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BREEDING AND SEED PRODUCTION OF GANGETIC PRAWN *MACROBRACHIUM GANGETICUM* (BATE)

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Introduction

Freshwater prawn farming in the world has registered an increase during the past decade. It has become an important component of global aquaculture production in terms of quantity and value. The total global production of freshwater prawns were nearly 4,10,474 with a value over US$ 1.6 billion (FAO, 2007). Aquaculture production in 37 countries is more than 30 000 ton per annum, Production is now valued above $1 billion annually. However, over 98% of production occurs in Asia (Saeed et al., 2009). The Ganga river prawn *Macrobrachium gangeticum* (Bate) is recognized as third largest growing freshwater prawn after *M acrobrachium rosenbergii* and *M acrobrachium malcolmsonii*. *M acrobrachium* species live in freshwater environments with links to saline waters since the larvae of the species require brackish water for complete development (Maria et al., 2011)

M. gangeticum is familiar with *M. chproi* (Tiwari). It attains weight up to 160 g in rivers (Tiwari & Holthouis, 1996; Kanaujia *et al*., 2001). The survey report of Tiwari (1949, 1955) and Jhingran (1956) indicated its migration up to Kanpur about 1300 Km away from the estuary of Bay of Bengal. It also occurs in the stretches of river Ganga, Padma, Hooghly and Bramhaputra passing through mass migration and recruitment of the juveniles are found between the border of Arunachal Pradesh and Assam. (Kewalramani *et al.,* 1971; Rajyalakhsmi, 1980). It is commercially important cultivable prawn species in North-East India, U.P, Bihar, Bengal, Bangaladesh and Assam. Efforts have been made to develop the hatchery technology for seed production of *M gangeticum* since 1972 along with other two species *M. rosenbergii* and Indian river prawn *M. malcolmsonii* . However, it met with limited success and till 1987; larvae could be reared up to Vth stage in 50% natural seawater. The effort was continued, since demand of freshwater prawn in local as well as in International market has been increased due to sit back of shrimp farming and business.

Macrobrachium gangeticum would have gained the popularity and rised into prominence for its farming. However, general decline in juvenile prawn fishery, mixed nature of the wild collected prawn seed and transport cost, are the main constraints experienced to establish prawn farming on a commercial scale. Hatchery operation for the Seed production of freshwater prawns needs to establish backyard / indoor hatchery with proper facilities of good quality freshwater and seawater, brood stock/ berried prawns feeds, power supply, suitable climatic and environmental conditions and labors for successful operation. Hatchery technology of this species developed at Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar is available for commercial seed production.

Brood Stock

Hatchery operations for the seed production of freshwater prawn depends upon the timely availability of healthy brood prawns which may be maintained either under pond condition or procured from the natural riverine sources. In both the cases it has got its own merits and demerits. However berried female of natural riverine stock carry more eggs and produce healthy post larvae with better survival and early post larval metamorphosis than the stock maintain under pond conditions. But availability of wild berried females is limited within the season depending upon the climatic position secondly a slight stress or hurt to the developing embryos in side the brood sac may cause the mortality to the hatched larvae reaching to the post-larval stage. Therefore it is always better to maintain the brood stock under captive conditions to obtain healthy berried females with the provision of proper food, water temperature (28^0 – 31^0 C) and other water quality parameters help to operate the year round hatchery for seed production.

An earthen pond with size ranged from 0.05 to 0.5-hectare area of 1 to 1.5 meter water depth are ideal to maintain brood stock. Pond should be free from the bottom silts. It is better to have the ponds with sandy or rocky bottom. It should also have inlet and outlet facilities to drain out the waste as per need. The bundh of the tank should be half meter higher from the water level to prevent the migration. Undrainable pond needs to be treated with fish poison like mahua oil cake @ 200-250 ppm. Or urea and bleaching powder @ 5 ppm nitrogen and chloride to eradicate the unwanted fish stock. In presence of insects and insects larvae oil –soap emulsion @ 18 Kg soap and 56-Kg oil per hectare water surface area may be applied to kill them. The juveniles or adult prawns may release with 50 – 70 mm size range in a ratio of 1 male and 5 female (1: 5) @20,000/ ha stocking density. It is always better to maintain the brood stock in cemented tank or plastic pools with the facility of proper aeration and water exchange or airlift biofilter recirculatory system. In such cases adult prawns may be reared @ male 1 and female 5 in one squire meter. Care should be taken to provide suitable feed to avoid wastage and water contamination. Any feed mixtures having 30-40% protein level along with required minerals and vitamins are good to feed the prawns. A mixture of ground nut oil cake and fish meal (1: 1) / poultry feed and poultry viscera (1:1) / a mixture of fresh fish / prawn / slaughter waste / mussel or snail meat along with other vegetable origin feed items in equal ratio prepared in pelleted form are equally good to feed the prawns. Feeding is done 5

– 10 % of total biomass twice a day or *add libitum* when reared in cemented tanks. Brood stock may also be maintained in green house under captive condition using 5000 to 50,000 liters cement tanks, FRP tank or plastic pool. In such cases water exchange may be needed daily or as when required. Pond may be fertilized initially with poultry dropping @ 1000 kg / ha and liming @ 200 kg / ha before stocking thereafter 50 kg fortnightly which may help for the growth and moulting of prawns.

Brood stock under pond condition needs following physico-chemical parameters recommended to maintain suitable water quality viz. water temperature 25 – 32 ^{0}C, pH 7 – 8.5, dissolved oxygen 5 – 6 ppm, ammoniacal nitrogen 0.01 – 0.20 ppm and hardness 90 – 115 ppm. The prawns attain 100 – 130 mm growth within six months where the males grow faster than the females. The sexual maturity generally occurs at the size of 60 – 70 mm and gonadal development starts from April. Matured and berried females appear during May and 90 % females carried developing eggs during July and August and available up till October. Females carry good number of eggs in the middle of the breeding season and the number of eggs decreases considerably by the end of October during last breeding.

Characteristics Of Sexually Matured Male And Female

Female attained 60 – 70 mm sizes within 5 – 7 months in natural habitat. Male and female are markedly distinguished through their external sexual dimorphism. The size of the matured male is considerably longer than the female, which has a pair of extremely long, strong and thick second thoracic (chelate) legs, big head, compact and narrow abdomen with little space between pleura (lateral abdominal plate). Second pleopods bear the appendix masculina present in between endopodite and appendix interna. A genital pore is present at the base of 5th thoracic legs. The female has a pair of short and slender second chelate legs. A spacious brood chamber is formed by the downward prolongation of first to fourth abdominal pleopods during egg laying. The basal segments of first four pairs of pleopods are prolonged with long ovegerous setae on its inner margin. These setae carry the eggs in bundles when they are released inside the brood chamber. Genital pore is present at the base of 3rd thoracic legs. In a live female, the ripe and fully developed ovaries are visible as a large green yellow-green colour mass occupying the dorsal and lateral parts of cephalothorax, which are visible through the transparent carapace.

Mating

The mating and spawning in the species is similar to other fresh water prawn species which may be observed watch while rearing of matured males and freshly molted female together in glass aquarium. Sexually matured males are able to mate at anytime, whereas females are ready to respond only after the completion of their pre-mating molt. Sexually matured female completes its pre-mating molts before mating and spawning. Just after shedding out of the chitinous shell, the weak and soft female is liable for attack by the other prawns; however, the presence of the male guards over it, until the body hardens. Perhaps the female prawn secretes a kind of substances (may be pheromones) just after the pre-mating molt, which strongly attract the males.

Mating and spawning occur with several activities. Initially the males fight for the establishment in new territory and the victorious male chases the female and move together. During mating male keeps female in between his two long second chelate legs and start becoming courting, mounting, displaying its masculine grace and strength by lifting head, raising body; weaving feelers, raising and extending his long powerful pincer legs in an embracing gesture, which are formed with the intermittent jerking movements. All these activities continue for a short while till then female surrounding to the male. The male holds the females in between the long chelate legs and actively clears the ventral portion of her thoracic shell with it other leg. The activity continues for a brief time and final mating takes place within few seconds. The male ejects and deposits the sperm in the form of gelatinous mass between the base of 3rd thoracic (walking) legs of the female. Thereafter, male and female are separated from each other.

Spawning

The process of egg laying takes place few hours after mating. Females move to safe place start ejecting the eggs through the genital pore and deposited in one mass on the ventral thoracic region between the thoracic legs, which are coated with a thin layer of gelatinous substance for protection. During the release of eggs, the female bends forward to keep contact with the ventral thoracic region, so that eggs are extruded through female genital pore at the base of third thoracic legs, directly into the brood chamber formed with the first to fourth pleopods (first on one side then other). The chamber formed with the fourth pleopods is filled first followed by third, second and first. The eggs are held in bundles like grapes by extremely thin and elastic membranous substance, which is held tightly to the fine ovegerous setae of the first to four pairs of pleopods. The release of eggs from the ovary to the brood chamber appears in a tabular form, which become rounded after leaving the genital pore. The eggs are fertilized while they come in contact with the sperm present in between the thoracic legs of the female. The unmated female prawns also lay eggs within few hours after pre-mating molt; however, they are dropped off or eaten by the female. The eggs are green yellow, slightly oval in shape ranging between 0.5 to 0.9 mm. The smaller specimen of 80 mm length and 5 g weight beard about 8000 eggs, whereas, a medium size female of 140 mm length and 40 g weight held 69,000 eggs. The maximum number of 80,000 eggs recorded from a female of 190-mm length. The females reared under controlled conditions lay eggs thrice within five months.

Embryonic Development

The berried females (egg bearing females) carry fertilized eggs with care till hatching; which may be reared either in freshwater or 5-ppt brackish water. The pleopods beat back and forth intermittently to provide aeration to the eggs through out the incubation period, which is depending on the temperature and food provided for the female. At optimum temperature 28 – 31 ^{0}C, the incubation period ranged from 12 – 13 days and with the fall of temperature (below 26^{0}C) incubation period may increased more than 13 days. Dead eggs and foreign materials are carefully removed from time to time by the sensitive and versatile first pair of thoracic legs. The just released eggs are yellow in colour, which becomes gradually lighter yellow to grey colour, which deepens gradually to slate grey colour when the first stage zoeae are fully developed and ready to hatch out.

Hatching

When the first stage larva (zoeae) is fully developed, it is ready to come out of the egg I shell to start active life. The hatching process is slow; continuous vibration of the mouth region of the larva gradually forces and stretches the rolled body. Vibration of the mouth region becomes more and more vigorous followed by further stretching of the body. The thoracic appendages also start to vibrate vigorously and intermittently. The intensity of vibration becomes more vigorous and continuous, resulting in the elongation of the head followed by the telson pressing outward. With the pressure, the egg I shell suddenly breaks the telson and head together emerges with a powerful flex and stretch. The entire body of the larva emerges out of the shell and starts swimming actively in water column within few minutes.

Water Quality Management

Aquaculture needs suitable culture media. Hence, precautionary measures are taken for successful larval rearing. Nitrogenous compounds, dissolved oxygen, pH, hardness, ionic composition, salinity, temperature, dissolved metals are the main chemical components, which play an important role in the growth, metamorphosis and survival of the larvae. Suitable ranges of the above factors contribute greatly for successful seed production. Nitrogenous compounds in a hatchery system are associated mainly with the excretion of ammonia and decomposition of organic compounds *viz.*, metabolites, molting skin and food. Slight increase in pH in water increases the unionized ammonia level and accelerate the formation of HNO_2 than nitrite. Low dissolved oxygen causes mortality of larvae. The increased in pH checked with the periodic application of calcium sulphate @5 ppm , while ammonia is controlled by nitrification through bio-filter (Kanaujia *et al.*, 1998). Dissolved oxygen is maintained through the establishment of an upward flow filtration. For maintenance of good water quality in the hatchery, the optimal ranges of the parameters are kept as under i.e. D.O. 4.0-6.0 ppm, pH 7.0- 9 ppm, ammonia 0.02-0.12 ppm, hardness 2000 –5000 ppm, salinity 12-16% and temperature 28 -30^0 C. The unutilized food, metabolites and molted skins are siphoned out daily and fresh brackish water is added to maintain the salinity and water level in the tanks. The water removed from the hatchery while cleaning the tanks should not be used further without proper treatment and aging, since it contains of organic compounds and suspended particles which may directly affect the larval metamorphosis. Sunlight is the most suitable source for water aging after chemical treatment of seawater. The organic compounds are dissolved in the water are utilized by the algal blooms and suspended colloidal components settled at the bottom. This water may be sand filtered should be used for larval rearing. The rainwater is most suitable sources of the freshwater for dilution of saline water.

Larval Biology

First stage zoea larvae and other larval stages of *Macrobrachium gangeticum* are planktonic in nature. Similar to *Macrobrachium malcolms onii* & *M acrobrachium rosenbergii* . Although they are photopositive and easily attracted towards the light, direct sunlight and strong illumination may result in sudden death of the larvae *Macrobrachium gangeticu m* completes larval cycle in brackish water. In nature the larvae present in riverine water drifted out down along with water current to estuarine brackish water to complete all the eleven larval stages immediately after attaining post – larval stage, post larvae leave the saline zone moves towards freshwater against the water current. However under freshwater pond condition, larvae may attain stage II and survive for few days only. The developing larval stages need brakishwater 12– 16 ppt salinity to attain the post – larval stage. All the eleven larval stages swim tail first ventral side up and dorsal side down, head rather lower than the tail at an oblique angle (upside down). The early larval stage I and II, cling to the side of the tank .as swim together in water column. The larval stages and number of moults there after they found more closer to *M. malcolms onii* as more than 5 moults recorded between stages V to VI. However duration for larval cycle recorded much shorter (20 days) than *M.malcolmsonii* (40 days). The characteristic features of different larval stages were observed in detailed are presented in Table 1.

Table 1: Key to identification of different larval stages (Kanaujia, 1999)

Larval stages	Length mm.	Distinguishing characters
I	1.87	Eyes sessile, telson triangular and fan shaped
II	2.03	Eye stalked, telson triangular and fan shaped
III	2.19	One dorsal rostral tooth, uropod appeared.
IV	2.43	Two dorsal rostral teeth, uropod biramous and bare.
V	2.85	Telson rectangular more elongated and narrower.
VI	4.06	Pleopods bud appeared.
VII	4.76	Pleopods buds biramous and bare.
VIII	5.24	The pleopods with setae, mostly in large.
IX	7.58	Appendix interna occurred on the endopodite of second to fifth pleopods.
X	9.37	Presence of 2 - 4 minute teeth on the dorsal side of the rostrum.
XI	10.53	Presence of 2 big and 6 – 10 small dorsal rostral teeth on entire length of rostrum

Post larva	11.79	Rostrum with 11- 13 dorsal and 3 or 5 ventral teeth; behavior of swimming like adult

Larval Growth And Metamorphosis

The growth performance and duration of different larval stages are indicated shown in Table 1. The first stage zoea ranges between 1.5 and 4.8mm. The progressive subsequent molt and growth increment of the larvae from stage I and V is rather fast. Than the *M malcolms onii* and M *rosenbergii*. The larval stage V passes through 5 moults and maximum 5days duration to attain stage VI. The time taken by the other larval stages is more or less similar, except stage X and XI, which take longer duration 2 days to attain the stage XI and post larval stage. The post larval length ranges between 3.00 and 12.00 mm. Water quality and feed help to produce healthy post larvae. The first few post larvae may appear on 19 – 22 days with adoption of air-lift biofilter recirculatory system and production rate increases gradually with a peak during 30 –35 days. Total post larval metamorphosis in a batch takes more than 64days. Depending upon the food, water temperature and water quality. The variation of periods in different larval stages for the same batch due to the larval efficiency in feeding. The larvae which are more efficient, consume sufficient food, grow faster and attain subsequent stages quickly; and metamorphose into post larva in relation to environmental conditions particularly temperature. In a healthy and well fed batch, the first few post larvae are expected within 19 – 22 days at 28 ± 2^{o}C water temperature, and most of the larvae (95%) metamorphose into post larvae by 35 – 40 days.

Larval Food

Newly hatched zoea larvae of *Macrobrachium gangeticum* range between 1.5 – 2.00mm in size. The larvae feed voraciously, grow faster and pass through all eleven larval stages attaining the post-larval stage within 20 and 26 days which is more or less similar to *M. rosenbergii* (New and Singholka, 1985). The early larval stages feed on newly metamorphose *Artemia nauplii* for one week morning (6.00AM) and evening (13.0PM). The earthworms and tubificid worm, egg custard,etc may also be provided . Brackishwater *Chlorella* and *Scenedesmus* species contain high level of protein may also be provided as larval food. Such feed may also reduce the concentration of toxic ammonia from the rearing medium. Brine shrimp nauplii are the excellent larval food for the early stages from I to V, which contains 50% protein and 20% fat. Two grams dry cysts are soaked in freshwater for about half an hour and then transferred to 10 l glass jar filled with 20% saline water. The medium containing suspended cysts is aerated vigorously. After 24 hours nauplii may hatched out at 28 –30^0 C water temperature. A beam of table lamp light is kept one point of the jar where the larvae congregated and are harvested through siphoning. These larvae are treated with few drops of 0.05% copper sulphate solution and then washed before feeding the larvae in the tank. During the first 7 days, 30,000 larvae in 300 lit tank fed 2g freshly hatched brine shrimp nauplii twice per day till complete harvesting of post larvae. From the fifth larval stage of 7 days onwards, the food is supplemented with egg custard (25 ml) and fed twice a day starting from 8^{th} day onward and continued till the end of the larval period.

Survival

The survival of the larvae depends on food and water quality parameters. Berried females need special care during embryonic development till the hatching of the larvae. The development of larvae from 1^{st} stage to post-larval stage is considered as a very crucial period as they undergo number of molting and exhibit cannibalism reducing the number under unsuitable water qualities. The larval mortality of the larvae occurs while molting from stage II – VI. Thereafter mortality is not found except few larvae getting stranded to the side of the tank. On the basis of total harvesting, above 20 –45 % survival @ 20 –45 PL/L is recorded from the experimental tanks, which may further increased with better management practices

Harvesting

Harvesting of prawn post-larvae from the hatchery tanks is unique and complicated due to their crawling of their nature. Generally, turn down draining or siphoning methods are applied to harvest the post-larvae of freshwater prawns. However, this method is not practical for harvesting the post-larvae of spp. The post-larvae of *Macrobrachium gangeticum* appear between 19-22 days and metamorphosis completed within 40 days. If the post-larvae are allowed to remain in the tank for 20days more the growth of the developing larvae may be affected due to competition for food by the post-larvae and developing advanced larvae. The post-larvae become more cannibalistic during early stage and attack the early larvae and freshly molted post-larvae. There fore the post larvae may be harvested daily by the shell-string developed at CIFA, Kausalyaganga. A number of string shells are prepared with the shells of freshwater mussel; plastic beads and nylon threads and these are hung into the side of the larval rearing tank with the support of sticks. The larval stage XI needs suitable place while molting into post-larval stages. The beds of the string shells are used for this purpose. After molting the post-larvae hide and rest on the shell beds. The string shells are lifted out slowly and carefully one by one from the rearing tank and kept into a big plastic tub half filled with the same water of the rearing tank. As soon as the string shell is kept into the tub, the post-larvae come out of the shell bed and start crawling like adult prawn. By this process daily harvest of post-larvae continues till the last molt without disturbing the water there by saving much time and labour.

Acclimatization

The newly metamorphosed post-larvae after passing their larval life in a medium of higher salinity 3 12 –16ppt requires to be acclimatized to freshwater before stocking them in freshwater medium for raising juveniles. In nature, after metamorphosis, post-larvae leave the brackish water and reach to the freshwater riverine zone for further growth and development. Under hatchery condition after harvesting of post-larvae, the saline water of the tub is gradually siphoned out and freshwater is added slowly. The process of siphoning and adding of water is repeated several times until the medium becomes fresh water. The entire process of acclimatization is done within an hour and then the post-larvae are released in the nursery tanks for the juvenile's seed production.

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