*J. Aqua.*, **21** (2013): 19-26

# EFFECT OF DIFFERENT LEVELS OF DIETARY LIPIDS ON REPRODUCTIVE PERFORMANCE OF CAPTIVE BROODSTOCK OF *MACROBRACHIUM ROSENBERGII*

**Swagatika Mohanty, Bindu R. Pillai and P. V. Rangacharyulu**

*Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751002, India*

*Corresponding author: swagatikacifa@gmail.com*

The present paper reports the results of a laboratory experiment to study the effect of different levels of dietary lipids on reproductive performance of captive broodstock of giant freshwater prawn *Macrobrachium rosenbergii*. Three formulated pellet diets were prepared containing 2% (D1), 4% (D2) and 6% (D3) lipid and fed to adult females of *M. rosenbergii* (Av. body weight - 28 g) at 5% of the biomass per day for 60 days. Prawns fed D3 showed significantly higher ($P<0.05$) weight gain (221.16±6.48 mg/day) compared to those fed D1 and D2. Similarly, significantly higher ($P<0.05$) reproductive parameters like fecundity (eggs/ g body weight) (1153.6±174.25), egg clutch weight (g) (4.9±0.25) and gonado-somatic index (6.82±0.27) were also found in prawns fed D3. Reproductive effort (g eggs/ female x100), post–hatch fecundity (nos. of larvae/ g female) and mid gut gland somatic index (%) also showed a similar trend. The present results clearly indicated that dietary lipid level of 6% significantly enhances reproductive performance of captive stock of *M. rosenbergii* compared to 2% and 4% lipid levels.

## INTRODUCTION

The giant freshwater prawn *Macrobrachium rosenbergii* is one of the most important cultured freshwater prawns worldwide. Its production in India increased from less than 178 t in 1996 to 42,820 t in 2005. However, since 2006 the production is declining and in 2010 it was only 13,525 tons (FAO, 2012). Poor seed quality is considered as one of the main causes of poor production. Currently commercial seed production depends on broodstock collected from rivers or from grow-out ponds. Unpredicted supply associated with the capture of gravid females has become a major bottleneck in the development of *M. rosenbergii* hatcheries. As the grow-out feed may not provide adequate nutrition necessary for the egg development, mother prawns from grow–out ponds generally yield poor quality eggs. Therefore, the use of captive broodstock has been proposed for seed production in hatcheries (Menasveta *et al.,* 1994; Ramos *et al.,* 1995; Palacios *et al.,* 1999a; 1999b; Preston *et al*., 1999).

The reproductive performance of captive broodstock can be improved by nutritional manipulations. Information on broodstock nutrition is limited (Takeuchi *et al.,* 1981; Watanabe *et al*., 1984a; 1984b; Watanabe, 1985; SEAFDEC, 1987; Ganeswaran, 1989; Ang *et al.*, 1992; Bromage *et al*., 1992; Das *et al.*, 1993 and Xu *et al*., 1994). It is generally accepted that egg and larval quality are partly controlled by maternal diet. Lipids play major roles in several reproductive processes of crustaceans and some studies have demonstrated that levels and composition of dietary lipids profoundly affect ovarian maturation and reproductive success (Harrison, 1997). Crustaceans have a limited ability to biosynthesise phospholipid *de novo* (Shieh, 1969). Mature females of *M. rosenbergii* are characterized by a fast rate of ovarian development (Damrongphol *et al.,* 1991) and ovaries with high lipid content and a limited lipid-storage capability for ovarian utilization (Cavalli *et al.*, 1999). *M. rosenbergii* also shows a relatively long period of embryonic development, which is affected by the availability and transfer of essential nutrients to the embryos. Therefore, broodstock diet of this species requires a higher content of dietary lipid. For the freshwater prawn however, most studies that have investigated lipid metabolism and requirements have been restricted to larval and juvenile stages (D'Abramo and Sheen, 1993). Hence the present work was undertaken to study the effect of varying dietary lipid levels on the breeding performance of captive broodstock of *M. rosenbergii*.

## MATERIALS AND METHODS

Adult *M. rosenbergii* were collected from the prawn farm of Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar. Apparently healthy prawns with no visible injury were selected for the experiment. Prawns were acclimated to the laboratory conditions for seven days prior to experiment. Three replicate tanks (3 m x 1.2 m x 1 m) were set up for each treatment. After one-week of acclimation, prawns were segregated sex wise and individually measured for length and wet weight and, those in inter-moult stage were selected for the experiment. Six immature females and two males were released into each tank.

Three iso-nitrogenous feeds with a crude protein content of 36% and lipid content of 2% (D1), 4% (D2) and 6% (D3) were prepared. The feed with 2% lipid served as control as the lipid content of grow out diet is nearly 2%. Composition of ingredients in test diets is given in Table 1. The diets were prepared by thoroughly mixing the powdered ingredients with oil and then adding required quantity of water until a stiff dough was formed. This was then passed through a pelletiser with a 3 mm die, dried for 24 hrs at 50°C and stored at -20 °C until use.

The prawns were fed with the test diets at 5% of the body weight twice daily in two divided doses at 0800 and 1700 h throughout the experimental period. Continuous aeration was provided in each tank. Cut pieces of perforated PVC pipes were placed at the bottom of the tanks to provide shade and shelter to the moulting prawns. Every morning, uneaten feed and faecal matter were siphoned out. Water was exchanged at 50% twice a week. The tanks were checked daily for molts, ovarian maturation and occurrence of berried prawns.

Table 1. Ingredient and proximate (%DM) composition of the experimental diets

| Ingredients (%) | D1 | D2 | D3 |
|---|---|---|---|
| Groundnut de oiled cake | 32 | 32 | 32 |
| Soybean de oiled cake | 22 | 22 | 22 |
| Prawn meal | 20 | 20 | 20 |
| Fish meal | 10 | 10 | 10 |
| Wheat flour | 13.1 | 11.1 | 9.1 |
| Vegetable oil | 0.5 | 2.3 | 4.1 |
| COD liver oil[a] | 0.2 | 0.4 | 0.6 |
| Vitamin mineral mixture[b] | 1.5 | 1.5 | 1.5 |
| Mussels (dried) | 0.2 | 0.2 | 0.2 |
| Carboxymethyl cellulose[c] | 0.5 | 0.5 | 0.5 |
| ***Proximate composition*** | | | |
| Crude protein | 36.4 | 36.2 | 36.8 |
| Ether ext. | 2.0 | 4.0 | 6.0 |
| Crude fiber | 10.5 | 11.10 | 10.9 |
| Ash | 10.8 | 10.2 | 11.10 |
| Nitrogen Free Extract | 40.3 | 38.5 | 35.2 |

*a. COD liver oil (SEVENSEAS, Universal Medicare Pvt. Ltd., India)*

*b. Vitamin mineral mixture (Supplevite – M; Sarabhai Zydus, India)*

*Composition: Vitamin A – 50,00,000 I.U., Vitamin D3 – 10,00,000 I.U., Vitamin B2 – 2.0g, Vitamin E – 750 units, Vitamin K – 1.0g, Calcium pantothenate – 2.5g, Nicotinamide – 10.0g, Vitamin B12 – 6.0mg, Choline chloride – 150g, Calcium – 750g, Manganese – 27.5g, Iodine – 1.0g, Iron – 7.5g, Zinc – 15g, Copper – 2.0g, Cobalt – 0.45g.*

*c. Carboxymethyl cellulose (MERCK, India)*

Water quality parameters such as pH, dissolved oxygen (DO) and ammonia ($NH_4$-N) were measured weekly once following standard procedures (APHA, 1995). Water temperature was measured twice daily using a mercury thermometer. Observations were made on growth, moult duration and selected reproductive parameters, like fecundity, egg clutch weight, occurrence of berried females, post-hatch fecundity, reproductive effort and GSI. The duration of the experiment was 60 days.

Data were analyzed using One Way Analysis of Variance. Means were compared using Duncan's multiple range test (Duncan, 1955). Significance was tested at the $P<0.05$ levels.

**RESULTS AND DISCUSSION**

Average values of water temperature, dissolved oxygen (DO) and ammonia nitrogen ($NH_4$-N) were 27.4±1.2 °C, 5.4±0.3 mg $l^{-1}$ and 0.02±0.005 mg $l^{-1}$, respectively and pH levels ranged from 7.1 to 7.8. All the water quality parameters recorded were well within the optimum range reported for the species. No significant difference (P>0.05) were observed in the water quality parameters among different treatments.

Final survival rate in different treatments ranged from 81.3% to 93.8% (Table 2). No significant difference (P>0.05) in survival rate was found among treatments. Sandifer and Smith (1978) reported 62% survival for *M. rosenbergii* broodstock reared in a recirculatory system for five months. Our results were higher, probably due to the shorter duration of experiment compared to the above authors. In a 180 day experiment, Cavalli *et al*. (2000) reported that the survival of *M. rosenbergii* adults were not affected by different phospholipid dietary treatments.

Table 2. Growth parameters, intermoult period and survival of *M. rosenbergii* fed diets containing different dietary lipid levels. Values are Mean ± SD.

| Parameters | D1 | D2 | D3 |
|---|---|---|---|
| Initial body wt. (g) | 28.19 ± 1.01 | 28.12 ± 2.17 | 28.35 ± 2.49 |
| Final body wt. (g) | 38.37 ± 1.53 | 39.75 ± 2.35 | 41.62 ± 2.88 |
| Av. daily gain (mg) | 169.67 ± 8.60[a] | 193.84 ± 2.94[a] | 221.16 ± 6.48[b] |
| Intermoult period (days) | 32.14 ± 3.02[a] | 25.33 ± 1.88[b] | 22.9 ± 1.54[b] |
| Survival (%) | 93.75 ± 8.83 | 81.25 ± 8.83 | 87.5 ± 17.67 |

Different superscripts within rows represent significant differences (P<0.05).

Highest average daily weight gain and specific growth rate was found in prawns fed D3 and the lowest values were found in prawns fed D1 (Table 2). Earlier workers reported similar results. Kanazawa *et al.* (1977) and Deshimaru *et al.* (1979) reported that the growth performance of prawn (*Fenneropenaeus indicus*) on lipid-free diet was very poor and was improved significantly by inclusion of lipids in diets at 6%. The steady increase in growth with the increase in dietary level of lipid could be ascribed to the protein sparing action of dietary lipids (Shivaram and Raj, 1997). The increased level of lipid in D3 might have provided the energy required for metabolic activities of the animal, thereby more and more protein had been spared for growth.

Moulting is an indispensable phenomenon in Crustacea and the involvement of lipid during moulting has been well established (Read, 1977). The process of ecdysis requires large amount of energy amounting to 25.6% of the total energy gained during intermoult period (Read and Caulton, 1980). In the present study, intermoult duration was longest in prawns fed D1 (32.14±3.02) and shortest in prawns fed D3 (22.9±1.54). Higher lipid levels in D3 might have provided sufficient energy for faster rate of growth and thus shortened the moult duration.

Table 3 provides the selected reproductive parameters of prawns fed with test feeds. Highest fecundity was found in prawns fed D3 (1153.6±174.25) that was significantly higher ($P<0.05$) than that of D1 and D2 fed groups. Similarly post hatch fecundity of females showed an increasing trend with the increase in the lipid content. Highest post hatch fecundity was found in prawns fed D3 (763.75±99.73) and lowest in prawns fed D1 (601.5±90.15). A lower fecundity has been commonly observed for pond reared shrimp broodstock, probably due to the fact that the feed provided during grow-out contain lower lipid levels (Menasveta *et al*., 1993; 1994; Cavalli *et al*., 1997; Palacios *et al*., 2000). Bray *et al*. (1990) fed *Litopenaeus stylirostris* with three different total lipid levels and found that broodstock feed requires approximately 3% higher total lipid than in grow-out feed for shrimp. As lipids constitute nearly 40% of the eggs (Cavalli *et al.* (1999) higher lipids levels are likely to help in higher egg production resulting in increased fecundity. Cavalli *et al.* (2000) however, reported that there was no significant difference in fecundity among the three dietary treatments of low, medium and high phospholipid levels.

Table 3. Reproductive parameters, gonado-somatic index (GSI) and mid gut gland somatic index (MSI) of freshwater prawn, *M. rosenbergii* females fed diets containing different dietary lipid levels. Values are Mean ± SD.

| Parameters | D1 | D2 | D3 |
|---|---|---|---|
| Fecundity (eggs/g female) | 971.9±59.38[a] | 1074.95±139.55[a] | 1153.6±174.25[b] |
| Occurrence of berried females (%) | 50±8.5 [a] | 62.5±11 [a] | 87.5±9 [b] |
| Nos. of larvae/ g female | 601.5±90.15[a] | 688.1±114.07[a] | 763.75±99.73[b] |
| Egg clutch weight (g) | 3.9±0.31[a] | 4.07±0.25[a] | 4.9±0.25[b] |
| Reproductive effort (g eggs/female ×100) | 10.17±0.82 | 10.38±0.63 | 11.77±0.61 |
| GSI (%) | 5.92±0.13[a] | 6.33±0.16[a] | 6.82±0.27[b] |
| MSI (%) | 4.49±0.67 | 4.6±0.67 | 4.83±0.43 |

Different superscripts within rows represent significant differences ($p<0.05$).

Highest levels of mid gut gland somatic index (MSI) were found in females fed D3 (4.83±0.43) and lowest in females fed D1 (4.49±0.67). In *M. rosenbergii*, the mid gut gland (MG) is the main lipid storage and processing organ (D'Abramo and Sheen, 1993) and perhaps this is reflected in the MSI values of different dietary groups. Other indicators of reproductive performance evaluated in the present study namely egg clutch weight, reproductive effort and gonado-somatic index (GSI) were also higher in prawns fed D3. Earlier studies have noted increase in hatch rate with increase dietary lipid (Millamena, 1989; Bray and Lawrence, 1990). Clarke *et al*. (1990) reported that saturated and monounsaturated fatty acids may serve as a source of energy during embryogenesis and perhaps, early larval development. Higher hatch rate will result in higher post hatch fecundity. Higher lipid levels in D3 probably increased the hatch rate and therefore enhanced the post hatch fecundity.

Our results clearly demonstrate the significance of higher dietary lipid levels for better reproductive performance of the captive broodstock of the *M. rosenbergii* as 6% dietary lipid significantly improved all the evaluated reproductive parameters. Further studies on dietary lipid quality on the reproductive performance would go a long way in developing dedicated captive stock of this very important species.

## ACKNOWLEDGEMENT

The authors express sincere gratitude to Director, CIFA for encouragement and providing all necessary facilities. The authors also gratefully acknowledge Dr. A. K. Sahu, Head of Division, Aquaculture Production and Environment Division, CIFA for his keen interest and constant support in carrying out this work.

## REFERENCES

Ang, K. J., Y. M. Lee and A. T. Law, 1992. Protein requirements for broodstock *Macrobrachium rosenbergii* (Abstract PN–08). *In*: Abstracts of the Third Asian Fisheries Forum, Singapore, 26 – 30, October 1992, Asian Fisheries Society, Manilla, pp. 188.

APHA (American Public Health Association), American Water Works Association, Water Pollution Control Federation, 1995. Standard Methods for the Examination of Water and Wastewater (20th Edn.) American Public Health Association, Washington DC 3.

Bray, W. A. and A. L. Lawrence, 1990. Reproductive of eyestalk-ablated *Penaeus stylirostris* fed various levels of total dietary lipids. *J. World Aquacult. Soc.*, **21**: 41–52.

Bray, W. A., A. L. Lawrence and J. R. Leung-Trujillo, 1990. Reproductive performance of ablated *Penaeus stylirostris* fed a soybean lecithin supplement. *J. World Aquacult. Soc*., **20**: 19A.

Bromage, N., J. Jones, C. Randall, M. Thrush, B. Davies, J. Springate, J. Duston and G. R. Barker, 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Onchorhynchus mykiss*). *Aquaculture*, **100**: 141–166.

Cavalli, R. O., G. Menschaert, P. Lavens and P. Sorgeloos, 2000. Maturation performance, offspring quality and lipid composition of *Macrobrachium rosenbergii* females fed increasing levels of dietary phospholipids. *Aquacult. Int.*, **8**: 41-58.

Cavalli, R. O., M. P. Scardua and W. Wasielesky Jr., 1997. Reproductive performance of different sized wild and pond-reared *Penaeus paulensis* females. *J. World Aquacult. Soc.*, **28**: 260–267.

Cavalli, R. O., P. Lavens and P. Sorgeloos, 1999. Performance of *Macrobrachium rosenbergii* broodstock fed diets with different fatty acid composition. *Aquaculture*, **179**: 387–402.

Clarke, A., J. H. Brown and L. J. Holmes, 1990. The biochemical composition of eggs from *Macrobrachium rosenbergii* in relation to embryonic development. *Comp. Biochem. Physiol.*, **96B**: 505–511.

D'Abramo, L. R. and S. S. Sheen, 1993. Polyunsaturated fatty acid nutrition in juvenile of freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture*, **115**: 63-86.

Damrongphol, P., N. Eangchuan and B. Poolsanguen, 1991. Spawning cycle and oocyte maturation in laboratory maintained giant freshwater prawn (*Macrobrachium rosenbergii*). *Aquaculture*, **95**: 347-357.

Das, N. N., C. R. Saad, K. J. Ang, A. T. Law and S. A. Harmin, 1993. Some aspects of protein requirements for broodstock *Macrobrachium rosenbergii* (de Man). Paper presented at the International conference of Fisheries and Environment: Beyond 2000, Serdang, Selangor, Malaysia, 6 – 9 December 1993.

Deshimaru, O., K. Kuroki and Y. Yone, 1979. Studies on a purified diet for prawn. XV: The composition and level of dietary lipid appropriate for growth of prawn. *Bull. Jpn. Soc. Sci. Fish.*, **45**: 591-594.

Duncan, D. B., 1955. Multiple range and multiple 'F' tests. *Biometrics*, **11**: 1-42.

FAO, 2012. The State of World Fisheries & Aquaculture 2012, FAO Fisheries and Aquaculture Department, Rome.

Ganeswaran, K. N., 1989. Reprodutive performance of the giant freshwater prawn *Macrobrachium rosenbergii* with special reference to broodstock age, size and nutrition, egg production and larval quality. Ph.D. Thesis, University of Stirling.

Harrison, K., 1997. Broodstock nutrition. *In*: Crustacean nutrition (Eds. L. R. D'Abramo, D. E. Conklin and D. M. Akiyama). World Aquaculture Society, Baton Rouge, Lousiana, USA, pp. 85-107.

Kanazawa, A., S. Teshima and S. Tokiwa, 1977. Nutritional requirements of prawn. VII: Effect of dietary lipids on growth. *Bull. Jpn. Soc. Sci. Fish.*, **43**: 849-856.

Menasveta, P., S. Piyatiratitivorakul, S. Rungsupa, N. Moree and A. W. Fast, 1993. Gonadal maturation and reproductive performance of giant tiger prawn (*Penaeus monodon* Fabricius) from the Andaman sea and pond-reared sources in Thailand. *Aquaculture*, **116** : 191–198.

Menasveta, P., S. Sangpradub, S. Piyatiratitivorakul and A. W. Fast, 1994. Effects of broodstock size and source on ovarian maturation and spawning of *Penaeus monodon* Fabricius from the Gulf of Thailand. *J. World Aquacult. Soc.*, **25**: 41-49.

Millamena, O. M., 1989. Effect of fatty acid composition of broodstock diet on tissue fatty acid patterns and egg fertilization and hatching in pond-reared *Penaeus monodon*. *Asian Fish. Sci.*, **2**: 127-134.

Palacios, E., A. M. Ibarra and I. S. Racotta, 2000. Tissue biochemical composition in relation to multiple spawning in wild and pond-reared *Penaeus vannamei* broodstock. *Aquaculture*, **185**: 353–371.

Palacios, E., C. I. Perez–Rostro, J. L. Ramirez, A. M. Ibarra and I. S. Racotta, 1999a. Reproductive exhaustion in shrimp *(Penaeus vannamei*) reflected in larval biochemical composition, survival and growth. *Aquaculture*, **171**: 209–221.

Palacios, E., M. C. Rodríguez-Jaramillo and I. S. Racotta, 1999b. Comparison of ovary histology between different-size wild and pond-reared shrimp *Litopenaeus vannamei* (=*Penaeus vannamei*). *Invertebr. Reprod. Dev.*, **35**: 251–259.

Preston, N. P., D. C. Brennan and P. J. Crocos, 1999. Comparative costs of postlarval production from wild or domesticated Kuruma shrimp *Penaeus japonicus* (Bate) broodstock. *Aquacult. Res.*, **30**: 191-197.

Ramos, L., M. Espejo, S. Samada and L. Perez., 1995. Maturation and reproduction of pond-reared *Penaeus schmitti*. *J. World Aquacult. Soc.*, **26**: 183-187.

Read, G. H. L. and M. S. Caulton, 1980. Changes in mass and chemical composition during the molt cycle and ovarian development in inmature and mature *Penaeus indicus* Milne Edwards. *Comp. Biochem. Physiol*., **66A**: 431–437.

Read, G. H. L., 1977. Aspects of lipid metabolism in *Penaeus indicus* Milne Edwards. M. Sc. thesis, University of Natal, Pietermaritzburg.

Sandifer, P. A. and T. I. J. Smith, 1978. Aquaculture of Malaysian prawns in controlled environments. *Food Technol.*, **32**: 36–38.

SEAFDEC (Southeast Asian Fisheries Development Centre), 1987. Aquaculture Department Annual Report, SEAFDEC, Tigbauan. Iloilo City, Philippines.

Shieh, H. S, 1969. The biosynthesis of phospholipids in the lobster *Homarus americanus*. *Comp. Biochem. Physiol.*, **30**: 679-684.

Shivaram, C. G. and R. P. Raj, 1997. Dietary lipid requirement of the juveniles of Indian white prawn *Penaeus indicus*, H. Milne Edwards. *J. Aqua. Trop*., **12**: 165-180.

Takeuchi, T., T. Watanabe, C. Ogino, M. Saito, K. Nishimura and T. Nose, 1981. Effects of low protein-high calorie diets and deletion of trace elements from a fish meal diet on reproduction of rainbow trout. *Bull. Jpn. Soc. Sci. Fish.*, **47**: 645-654.

Watanabe, T., 1985. Importance of the study of broodstock nutrition for further development of aquaculture. *In*: Nutrition and feeding in fish. (Eds. C. B. Cowey, A. B. Mackie and J. G. Bell). Academic Press, New York, pp. 395–414.

Watanabe, T., T. Arakawa, C. Kitajima and S. Fujita, 1984a. Effect of nutritional quality of broodstock diets on reproduction of red seabream. *Bull. Jpn. Soc. Sci. Fish.*, **50**: 495-501.

Watanabe, T., T. Takeuchi, M. Saito and K. Nishimura, 1984b. Effect of low protein–high calorie or essential fatty acid deficient diet on reproduction of rainbow trout. *Bull. Jpn. Soc. Sci. Fish.*, **50**: 1207–1215.

Xu, X. L., W. J. Ji, J. D. Castell and R. K. O'Dor, 1994. Influence of dietary lipid sources on fecundity, egg hatchability and fatty acid composition of Chinese prawn (*Penaeus chinensis*) broodstock. *Aquaculture*, **119**: 359–370.