA). The optimum level of crude protein requirement was derived out of the second-order

polynomial regression curve with mean values of response parameters (SGR and daily body protein gain) vs. dietary protein levels (Shearer 2000) using ORIGIN-7.0 (OriginLab, MA, USA).

Results

Survival

The dietary protein levels had a significant ($P < 0.05$) influence on the survival of juvenile crabs, resulting in 100% mortality of the crabs fed with the low-protein diet CP-15. The crabs fed with CP-20 showed 12.5% mortality, whereas all other treatments recorded 100% survival throughout the trial (Table 4). The mortality of crabs in low-protein treatments was associated with a significantly delayed first moulting (32–46 days). The crabs fed with lower protein diets (CP-15 and CP-20) were unable to moult fully. The dead crabs were found to be in a partly moulted condition.

Growth response

Because crabs fed with diet CP-15 showed 100% mortality, the growth parameters were recorded only for the remaining treatments (CP-20, CP-25, CP-30, CP-35, CP-40, CP-45, CP-50 and CP-55). Live weight gain (% of initial weight), CWG (% of initial carapace width), SGR (% day^{-1}), VFI (g kg^{-1} average live weight day^{-1}), FCR, PER and av.IMD (days moult^{-1}) were taken as growth parameters to assess the best growth response (Table 4). The above growth response parameters were found to be significantly ($P < 0.05$) affected by different dietary treatments.

Live weight gain, CWG and SGR were found to be increased with increasing dietary protein levels up to 45% ($P/E = 26.35$ mg kJ^{-1}). A further increase in dietary protein up to 50% ($P/E = 29.39$ mg kJ^{-1}) and 55% ($P/E = 31.90$ mg kJ^{-1}) resulted in a slight decline in LWG and SGR, but were higher than that of diets fed with lower dietary protein levels (20–40%). The LWG was significantly different ($P < 0.05$) among different treatments, whereas significant differences in SGR were observed in all treatments, except CP-50 and CP-55 ($P > 0.05$). Carapace width gain did not show any significant difference ($P > 0.05$) among CP-45, CP-50 and CP-55, although all other treatments showed significant differences ($P < 0.05$).

The highest ($P < 0.05$) av.IMD was noted in juvenile crabs fed with CP-15, followed by CP-20, and the duration kept on decreasing with an increase in the protein level up to 45%. However, treatments CP-40–CP-55 did not show any significant difference in av.IMD values.

Table 4 Growth performance of *Scylla serrata* juveniles fed with graded protein levels

	Dietary protein levels									
	CP-15	CP-20	CP-25	CP-30	CP-35	CP-40	CP-45	CP-50	CP-55	PSEM
Survival (%)*	0	87.5	100	100	100	100	100	100	100	
LWG (%)†	ND	199.11^{a}	518.82^{b}	709.84^{c}	1187.19^{d}	1325.10^{e}	1874.09^{f}	1698.89^{g}	1568.62^{h}	13.09
CWG (%)‡	ND	112.43^{a}	128.36^{b}	139.33^{c}	160.45^{d}	210.56^{e}	225.73^{f}	225.37^{f}	223.03^{f}	0.88
SGR (% day^{-1})§	ND	1.09^{a}	2.61^{b}	3.11^{c}	3.93^{d}	4.10^{e}	4.65^{f}	4.50^{g}	4.37^{g}	0.02
av.IMD (days moult^{-1})¶	46.0^{a}	32.0^{b}	17.7^{c}	13.3^{d}	13.3^{d}	10.6^{e}	10.2^{e}	10.4^{e}	10.6^{e}	0.37
VFI (% ABW day^{-1})‖	ND	3.05^{a}	5.15^{b}	4.83^{c}	4.75^{c}	4.50^{cd}	4.34^{d}	4.74^{c}	4.78^{c}	0.05
FCR**	ND	2.90^{a}	2.40^{b}	2.02^{c}	1.77^{d}	1.65^{e}	1.52^{f}	1.68^{e}	1.71^{de}	0.01
PER††	ND	1.69^{a}	1.66^{ab}	1.64^{ab}	1.61^{b}	1.53^{c}	1.47^{c}	1.19^{d}	1.08^{e}	0.01

Means ($n = 21$) in the same row with different superscripts are significantly different ($P < 0.05$).

*Survival = 100 × (final number of crabs/initial number of crabs), during 63 days of the experimental period.

†Live weight gain = 100 × [(final mean body weight − initial mean body weight)/initial mean body weight].

‡Carapace width gain = 100 × [(final mean carapace width − initial mean carapace width)/initial mean carapace width].

§Specific growth rate = 100 × [($\log_e$ final body weight − $\log_e$ initial body weight)/days].

¶Average intermoult duration = (duration in days between first and last moult/number of moults), during 63 days of the experimental period.

‖Voluntary feed intake = 100 × [feed intake ABW day^{-1}], where average body weight (ABW) = [initial body weight + final body weight]/2.

**Feed conversion ratio = dry feed fed/wet weight gain.

††Protein efficiency ratio = wet weight gain/total protein fed.

ND, due to 100% mortality, parameters not determined.

PSEM, pooled standard error of mean.

The crabs fed with CP-20 (20% dietary protein) showed the lowest VFI of 3.05% average body weight (ABW) day^{-1}, whereas those fed with CP-25 showed the maximum VFI (5.15% ABW day^{-1}). A progressive reduction in VFI could be observed from the dietary protein levels 25–45%. A further increase in dietary protein levels resulted in a slow increase in VFI, but was not significantly different ($P > 0.05$) from juvenile crabs fed with 30–40% dietary protein. Similarly, VFIs recorded for crabs fed with CP-40 and CP-45 were not significantly different ($P > 0.05$) from each other (Table 4).

Significantly higher ($P < 0.05$) FCR was obtained in crabs fed with CP-20. The FCR was found to decrease with an increasing dietary protein level up to 45% (CP-45). The FCRs of the crabs fed with CP-50 and CP-55 were higher ($P < 0.05$) than CP-45, but were not significantly different ($P > 0.05$) from the crabs fed with diet CP-40. The highest PER was obtained by feeding the crabs with CP-20, and the PER showed a progressive reduction as the dietary protein levels were increased.

Biochemical composition of experimental crabs

Whole-body protein, lipid, ash and energy levels of juvenile *S. serrata* fed with different diets are given in Table 5. The moisture content of the crabs ranged from 68.62% to 70.66%, and did not show any significant difference ($P > 0.05$) among different dietary protein levels. The whole-body protein levels showed only a marginal difference among the crabs fed with a 20–40% dietary protein level. However, the crabs fed with 45% dietary protein showed a steep increase in body protein level with little or no significant increase at 50–55% dietary protein levels. The lipid content of the crabs showed an increasing trend up to CP-45 and thereafter exhibited a decreasing trend. However, carcass lipid levels for the treatments CP-45 and CP-50 were not statistically different ($P > 0.05$). The ash content of the juvenile crabs fed with different dietary protein levels showed no significant difference ($P > 0.05$), except for the marginal differences observed in crabs fed with CP-20 and CP-55. The whole-body energy levels of juvenile crabs showed an increasing trend in accordance with the increase in the dietary protein level up to 50%. The dietary treatment CP-45 and CP-50 did not show any significant difference ($P > 0.05$) in the whole-body protein, lipid, ash and energy content.

Protein, lipid and energy turnover

Protein intake was significantly different ($P < 0.05$) among all the treatments and showed an increasing trend from lower to higher dietary protein levels. The highest protein retention was recorded at 40% (CP-40) dietary protein level, and showed a diminishing trend at the levels below and beyond. However, the protein retention among the treatments from 30% to 45% dietary protein levels (CP-30, CP-35, CP-40 and CP-45) did not show any significant difference ($P > 0.05$). The maximum protein gain was recorded in crabs fed with CP-50, while a statistical difference ($P > 0.05$) could not be observed for the treatments from CP-40 to CP-55. The highest lipid intake was recorded in crabs fed with CP-20 and was negatively correlated with increasing dietary protein levels up to 45%. The crabs fed with dietary protein levels above 45% showed only a marginal increase in lipid intake with no statistical significance ($P > 0.05$). The crabs fed with CP-45 showed significantly ($P < 0.05$) higher gain as well as retention in body lipids when compared with the crabs fed diets containing <45%

Table 5 Biochemical composition (% live weight basis) of *Scylla serrata* juveniles fed with graded protein levels

	Dietary protein levels									
	Initial	CP-20	CP-25	CP-30	CP-35	CP-40	CP-45	CP-50	CP-55	PSEM
Moisture	69.59	70.66	70.44	70.17	69.35	69.1	68.75	68.55	68.62	0.326
Protein	13.92	12.26^{a}	12.89^{a}	12.85^{a}	13.56^{b}	13.96^{bc}	14.35^{c}	14.52^{c}	14.51^{c}	0.191
Lipid	2.9	2.00^{a}	2.18^{b}	2.93^{c}	3.46^{d}	3.58^{d}	3.79^{e}	3.74^{e}	3.50^{d}	0.037
Ash	10.06	10.9	10.41	9.95	9.74	9.63	9.38	9.5	9.87	0.194
GE (MJ kg^{-1})	4.72	4.12^{a}	4.31^{a}	4.60^{b}	4.93^{c}	5.03^{cd}	5.20^{d}	5.21^{d}	5.08^{cd}	0.086

Means ($n = 3$; pooled samples having 21 crabs per treatment) in the same row with different superscripts are significantly different ($P < 0.05$).

PSEM, pooled standard error of mean.

Table 6 Protein, lipid and energy turnover in *Scylla serrata* juveniles fed with graded protein levels

	Dietary protein levels (%)								
	CP-20	CP-25	CP-30	CP-35	CP-40	CP-45	CP-50	CP-55	PSEM
Protein intake (g kg^{-1} ABW day^{-1})	6.22^{a}	12.94^{b}	14.54^{c}	16.61^{d}	17.83^{e}	19.34^{f}	23.68^{g}	25.87^{h}	0.17
Protein retention (%)*	16.56^{ad}	20.59^{b}	20.48^{b}	21.53^{c}	21.87^{c}	21.35^{c}	17.34^{d}	15.71^{a}	0.12
Protein gain (g kg^{-1} ABW day^{-1})†	1.10^{a}	2.71^{b}	3.03^{c}	3.62^{d}	3.90^{e}	4.10^{e}	4.11^{e}	4.06^{e}	0.04
Lipid intake (g kg^{-1} ABW day^{-1})	2.45^{a}	4.13^{e}	3.88^{de}	3.81^{cd}	3.61^{bcd}	3.47^{b}	3.81^{cd}	3.56^{bc}	0.04
Lipid retention (%)*	4.46^{a}	10.40^{b}	18.07^{c}	24.90^{d}	28.68^{e}	31.61^{f}	28.02^{e}	27.75^{e}	0.15
Lipid gain (g kg^{-1} ABW day^{-1})†	0.11^{a}	0.43^{b}	0.70^{c}	0.95^{d}	1.04^{ef}	1.10^{g}	1.07^{fg}	0.99^{de}	0.01
Energy intake (MJ kg^{-1} ABW day^{-1})	0.51^{a}	0.87^{b}	0.81^{bc}	0.80^{c}	0.76^{cd}	0.72^{d}	0.78^{c}	0.79^{c}	0.01
Energy retention (%)‡	7.07^{a}	10.42^{b}	13.44^{c}	16.58^{d}	18.71^{e}	20.76^{f}	18.86^{e}	17.98^{e}	0.11
Energy gain (MJ kg^{-1} ABW day^{-1})§	0.04^{a}	0.09^{b}	0.11^{c}	0.13^{d}	0.14^{d}	0.15^{de}	0.15^{de}	0.14^{d}	0.01

Means ($n = 4$; each replicate is a plastic trough stocked with four crabs in individual partitions, i.e. 16 crabs per treatment) in the same row with different superscripts are significantly different ($P < 0.05$).

*Nutrient retention = 100 × (nutrient retained/total nutrient intake).

†Nutrient gain = nutrient retained/average body weight/days, where average body weight (ABW) = [initial body weight+final body weight]/2.

‡Energy retention = 100 × (energy retained/total energy intake).

§Energy gain = nutrient retained/average body weight/days.

PSEM, pooled standard error of mean.

crude protein. But it was not significantly different ($P > 0.05$) from those fed with CP-50. However, a further increase in the dietary protein level up to 55% resulted in a significantly lower ($P < 0.05$) body lipid gain and retention. Similarly, significantly ($P < 0.05$) lower energy intake, followed by the highest energy gain and energy retention was recorded in crabs fed with 45% dietary protein (CP-45). A detailed depiction of the protein, lipid and energy turnover is provided in Table 6.

Amino acid profile, *A/E* ratio and EAAI – diets and experimental crabs

The amino acid profile (% as fed, Table 3) of the diets showed a graded increase in levels in accordance with the increase in dietary protein levels. The ratios between essential (EAA) and non-essential amino acids (NEAA) in all the diets were the same (0.98). The *A/E* ratios of individual EAA of different diets are given in Table 3. The amino acid profiles (as percentage of body protein, Table 7) of the juvenile crabs fed with graded protein levels did not show any significant ($P > 0.05$) difference. The *A/E* ratio of individual EAA in crabs fed with different diets showed only a marginal difference among different treatments (Table 8). The essential amino acid index (Peñaflorida 1989), derived as *n*th root of *A/E* ratios of different diets to that of crabs fed with those diets, ranged from 0.967 to 0.977 for different treatments (Table 9).

EAA retention and gain

Significantly higher ($P < 0.05$) amino acid gain was recorded in crabs fed with CP-45, followed by those fed with CP-50 (Table 10). Juvenile crabs fed with CP-40 (40% dietary protein) recorded the highest retention of all the EAAs. But no significant differences ($P > 0.05$) were observed among the crabs fed with 25–45% dietary protein (Table 11).

Optimum protein requirement

Second-order polynomial regression curve of SGR vs. dietary protein level (Fig. 1) showed the optimum dietary protein requirement of the juvenile crabs. Formulated pellet diet containing 46.90% protein ($P/E = 27.44$ mg kJ^{-1}; P/E vs. dietary protein, $Y = 0.7836 + 0.5684X$, $R = 0.999$, figure not shown), when fed to the juvenile crabs, assuring a daily protein intake of 21.76 g kg^{-1} ABW day^{-1} (daily protein intake vs. dietary protein level, $Y = -1.3404 + 0.4926X$, $R = 0.975$, figure not shown), can yield the best SGR of 4.59% day^{-1}. A dietary protein level of 16.2% ($P/E = 9.14$ mg kJ^{-1}) corresponding to the daily maintenance ration of 6.64 g kg^{-1} ABW day^{-1} is required by the crabs for minimum maintenance at SGR_0. Another polynomial regression curve (Fig. 2), depicting the relationship between daily body protein gain and dietary protein levels, showed that the maximum protein gain of 4.19 g kg^{-1} ABW day^{-1} can be obtained at 47.03% dietary protein level ($P/E =$ 27.52 mg kJ^{-1}; daily protein intake = 21.83 g kg^{-1}

Table 7 Amino acid profile of *Scylla serrata* juveniles fed with graded levels of protein

	Dietary protein levels									
	Initial	**CP-20**	**CP-25**	**CP-30**	**CP-35**	**CP-40**	**CP-45**	**CP-50**	**CP-55**	**PSEM**
EAA										
Arg	5.02	5.01	5.03	5.02	5.03	5.04	5	5.02	5.02	0.007
His	2.74	2.75	2.79	2.77	2.75	2.76	2.74	2.78	2.75	0.010
Ile	5.93	5.92	5.91	5.95	5.94	5.93	5.97	5.92	5.95	0.012
Leu	10.71	10.76	10.72	10.73	10.74	10.7	10.74	10.72	10.71	0.011
Lys	5.16	5.17	5.18	5.12	5.15	5.16	5.11	5.19	5.14	0.016
Met	1.87	1.86	1.86	1.82	1.89	1.87	1.85	1.86	1.88	0.012
Thr	4.54	4.51	4.59	4.53	4.55	4.57	4.54	4.52	4.53	0.015
Trp	0.41	0.44	0.43	0.42	0.45	0.41	0.4	0.41	0.4	0.011
Phe	7.15	7.18	7.19	7.15	7.14	7.13	7.12	7.13	7.11	0.016
Val	5.98	5.99	5.98	5.96	5.93	5.95	5.92	5.94	5.9	0.017
NEAA										
Ala	6.98	6.99	7.02	6.97	6.95	6.94	6.96	6.94	6.92	0.018
Asp	4.32	4.3	4.31	4.3	4.29	4.26	4.28	4.27	4.27	0.010
Cys	0.48	0.48	0.49	0.47	0.46	0.44	0.46	0.47	0.45	0.009
Glu	8.89	8.85	8.88	8.86	8.82	8.78	8.79	8.75	8.79	0.026
Gly	11.88	11.75	11.79	11.83	11.85	11.84	11.78	11.77	11.75	0.023
Pró	8.99	8.9	8.95	8.94	8.9	8.89	8.86	8.86	8.88	0.019
Ser	5.59	5.57	5.59	5.55	5.61	5.53	5.51	5.54	5.5	0.022
Tyr	3.46	3.47	3.49	3.46	3.42	3.4	3.41	3.4	3.42	0.020

Means of three analysis ($n = 3$); pooled samples having 21 crabs per treatment.
EAA, essential amino acid; NEAA, non-essential amino acid; PSEM, pooled standard error of mean.

Table 8 A/E* ratio of *Scylla serrata* fed with graded protein levels

		Dietary protein levels							
EAA	**Initial†**	**CP-20**	**CP-25**	**CP-30**	**CP-35**	**CP-40**	**CP-45**	**CP-50**	**CP-55**
Arg	10.05	10.25	10.11	10.31	10.17	10.06	10.72	10.32	10.64
His	5.50	5.54	5.44	5.71	5.58	5.56	5.47	5.50	5.48
Ile	11.89	11.38	11.66	11.43	11.49	12.24	11.90	11.89	11.75
Leu	21.44	20.89	21.33	21.31	22.03	21.41	21.44	21.71	21.47
Lys	10.35	10.35	10.50	10.07	9.93	10.28	10.33	10.48	10.83
Met	3.73	3.87	3.94	3.87	3.74	3.78	3.72	3.73	3.73
Thr	9.46	9.12	9.04	9.30	9.22	8.95	8.97	9.09	8.99
Trp	0.83	0.88	0.83	0.87	0.85	0.85	0.83	0.83	0.82
Phe	14.77	14.96	14.58	14.92	14.70	14.60	14.27	14.46	14.43
Val	11.99	12.75	12.59	12.20	12.29	12.28	12.34	11.99	11.85

Mean of four observations ($n = 4$; each replicate is a plastic trough stocked with four crabs in individual partitions, i.e. 16 crabs per treatment).
*$A/E = 100 \times$ (individual EAA/total EAAs).
†A/E ratio of the initial whole body EAA of juvenile crabs.

ABW day^{-1}). A minimum dietary protein level of 14.70% ($P/E = 9.99$ mg kJ^{-1}; daily protein intake = 5.90 g kg^{-1} ABW day^{-1}) is found to be required for maintenance metabolism.

Discussion

After 63 days of a feeding trial, the juvenile mud crabs fed with diets containing 45% protein recorded the highest LWG, CWG, SGR and FCR compared with the crabs fed with other diets. However, the highest body nutrient gains (protein, lipid, amino acid and energy) was obtained in crabs fed with 45% and 50% dietary protein, but were not statistically different (DMRT test) from each other. The SGR as well as protein requirement recorded in the present study was higher than those reported by Catacutan (2002), for the same species. It is reported that faster

Table 9 Ratio of essential amino acids (EAAs) in feedstuffs to that of juvenile *Scylla serrata* (aa/AA)* fed with graded protein levels and essential amino acid index (EAAI)†

	Dietary protein levels							
EAA	**CP-20**	**CP-25**	**CP-30**	**CP-35**	**CP-40**	**CP-45**	**CP-50**	**CP-55**
Arg	0.98	0.99	0.97	0.99	1.00	0.94	0.97	0.94
His	0.98	1.00	0.95	0.98	0.98	0.99	0.99	0.99
Ile	1.00	0.98	1.00	0.99	0.93	0.96	0.96	0.97
Leu	1.00	0.98	0.98	0.95	0.98	0.97	0.96	0.97
Lys	0.96	0.95	0.99	1.00	0.97	0.96	0.95	0.92
Met	0.96	0.95	0.96	1.00	0.98	1.00	1.00	1.00
Thr	0.98	0.99	0.96	0.97	1.00	1.00	0.98	0.99
Trp	0.93	0.99	0.94	0.96	0.97	0.99	0.99	1.00
Phe	0.95	0.98	0.96	0.97	0.98	1.00	0.99	0.99
Val	0.93	0.94	0.97	0.96	0.96	0.96	0.99	1.00
EAAI	0.967	0.974	0.968	0.977	0.974	0.977	0.977	0.977

Mean of four observations ($n = 4$; each replicate is a plastic trough stocked with four crabs in individual partitions, i.e. 16 crabs per treatment).

*aa/AA = *A*/*E* ratio in feed/*A*/*E* ratio in juvenile *S. serrata*. aa/AA ratio are normalized to have 1 maximum (Peñaflorida 1989).

†EAAI $= \sqrt[n]{aa_1/AA_1 \times aa_2/AA_2 \times \cdots\cdots\cdots \times aa_n/AA_n}$, where 'aa' is the *A*/*E* ratio in the feed; 'AA' is the *A*/*E* ratio in the crabs; *n* is the number of EAAs.

Table 10 Essential amino acid (EAA) gain* in *Scylla serrata* fed with graded protein levels (% of initial mean body level)

	Dietary protein levels								
EAA	**CP-20**	**CP-25**	**CP-30**	**CP-35**	**CP-40**	**CP-45**	**CP-50**	**CP-55**	**PSEM**
Arg	69.71^{a}	356.54^{b}	524.29^{c}	1010.7^{d}	1216.83^{e}	1776.33^{f}	1617.27^{g}	1437.83^{g}	44.90
His	70.95^{a}	360.63^{b}	536.60^{c}	1029.0^{d}	1223.23^{e}	1789.30f	1635.63^{g}	1498.27^{h}	47.06
Ile	68.10^{a}	342.76^{b}	534.48^{c}	1020.2^{d}	1211.33^{e}	1765.70^{f}	1621.93^{g}	1482.23^{g}	39.51
Leu	66.53^{a}	351.20^{b}	530.10^{c}	1015.6^{d}	1218.50^{e}	1773.00^{f}	1626.27^{g}	1452.13^{g}	39.99
Lys	64.36^{a}	339.28^{b}	521.30^{c}	1012.5^{d}	1206.43^{e}	1761.73^{f}	1611.43^{g}	1446.43^{g}	39.88
Met	71.02^{a}	369.32^{b}	542.50^{c}	1024.3^{d}	1243.13^{e}	1803.20^{f}	1661.53^{g}	1480.73^{h}	40.31
Thr	62.53^{a}	343.20^{b}	500.33^{c}	967.4^{d}	1153.80^{e}	1682.13^{f}	1541.97^{g}	1409.47^{h}	38.16
Trp	87.37^{a}	396.70^{b}	529.90^{b}	1116.4^{c}	1291.23^{d}	2028.03^{e}	1632.23^{f}	1517.60^{f}	40.30
Phe	64.87^{a}	348.00^{b}	512.07^{c}	981.9^{d}	1173.93^{e}	1725.20^{f}	1552.87^{g}	1434.30^{g}	39.29
Val	68.60^{a}	360.97^{b}	531.00^{c}	1013.1^{d}	1212.30^{e}	1770.73^{f}	1603.33^{g}	1477.07^{g}	40.50

Means ($n = 4$; each replicate is a plastic trough stocked with four crabs in individual partitions, i.e. 16 crabs per treatment) in the same row with different superscripts are significantly different ($P < 0.05$).

*EAA gain = 100 × [(final mean body amino acid level − initial mean body amino acid level)/initial mean body amino acid level].

PSEM, pooled standard error of mean.

growth rates may reflect faster metabolic rates, which in turn can be related to the higher protein requirements in earlier stages of *S. serrata* (D'Abramo & Sheen 1996; Campaña 2001).

The intake of dietary protein above the optimum level showed a negative correlation with the growth response of *S. serrata* juveniles in the present study. Apart from creating an imbalance in the nutrient profile of the diets, a higher dietary protein level leads to catabolism of excess protein. This in turn may generate higher ammonia levels in the haemolymph of crustaceans as an excretory product (Rosas, Diaz, Soto, Gaxiola, Brito, Baez & Pedroza 1995). The accumulation of ammonia nitrogen in the haemolymph may negatively affect metabolic processes such as oxygen transport and osmotic pressure balance (Schmitt and Santos 1998; Guzman, Gaxiola, Rosa & Torre-blanco 2001). It can further add to the metabolic cost of nitrogen excretion (Jauncey 1982; Vergara, Fernandez-palacios, Robaina, Jauncey, De La Higuera & Izquierdo 1996), compromising normal growth in crustaceans. Another explanation by Har-

Table 11 Essential amino acid (EAA) retention* in *Scylla serrata* fed with graded protein levels (% of intake)

	Dietary protein levels								
EAA	**CP-20**	**CP-25**	**CP-30**	**CP-35**	**CP-40**	**CP-45**	**CP-50**	**CP-55**	**PEM**
Arg	12.10^{a}	15.03^{b}	14.90^{b}	15.77^{c}	15.83^{c}	15.33^{c}	12.57^{a}	11.23^{a}	0.222
His	18.23^{a}	22.60^{b}	22.53^{b}	23.80^{c}	23.83^{c}	23.10^{c}	18.87^{a}	17.00^{a}	0.337
Ile	23.70^{a}	29.37^{bc}	29.20^{b}	30.87^{c}	30.97^{c}	30.00^{c}	24.57^{a}	22.03^{a}	0.433
Leu	23.87^{a}	29.57^{b}	29.47^{b}	31.13^{c}	31.20^{c}	30.27^{c}	24.77^{a}	22.23^{a}	0.437
Lys	11.07^{a}	13.73^{b}	13.67^{b}	14.43^{c}	14.50^{c}	14.00^{bc}	11.47^{a}	10.30^{a}	0.199
Met	13.20^{a}	16.43^{b}	16.33^{b}	17.27^{c}	17.30^{c}	16.77^{bc}	13.70^{a}	12.33^{a}	0.239
Thr	15.67^{a}	20.63^{b}	20.60^{b}	21.87^{c}	21.97^{c}	21.30^{c}	17.43^{ab}	15.63^{a}	0.319
Trp	1.60^{a}	2.00^{bc}	1.93^{b}	2.10^{c}	2.07^{c}	2.00^{bc}	1.60^{a}	1.50^{a}	0.121
Phe	27.87^{a}	35.90^{c}	35.87^{c}	37.97^{c}	38.10^{c}	36.97^{c}	30.23^{b}	27.17^{a}	0.550
Val	18.67^{a}	23.07^{c}	23.03^{c}	24.30^{c}	24.33^{c}	23.60^{c}	19.30^{b}	17.33^{a}	0.341

Means ($n = 4$; each replicate is a plastic trough stocked with four crabs in individual partitions, i.e. 16 crabs per treatment) in the same row with different superscripts are significantly different ($P < 0.05$).
*EAA retention = 100 × (EAA retained/EAA intake).

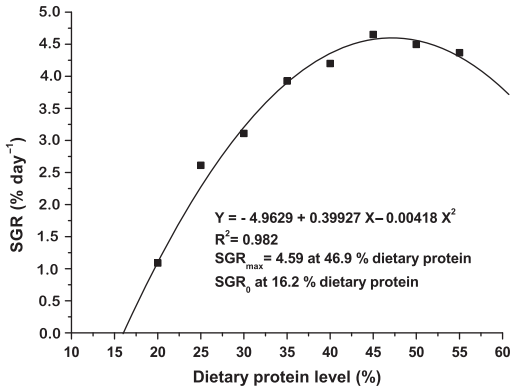

Figure 1 Second-order polynomial regression curve, specific growth rate (SGR; % day^{-1}) vs. dietary protein level (%) in juvenile *Scylla serrata* fed graded levels of protein for 63 days.

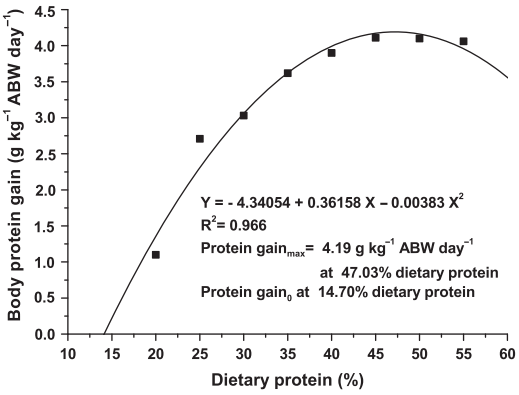

Figure 2 Second-order polynomial regression curve, body protein gain (g kg^{-1} ABW day^{-1}) vs. dietary protein level (%) in juvenile *Scylla serrata* fed graded levels of protein for 63 days.

per, Benevenga and Wohleuter (1970) is that, at excessively higher dietary protein levels, free amino acids accumulated in the body fluids may become toxic, affecting the normal metabolism and growth.

In the present study, the apparent digestibility of protein and gross energy ranged from 79.0% to 86.1% and 82.3% to 89.8% respectively. Lower apparent digestibility of protein as well as gross energy was recorded in crabs fed with CP-15 and CP-20. No other treatments showed any significant difference. The poor digestibility of diets CP-15 and CP-20 can be attributed to the high NFE (carbohydrate) levels in the form of dextrin, together with a severe dietary nutrient imbalance. In the present study, a dietary NFE level above 48% is found not to be tolerated by the juvenile crabs to a greater extent, resulting in the poor apparent digestibility of low-protein diets (CP-15 and CP-20). This is in agreement with the earlier report of Pavasovic, Richardson, Anderson, Mann and Mather (2004) that the mud crabs can digest up to 47% of carbohydrate in the diet. Truong *et al.* (2008) reported that a higher starch digestibility was obtained when 45% wheat starch was included in the diet, which is further confirmed in the present study.

The FCR recorded in this study ranged from 1.52 (CP-45) to 2.90 (CP-20), which is much ideally lower than that reported previously for the same species (Catacutan 2002) and *E. sinensis* (Mu *et al.* 1998). This confirms the higher quality of diets used in the present study (Tacon 2001; Tacon, Nates & Mcneil

2004). The crabs fed CP-15 and CP-20 were observed to have a reduced appetite compared with the crabs fed other diets. The lowest VFI, calculated for crabs fed with diet CP-20 (20% dietary protein), further confirmed the above observation. It is known that animals avoid eating unbalanced diets (Vivas, Sanchez-vazquez, Garcia & Madrid 2003; Vivas, Rubio, Sanchez-vazquez, Mena, Garcia & Madrid 2006) that do not meet their nutritional requirements. This clearly explains the loss of appetite and reduced VFI of crabs fed with lower dietary protein levels (CP-15 and CP-20). Crabs fed with CP-25 recorded the highest VFI. A progressive reduction in VFI and FCR was observed in crabs fed with a dietary protein level up to 45%. A further increase in dietary protein was found to be affecting the feed efficiency negatively, which can be attributed to the imbalance in the nutrient profile of the diets (CP-50 and CP-55). Crabs fed with 25–45% dietary protein tend to adjust the feed intake to meet the dietary protein requirement by increasing the feed intake at a lower protein level and vice versa. Aquatic animals usually regulate feed intake to meet the energy requirements (Bureau, Kaushik & Cho 2002; Vivas *et al.* 2006). Within these limits, they also try to adjust feed intake to meet the protein requirements (Peres & Oliva-Teles 1999; Fournier, Gouillou Coustans, Metailler, Vachot, Guedes, Tulli, Oliva-teles, Tibaldi & Kaushik 2002; Vivas *et al.* 2006).

Zero percentage survival of the crabs fed with CP-15 (associated with incomplete moulting leading to death) confirms that the dietary protein levels did not comply with the minimum dietary protein requirement for the maintenance, moulting and growth. Also, crabs fed with 20% dietary protein suffered 12.5% mortality associated with moulting. The mortality of crabs fed with low-protein diets (CP-15 and CP-20) was associated with moulting. The crabs fed with low-protein diets (CP-15 and CP-20) moulted only once during the entire 63 days of the experiment, with a prolonged intermoult duration of 32–46 days. Reduced VFI, due to severe nutrient imbalance in low-protein diets, would have further added to the magnitude of dietary protein deficiency in low-protein diets.

The crabs fed with diet CP-45 showed the lowest av.IMD with a higher moulting frequency when compared with other treatments. However, av.IMDs of crabs fed with 40–55% dietary protein were statistically analogous, showing that at least 40% dietary protein is necessary for normal moulting. The impact of nutrition on the moulting frequency and growth of crustaceans was recognized by Guillaume (1997) and D'Abramo and New (2000). It is reported that crustaceans fed with nutritionally deficient diets resulted in poor growth, accommodating themselves to such diets by altering their moulting patterns (Jones, De Silva & Mitchell 1996).

Gain in carapace width is an important growth index in portunids (Pinheiro & Fransozo 1993; Takeuchi, Satoh, Sekiya, Shimizu & Watanabe 1999; Catacutan 2002). In the present study, carapace width was found to increase with the dietary protein level from 20% to 45%. A further increase in the dietary protein level did not show any improvement. Hence, 45% dietary protein level once again proved to be optimum for the best growth performance of juvenile mud crabs.

Protein efficiency decreased with increasing dietary protein from 20% to 55%. This clearly indicates that, at lower protein levels, crabs tend to conserve and utilize the available dietary protein for growth. Lowering of PER at higher dietary protein levels is indicative of crabs using more and more protein for catabolism. Similar results have been reported for other crustaceans such as *Penaeus monodon* (Hajra, Ghosh & Mandal 1988; Shiau & Peng 1992), *Litopenaeus vannamei* (Hu, Tan, Mai, Ai, Zheng & Cheng 2008), *E. sinensis* (Mu *et al.* 1998) and *Cherax quadricarinatus* (Corté, Jacinto, Villarreal-Colmenares, Civera-Cerecedo & Martínezcó Rdova 2003). In the present study, the diets used were iso-energetic, iso-lipidic with similar amino acid profiles. Therefore, differences in the response could be attributed to the protein levels, rather than dietary energy, lipid levels or protein quality.

The dietary energy content of different diets used in the present study (16.79–17.09 MJ kg^{-1}) was in the range close to that recommended for crustacean diets (Cuzon & Guillaume 1997). In the present study, the highest growth rate was observed at a *P/E* ratio of 26.35 mg kJ^{-1} (45% dietary protein, $\simeq$ 8% lipid), which is close to that observed (27.5 mg kJ^{-1}; 40% dietary protein, 6% dietary lipid) by Catacutan (2002) for the same species. Preliminary studies with larger-sized (600 g) mud crabs by Chin *et al.* (1992) also showed a comparable *P/E* ratio (calculated from the given feed composition) of 25.66 mg kJ^{-1} (35% dietary protein, 5.2% dietary lipid) for the best growth response. A similar range of the *P/E* ratio has been reported for other crustacean species such as *E. sinensis* (26.76 mg kJ^{-1}; 39% dietary protein, Mu *et al.* 1998), *P. monodon* (26.05–29.87 mg kJ^{-1}; 36–40% dietary protein, Shiau & Chou 1991), *Penaeus merguiensis* (26.29–31.07 mg kJ^{-1}; D'Abramo and Sheen 1994) and *Astacus astacus* (27.24–29.31 mg kJ^{-1}; Ackefors, Castell, Linda, Raty & Svensson 1992).

The body protein levels showed a marginal increment with an increase in dietary protein levels (12.26–14.52%). This is in consensus with the observation in *L. vannamei* (Hu *et al.* 2008) and *P. monodon* (Alava and Lim 1983). However, in the species under investigation (*S. serrata*), Catacutan (2002) could not find any significant differences in flesh protein of crabs fed different dietary protein levels. Similarly, no significant difference in body protein levels in response to dietary protein levels were identified in *E. sinensis* (Mu *et al.* 1998). In the present investigation, it is found that the whole-body lipid levels tended to increase with the increase in the *P*/*E* ratio of the diets up to 45% and remained invariant in CP-50. This clearly indicates that the crabs tend to show a preference for lipids as an energy source, when fed with dietary protein levels close to the maintenance level, in order to spare the protein (Cuzon and Guillaume 1997; Hu *et al.* 2008). Similar observations were found in *L. vannamei* (Rosas, Cuzon, Taboada, Pascual, Gaxiola & Wormhoudt 2001; Hu *et al.* 2008) and *Litopenaeus setiferus* (Rosas *et al.* 2001). Whole-body energy levels followed a similar pattern similar to that of the body lipid levels. It can be chiefly attributed to the increased accretion of body lipid levels, followed by body protein levels, from lower (20%) to optimum dietary protein levels (45–50%). This is in accordance with the increase in growth rates. Moisture as well as whole-body ash content did not show any significant difference among the treatments. Similar observations were made in the same species (Catacutan 2002) as well as in *L. vannamei* (Hu *et al.* 2008).

The dietary protein intake was found to be directly proportional to the availability of protein in different diets. Protein retention (percentage of intake) remained statistically invariant ($P > 0.05$) from 35% to 45% dietary protein levels, although protein retention showed a peak value at 40%. From the results, it is evident that owing to the better nutrient balance available in the dietary protein range 35–45%, higher protein retention was observed. At still higher dietary protein levels (50% and 55%), excess dietary protein available is diverted for catabolism, thereby reducing the protein retention (Guzman *et al.* 2001). Average body protein gain showed the highest value at 45% and remained statistically invariant at dietary protein levels above this. This has further confirmed the assumption that the juvenile crabs require a dietary protein level close to 45%. The lipid as well as energy intake followed a similar pattern of VFI. The highest daily lipid intake was recorded at the 25% dietary protein level and showed to decline towards the 45% dietary protein level, as crabs tend to increase the intake of feed (iso-energetic and iso-lipidic) to compensate for the protein requirement at lower protein levels (Peres & Oliva-Teles 1999; Vivas *et al.* 2003, 2006). Both gain and retention of body lipid as well as energy showed an inverse relation to that of the feed intake, with a higher deposition recorded at 45% dietary protein. Better growth response, substantiated by higher nutrient and energy deposition, once again confirms that among different dietary treatments, 45% dietary protein is the optimum for normal growth and metabolism in *S. serrata*.

The amino acid profile of *S. serrata* fed with different protein levels did not show any significant difference. The EAA retention followed a pattern similar to that of the body protein retention, with a better retention at 35–45% ($P > 0.05$) dietary protein levels. Essential amino acid gain (as percentage of the initial body level) of crabs fed with different diets was in agreement with the gain in total body protein (as percentage of the initial body level). The highest body weight gain as well as amino acid gain was recorded at the 45% dietary protein level, which in turn reflects the highest protein deposition. At lower dietary protein levels (20–30%), it is likely that the percentage of intake is channelled towards maintenance metabolism, which would have resulted in a lower retention (Teshima, Alam, Koshio, Ishikawa & Kanazawa 2002). It is known that surplus EAA available at higher protein levels above the optimum will be channelled for catabolism and energy production, thereby reducing the retention efficiency (Cuzon & Guillaume 1997). Similar observations were reported in *Marsupinaeus japonicus* (Teshima *et al.* 2002) and *Paralichthys olivaceus* (Alam, Watanabe, Carroll & Rezek 2009).

The *A*/*E* ratio of individual amino acids in the whole crab (control – for estimating initial nutrient levels) and that of the diets are highly comparable in the present study. Also, the *A*/*E* ratios of whole-body EAA of crabs at the end of different treatments (as well as that of the control crabs – for estimating the initial nutrient levels) did not show any noticeable variation. Similarly, the EAAI values calculated as described by Peñaflorida (1989), for each EAA, in different diets were in the ideal range (0.967–0.977). The similarity in the *A*/*E* ratios of diets to that of whole crabs and the ideal EAAI ratios of diets clearly confirms that the protein source used in the present study is of superior quality for *S. serrata* (Peñaflorida 1989; Mente, Coutteau, Houlihan, Davidson & Sorgeloos 2002).

In order to probe more into the optimum dietary protein requirement, second-order polynomial regression was performed on dietary protein vs. selected growth parameters. Shearer (2000) suggested that the quadratic regression method can be superior to the conventional broken-line analysis to obtain a more accurate estimation of the optimum dietary nutrient level, supporting the best growth response. The second-order polynomial regression fitting on the curves of SGR and daily body protein gain vs. dietary protein levels clearly indicate that an optimum dietary protein level ranging from 46.90% to 47.03% (daily protein intake of 21.76–21.83 g kg^{-1} ABW day^{-1}; P/E = 27.44–27.52 mg kJ^{-1}) is required for the best growth response and protein deposition in juvenile *S. serrata*. An extrapolation to 'SGR_0' and 'zero daily protein gain' along the curves showed that a minimum dietary protein level of 14.70–16.20% (daily protein intake of 5.90–6.64 g kg^{-1} ABW day^{-1}; P/E = 9.14–9.99 mg kJ^{-1}) is necessary for the minimum maintenance metabolism. In the current investigation, crabs fed with the lowest protein diets (15%) died during moulting, after passing through a very long intermoult duration. This corroborates with the assumption that the lowest protein diet (CP-15) would have supported the minimum maintenance metabolism, but was deficient to support an increased metabolic requirement (Stern & Cohen 1982) associated with moulting. Higher protein requirement values obtained in the present study can be attributed to the more carnivorous nature of the juvenile crabs when compared with the adults. This can be further attributed to the higher metabolic requirements of protein to meet faster growth rates in juvenile crabs.

In the current investigation, the authors used smaller crabs with a higher number of juveniles per treatment. Also, the present feeding experiment used a wider range of dietary protein levels. This was helpful in obtaining a more precise estimation of growth response parameters. The results of the present study were strengthened with the evaluation of more parameters (protein, amino acid, lipid and energy turnover, A/E, EAAI, etc.). The use of a superior-quality protein source in the diet (EAAI = 0.97) could assure a more accurate estimation of the protein turnover in juvenile mud crabs.

The present study has used practical diets formulated using locally available ingredients, with a protein source very close to the amino acid profile of *S. serrata*. The results discussed in the study have enhanced the scope for developing nutritionally balanced and cost-effective formulated practical diets for *S. serrata*, which can also ensure better water quality in the farms. Further, it can replace the currently used fresh feeds (trash fish, molluscan meat, meat entrails, etc.), which have uncertainties in quality and continuous supply.

Acknowledgments

We are grateful to National Agricultural Technology Project (Joint funding programme of Indian Council of Agricultural Research and World Bank - ICAR/NATP/CAES-PSR/9903) for funding this investigation and providing fellowship to the first author during the tenure of his Doctoral research. Expert assistance in identifying the species in new taxonomic lines by Dr C. P. Keenan and Dr David Mann, Department of Primary Industries, ACIAR, Australia, is duly acknowledged. We thank Mrs Rosalie Shaffer, NMFS, Florida, USA, for providing bibliographic support. Technical assistance in amino acid analysis provided by Mrs G. Shailaja, CMFRI, Cochin, India, is gratefully appreciated. Assistance in English language editing provided by Mrs. Vijayalekshmi Nair and Mrs. Shailaja Menon is also acknowledged.

References

Ackefors H., Castell J.D., Linda D.B., Raty P. & Svensson M. (1992) Standard experimental diets for crustacean nutrition research: II. Growth and survival of juvenile crayfish *Astacus astacus* (Linne) fed diets containing various amounts of protein, carbohydrate and lipid. *Aquaculture* **104**, 341–356.

Alam M.S., Watanabe W.O., Carroll P.M. & Rezek T. (2009) Effects of dietary protein and lipid levels on growth performance and body composition of black sea bass *Centropristis striata* (Linnaeus 1758) during grow-out in a pilot-scale marine recirculating system. *Aquaculture Research* **40**, 442–449.

Alava V. & Lim C. (1983) The quantitative protein requirements of *Penaeus monodon*. *Aquaculture* **30**, 53–61.

AOAC (1990) *Official Methods of Analysis*, 15th edn. Association of Official Analytical Chemists, Washington, DC, 1094pp.

Boyd C. & Clay J. (1998) Shrimp aquaculture and the environment. *Scientific American* **278**, 42–48.

Bureau D.P., Kaushik S.J. & Cho C.Y. (2002) Bioenergetics. In: *Fish Nutrition*, 3rd edn, (ed. by J.E. Halves & R.W. Hardy), pp. 1–59. Academic Press, San Diego, CA, USA.

Campaña T.A. (2001) *Digestibility of vegetal and animal ingredients and diets for juvenile and pre-adult redclaw Cherax*

quadricarinatus. Master of Science Thesis, CIBNOR, S.C. La Paz, B.C.S. México.

Carter J.F., Bolin D.W. & Erickson D. (1960) Evaluation of forages by the agronomic "difference" method and the chromogen oxide "indication" techniques. *North Dakota Agriculture Extension Bulletin* **426**, 55pp.

Catacutan M.R. (2002) Growth and body composition of juvenile mud crab, *Scylla serrata*, fed different dietary protein and lipid levels and protein to energy ratios. *Aquaculture* **208**, 113–123.

Catacutan M.R., Eusebio P.S. & Teshima S. (2003) Apparent digestibility of selected feedstuffs by mud crab, *Scylla serrata*. *Aquaculture* **216**, 253–261.

Chen L.Q., Du N.S. & Lai W. (1994) The influence of dietary protein on growth of Chinese mitten crab juvenile *Eriocheir sinensis*. In: *The 60th Anniversary of Chinese Zoological Society, Treatise on the 100th Anniversary in Memory of Prof. Chen Zhen*, Vol. 9, pp. 512–518. Beijing, China (in Chinese, with English abstract).

Chin H.C., Gunasekera U.P.D. & Amandokoon H.P. (1992) Formulation of artificial feeds for mud crab culture: a preliminary biochemical, physical and biological evaluation. In: *Report of the Seminar on the Mud Crab Culture and Trade, Thailand, 5–8 November 1991* (ed. by C.A. Angell), pp. 179–184. Bay of Bengal Programme for Fisheries Development, Madras, India.

Cortés-Jacinto E, Villarreal-Colmenares H., Civera-Cerecedo R. & Martínez-córdova R. (2003) Effect of dietary protein level on growth and survival of juvenile freshwater crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *Aquaculture Nutrition* **9**, 207–213.

Cuzon G. & Guillaume J. (1997) Energy and protein: energy ratio. In: *Crustacean Nutrition – Advances in World Aquaculture VI* (ed. by L. D'Abramo, D. Conklin & D. Akiyama), pp. 51–70. World Aquaculture Society, Los Angeles, USA.

D'Abramo L. & Sheen S.D.J. (1996) Requerimientos nutricionales, formulación de dietas, y prácticas alimenticias para el cultivo intensivo del langostino de agua dulce *Macrobrachium rosenbergii*. In: *Memorias del Segundo Simposium Internacional de Nutrición Acuicola*, Universidad Autónoma de Nuevo León, Nuevo León, Mexico (ed. by R. Mendoza, S.E. Cruz & D. Ricque), pp. 81–101, World Aquculture Society, Los Angeles, USA.

D'Abramo L. & New M. (2000) Nutrition, feeds and feeding. In: *Freshwater Prawn Culture. The Farming of Macrobrachium rosenbergii* (ed. by M. New & W. Cotroni), pp. 203–220. Blackwell Science, London, UK.

D'Abramo L.R. & Sheen S.-S. (1994) Nutritional requirements, feed formulation, and feeding practices for intensive culture of the freshwater prawn *Macrobrachium rosenbergii*. *Reviews in Fisheries Science* **2**, 1–21.

Duncan D.B. (1955) Multiple range and multiple F-tests. *Biometrics* **11**, 1–42.

Fournier V., Gouillou Coustans M.F., Metailler R., Vachot C., Guedes M.J., Tulli F., Oliva-Teles A., Tibaldi E. & Kaushik S.J. (2002) Protein and arginine requirements for maintenance and nitrogen gain in four teleosts. *British Journal of Nutrition* **87**, 459–469.

Genodepa J., Zeng C. & Southgate P.C. (2004) Preliminary assessment of a microbound diet as an Artemia replacement for mud crab, *Scylla serrata*, megalopa. *Aquaculture* **236**, 497–509.

Gillespie N.C. & Burke J.B. (1992) Mud crab storage and transport in Australian commerce. In: *Report of the Seminar on the Mud Crab Culture and Trade*, held at Surat Thani, Thailand, 5–8 November 1991 (ed. by C.A. Angell), pp. 207–210. Bay of Bengal Program, BOBP/REP/51, Madras, India.

Guillaume J. (1997) Protein and amino acids. In: *Crustacean Nutrition. Advances in World Aquaculture Society 6* (ed. by R.L. D'Abramo, E.D. Concklin & D.M. Akiyama), pp. 26–41. World Aquaculture Society, Baton Rouge, LA, USA.

Guzman C., Gaxiola G., Rosa C. & Torre-Blanco A. (2001) The effect of dietary protein and total energy content on digestive enzyme activities, growth and survival of *Litopenaeus setiferus* (Linnaeus 1767) postlarvae. *Aquaculture Nutrition* **7**, 113–122.

Hajra A., Ghosh A. & Mandal S.K. (1988) Biochemical studies on the determination of optimum dietary protein to energy ratio for tiger prawn, *Penaeus monodon* juveniles. *Aquaculture* **71**, 71–79.

Hamasaki K., Suprayudi M.A. & Takeuchi T. (2002) Mass mortality during metamorphosis to megalops in the seed production of mud crab, *Scylla serrata* (Crustacea, Decapoda, Portunidae). *Fisheries Science* **68**, 1226–1232.

Harper A.E., Benevenga N.J. & Wohleuter R.M. (1970) Effects of ingestion of disproportionate amounts of amino acids. *Physiological Review* **50**, 428–558.

Harris E.L.V. (1988) Amino acid analysis by precolumn derivatization. In: *Methods in Molecular Biology, Vol. 3, New Protein Techniques*, pp. 33–47. Springer, NJ, USA.

Holme M.H., Zeng C. & Southgate P.C. (2006) The effect of supplemental dietary cholesterol on growth, development and survival of mud crab *Scylla serrata*, megalopa fed semi-purified diets. *Aquaculture* **261**, 1328–1334.

Holme M.H., Southgate P.C. & Zeng C. (2007) Assessment of dietary lecithin and cholesterol requirements of mud crab, Scylla serrata, megalopa using semi-purified microbound diets. *Aquaculture Nutrition* **13**, 413–423.

Hu Y., Tan B., Mai K., Ai Q., Zheng S. & Cheng K. (2008) Growth and body composition of juvenile white shrimp, *Litopenaeus vannamei*, fed different ratios of dietary protein to energy. *Aquaculture Nutrition* **14**, 499–506.

Jauncey K. (1982) The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). *Aquaculture* **27**, 43–54.

Jones P.L. & De Silva S.S. (1997) Apparent nutrient digestibility of formulated diets by the Australian freshwater cray-

fish *Cherax destructor* Clark (Decapoda, Parastacidae). *Aquaculture Research* **28**, 881–891.

Jones P.L., De Silva S.S. & Mitchell D.B. (1996) Effect of dietary protein content on growth performance, feed utilization and carcass composition in the Australian freshwater crayfish, *Cherax albidus* Clark and *Cherax destructor* Clark (Decapoda, Parastacidae). *Aquaculture Nutrition* **2**, 141–150.

Kanasawa A., Teshima S., Matsumoto S. & Nomura T. (1981) Dietary protein requirement of the shrimp *Metapenaeus monoceros*. *Bulletin of Japanese Society of scientific Fisheries* **47**, 1371–1374.

Kanazawa A., Shimaya M., Kawasaki M. & Kashiwada K. (1970) Nutritional requirements of prawn: I. Feeding and artificial diets. *Bulletin of Japanese Society of scientific Fisheries* **36**, 949–954.

Keenan C.P. (1999) Aquaculture of the mud crab, Genus *Scylla* – past, present and future. In: *Mud Crab Aquaculture and Biology, Proceedings of an International Scientific Forum, ACIAR Proceedings No. 78* (ed. by C.P. Keenan & A. Blackshaw), pp. 9–13. Darwin, Australia.

Keenan C.P., Davie P.J. & Mann D.L. (1998) A revision of the Genus *Scylla* de Haan, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). *Raffles Bulletin of Zoology* **46**, 217–245.

Marichamy R. & Rajapackiam S. (2001) The aquaculture of *Scylla* species in Índia. *Asian Fisheries Science* **14**, 231–238.

Mente E., Coutteau P., Houlihan D., Davidson I. & Sorgeloos P. (2002) Protein turnover, amino acid profile and amino acid flux in juvenile shrimp *Litopenaeus vannamei*: effects of dietary protein source. *The Journal of Experimental Biology* **205**, 3107–3122.

Millikin M.R., Fortner A.R., Fair P.H. & Sick L.V. (1980) Influence of dietary protein concentration on growth, feed conversion and general metabolism of juvenile prawn (*Macrobrachium rosenbergii*). *Proceedings of the World Mariculture Society* **11**, 382–391.

Mu Y.Y., Shim K.F. & Guo J.Y. (1998) Effects of protein level in isocaloric diets on growth performance of the juvenile Chinese hairy crab, *Eriocheir sinensis*. *Aquaculture* **165**, 139–148.

Obaldo L.G., Divakaran S. & Tacon A.G. (2002) Method for determining the physical stability of shrimp feeds in water. *Aquaculture Research* **33**, 369–377.

Pavasovic M., Richardson N.A., Anderson A.J., Mann D. & Mather P.B. (2004) Effect of pH, temperature and diet on digestive enzyme profiles in the mud crab, *Scylla serrata*. *Aquaculture* **242**, 641–654.

Peñaflorida V.D. (1989) An evaluation of indigenous protein sources as potential component in the diet formulation for tiger prawn, *Penaeus monodon*, using essential amino acid index (EAAI). *Aquaculture* **83**, 319–330.

Peres M.H. & Oliva-Teles A. (1999) Effect of dietary lipid level on growth performance and feed utilization by European sea bass juveniles (*Dicentrarchus labrax*). *Aquaculture* **179**, 325–334.

Pinheiro M. & Fransozo A. (1993) Relative growth of the speckled swimming crab *Arenaeus cribrarius* (Lamarck, 1818) (Brachyura Portunidae), near Ubatuba, State of Sao Paulo, Brazil. *Crustaceana* **65**, 365–376.

Rosas C.S., Diaz E., Soto L.A., Gaxiola G., Brito R., Baez M. & Pedroza R. (1995) Oxygen consumption and ammonia excretion of *Penaeus setiferus*, *P. schmitti*, *P. duorarum* and *P. notialis* postlarvae fed purified test diets: effect of protein levels on substrate metabolism. *Aquatic Living Resources* **8**, 161–169.

Rosas C., Cuzon G., Taboada G., Pascual C., Gaxiola G. & Wormhoudt V.A. (2001) Effect of dietary protein and energy levels on growth, oxygen consumption, haemolymph and digestive gland carbohydrates, nitrogen excretion and osmotic pressure of *Litopenaeus vannamei* (Boone) and *L. setiferus* (Linne) juveniles (Crustacea, Decapoda; Penaeidae). *Aquaculture Research* **32**, 531–547.

Sastry C.S.P. & Tummuru M.K. (1985) Spectrophotometric determination of tryptophan in proteins. *Journal of Food Science and Technology* **22**, 146–147.

Schmitt A.S.C. & Santos E.A. (1998) Ammonia-N efflux rate and nutritional state of juvenile pink shrimp, *Penaeus paulensis* (Perez-Farfante), in relation to food type. *Aquaculture Research* **29**, 495–502.

Shearer K.D. (2000) Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: a critical review. *Aquaculture Nutrition* **6**, 91–102.

Sheen S.S. (2000) Dietary cholesterol requirements of juvenile mud crab *Scylla serrata*. *Aquaculture* **189**, 277–285.

Sheen S.S. & Wu S.W. (1999) The effect of dietary lipid levels on the growth response of juvenile mud crab, *Scylla serrata*. *Aquaculture* **175**, 143–153.

Sheen S.S. & Wu S.W. (2003) Essential fatty acid requirements of juvenile mud crab, *Scylla serrata* (Forskål, 1775 (Decapoda, Scyllaridae). *Crustaceana* **75**, 1387–1401.

Shiau S.-Y & Chou B.-S. (1991) Effects of dietary protein and energy on growth performance of tiger shrimp *Penaeus monodon* reared in sea water. *Nippon Suisan Gakkaishi* **57**, 2271–2276.

Shiau S.Y. & Peng C. (1992) Utilisation of different carbohydrates at different dietary protein levels in grass prawn, *P. monodon* reared in seawater. *Aquaculture* **101**, 241–250.

Snedecor G.W. & Cochran W.G. (1967) *Statistical Methods*. Oxford Publishing Company, New Delhi, India, 593pp.

Spyridakis P., Metailler R. & Gabaudan J. (1989) Studies on nutrient digestibility in European sea bass (*Dicenntrarchus labrax*): 1. Methodological aspects concerning faeces collection. *Aquaculture* **77**, 61–70.

Stephen W. & Campbell B. (1960) The Australian Portunids (Crustacea: Portunidae): IV remaining genera. *Australian Journal of Marine and Fresh Water Research* **11**, 73–112.

Stern S. & Cohen D. (1982) Oxygen consumption and ammonia excretion during the molt cycle of the freshwater prawn *Macrobrachium rosenbergii* (De Man). *Comparative Biochemistry and Physiology* **73A**, 417–419.

Suprayudi M.A., Takeuchi T. & Hamasaki K. (2004) Essential fatty acids for larval mud crab, *Scylla serrata*: implications of lack of the ability to bioconvert C18 unsaturated fatty acids to highly unsaturated fatty acids. *Aquaculture* **231**, 403–416.

Tacon A.G.J. (2000) Aquatic feeds and nutrition: feed development guidelines for closed indoor farming systems. *The Global Aquaculture Advocate* **3**, 16–18.

Tacon A.G.J. (2001) Aquatic feeds and nutrition: minimizing environmental impacts of shrimp feeds. *The Global Aquaculture Advocate* **4**, 34–35.

Tacon A.G.J., Nates S.F. & McNeil R.J. (2004) Dietary feeding strategies for marine shrimp: a review. In: *Proceedings of the VII International Symposium on Aquaculture Nutrition*, Hermosillo, Mexico, 16–19 November 2004, Programa Maricultura, Facultad de Ciencia Biologicas, Universidad Autonoma de Nuevo Leon, Mexico (ed. by S.L.E. Cruz, M.D. Ricque, L.M.G. Nieto, D.A. Villarreal Cavazos, U. Scholz & M.L. Gonzalez Felix), pp. 695–704. World Aquaculture Society, Los Angeles, USA.

Takeuchi T., Satoh N., Sekiya S., Shimizu T. & Watanabe T. (1999) The effect of dietary EPA and DHA on the molting rate of larval swimming crab *Portumus trituberculaus*. *Nippon Suisan Gakkaishi* **65**, 998–1004.

Teshima S., Alam M.S., Koshio S., Ishikawa M. & Kanazawa A. (2002) Assessment of requirement values for essential amino acids in the prawn, *Marsupenaeus japonicus* (Bate). *Aquaculture Research* **33**, 395–402.

Truong P., Anderson A., Mather P., Paterson B. & Richardson N. (2008) Effect of selected feed meals and starches on diet digestibility in the mud crab. *Scylla serrata Aquaculture Research* **39**, 1778–1786.

Tuan V., Anderson A., Luong-van J., Shelley C. & Allan G. (2006) Apparent digestibility of some nutrient sources by juvenile mud crab, *Scylla serrata* (Forskal 1775). *Aquaculture Research* **37**, 359–365.

Vergara J.M., Fernandez-Palacios H., Robaina L., Jauncey K., De La Higuera M. & Izquierdo M. (1996) The effects of varying dietary protein level on the growth, feed efficiency, protein utilization and body composition of gilthead sea bream. *Fisheries Science* **62**, 620–623.

Vivas M., Sanchez-Vazquez F.J., Garcia B.G. & Madrid J.A. (2003) Macronutrient self-selection in European sea bass in response to dietary protein or fat restriction. *Aquaculture Research* **34**, 271–280.

Vivas M., Rubio V.C., Sanchez-Vazquez F.J., Mena C., Garcia B.G. & Madrid J.A. (2006) Dietary self-selection in sharpsnout seabream (*Diplodus puntazzo*) fed paired macronutrient feeds and challenged with protein dilution. *Aquaculture* **251**, 430–437.

Yalin S. & Qingsheng L. (1994) Present status of mangrove crab *Scylla serrata* (Forskål)) culture in China. *NAGA, the ICLARM Quarterly* **17**, 28–29.