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Breeding Behavior and Effect of Salinity and Osmolarity on Incubation and Hatching of *Macrobrachium malcolmsonii* (H. Milne Edwards) Under Laboratory Conditions

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Abstract: The second largest palaemonid prawn, *Macrobrachium malcolmsonii* has great potential for aquaculture. In the present study the breeding behavior and hatching mechanism was studied. The berried females were kept in different salinities until the larvae hatched out. The developed eggs were kept in bowls containing respective salinity. The incubation period was 14 days in freshwater but it decreased to 11 days with the addition of brackishwater of 7 ppt salinity. Hatching percentage increases when the berried females were reared in 7 ppt salinity than freshwater. The eggs of *M. malcolmsonii* hatched in tap water, pond water and distilled water (control) but not in the different concentrations of sucrose solutions (0.01, 0.02, 0.03, 0.04 and 0.1 M).

Key words: *Macrobrachium malcolmsonii*, incubation, hatching, sucrose solutions, ovigerous females, mating

INTRODUCTION

Freshwater prawn farming is expanding fast all over the world and therefore concerted efforts are being made to increase the seed production. The Godavari river prawn, *M. malcolmsonii*, is the second largest freshwater prawn of the family palaemonidae. For seed production, wild gravid females are caught and maintained in captivity for hatching. It is very difficult to obtain gravid females in non-monsoon months and therefore mating in captivity is necessary. Mating of *Macrobrachium* sp. has been accomplished under laboratory conditions and described by several authors; *M. rosenbergii* (Ling, 1969a; Chow *et al.*, 1982), *M. acanthurus* (Choudhury, 1971) and *M. heterochirus* (Ching and Velez, 1985) *M. malcolmsonii* and *M. rosenbergii* (Soundarapandian and Kannupandi, 2000). A simple osmotic hatching mechanism in which the egg membrane of developing eggs ruptures due to the imbibitions of water. This swelling and then breaking of egg membrane has been described for *Homarus americanus* (Davis, 1964); *M. idae* (Katre and Pandian, 1972); *Chirocephalus diaphanus* (Hall and Donald, 1975) and *Streptocephalus dichotomus* (Sam and Krishnaswamy, 1979). The present work is designed to investigate the breeding behavior and hatching mechanism of the freshwater prawn, *M. malcolmsonii*.

MATERIALS AND METHODS

Thirty mature males (Length 155 mm and weight 33 g) and equal number of mature females (Length 150 mm and weight 31.4 g) of *M. malcolmsonii*, were collected from the freshwater tanks of Manampadi (Lat. 11°29 N and Long 79°46 E) Tamil Nadu, India. They were acclimatized to laboratory conditions (salinity 0.5 ppt, temperature 28±2°C, pH 8.0-8.5, DO 5 ppm and photophase

12/12 h L/D) and maintained in a 180×60 cm fiberglass tank. One third of the water was changed daily and the prawns were fed with clam meat. Twenty four hours after mating (John Samuel *et al.*, 1997) ovigerous females were separated and kept one each in five fibreglass tanks (50 l) containing experimental salinities (0.5, 3.5, 7, 10.5 and 14 ppt) and were reared until their larvae hatched. Five replicates were maintained for each salinity. Hatching percentage was calculated from the number of eggs in a brood and the number of larvae hatched out (*M. malcolmsonii* of length 150 mm and weight 31 g have 42.896 eggs (Ibrahim, 1967).

To investigate the role of osmotic concentrations on hatching, well-developed eggs were ceased and separated from the mother prawn (John Samuel *et al.*, 1997) and were kept in bowls containing distilled water as a control and sucrose solutions (0.01, 0.02, 0.03, 0.04 and 0.1 M). In each bowl 130 eggs were introduced containing 40 mL (temperature 28±2°C, pH 8.0-8.5, DO 5 ppm and photophase 12/12 h L/D). To know the statistical significance, regression analysis, ANOVA and the Newman-Keuls multiple range test was attempted as per Zar (1974).

RESULTS

Mating takes place after a male protects a newly moulted female from aggression by other individuals in the tank. Four to 5 h after the pre-mating moult, the male started his courtship display, which continued for about 5 to 10 min. The male then grasps the female and begins to mount her. Subsequently, he begins to search the sternum of the female using the dactylae of his third and fourth pereopods. When he recognizes her sternum near the bases of her last three pairs of pereopods, he begins to turn her upside down using his first, third and fourth pereopods in a way that her ventral side was up. The male then pressed down from above, bringing its genital pores in close contact with the ventral thoracic region of the female. With a vigorous vibration of pleopods, the sperm was ejected and deposited in the females ventral median thoracic region (John Samuel *et al.*, 2000).

Within 5 to 12 h after mating, the eggs were deposited on the first 4 pairs of pleopods. Unfertilized eggs deposited in the pleopods dropped off within two or three days. The incubation period was about 14 days when the berried female was reared in freshwater (salinity 0.5 ppt). This incubation period was statistically similar to the incubation period observed for other salinities (3.5, 10.5 and 14.0 ppt). However, it reduced significantly to 11 days when the berried female was reared in brackish water with 7 ppt salinity (Table 1). The regression equation is $Y = 13.52 - 0.063X$ and there is no direct linear relationship between the salinity and incubation period. A significant higher hatching percentage (94.6%) was observed when the berried female was kept in the tank containing 7 ppt brackish water rather than freshwater (Table 1). No significant variation in hatching percentage was observed in the gravid females reared in freshwater and at 10.5 ppt salinity. Simple regression ($Y = 80.81 - 0.844X$) confirmed that there is no direct linear relationship between salinities and hatching percentage.

From Table 2, it was evident that the hatching is observed only in distilled water in which the larvae hatched out in 24 h and survived for 2 days. In 0.01 and 0.03 M sucrose solution no hatching

Table 1: Period of incubation and hatching percentage in gravid females of *Macrobrachium malcolmsonii* at different salinities

Salinity (ppt)	Incubation period (days)	Hatching percentage (%)
00.5	14.0±0.54 ^a	72.20±0.75 ^a
03.5	13.9±0.66 ^{ab}	75.50±2.41 ^a
07.0	11.1±0.20	94.60±1.02
10.5	13.1±0.68 ^{bc}	71.16±2.64 ^a
14.0	13.3±0.75 ^{abc}	60.60±1.63

In each column the values with same superscript are not significantly different (p>0.05)

Table 2: Osmotic concentration's effect on egg when exposed to different molar sucrose solution

Medium	Survival time (h)	Observation
DW	72	In all the cases hatching was observed. Hatched zoea lived upto 2 days.
0.01 M SS	32	No hatching bursting of eggs.
0.02 M SS	24 ^a	No hatching bursting of eggs.
0.03 M SS	24 ^a	No hatching bursting of eggs.
0.04 M SS	20	No clear shrinkage.
0.01 M SS	12	Shrinkage of eggs.

DW: Distilled Water, SS: Sucrose Solution, In each column the values with same superscript are not significantly different ($p > 0.05$)

was observed but bursting of eggs were observed after 32 and 24 h. In 0.02 and 0.03 M sucrose solutions, the survival period was statistically similar. In 0.1 M sucrose solutions shrinkage of eggs were observed at 12 h of exposure and no clear shrinkage was observed in 0.04 M solution even after 24 h of exposure. Simple regression ($Y = 44.42 - 412.63X$) confirmed that the sucrose solutions in different concentrations on egg hatching are not linear.

DISCUSSION

In *M. malcolmsonii*, the duration of courtship display was only for about 5 to 10 min and the incubation period was 14 days. But in *M. rosenbergii*, the duration courtship display was 15 to 20 min and the incubation period was 19 days (Ling, 1969a). The shorter incubation period observed in the present study was probably due to 7 ppt salinity in the tank containing berried female. This indicates that the brackish water at 7 ppt salinity accelerates the embryonic development of *M. malcolmsonii*. The result obtained by Damrongphol *et al.* (1990) in *in vitro* embryo culture of *M. rosenbergii* support our findings. Fertilized eggs were deposited 5 to 12 h after mating in *M. malcolmsonii* whereas; in *M. rosenbergii* it was between 16 to 20 h (Ling, 1969b) and between 5 to 24 h in *M. heterochirus* (Ching and Velez, 1985).

From the present study it was concluded that the eggs of *M. malcolmsonii* hatched in tap water, pond water and also in distilled water but not in an osmotically concentrated sucrose solutions (0.01 to 0.1 M). Therefore, the osmotic pressure of the developing *M. malcolmsonii* eggs is below or equivalent to 0.01 M sucrose solution. That is osmotic pressure of the liquid inside the egg membrane is below or equivalent to 0.3 atm; the Δf : 0.5°C. So this higher osmotic pressure of the medium inhibits the hatching of *M. malcolmsonii* eggs. This was agreement to the findings of Hall and Donald (1975) for *Chirocephalus diaphanus* and Sam and Krishnaswamy (1979) for *Streptocephalus dichotomus*.

Since, the osmotic concentrations did not seems to be the immediate causative factor for the hatching of *M. malcolmsonii* eggs and also the fastest beating of pleopods observed just prior to hatching followed by the break of egg membrane and larval release, it was conceived that the constant beating of pleopods of the mother prawn had something to do with hatching. The above observation was supported by the findings of Davis (1964) in American lobster in which the breakage of second egg membrane was by the action of mother's swimmerets and the first membrane by osmotic swelling. Simple conclusions was drawn from the results of the present study that the breaking the *M. malcolmsonii* of egg membrane is by the action of mothers swimmerets and the internal pressure developed by the continuous jerking movements of the embryo, accompanied by stretching of the rolled up body and increasing volume of the larvae pushes the zoea out.

Most adult *Macrobrachium* sp., are known to migrate to the brackish water for breeding purpose (Panikkar, 1967). Ling (1969b) found that the presence of small amount of brackish water (4 to 6 ppt) provides a better media for hatching of *M. rosenbergii* eggs. Katre and Pandian (1972) confirmed that the egg of *M. idea* is able to Pick up salts from brackish water more readily than from freshwater.

Likewise, the higher hatching percentage of *M. malcolmsonii* eggs in water containing 7 ppt salinity than freshwater may be due to the absorption of salts, which results in more internal pressure, facilities easy rupture of the egg membrane.

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