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Studies on mating, spawning and development of egg in *Macrobrachium nobilii* (Henderson and Mathai)

C BALASUNDARAM and A K KUMARAGURU*

School of Biological Sciences, *School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai 625021, India.

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Abstract. The chronomorphological events during the development of the egg of the freshwater caridean prawn, *Macrobrachium nobilii*, were followed from spawning to hatching. The female is receptive only for a 30-minute period after the premating moult. After mating, spawning ensued within 9 ± 3 hours and was completed within 15–20 seconds. Unmated females also spawned but the eggs did not survive. The rate of egg development increased 2·5x for a temperature rise of 10°C. Hatching was synchronised under *in vitro* conditions indicating uniform development of a clutch.

Keywords. *Macrobrachium nobilii*; mating; spawning; egg development.

1. Introduction

Macrobrachium nobilii, a caridean freshwater prawn moults and breeds once in 19 days. In carideans, there is a premating moult before spawning. A freshly moulted female remains receptive to a male for only a limited period (Ling 1964). If a male is not available during the receptive period, then fertilization of a brood will be missed and such a brood will be lost. Hence, it is important for culture purpose to know the receptive period. Therefore, studies were carried out on the sexual receptiveness and mating of *M. nobilii*.

Like most other decapods, *M. nobilii* incubates the developing eggs in the ovigerous setae of the pleopods till hatching. However, incubation by the mother is less productive in terms of larvae for culture needs (Balasundaram 1980). To circumvent the disadvantages associated with incubation, the eggs can be hatched under artificial conditions, simulating the ventilation technique of the mother (Balasundaram and Pandian 1981). An average female (39 ± 5 mm : total length from tip of rostrum to telson) incubates about 2,200 eggs/clutch. For mass incubation, eggs from more than one female are needed. Incubation of egg masses of identical age will enable simultaneous hatching. The percentage of hatching is influenced by the age of egg mass at the time of relieving (Balasundaram and Pandian 1981).

Hence, it is advantageous to procure egg masses of identical age for mass incubation under *in vitro* conditions. Therefore, an attempt has been made in this study to know the age of eggs based on simple chronomorphological features of the developing egg from 0 hour to hatching.

In general, temperature is known to influence egg development of decapods (Wear 1974). This indicates that the time required for development can be accelerated by

controlling the temperature. Hence, the effect of temperature on development of egg and hatching also was studied.

2. Material and methods

Healthy individuals of *M. nobilii* were collected from the river Cauvery at Tiruchi (10°5′N; 78°43′E). They were maintained in 90 litre/laboratory tanks at a density of 1 male to 7 females. The water temperature was 28 ± 2°C (mean ± SD). The pH and dissolved oxygen content were 7·5 ± 1 and 6 ± 1 ml/l respectively. The photo-period was 14L : 10D. The prawns were fed once daily, *ad libitum*, with a mixture of beef, goat liver and boiled Bengal gram (*Cicer arietinum*). Water was changed once a day. The female about to moult and spawn was identified by its fully developed dark green ovaries beneath the transparent carapace extending upto the third rostral spine anteriorly and into the first abdominal somite posteriorly (Balasundaram 1980). Such females were transferred into circular troughs at a density of 1 male to 1 female and observed for moulting and spawning.

Sexual receptivity of the female was studied by introducing several freshly moulted individuals in separate troughs. Males were released individually into each one of these troughs at different time intervals (5, 10, 15, . . . minutes) after moult, till sexual attractiveness ceased to exist when the prawns ignored each other.

Two different techniques were tried to obtain eggs from the mother, for *in vitro* incubation. In the first technique, 6 of the freshly moulted and mated females were selected. Each one was gently stretched and enveloped with a flexible aluminium wire mesh (0·5 cm mesh) around the body. This prevented the animal from bending its abdomen to form the brood chamber. However, the enclosure was loose enough to allow appendage movements. This arrangement enabled the eggs, on release, to pass through the wire mesh to be collected at the bottom of the trough. The collected eggs were washed with sterile water and transferred to incubating chambers. The incubating chambers were 100 ml conical flasks with 50 ml water disinfected by boiling. Each incubating chamber contained 10 eggs. The flasks were then covered loosely with cotton plugs to avoid atmospheric contamination.

The second technique involved the removal after 3 hours of berrying of small egg masses (25 ± 5 eggs/mass) from a clutch. These small masses were then carefully teased manually with the help of needles into individual eggs and incubated as in the previous method.

A batch of 6 berried females were allowed to incubate their eggs normally. Eggs were removed from these females and examined under a light microscope once an hour. The morphological stages attained by these eggs were compared with those of similar age incubated *in vitro*.

To study the effect of temperature on egg development and hatching, samples of egg mass from freshly berried females (3 hour old), reared at room temperature of 28 ± 2°C, were teased individually and transferred into disinfected water in incubation chambers as described above. The chambers were kept at 19, 22, 25, 29, 32 and 34 ± 0·5°C at normal photoperiod (14L : 10D). Six replicates were maintained at each temperature till the eggs hatched. The water in the chambers was carefully decanted and fresh disinfected water at the same temperature was added everyday.

3. Results and discussion

3.1 *Mating and spawning*

When a male was introduced into a trough containing a freshly moulted female with ripe ovaries, the male quickly rushed to the female, turned it over and mated. In less than 5 minutes the female, lying between the maxillipeds of the male, started moving freely and kept away from the male darting backwards even on chance encounters. Spawning occurred within 9 ± 3 hr after mating and lasted for a period of 15–20 seconds. The eggs on release were fertilized externally and passed into the brood chamber. The pleopods secreted a glue-like substance into which the eggs were enveloped and finally kept attached to the ovigerous setae of the pleopods as observed by Ling (1969) in *M. rosenbergii.*

The female after undertaking the premating moult, remained receptive only for 30 minutes. After this period when a male was introduced, they ignored each other. In *M. rosenbergii* such attractiveness persisted for 3 hours (Ling 1964) and *Palaemonetes* was receptive only for 20 minutes (Burkenroad 1947).

Females which were not mated also spawned and got berried as usual but these unfertilized eggs turned golden yellow owing to cytolysis and were lost subsequently. Such females with golden egg mass have also been collected from the field (3 out of 456 females over a period of one year). These eggs when examined under a light microscope were similar to the unfertilized eggs obtained from unmated females in the laboratory. This clearly reveals the importance of the male availability at the right time for mating to ensure fertilisation of the eggs.

Table 1. *Macrobrachium nobilii*: Chronomorphological features of the developing egg from 0 hour to hatching

Time: Hour	Identification features
0	Egg-oval in shape and measures 570 μ long and 437 μ wide. Uniformly granulated.
7	Cleavage commences as furrows at 4 equidistant points—8-celled stage.
14	Many hexagonal cells are seen—Egg pale green in colour.
25	Egg appears olive green in the centre due to the presence of large yolk cells and pale green ectodermal cells in the periphery (see also Anderson 1973)
35	White opaque blastoderm appears
60	Blastoderm extends anteriorly
80	Blastoderm occupies 8·3% of the egg's surface area
120	Blastoderm occupies 16·7% of the egg's surface area
160	Blastoderm occupies 36·7% of the egg's surface area
170	Heart beat begins—Eyes appear as crescentic streaks
205	Crescentic eye becomes oval in shape—Carapace appears
220	Length of egg increases from 570 to 608 μ—Breadth from 437 to 497 μ
240	Cornea formed—Mouth parts twitch occasionally
250	Setae of exopodite extend beyond carapace posteriorly
270	The outer and inner flagellae of antennule, their aesthetes and setae are seen distinctly—larva twitches often and tries to stretch out abdomen—Hatching imminent.

3.2 *Egg development*

The eggs on release were fertilized externally and passed into the brood chamber. The pleopods secreted a glue-like substance into which the eggs were enveloped and kept attached to the ovigerous setae. Ling (1969) made similar observations in *M. rosenbergii*. Eggs removed from the pleopods within the first 3 hours of berrying developed abnormally. This timing appears to vary in different species. For example in *Homarus* it takes at least 29 hours before the eggs can be removed for normal development to proceed (Templeman 1940). Patel and Crisp (1960) too observed that initial development stages are highly sensitive and develop abnormally when incubated *in vitro*.

The chronomorphological events occurring in the developing egg of *M. nobilii* incubated at 29 + 0·5°C is summarised in table 1. The segmentation of the egg was completed on the first day and the blastoderm appeared on the second day. On the seventh day the eyes appeared and heart beat began (figure 1b). The size of the egg

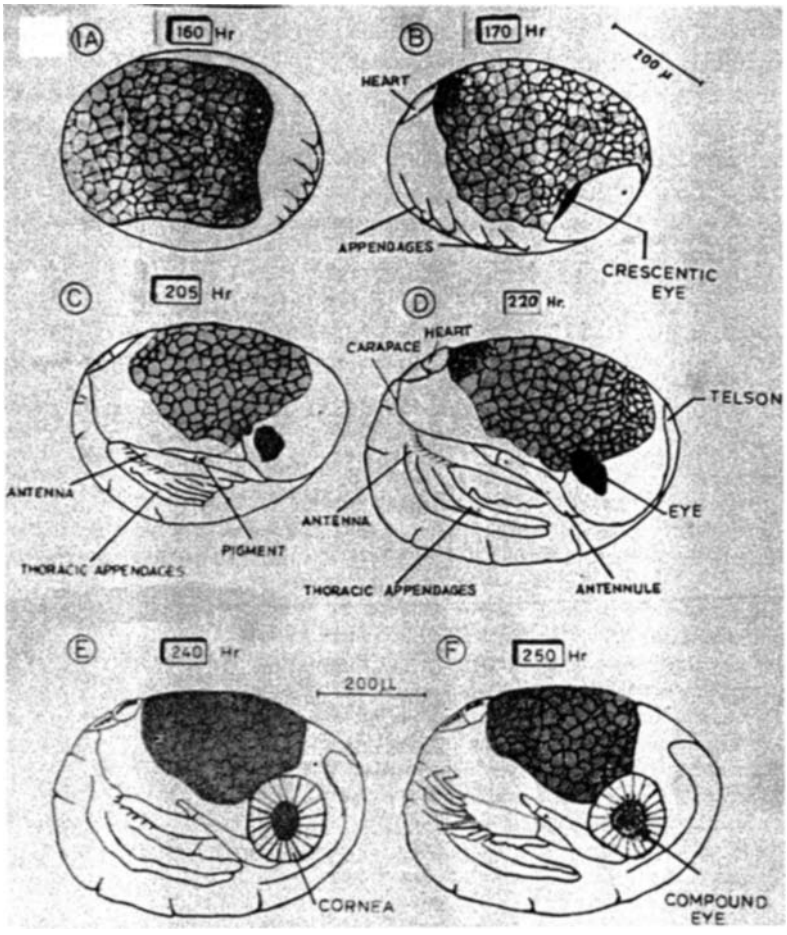

Figure 1. *Macrobrachium nobilii*: Chronomorphological features of the developing egg.

increased 1·6x on the tenth day (figure 1d). Such volumetric expansion of eggs due to imbibition of water has been reported by Pandian (1984) for several species of decapods and range from 1·2x in *Palirurus gammarus* (Berry 1971) to 5·4x in *Petrolisthes elongatus* (Greenwood 1965). On the 12th day the egg hatched as zoea (figure 1f).

3.3 *Effect of temperature*

The developmental stages attained by the eggs of *M. nobilii* at 22, 25, 29 and 32°C were observed. The eggs required 333, 300, 270 and 173 hours respectively to complete development to hatch (table 2). At any chosen temperature once the egg development is completed, hatching of simultaneously incubated eggs lasts for a period of 6 hours irrespective of the time of the day. The regression lines fitted by the method of least squares bear a linear relationship between the stages attained and time taken for development (figure 2). However, the percentage hatchability did not vary significantly and ranged from 73–78 % ($P < 0{\cdot}05$) (table 2). At 19 and 34°C the eggs failed to develop and suffered total mortality due to cytolysis within 9 ± 3 hours of incubation.

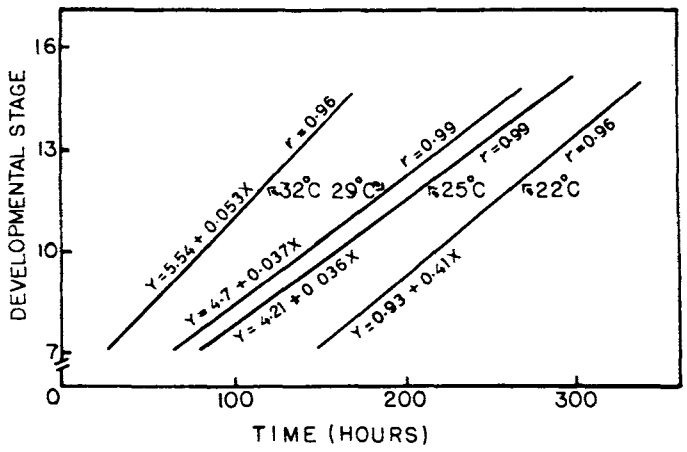

Figure 2. *Macrobrachium nobilii*: Effect of temperature on egg development.

Table 2. Effect of temperature (±0·5°C) on incubation period and hatchability of eggs of *Macrobrachium nobilii*.

Temperature (°C)	Time Hour	Hatchability (%)
22	333±6·6	73±9·1
25	300±7·1	77±5·2
29	270±6·9	78±6·4
32	173±6·6	75±8·3

Each value (mean ± SD) is based on 6 observations

The development of egg at the lowest temperature (22°C) was the least. The rate increased 2·5x times for a 10°C rise in temperature. This is comparable to the period required for the development of several species of barnacles (Patel and Crisp 1960).

Under *in vitro* conditions all the eggs of a single batch incubated at any particular temperature hatched simultaneously. This indicates the synchronous nature of the development of all the eggs. The rate of egg development when incubated by the mother (29 ± 1°C) was also uniform as in amphipods (Fish 1975), Cirrepeds (Patel and Crisp 1960) and decapods (Bensam and Kartha 1967).

The present study indicates that whether incubated by the mother *in situ* or under *in vitro* conditions, all the eggs of a brood commence development at the same time. They are at the same stage of development at any given time during incubation. However, when incubated by the mother the eggs are hatched in intermittent batches (Balasundaram and Pandian 1981).

Information on the chronomorphological events during the development will help to procure egg masses of identical age so that hatching can be synchronised. At room temperature (28 ± 2°C) the eggs take 12 days to hatch whereas at 32°C they need only 8 days. This indicates that hatching can be timed to suit the need for larvae.

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