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Effect of eyestalk ablation on ovarian maturation in the banana prawn, *Fenneropenaeus merguiensis* de Man under different environmental conditions

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Abstract

Investigations were made on the ovarian maturation of eyestalk ablated *Fenneropenaeus merguiensis* maintained under different environmental conditions. The results obtained indicated no signs of maturation when females of size smaller were ablated. Larger ablated females showed a delay in maturation during the colder months in contrast to those ablated during the warmer months. The study suggests that a system incorporating unilateral eye stalk ablation, high salinity, good water quality and optimum temperature are sufficient to induce maturation in captivity in females above the length of first maturity.

Reproduction, in crustaceans has been hypothesised to be controlled by dual endocrine factors - a gonad-inhibiting hormone (GIH) from the X organ-sinus gland complex and a gonad-stimulating hormone (GSH) secreted by the brain/ thoracic ganglion of maturing females, the actions of which are antagonistic to each other (Adiyodi and Adiyodi, 1970). In the majority of malacostracan crustaceans, eyestalk is the pivotal organ for housing various neuropeptides responsible in regulating maturation. Eyestalk ablation reduces the titre of GIH in females causing accelerated ovarian growth. Thus, endocrine manipulation to induce gonadal maturation has so far been synonymous with unilateral eyestalk ablation and has a far-reaching impact on crustacean aquaculture.

Eyestalk ablated shrimps respond to their operation with a rapid and unstoppable gonadal development thus augmenting total egg production in a given time.

Eyestalk ablation, often, is associated with problems such as deterioration in spawn quality and quantity over time (Emmerson, 1980; Primavera, 1985) and manifesting conflicting results on spawn size, hatching success and other variables (Browdy, 1992). Eyestalk ablation suspends the controls on a large number of body functions, so that females so treated divert all their energies into ever more frequent bouts of egg production, leading to a loss in egg quality and eventual death. However, many commercial hatcheries prefer eyestalk ablation to induce maturation in penaeid shrimps in spite of the associated

disadvantages of increased mortality, disruption of the shrimp's endocrine system and decreased larval viability with repeated spawning. This is mainly due to the predictable peaks in maturation and spawning with eyestalk ablation, which facilitates the setting up of production schedules, in contrast to scattered spawns from unablated females. The present study was undertaken to investigate the effect of eyestalk ablation on the ovarian maturation of *Fenneropenaeus* (*Penaeus*) *merguiensis* under different environmental conditions.

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Material and methods

Healthy specimens of *Fenneropenaeus merguiensis* were collected on the basis of their sex and stage of ovarian maturity. They were disinfected with 50mg/l formalin for 1-2 minutes in the laboratory and were transferred to aerated plastic pools containing seawater (salinity 25 ± 2ppt; temperature: 29 ± 2°C). The shrimps were fed with fresh clams and squids *ad libitum* once daily at the rate of 10% of their body weight.

Experimental protocol: After an initial period of acclimatisation the females were subjected to eyestalk ablation and transferred to experimental units (velon-screen net cages of 2x1x1 m; mesh size - 225 mesh /sq. inch) installed at Karwar Bay. In the first series of experiments, five female shrimps smaller than the reported minimum size of first maturity, collected from filtration ponds (total length 80-110 mm; carapace length 18-20mm) were subjected to eyestalk ablation and introduced into the experimental units (Temperature: 29°C ± 2°C; Salinity: 27- 33ppt). In the second series, an attempt was made to assess the effect of eyestalk ablation on ovarian maturation in the species during the colder months in nature. Five adult females (total length 120-150 mm; carapace length 23-35mm) with immature ovaries were subjected to unilateral eyestalk ablation during the month of December and introduced into the units established in the bay. The temperature during the daytime varied between 27° C and 29° C and during night, it fell to 18°C to 22° C. Animals collected from the same area, with similar biological characters and reared under similar experimental conditions, but without eyestalk ablation served as controls in the experimental trials. Males were introduced into the rearing units in the ratio of one male to two females. The total experimental duration was 10 days.

The development of gonad was determined externally by holding each female against a powerful light and observing the state of ovary through the cephalothorax and abdomen. At the termination of the experiment, the females were removed and weighed and the gonadosomatic index was determined using the formula

$$\text{Gonadosomatic index} = \frac{\text{Wet weight of the ovary}}{\text{Wet weight of the shrimp}} \times 100$$

Histology: Morphological features such as colour, nature and development of ovarian lobes were noted. Samples from the middle lobe of the ovary were fixed in Bouin's solution and prepared for histological observation. After 24hr of fixation, the ovaries were dehydrated in an alcohol series and embedded in paraffin (mp 56-58°C). Seven-micrometer sections were prepared and stained with haematoxylin and eosin. One hundred oocytes from each group were measured to determine their diameter by using ocular micrometer with a light microscope. Staging of gonadal development was done according to Rao (1967).

Results

Eyestalk ablation (ESA) in smaller prawns

In the first series of eyestalk ablation experiments, females smaller than the minimum size of first maturity were subjected to eyestalk ablation. On termination of the experiment, both control and the experimental groups showed no signs of ovarian development. Ovary was not visible through the dorsal exoskeleton. The eyestalk ablated females were observed to be still in the first or previtellogenic stage of gonadal development and eyestalk ablation did not induce any progressive change in ovarian maturation during the experiment. Ovarian lobes were translucent and histologically the ovarian lobes were packed with the developing oogonia and previtellogenic oocytes (average oocyte diameter 30μ), which are characteristic features of immature ovaries.

Influence of unilateral eyestalk ablation during the colder months

No obvious changes were observed in the ovaries till the fifth day, both in the eyestalk ablated and control females. However, on the seventh day, signs of ovarian maturation were visible in all the eyestalk ablated females, while no signs of ovarian maturation were observed in the control females. The ovaries of the ablated females appeared greenish yellow to olive green in colour, but were restricted only to the anterior end. Thus the ovaries showed a minimum growth. Hundred percent survival was observed during the experiment. No further development was observed till the termination of the experiment on the 10th day. The animals were sacrificed and the GSI was calculated (1.2- 2). Light microscopic studies revealed that these yolky oocytes were in late vitellogenic stage. The average oocyte diameter was 90μ.

Discussion

The minimum size for maturation in *F. merguiensis* was reported to be 23mm carapace length (CL) (Tuma, 1967). In the first series of experiments where females below the reported size for maturation were subjected to eyestalk ablation showed no response in ovarian maturation. Larger females subjected to eyestalk ablation and maintained in the same conditions were reported to show visible signs of ovarian maturation within three to five days and spawn within a period of 5-8 days (Sherly and Kakati, 2001). The results indicated the need of older and larger females for

successful production of postlarvae from pond reared brood stock. But larger females ablated in the colder months showed a delay in response (after 7 days) to eyestalk ablation technique.

Studies indicate that a reproductive response is produced through a relative interaction of environmental factors especially temperature, light, salinity and food and endogenous factors of an organism (Emmerson 1980, 1983). Thus, the pattern of reproductive cycle in a species is apparently determined through the co-ordination of the successive reproductive events with changes in the external environment. The influence of some environmental factors on the growth of gonad has been determined experimentally. Temperature and light are considered to be the most important physical factors influencing gonad maturation of marine organisms and the biological effects of these factors are complex and wide ranging. Nevertheless, the mechanism co-ordinating the physiological process underlying the events within the organism and the changes in the environment are not clearly understood. However, the ovarian maturation in *F. merguiensis* as evident from the results of the present experiment, was slow in eyestalk ablated females during the colder months in contrast to that obtained during the warmer months where females showed signs of ovarian maturation within 3-5 days indicating the need for higher temperature of warmer period for gonadal development. The results also suggest that a system incorporating unilateral eye stalk ablation, high salinity, good water quality and reduced light intensity and optimum temperature are sufficient to induce maturation in females above the length of first maturity of *F. merguiensis* in captivity.

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