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Effect of unilateral eyestalk ablation on ovarian maturation of female freshwater prawn, *M. lamarrei lamarrei* {H. Milne Edwards, 1837}

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Abstract

The study was done in laboratory for a period of 30 days to know the effect of unilateral eyestalk ablation (ULEA) on the ovaries of female freshwater prawn, *M. lamarrei lamarrei*. Morphologically good looking and apparent healthy specimens of *M. lamarrei lamarrei* (28- 35 mm) were selected for experimental work. ULEA was done by cutting one eyestalk (left) away at their bases with sterilized scissors. The result of study indicated positive effects of ULEA on ovarian maturation of female freshwater prawn, *M. lamarrei lamarrei*. The microscopic examination of different sections of ovary of female prawn which were ablated showed post vitellogenic oocytes while as females with both eyestalks intact never developed beyond pre vitellogenic oocytes during the experimentation. G.S.I. also shows that much ovarian indices develop better and rapidly as compared to control and on the basis of these facts, it is concluded that eyestalk of *M. lamarrei lamarrei* containing ovary inhibiting factor results in acceleration of ovarian maturation.

Keywords: Eyestalk ablations, ovary, vitellogenesis, ablation

1. Introduction

For reproductive dynamics of economically important species one should be aware of spawning. Eyestalk ablation technique is most common and widely known for manipulating ovarian development and maturation in captivity and is practiced commercially in prawn hatcheries, particularly with prawns that don't spontaneously mature and spawn [1]. Reproduction in decapod crustaceans is regulated and controlled by various neurosecretions that are secreted from X-organ sinus gland complex located in eyestalk of various crustaceans' species [2, 3]. Various physiological processes else than the reproduction, are affected by the ablation of eyestalk as it contains sinus gland(X-organ) [4].

Since from the last few decades biologists are very much engaged in research on these economically important crustaceans and recently a neuropeptides that regulates Methyl ferrioxalate synthesis by mandibular organs have been isolated from sinus gland sequenced and named as (MO-IH), Mandibular organ inhibiting hormone and scientifically considered as a member of (CHH), crustacean hyperglycemic hormone family [5]. CHH is very common hormone in arthropods phylum [6]. CHH in various decapod crustaceans is associated with various biological features [7].

Eyestalk ablation is mostly adapted technique by various biologists for induced maturation of gonads in crustaceans. Significant use of eyestalk ablation technique in aquaculture farming of prawn and shrimps has put forth both its positive and negative effects on the quality and quantity of spawning and fecundity [8]. Gonadal development in freshwater prawn *M. dayanum* can be accelerated by incorporating unilateral eyestalk ablation [2].

Most penaeid prawns, there is natural inhibition of gonadal maturation and spawning. In aquaculture practices this inhibition is removed by the removal of eyestalk. This is true for all other penaeid prawn species, that use of eyestalk ablation technique is very much beneficial as it reduces interbreeding time significantly [9], also effecting fecundity as well at a particular time. In penaeid prawns ovarian development as well as spawning starts after 14- 28 days after eyestalk ablation technique [10].

Knowledge concerning the effects of eyestalk ablation in candidate prawn is very important for understanding reproductive biology and growth. Hence prime aim of the study was to investigate reproductive activities (ovarian maturation), using eyestalk ablation technique in female prawn, *M. lamarrei lamarrei*.

2. Material and Methods

Live freshwater prawn *Macrobrachium lamarrei lamarrei* were collected from Upper Lake Bhopal during early hours of the day and brought to the laboratory. Before experimentation, prawns were treated with 0.1 KMnO₄ solutions to obviate any dermal infection and acclimatized in the laboratory for 3 days. Prawns were kept in glass aquarium (55 L) and fed with commercially available food. The excess feed and fecal matter has been siphoned out every day to avoid contamination of water. The water is changed after 5 days. 40 specimens of *M. lamarrei lamarrei*, looking apparently healthy, in the size group of 28- 35mm in total length and 0.325g in total weight with uniform ovarian condition (immature) were selected for the experiment. The total duration of the experiment investigated in the laboratory was 30 days. The experimental prawns were divided into the following two groups each having 20 specimens.

Group A

Control: This group consists of 20 specimens of freshwater female prawns of *M. lamarrei lamarrei* with uniform immature ovarian condition.

Group B

Eyestalk ablated group: This group consists of another 20 specimens of freshwater female prawns with uniform immature ovarian condition which were undergone unilateral eyestalk ablation. For this sterilized scissors was used for the ablation technique and eye stalks were cut from the bases in cold water bath (temperature 2-5°C) as to reduce cardiac peace and prevent loss of haemolymph. Care was taken during ablation; the eyestalk was removed after holding the prawn in cooled water to reduce the heart beat rate and loss of haemolymph.

Shelter in both the groups was provided artificially by placing small plastic pipe pieces in to the aquaria's.

2.1 Statistical Analysis

The Gonado- somatic indices (ovarian indices) were determined using the standard formula in both the groups.

$$GSI = 100 \times (\text{Wet weight of the gonad (g)} / \text{Wet weight of Prawn (g)})$$

Similarly, statistical analysis was subjected to 't'- Test of significance.

The oocytes dimensions were determined with the aid of oculometer. Female reproductive functions were determined by ovarian indices, oocytes diameter and histological studies of ovaries.

2.2 Histological Analysis

After the completion of the experiment prawns from both control as well as experimental group were sacrificed for the ovaries. Ovaries were then weighed and processed for histological studies. Paraffin blocks of ovaries were prepared and sections were cut at 6-7µm in thickness, stained by hematoxylin and eosin stain. Ovarian sections were examined under compound microscope.

3. Results

During the present research, prawn group subjected to eyestalk ablation technique shows enhanced ovarian maturation as compared to that of control group. The results of the study for both the control group and eyestalk ablated group are discussed below

Group A: The prawns in this group have lower ovarian indices value i.e. 0.674 ± 0.241 Table 1. Histological observation of ovaries in this group revealed an abundance of oogonial cells (OG) and pre-vitellogenic oocytes (PO) near germinal zone Fig. 1. They are basophilic in nature. Also, oocytes diameter value was also found to be lowest ($75.50 \pm 13.84 \mu\text{m}$) as shown in the Table 1.

Group B: The prawns in this group were unilaterally eyestalk ablated and their ovarian indices value is recorded (1.364 ± 0.021) which is higher than the control group. Histological observation was made and it was found that there was large number of post-vitellogenic oocytes. Yolk deposition is almost in all these oocytes and hence oocytes having large number of yolk globules Fig. 2. The entire cytoplasm becomes eosinophilic. The oocytes diameter value was found to be highest ($332.65 \pm 21.98 \mu\text{m}$) than the control group.

And after applying 't'- Test of significance it shows that the control and the eyestalk ablated group differs significantly at 5% level of significance as t_{tab} is 2.306 and t_{cal} is 42.628 hence, it differs significantly.

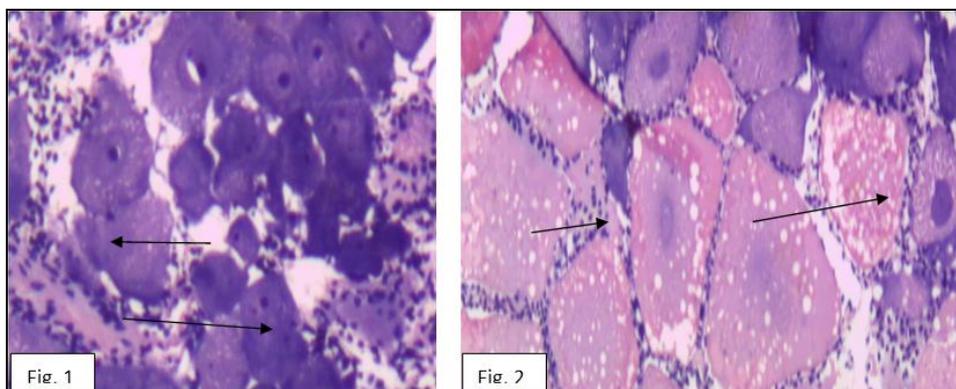


Fig 1 & 2: showing the ovarian maturation stages in freshwater prawn, *M. lamarrei lamarrei* in control and eyestalk ablated group after the course of experiment (arrow heads showing germinative zone and pre vitellogenic oocytes in Fig. 1 & in Fig. 2 showing post-vitellogenic oocytes).

Table 1: Showing variation in ovarian indices and oocyte diameter in control and eyestalk ablated group in *M. lamarrei lamarrei*

Animal Group	Ovarian Indices (Mean \pm S.D)	Oocyte Diameter (Mean \pm S.D) (In Mm)
Control	0.674 \pm 0.241	75.50 \pm 13.84
Eyestalk Ablated	1.364 \pm 0.021	332.65 \pm 21.98

4. Discussion

In the present study, the investigation is done on the effects of eyestalk ablation on ovarian maturation of *M. lamarrei lamarrei* by using various parameters ovarian indices and oocytes diameter.

It had been told that the gonadal maturation in crustaceans is either controlled by inhibitory functions of X- organ neurosecretory cells of the eye-stalk or by the stimulatory effect of thoracic ganglion and the brain [2]. Similar work has been done by many who also have taken G.S.I or ovarian indices as a great indicator for the gonadal maturation [11]. Had predicted that due to eyestalk ablation there was an increase in the gonadal maturation of the prawns that can be proven by their G.S.I or ovarian indices value which is very high in eyestalk ablated group than in the control [2]. while working on the studies on the effect of unilateral eyestalk ablation in maturation of gonads of fresh water prawn, *M. dayanum* had clearly stated that due to excising the eyestalk there is an enhancement of the reproductive activity of the prawn, thereby increase in the value of G.S.I or ovarian indices of the eyestalk ablated group by 2.856 \pm 1.11 than in the control by 0.910 \pm 0.2. Also, by comparing oocytes diameter of both control and eyestalk ablated group it is evident that more post- vitellogenic oocytes were found in eyestalk ablated group than in the control. The oocytes diameter of the eyestalk ablated group is 319.46 \pm 4.98 which is higher than the control group whose oocytes diameter is 86.12 \pm 367 [12]. Stated that there is an increase in the ovarian indices and oocytes diameter of the eyestalk ablated females than compare to the control group [13]. While working on the effects of unilateral eyestalk ablation on the gonads of *Penaeus monodon* concluded that the eyestalk ablated group is having higher ovarian indices as well as oocytes diameter values than the control. The value of ovarian indices in the eyestalk ablated group and control group is 3.783 \pm 1.65 and 1.894 \pm 2.46 respectively. Whereas the oocytes diameter of eyestalk ablated group and the control group is 364.53 \pm 3.65 and 96.54 \pm 6.54 respectively. These investigations give similar results with regard to [14] in *M. kistensis* and [15] in *M. affinis*. Thus, it shows that eyestalk ablation have great effect on the gonadal maturation of the prawn.

5. Conclusion

Hence, it is finally concluded that ovarian maturity can be easily attained by doing unilateral eyestalk ablation. Although ablation technique results in permanent detachment of eyestalk from the animal, so always preference should be given to unilateral ablation technique. As eyestalk ablation technique can be use for large scale ponds or lakes which is impractical in the case of the photoperiodism, the ovarian maturation can be enhanced by the eyestalk ablation technique either physiological or histological studies can be done.

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