Effect of unilateral eyestalk ablation on ovarian maturation and occurrence of berried females in *Macrobrachium rosenbergii* (de Man)

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ABSTRACT

The efficacy of unilateral eyestalk ablation technique as a means for synchronising ovarian maturation in adult females of giant freshwater prawn *Macrobrachium rosenbergii* was evaluated in the present study. The experiment was conducted in six out-door cement tanks (7.5x1.75x1.2 m) for a period of six weeks. Experimental group of 18 females was subjected to unilateral eyestalk ablation and was equally distributed in to three tanks. Another group of 18 intact females stocked in three tanks (@ 6 nos. per tank) acted as control group. One adult blue clawed male was released to each of the six tanks for breeding purpose. Prawns were fed twice daily with pellet feed @5% of biomass. Prawns were sampled at weekly intervals to determine the growth, ovarian maturation and health. Results showed a positive effect of unilateral eyestalk ablation on growth and ovarian maturation in *M. rosenbergii*. Specific growth rate (%) of unilaterally eye-ablated prawns (0.965±0.525) was significantly higher (p<0.05) than that of control prawns (0.869±0.474). Survival rate (%) was higher in control non-ablated prawns (72.22±9.62) than that of ablated prawns (66.66±16.66). Increased number of maturing females in ablated group after six weeks indicated re-maturation after spawning. Unilateral eyestalk ablation has thus accelerated the pace of ovarian maturation however, this was not strong enough to synchronise ovarian maturation in *M. rosenbergii*.

Keywords: Eyestalk ablation, *Macrobrachium rosenbergii*, Ovarian maturation, Synchronisation

Introduction

Giant freshwater prawn *Macrobrachium rosenbergii* is widely cultured in many tropical and sub-tropical countries around the world. Aquaculture production of this species has shown dramatic increase since mid-nineties from 19,035 t in 1994 to 2,13,274 t in 2007 (FAO, 2009). Despite the potential of increased production, the sustainability of freshwater prawn farming is currently threatened by low production efficiency and vulnerability of the farmed stocks to diseases as in the case of marine shrimp (Vijayan et al., 2005). This has created renewed interest to undertake selective breeding programmes to improve the growth rate and disease resistance of the species.

Assured supply of ready to spawn breeders is a pre-requisite for selective breeding programmes. *M. rosenbergii* breeds throughout the year if the water temperature remains above 26 °C (New, 2005). However, even during the peak breeding season only about 30-40% of the females will be in ready to spawn condition. Hence very large numbers of adult females have to be maintained to obtain the required numbers of ready to spawn breeders, thereby increasing the cost of operation dramatically. One solution to this problem is synchronisation of ovarian maturation through hormonal or environmental manipulations. Eyestalk ablation is widely used in shrimp breeding for induced maturation (Arnstein and Beard, 1975; Santiago, 1977; Primavera, 1978; Muthu and Laxminarayana, 1982; Browdy and Samocha, 1985; Palacios et al., 2000; Venkitaraman et al., 2004). X organ-sinus gland complex (XO-GO) is a major neuro-endocrine center situated in the eyestalk of shrimps and prawns. The hormones produced by the XO-GO are reported to have gonad inhibiting and growth inhibiting function (Mohamed and Diwan, 1991). Hence, eyestalk ablation has been shown to result in precocious moulting or precocious gonad development. Unilateral eyestalk ablation (UEA) has been employed to induce both ovarian maturation and spawning with varying success in many species (Santiago, 1977; Primavera,1978; Zaib, 2001). UEA is reported to be safer than bilateral eyestalk ablation as the survival and growth are much higher in former than the latter (Maynard and Sallee, 1970; Venkitaraman et al., 2004). Earlier studies have reported that both bilateral and unilateral eye-stalk ablation induce ovarian maturation in *M. rosenbergii* (Okumura and Aida, 2001). In the present study, the effect of unilateral eyestalk ablation as a means of synchronisation of ovarian maturation in *M. rosenbergii*, was evaluated.
Materials and methods

Live adult prawns for the experiment were collected from earthen grow-out ponds of the Central Institute of Freshwater Aquaculture, Bhubaneswar, India and stocked in acclimation tanks for seven days. After acclimation, a total of 36 immature females in inter-moult stage were divided into two groups of 18 prawns each. One group was subjected to unilateral eyestalk ablation and distributed equally in 3 experimental tanks (7.5 x 1.75 x 1.2 m) at the rate of 6 nos./tank. The second group was treated as the control group and were released in to three separate tanks of similar dimensions @ 6 nos./tank. Eyestalk ablation was performed using sterile surgical blades. After ablation, the prawns were held in pre-cooled water to reduce heart beat rate and loss of haemolymph. Thereafter the specimens were disinfected with 1 ppm potassium permanganate solution by giving a dip bath and then released in the respective tanks. One adult male prawn (blue claw morphotype) was released to each experimental tank for breeding purpose. Prawns were individually measured for total length and wet weight prior to release in the tanks. Prawns were fed twice daily with grow-out feed (3 mm pellet) @ 5% of the biomass per day. Cut pieces of PVC pipes (4” dia; 12” length) were placed in each tank as shelter to molting prawns.

Temperature and dissolved oxygen of the water in the tanks were measured twice-daily using digital DO meter (Eutech Instruments, Singapore). pH was measured using pH meter (Thermo Orion, USA) and ammonia was analysed by phenol hypochlorite method of Solorzano (1969) at pH meter (Thermo Orion, USA) and ammonia was analysed by phenol hypochlorite method of Solorzano (1969) at weekly intervals. Water exchange was done at the rate of 50%, once every week.

Prawns were sampled weekly once to assess the growth, ovarian maturation and health. The stages of ovarian development were monitored by observing the changes in egg colour and increase in the size of ovary in relation to the size of the carapace. The prawns were classified into either one of the four female maturity stages, i.e., stage I (immature – translucent, small ovaries), stage II (early maturing – slightly orange and small confined to less than one third of the carapace), stage III (maturing – bright orange, larger and occupying nearly two third of the carapace), stage IV (ripe-bright orange, very large and almost fully occupies the carapace cavity). Specific growth rate was calculated as per the formula:

\[
\text{Specific growth rate} = \frac{\ln \text{ final body wt} - \ln \text{ initial body wt}}{\text{Duration of experiment period}} \times 100
\]

Growth, survival and maturation data were statistically analysed using Student’s t test to test the treatment effect at 5% level of significance.

Results and discussion

The water quality parameters recorded during the study period were: temperature 27.8-30.6 °C; pH 8.5 - 8.9; dissolved oxygen 7.1-7.6 mg 1⁻¹; total ammonia nitrogen 0.008-0.018 mg 1⁻¹. None of water quality parameters differed significantly between treatments and remained within optimum range reported for the growth of the species (New, 2005).

The specific growth rate of unilaterally eye-ablated prawns (0.965±0.525) was significantly higher (p<0.05) than that of control prawns (0.869±0.474) (Table 1). Karplus and Hulata (1996) while evaluating the effect of UEA on jumpers and laggards of M. rosenbergii found a highly significant effect of UEA on the growth of male and female laggards manifested in both increase in size increments per moult and shortening of the moult interval. In Metapenaeus dobsoni, Venkitaraman et al. (2004) also reported an increase in dry weight and food conversion efficiency leading to 84% higher production in ablated animals. In the present study, survival rate was higher in control non-ablated prawns than that of ablated prawns (Table 1). However, the observed difference in survival rate was not statistically significant (p>0.05). In Penaeus monodon, Santiago (1977) also reported highest survival among the control group when compared to unilateral and bilateral ablated group.

Table 1. Growth and survival data of unilateral eyestalk ablated and control prawns

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-ablated prawns (Control) (mean ± SE)</th>
<th>Unilateral eyestalk ablated prawns (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>25.64±3.5</td>
<td>27.54±0.73</td>
</tr>
<tr>
<td>Duration (d)</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>37.16±4.07</td>
<td>42.10±10.35</td>
</tr>
<tr>
<td>Specific growth rate (%)</td>
<td>0.869±0.474</td>
<td>0.965±0.525 *</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>72.22±9.62</td>
<td>66.66±16.66</td>
</tr>
</tbody>
</table>

*Significantly different at p<0.05

Fig. 1 shows the percentage of maturing females (stage III and IV) in ablated and non-ablated groups at weekly intervals. Ovarian maturation was noticed in both ablated and control groups in the first week itself. Percentages of maturing females were higher in ablated group seven days after eyestalk ablation, and by third week nearly 50% of the ablated females showed ovarian maturation indicating the fast pace of ovarian maturation in the ablated group. Thereafter due to spawning there was a decline in the number of maturing females in the ablated group till fifth week, and increased again in the sixth week indicating re-maturation after spawning. Thus it appeared that UEA has accelerated the pace of ovarian maturation in M. rosenbergii females.
On the other hand, in control prawns, the percentage of maturing females showed an increasing trend up to the second week and remained more or less stable till fourth week and then showed a decline due to spawning. There was no further increase in the number of maturing females till the termination of the experiment. The observed differences in the number of maturing females between the ablated and non-ablated groups were however, not statistically significant (p>0.05).

In decapods, crustaceans the X organ–sinus gland complex present in the eyestalk produces the neurohormones that regulate various physiological processes including moulting and reproduction (Keller, 1992; Okumura and Aida, 2001). Growth and reproduction in crustaceans is under the control of inhibitory hormones called moult-inhibiting hormone (MIH) and gonad-inhibiting hormone (GIH) produced by the XO-SG. The GIH is also called the vitellogenesis–inhibiting hormone (VIH) because of its vitellogenesis–inhibiting activity in female crustaceans (De Kleijn and Van Herp, 1995). Presence of VIH like peptides has been reported in _M. rosenbergii_ (Yang and Rao, 2001). Eyestalk ablation has been reported to accelerate moulting and ovarian maturation in a number of decapod crustaceans (Cooke _et al._,1985; Murugadas _et al._,1987; Vadher, 2004). The present study also showed a positive effect of unilateral eyestalk ablation on growth and ovarian maturation in _M. rosenbergii_. Removal of eyestalk lowers the titre of the vitellogenin-inhibiting hormone in the haemolymph thereby removing the inhibitory effect on vitellogenin synthesis. This leads to vitellogenin synthesis and maturation of ovary. Okumura and Aida (2001) reported that bilateral eyestalk ablation in _M. rosenbergii_ resulted in a significant increase in prawns undergoing reproductive moult. Pandey and Kumar (2007) reported an increase in GSI and ova diameter in unilaterally ablated females compared to control prawns. Murmu _et al._ (2007) observed higher GSI values in eyestalk ablated _M. rosenbergii_ compared to non-ablated group indicating a positive effect of unilateral eyestalk ablation on ovarian maturation.

Increased number of maturing females in ablated group in the present study after six weeks post-ablation indicated re-maturation after spawning. Unilateral eyestalk ablation has thus found to accelerate the pace of ovarian maturation in _M. rosenbergii_ probably by shortening the moult cycle as reported by Browdy and Samocha (1985) and Murmu _et al._ (2007).

Percentage of berried females in ablated and non-ablated groups at weekly intervals is presented in Fig. 2. Berried females could be observed in both ablated and non-ablated groups three weeks after initiation of the experiment. During the third week, the percentage of berried females were slightly higher in control group whereas in fourth and fifth week it was higher in ablated group. Sixth week after ablation, percentage of berried females in ablated group were significantly lower than control due to egg hatching. The ablated females thus became berried earlier than the control females and released the larvae also earlier. Unlike other studies which limited their observations to GSI and ova diameter to confirm ovarian maturation status, the present study has also monitored the percentage occurrence of berried prawns in both the groups. Occurrence of berried females indicates the successful completion of ovarian cycle and hence appears to be a better indicator of the pace of ovarian maturation. The percentage of berried females recorded in the present study also indicated a positive effect of unilateral eyestalk ablation in _M. rosenbergii_.

![Fig. 1. Weekly variations in the ovarian maturation (%) in unilaterally eyestalk ablated and non-ablated _Macrobrachium rosenbergii_.](image1)

![Fig. 2. Weekly variations in the occurrence of berried females (%) in unilaterally eyestalk ablated and non-ablated _Macrobrachium rosenbergii_.](image2)
Though the present study has showed that UEA has accelerated the pace of ovarian maturation in adult females of *M. rosenbergii*, this has not resulted in synchronisation of ovarian maturation.

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**References**


