Indian J. Fish., 61(2) : 67-72, 2014

Effect of dietary protein levels in the formulated diets on growth and survival of juvenile spiny lobster *Panulirus homarus* (Linnaeus)

MARGARET MUTHU RATHINAM[1], JOE K. KIZHAKUDAN[1], P. VIJAYAGOPAL[2] VIDYA JAYASANKAR[1], V. A. LESLIE[3] AND R. SUNDAR[1]

[1]*Madras Research Centre of Central Marine Fisheries Research Institute, 75, Santhome High Road R. A. Puram, Chennai, Tamil Nadu, India*

[2]*Central Marine Fisheries Research Institute, Kochi - 682 018, Kerala, India*

[3]*Vizhinjam Research Centre of Central Marine Fisheries Research Institute, Vizhinjam, Thiruvananthapuram - 695 521 Kerala, India*

e-mail: amrathinam@rediffmail.com

ABSTRACT

Effect of dietary protein level on the growth and survival of juvenile spiny lobster *Panulirus homarus* was evaluated. Pellet diets containing three different protein levels of 340 g kg^{-1} (diet A), 400 g kg^{-1} (diet B) and 460 g kg^{-1} (diet C), and a control diet of clam meat (diet D) with 470 g kg^{-1} protein were used. The duration of the experiment was 90 days. Survival was 100% for lobsters fed on diet C, 95% for lobsters fed on diet A, and 85% and 60% respectively for lobsters fed diets B and D. The results indicated that diet C gave the highest growth of 0.088 (± 0.10) mm CL followed by diet B with 0.055 (± 0.12) mm and diet A with 0.044 (± 0.10) mm in comparison to the control diet (diet D) with 0.077 (± 0.12) mm growth per day which indicated that next to clam meat, diet C with 460 g kg^{-1} protein level gave highest growth. The specific growth rate (SGR) was 0.627 ±0.03, 0.547 ± 0.03 and 0.354 ± 0.01 respectively for the diets C, B and A in comparison to the SGR of the clam diet (diet D) which was 0.685 ± 0.05. Diet C with a protein level of 460 g kg^{-1} gave good survival, SGR and FCR next to clam meat diet (D).

Keywords: Dietary protein, Growth, *Panulirus homarus,* Pellet diet, Spiny lobster

Introduction

Success of intensive commercial culture of lobsters depends heavily on the availability of relatively inexpensive feed (D'Abramo *et al*., 1981). Feed quality and cost are directly related and the feed cost ranges from 50-70% of the total variable cost of production (Akiyama and Chwang, 1989). There are several lacunae in the knowledge on feed requirement of cultured spiny lobsters. The suitability of experimental diet is evaluated on the basis of two main criteria: (i) optimal performance in terms of growth rate and survival and (ii) best feed conversion ratio. Good growth was obtained for *Panulirus homarus* under captive rearing with conventional diets like meat of the clam *Meretrix casta* (Vijayakumaran and Radhakrishnan, 1984; Radhakrishnan and Devarajan, 1986) and brown mussel *Perna indica* (Mohamed and George, 1968; Thomas, 1972).

Feed is one of the important components involving high cost in the operation of an aquaculture enterprise (Cruz-Suarez *et al*., 2002) and this can reach upto 50% of total expenses (Akiyama and Chwang, 1989; Martinez-Cordova *et al*., 2003). The nutrition and energy requirements of aquatic animals can be provided by protein, carbohydrate and lipid sources. Protein is the most expensive component in balanced feeds and is probably the most important feed element for growth of cultured species (Cortes-Jacinto *et al*., 2003a; Johnston *et al*., 2003; Ward *et al*., 2003). Studies on the nutritional requirements, feed conversion efficiency and growth rate of lobsters using different compounded diets are essential for formulating a nutritionally balanced and economical feed that would yield a higher growth rate. This study was conducted to evaluate the effect of dietary protein in formulated diets on the growth and survival of juvenile spiny lobster *Panulirus homarus*.

Materials and methods

Live spiny lobsters *Panulirus homarus* of the required size range of 45 ± 2 mm CL, weighing 88±5 g

were collected from the coastal areas of Kovalam. The live samples were transported to the laboratory in aerated seawater within one hour after catch. The animals were sorted based on size groups and acclimatised for 24 h in seawater with continuous aeration and conditioned further for feeding experiments.

Selection of feed ingredients

Preliminary trials in the laboratory have shown that the lobsters prefer molluscan meal, especially cephalopods and to an extent fish meal and crustacean meal. Formulated diets developed with appropriate attractants led to improved consumption and assimilation in lobsters. A formula for combined fish meal (CFM) has been developed comprising of cephalopod meal (20%), clam (*Meretrix casta*) meal (15%), sardine (*Sardinella longiceps*) meal (50%), trash meal (10%) and shrimp head meal (5%) (Table 1). Test diets A, B and C were formulated with CFM percentages of 30, 50 and 67.7% respectively to give final crude protein levels of 340 g kg^{-1}, 400 g kg^{-1} and 460 g kg^{-1} respectively. Wheat flour was used as a substitute to adjust the protein level in final composition of the feed. CFM was prepared using the required quantity of ingredients collected from the landing centre and processed properly by washing, drying, powdering and sieving.

The composition of the formulated feed is given in Table 2. Along with CFM, other ingredients like cod liver oil, polycheate worm paste, vitamin premix, oyster shell powder, earth mix, soya lecithin and cholesterol were added and mixed thoroughly (Kandasami *et al*., 1987; Easterson *et al*., 1989). Wheat gluten and gelatin were added in boiling water and a paste was prepared. This paste was added to the mixture and a dough was prepared with proper blending and extruded immediately using a hand pelletiser using a suitable die producing pellets of 3 ± 0.1 mm thickness. The pellets were cut into uniform sizes of approximately 1.5 cm length (Fig. 1). The pellets were oven dried at 50 °C for 48 h, and stored in a desiccator.

Stability of the feed

The hydrostability of the feed was tested by weighing 5 g of each experimental diet in preweighed net pouches in triplicate and immersing in filtered seawater. The pouches were removed at an interval of 15, 30, 45 and 60 min, rinsed carefully in double distilled water, dried in a hot air oven at 60 °C and weighed. Leaching was minimal up to a time limit of 45 min. All the feeds were stable, retaining the shape and texture for about one hour. Dry matter loss recorded for all three diets was only 8-10% at the end of one hour.

Table 1. Percentage composition of ingredients in combined fish meal

Ingredients	Weight in %
Cephalopod meal	20
Clam meal	15
Sardine meal	50
Trash fish meal	10
Shrimp head meal	5

Table 2. Percentage composition of ingredients in formulated feeds A, B and C

Ingredients	Diet A (%)	Diet B (%)	Diet C (%)
Combined fish meal	30.0	50.0	67.7
Wheat gluten	10.0	10.0	5.0
Cod liver oil	2.0	2.0	2.0
Sea sand worm	5.0	5.0	5.0
Vitamin and mineral mix*	2.0	2.0	2.0
Oyster shell powder	7.0	7.0	5.0
Earth mix	7.0	7.0	5.0
Soya lecithin	1.5	1.5	1.5
Cholesterol	0.3	0.3	0.3
Gelatine	0.5	0.5	0.5
Wheat flour	30.0	10.0	1.6
Bentonite	4.7	4.7	4.4

*Vitamin 2% (Smith *et al.,* 2005)

Fig. 1. Formulated pellet feed used in the study

Experimental setup

Black coloured rectangular FRP tanks with a floor area of 1 m^2 and volume of 1 ton were used to hold 12 lobsters each. The experiment was conducted in duplicate using 8 similar tanks (n = 96) placed at random. Filtered and treated seawater (35±8 ppt salinity, 8.2 ± 0.02 pH, 5 ± 0.5 mg DO, < 1ppm NH_3, < 0.1 ppm NO_2, 0.1 ppm H_2S) was used as rearing media throughout the experiment. The control of ammonia, sulphides and nitrite was achieved by circulating the water through active biofilters and also by minimal (20-30%) water exchange. When the animals were introduced into the tanks, each lobster was tagged using coloured wires at the base of antennae. Regular morphometric measurements and health monitoring were also carried out. Moults were recovered and the newly moulted animals were retagged. The initial mortalities (first 10 days of culture) were substituted with healthy animals of the same size range.

The variable among the treatments was in the protein content with diet A having 340 g kg^{-1} protein, Diet B - 400 g kg^{-1}, Diet C - 460 g kg^{-1} and raw clam meat diet with protein content at 470 g kg^{-1}. The experiments were carried out for 90 days. Growth was recorded (carapace length increment and total length increment) using calibrated vernier calipers.

The feeding schedule was fixed as three rations per day (30% were fed at 08:00 hrs, 30% at 14:00 hrs and 40% at 20:00 hrs) (Fig. 2). In the control tank (D), live clams were cut open and fed to the lobsters.

Fig. 2. *P. homarus* feeding on formulated pellet feed

Uneaten feed and fecal matter from the tanks were removed after each feeding. Uneaten feed was siphoned out to a 1 mm mesh screen, rinsed with freshwater to remove residual salts, dried at 100 °C for 16 to 20 h, weighed and subjected to proximate analysis as per AOAC (1995). The fecal matter was collected separately on to a piece of bolting silk and processed similarly. The feed consumed and assimilated were calculated.

The initial proximate composition of lobster muscle tissue and hepatopancreas were analysed prior to the experiment. At the end of the experiment, the lobsters from the experimental tanks and control tanks were harvested and the proximate composition of muscle tissue and hepatopancreas were analysed. Food conversion ratio (FCR), protein efficiency ratio (PER), apparent digestibility coefficient (ADC) and specific growth rate (SGR) were estimated following Cortes-Jacinto *et al*. (2004), Jover *et al*. (1999) and Bureau *et al*. (1999). Growth performance and feed utilisation efficiency were calculated as follows:

Digestible energy (kJ) = 23.0 kJ g^{-1} for protein, 16.7 kJ g^{-1} for carbohydrate and 37.6 kJ g^{-1} for lipid.

Food assimilation = 100 x (food consumed (g) – fecal matter (g) / food consumed (g).

Food conversion ratio (FCR) = Food intake (g dry weight / Weight gain (g wet weight).

Specific growth rate (SGR) = Ln Wr - Ln Wi x 100/T; where Wr = final mean weight; Wi = initial mean weight and T = feeding period.

Protein efficiency ratio (PER) = Weight gain (g) / protein intake. The data were statistically analysed using one way analysis of variance (ANOVA) (Snedecor and Cochran, 1967).

Results and discussion

The survival was 100% for the lobsters which fed on diet C, followed by A (95.8%), B (83.3%) and control D (62.5%) (Table 3). Survival rate of more than 80% is considered as good in crustacean studies (Cuzon and Guillaume, 1997). Crear *et al*. (2000) obtained a survival rate of 98% for *Jasus edwardsii,* while Saez-Rouela *et al*. (2002) reported survival rate of only 50.5% for the juvenile white-clawed crayfish with pelleted feed. Water quality parameters like ammonia, nitrite and hydrogen sulphide were recorded for the experimental and control tanks and were within the permissible range.

The protein energy ratio (PER) and the digestible energy of the diets were almost equal (15.23 to 20.01 kJ g^{-1}), however the efficiency of the diets differed from one another. The apparent digestibility coefficient was high (≈ 85.0) for all the diets (Table 4). Jones and De Silva (1997) reported ADC of 80.2% to 94% for the Australian crayfish *Cherax destructor*. Smith *et al*. (2005) reported that the adult lobsters (*Panulirus ornatus*) showed no significant difference in digestibility across a series of diets.

Maximum increase in carapace length and body weight was recorded in lobsters fed on diet C (Table 3). Clam fed lobsters showed maximum increase in body weight and the carapace length increment was comparable to that of diet C. The culture of lobsters aims at better survival as well as yield and the lobsters fed with diet C gave best results in this regard. Radhakrishnan and Vijayakumaran (1984) and Vijayakumaran and Radhakrishnan (1984) reported the growth of clam meat fed *P. homarus* (eyestalk ablated) as 0.30 ±0.02 g per day. Mohamed and George (1968) reported the growth of *P. homarus* as 0.21± 0.02 g per day by mark-recovery experiment. But in the present study, a higher growth rate was recorded for diet C (0.088 ± 0.10) and control diet (0.077±0.12) (Table 4). Cox and Davis (2009) reported faster growth rate

Table 3. Growth and survival of *P. homarus* fed on feeds A, B, C and control feed (D) at the end of culture period

Feed	Initial CL (mm)	Final CL (mm)	Growth per day (mm)	Initial TL (mm)	Final TL (mm)	Growth per day (mm)	Initial Wt (g)	Final Wt (g)	Growth (g per day)	Initial biomass (kg)	Final biomass (kg)	Net growth (g)	Survival rate (%)
A	45 ± 2	49 ± 2	0.044 ± 0.10	125 ± 5	129 ± 2	0.044 ± 0.13	88 ± 2	121 ± 3	0.367 ± 0.10	2.110	2.900	0.790	100
B	46 ± 3	51 ± 1	0.055 ± 012	128 ± 4	144 ± 3	0.177 ± 0.10	86 ± 3	141 ± 3	0.610 ± 0.12	2.064	2.820	0.756	83.3
C	45 ± 2	53 ± 3	0.088 ± 0.10	120 ± 5	141 ± 3	0.233 ± 0.13	87 ± 2	153 ± 2	0.733 ± 0.12	0.090	3.520	1.430	95.8
D	46 ± 2	53 ± 1	0.077 ± 0.12	130 ± 5	150 ± 4	0.222 ± 0.12	88 ± 2	163 ± 3	0.833 ± 0.10	2.110	2.450	0.340	62.5

Table 4. FCR, ADC, SGR and growth rate values of *P. homarus* on termination of the experiment

Parameters	Diet A	Diet B	Diet C	Diet D
Food conversion ratio (FCR)	1:7	1:6	1:3	1:2
Apparent digestibility coefficient (ADC)	85.05 (0.70)	85.55 (2.31)	85.38 (1.35)	85.03 (2.31)
Specific growth rate (SGR)	0.354 (0.01)	0.547 (0.03)	0.627 (0.03)	0.685 (0.05)
Growth in terms of CL (per day) (mm)	0.044 (0.10)	0.055 (0.12)	0.088 (0.10)	0.077 (0.12)

(*Values in parentheses are standard deviations*)

of 3.49% of weight gain per day in the culture of post-pueruli of spiny lobsters *P. argus*.

The specific growth rate (SGR) was 0.627 (±0.03), 0.547 (±0.03), and 0.354 (±0.01) respectively for the diets C, B and A, in comparison to the SGR of the control diet (D) with 0.685 (±0.05). The FCR of the diets A, B and C were 1:7, 1:6 and 1:3 respectively in comparison to the control (1: 2) (Table 4). Vijayakumaran and Radhakrishnan (1984) reported that the FCR of the lobster *P. homarus* fed with fresh clam meat ranged from1:2 to 1:6. The yield of lobsters fed on test diet C was maximum with FCR at 1:3 indicating a better conversion and economic viability.

Protein levels in the muscle tissue and hepatopancreas of lobsters fed on high protein diets B and C were higher than the initial values compared to that of diet A and control. The lipid profile in the hepatopancreas was observed to be lowest in the lobsters fed with diet C. The higher protein levels in the muscles and hepatopancreas of lobsters fed with diet C probably indicate the response to higher degree somatic conversion than other diets (Fig. 3 and 4). Increase in muscle weight and protein is the prime requirement sought while culturing food organisms.

Lobsters can be grown in alternate systems on artificial diets with a protein level of 460 g kg^{-1} (Smith *et al.,* 2003). The low survival rate with clam diet also supports the view that a supplemented balanced diet is the way forward in aquaculture of lobsters. Lobsters showed very good palatability for the formulated feeds and pellets were readily accepted. Among the three pelleted dry feeds tested, the diet C (460 g kg^{-1}) gave good survival, SGR and FCR next to clam meat diet D (470 g kg^{-1}). The one way ANOVA indicated that weight gain, protein efficiency ratio and food conversion

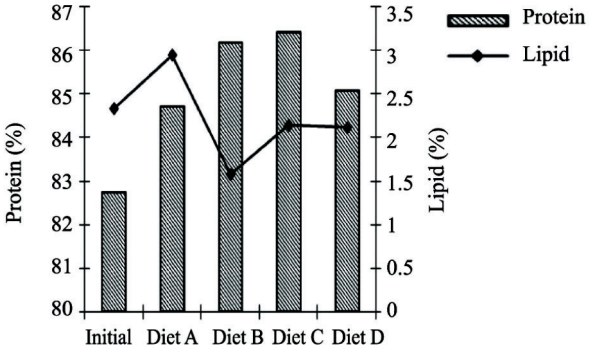

Fig. 3. Protein and lipid content in muscle tissue of *P. homarus* fed on pellet diets (A, B, C) and clam meat (D)

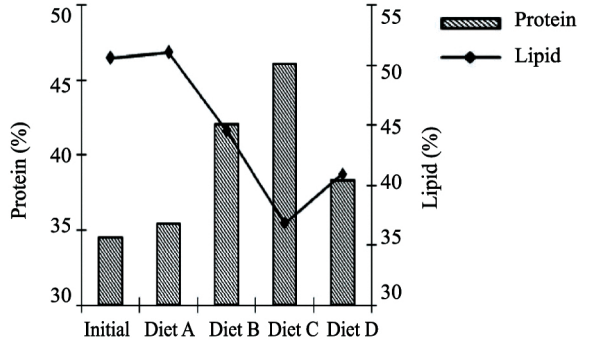

Fig. 4. Protein and lipid content in hepatopancreas of *P. homarus* fed on pellet diets (A, B, C) and clam meat (D)

ratio were significantly different among the four groups ($p<0.05$). On the other hand, food and protein consumption were not significantly different.

The harvested lobsters were cooked in brine and tested for comparing the quality and flavour in the meat following the standard organoleptic cum sensory evaluation procedure. The flavour and texture of the pellet

grown lobsters compared well with that of wild lobster meat.

The acceptance of formulated diets by the spiny lobsters and positive growth factors indicate that other than natural feeds, spiny lobsters can also be grown successfully on pellet feeds with improved survival rates. Survival and growth rates have been almost similar to that with clam or other wet feeds, even at higher densities.

Acknowledgements

The authors are thankful to the former Director of Central Marine Fisheries Research Institute, Dr. G. Syda Rao, for constant encouragement. The authors sincerely acknowledge the encouragements by the Scientist-in-Charge, MRC of CMFRI, Chennai in carrying out the study.

References

Akiyama, D. M. and Chwang, N. L. 1989. Shrimp feed requirements and feed management. In: Akiyama D. M. (Ed.), *Proceedings of the South-east Asia shrimp farm management workshop*, *Philippines, Indonesia, Thailand*, 26th July - 11th August 1989, American Soybean Association, republic of singapore, p. 75-82.

AOAC 1995. *Official methods of analysis*, 16th edn. Association of Official Analytical Chemists, Inc, Washington DC, USA.

Bureau, O. P., Harris, A. M. and Cho, C. Y. 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*), *Aquaculture*, 180: 345-385.

Cortes-Jacinto, E., Villarreal-Colmenares, H., Civera-Cerecedo, R. and Martinez-Cordova, R. 2003a. Effect of dietary protein level on growth and survival of juvenile freshwater crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *Aquacult. Nutr*., 9: 207-213.

Cortes-Jacinto, E., Villarreal-Colmenares, H. and Rendon-Rumualdo, M. 2003b. Effect of feeding frequency on growth and survival of juvenile crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *Hidrobiologica*, 13: 151-158.

Cortes-Jacinto, Villarreal-Colmenares, H., Civera-Cercecado, R. and Naranjo-Pavamo, J. 2004. Effect of dietary protein levels on the growth and survival of pre-adult freshwater cray fish *Cherex quadricarinatus* (Von Martens, 1968) in mono sex culture. *Aquacult. Res.*, 35: 71-79.

Cox, S. L. and Davis, M. 2009. An evaluation of potential diets for the culture of postpueruli spiny lobsters *Panulirus argus* (Palinuridae). *Aquacult. Nutr*., (15): 152-159.

Crear, B. J., Thomas, C. W., Hart, P. R. and Carter, C. G. 2000. Growth of juvenile southern rock lobsters, *Jasus edwardsii* is influenced by diet and temperature, whilst survival is influenced by diet and tank environment. *Aquaculture*, 190(1&2): 169-182.

Cruz-Suarez, E., Ricque-Marie, D., Tapia-Salazar, M., Marin-Zaldivar, L. F., Guajardo-Bardosa, C., Nieto-Lopez, M. and Salinas-Miller, A. 2002. Historia yestatus actual de la digesti-bilidad y algunas caracteristicas fisicoquimicas de los alimentos comerciales para camaron usados en Mexico. In: Cruz-Suarez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-cortes, M. and Simoes, N. G. (Eds.), *Avances en nutricion acucola VI. Memorias del VI Simposium Internacional de Nuticion Acuicola*, 3 al 6 Septiembre del 2002, Universidad Autonoma de Nuevo Leon, San Nicolas de Los Garza, Mexico, p. 1-22.

Cuzon, G. and Guillaume, J. 1997. Energy and protein energy ratio. In: D'Abramo, L. R., Conklin, D. E. and Akiyama, D. M. (Eds.), *Crustacean Nutrition.* World Aquaculture Society, Louisiana State University, Baton Rouge, L.A., p. 51-70.

D'Abramo, L. R., Conklin, D. E., Bordner, C. E., Baum, N. A. and Norman-Boundreau, K. A. 1981. Successful artificial diets for the culture of juvenile lobster. *J. World Maricult. Soc*., 12: 325-332.

Easterson, D. C. V., Kandasami, D. and Paul Raj, R. 1989. Dietary utilization of selected vegetable proteins by mullet, *Liza macrolepis*. *Indian J. Fish*., 36(1): 65-73.

Johnston, D. J., Calvert, K. A. Crear, B. J. and Carter, C. G. 2003. Dietary carbohydrate/lipid ratio and nutritional condition in juvenile southern rock lobster, *Jasus edwardsii. Aquaculture*, 220: 667-682.

Jones, P. L. and De Silva, S. S. 1997. Apparent digestibility of formulated diets by the Australian freshwater cray fish *Chernax destructor*, Clark (Decapoda, Parastacidae). *Aquacult. Res*., 28: 881-891.

Jover, M., Fernandez-Carmona, J., Del Rio, M. C. and Soler, M. 1999. Effect of feeding cooked- extruded diets, containing different level of protein, lipid

and carbohydrate on growth of red swamp cray fish (*Procambarus clarkii*). *Aquaculture,* 178: 127-137.

Kandasami, D., Paul Raj, R. and Easterson, D. C. V. 1987. Effect of selected levels of dietary protein on the growth and feed efficiency of mullet, *Liza macrolepis* fry. *Indian J. Fish*., 34(3): 306-311.

Martinez-Cordova, L. R., Campana-Torres, A. and Porchas-Cornejo, M. A. 2003. Dietary protein level and natural food management in the culture of blue (*Litopenaeus stylirostris*) and white shrimp (*Litopenaeus vannamei*) in microscosms. *Aquacult. Nutr*., 9: 155-160.

Mohamed, K. H. and George, M. J. 1968. Result of the tagging experiments on the Indian spiny lobster *Panulirus homarus* (Linneaus movement and growth. *Indian J. Fish*., 15(1&2): 15-26.

Radhakrishnan, E. V. and Devarajan, K. 1986. Growth of the spiny lobster *Panulirus polyphagus* (Herbst) reared in the laboratory. *Proc.eedings of the Symposium on Coastal Aquaculture*, Marine Biological Association of India, 4: 1164-1170.

Radhakrishnan, E. V. and Vijayakumaran, M. 1984. Effect of eyestalk ablation in spiny lobster *Panulirus homarus* (Linnaeus): 1. on moulting and growth. *Indian J. Fish*., 31(1): 130-147.

Saez-Rouela , M, Carral, J. M., Cealda, J. D. and Perez, J. R. 2002. Effect of shelter type and food supply frequency on the survival and growth of stage 2 juvenile white clawed cray fish (*Austropotamobius pallipes* Lareboullet) under laboratory condition. *Aquacult. Int*., 9(6): 489-497.

Smith, D., Williams, M. K. C., Irvin, S., Barclay, M. and Tabrett, S. 2003. Development of a pelleted feed for juvenile tropical spiny lobster (*Panulirus ornatus*) response to dietary protein and lipid. *Aquacult. Nutr*., 9: 231-237

Smith. D., Williams, K. C. and Irvin, S. J. 2005. Response of the tropical spiny lobster *Panulirus ornatus* to protein content of pelleted feed and to a diet of mussel flesh. *Aquacult. Nutr*., (11): 209-217.

Snedecor, G. W. and Cochran, G. W. 1967. *Statistical methods*, 6th edn., Oxford and IBH Publishing Co., New Delhi, 593 pp.

Thomas, M. M. 1972. Growth of spiny lobster *Panulirus homarus* (Linneaus) in captivity. *Indian J. Fish*., 19 (1): 125-129.

Vijayakumaran, M. and Radhakrishnan, E. V. 1984. Effect of eyestalk ablation in spiny lobster *Panulirus homarus* (Linnaeus): on feed intake and conversion. *Indian J. Fish*., 31(1): 148-155.

Ward, L. R., Carter, C. G., Crear, B. J. and Smith, D. M. 2003. Optimal dietary protein level for juvenile southern rock lobster *Jasus edwardsii* at two lipid levels. *Aquaculture*, 217: 483-500.

Date of Receipt : 11-06-2012
Date of Acceptance : 06-11-2013