

## Studies on the Effect of Unilateral Eyestalk Ablation in Maturation of Gonads of a Freshwater Prawn *Macrobrachium dayanum*

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**Abstract:** The effect of unilateral eyestalk ablation in freshwater prawn *Macrobrachium dayanum* was investigated in the laboratory for 15 days. Healthy specimens of *M. dayanum* were taken (3.5-7.0 cm in length). Specimens with uniform ovarian and testes conditions (immature) were selected for the experiment. The eye stalk ablation was done by cutting the eyestalk from their bases with the help of fine sterilized scissors. The results obtained indicated that the removal of the eyestalk has accelerated the gonad development. In males we observed an increase in the testicular index in *M. dayanum* after the eye-stalk ablation, increase in the size of testis, follicle diameter and the number of the follicles and the mature follicles/Spermatocytes, after the eye-stalk ablation, is the testimony of the fact that the removal of eye-stalk of *M. dayanum* contains testis inhibiting factors. In female animals also there is an increase in the ovarian index in eyestalk ablated prawns as compared to normal animals. The study indicated a positive effect, incorporating unilateral eyestalk ablation is sufficient to induce gonadal development in *Macrobrachium dayanum*.

**Key words:** Eyestalk ablation • Ovary • Testes • Induce

### INTRODUCTION

Decapod crustaceans represent a large, diverse biological group with significant potential as an aquaculture resource. Knowledge of spawning seasons and areas are important for management and hence to studies of the reproductive dynamics of commercially important species. The technique of eyestalk ablation has been widely used for manipulating ovary development and maturation in captivity and is commercially practiced in shrimp hatcheries, particularly with shrimp that do not spontaneously mature and spawn [1]. Reproduction in crustaceans is regulated by various neurohormones that are synthesized and released from the X organ-sinus gland complex located in the eyestalks of the species. In addition to reproduction, other physiological and metabolic processes are affected by removal of the X-organ sinus gland complex located in the eyestalk, [2]. With this technique, the endocrine system is directly affected by reducing the inhibitory control over reproduction. Crustacean eyestalk is known to have a

neurohaemal function due to the presence of X-organ sinus gland system. The X-organ sinus gland system which is located in the eyestalk of crustacean regulates the ovarian development and maturation [3 - 5]. It was found that excision of eyestalk removes the neuroendocrine system of prawns located in the eyestalk which influences growth, reproduction and other metabolic activities [6].

It has been reported that eyestalk ablation accelerated the development of female gonads, in parallel with transportation of reserves from the hepatopancreas to the ovaries through the hemolymph [7, 8]. It was demonstrated that in *P. semisulcatus* eyestalk ablation increases the number of spawning and correspondingly the number of eggs and nauplii produced per female in comparison to non-ablated females [9]. This is assumed to be due to lowering the level of GIH (gonad inhibiting hormone) and MIH (moult inhibiting hormone) in the hemolymph of the eyestalk ablated females. It was found that roles of GIH on ovarian development in various crustaceans have been firmly established that destroying

the eyestalk using various means induces precocious gonadal development in almost all crustaceans [10, 11]. This neurohormone is part of a multifunctional family of hormones related to the crustacean hyperglycemic hormone (CHH) that has been widely studied in several species [12].

In the present investigation an attempt was made to understand the basic aspects of reproductive physiology in relation to the changes occurring in the neuroendocrine system of *M. dayanum* and to know about the mechanism involved in the technique of eyestalk ablation for induced maturation.

## MATERIALS AND METHODS

**Procurement and Maintenance of Test Animals:** Freshwater prawn *Macrobrachium dayanum* (length 35-70mm, weight 0.7 - 1.0 g) were collected from local Lake of Sagar and were brought to the laboratory under live oxygen packing conditions. The prawns were kept in the glass aquarium to observe any visible pathological symptoms. Before introducing in the aquariums prawns were treated with 0.1 KMnO<sub>4</sub> solutions to obviate any dermal infection.

**Acclimatization of Test Animals:** Prawns were acclimatized to laboratory conditions for a period of Two weeks and no mortality was recorded during this period. 20 prawns were kept in each aquarium, which contained 60 liters of water.

**Experimental Setup:** 80 specimens of *M. dayanum*, looking apparently healthy, in the size group of 45-55 mm in total length, with uniform ovarian condition (immature) were selected for the experiment. The total duration of the experiment investigated in the laboratory was 30 days. The eye-stalk ablation was done by cutting the eye-stalks from their bases with the help of fine sterilized scissors. During ablation, the eyestalk was removed after holding the prawn in precooled water to reduce the heart beat rate and loss of hemolymph. The ablated prawns were then released in the aquariums. The gonadal indices were determined using the standard formula.

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

All the groups were fed daily on minced meat/ potato/ carrot. Prior to feeding exuviae (fecal matter)

were collected with a net, presence of any dead prawn was recorded and any excess of food removed to preserve water quality. The water is changed after 2-3 days.

## RESULTS

As per our present knowledge, two factors control the gonadal maturation in crustaceans. One is inhibitory in function which is produced by the X-organ neurosecretory cells of the eye-stalk and the other is/are stimulatory which is/are produced by the thoracic ganglion and the brain. The induction of ovarian maturation and spawning in *Macrobrachium dayanum* is mainly carried out using the unilateral eyestalk ablation technique. Unilateral eyestalk ablation technique is more efficient due to the anticipation of the first spawn, repeatability between spawns, expressive rate of ovigrous females and survival that favored its applicability.

In the present study, an increase in the Gonadal index, was observed after the eye-stalk ablation in *M. dayanum* which indicated increased reproductive activity due to the removal of the gonad inhibiting gonadotropin present in the X-organ of the eye-stalk. Removal of the eye-stalks has accelerated the gonad development, it may well be stated that the eye-stalks of *M. dayanum* contain the gonad inhibitory factors, the supply of which ceases to be by the removal of the eye-stalks, thereby increasing the activity of gonad development. Thus, the findings of the present study revealed that the neurohormones produced by the eye-stalk do not only perform the function of inhibiting the gonad development but they are also equally important for regulating the process of Oviposition in the females.

In the present investigation, we observed an increase in the testicular index in *M. dayanum* after the eye-stalk ablation, indicates increased reproductive activity due to the removal of gonad inhibiting factor present in the X-organ of the eye-stalk. The increase in the size of testis, follicle diameter and the number of the follicles and the mature follicles/spermatocytes, after the eye-stalk ablation, are the testimony of the fact that the eye-stalks of *M. dayanum* contain testis inhibiting factors as is seen clearly in the Table 1. From the Table 2 we conclude that there is a significant increase in the ovarian index and the Oocyte diameter in the animals belonging to Group 2 as compared to Group 1.

Table 1: Effect of eye-stalk ablation on the Testicular development of *M. dayanum*

Animal group		Testicular indices $\pm$ SD	No. of follicle ( $\mu\text{m}$ ) $\pm$ SD	Follicle diameter ( $\mu\text{m}$ ) $\pm$ SD	No. of mature follicles/ Spermatoocytes
Group-1	Normal	0.750 $\pm$ 0.415	6.89 $\pm$ 1.45	149.22 $\pm$ 7.09	71.25 $\pm$ 7.050
Group-2	Eye-stalk ablated	1.912 $\pm$ 0.320	17.34 $\pm$ 3.05	234.05 $\pm$ 5.02	137.04 $\pm$ 12.07

Table 2: Effect of eye-stalk ablation on the ovarian development of *M. dayanum*

Animal group		Ovarian indices $\pm$ SD	Oocyte diameter ( $\mu\text{m}$ ) $\pm$ SD
Group-1	Normal	0.910 $\pm$ 0.21	86.12 $\pm$ 3.67
Group-2	Eye-stalk ablated	2.856 $\pm$ 1.11	319.46 $\pm$ 4.98

## DISCUSSION

The present study clearly indicated that, with few exceptions, the growth and the function of the testes are under the control of the hormones produced by the eye-stalks. Which in turn is controlled by a number of extrinsic factors, out of which photoperiodism plays an important role. Therefore cutting away of light by keeping the prawns in dark, eye-stalk removal cuts off the supply of the gonad inhibiting factor resulting in the rapid development of the testes and vasa deferentia and accelerating the process of spermatogenesis. Since the eye-stalk extract injections exhibit testis inhibitory effect in the eye-stalk ablated prawns, it can also be confirmly claimed that the eye-stalks contain the testis inhibitory hormones in *M. dayanum*. The results of this investigation support the views, in this regard, forwarded by Sarojini, *et al.* [13, 14] in *M. kistnensis* and Rao, *et al.* [15] in *M. affinis*.

The time of spawning and recruitment and the mean length, or age at which these events happen are important to aquaculture studies, particularly the cycle of events leading to reproduction and the timing of gamete release [16]. Removal of eyestalk leads to weight gain and osmolality decrease of hemolymph [17] and unilateral eyestalk ablation has been used to induce ovarian maturation and spawning with success in many crustacean species [18-21]. The presence of advanced yolky oocytes (YO) in the ovaries of ablated females just a few hours after spawning may indicate a relatively faster rate of ovarian maturation in these female [22]. It is well established that crustacean reproduction can be affected by eyestalk neuropeptides e.g. gonad-stimulating and gonad-inhibiting hormones [23, 24]. It was suggested that a system incorporating unilateral eyestalk ablation is sufficient to induce maturation in captivity in females above the length of first maturity [25].

Mortality was directly related to the degree of ablation. This was expected, considering the strong physiological stress caused by partial or total removal of

the main endocrine gland, the X-organ sinus gland complex. In an attempt to increase the frequency of reproduction through eyestalk ablation, it was revealed that the larger size group responded quickly than the smaller size group. Partial arresting of the gonad inhibiting hormone (GIH) by removing or ablating a single eyestalk of the female prawn is the simple method generally practiced in all commercial shrimp hatcheries, for inducing gonad maturation and spawning. Also, it was found that eyestalk ablation increased gonad size and doubled mating frequency of *P. vannamei* in comparison to normal [26]. However, [27] observed in their study on *Cherax quadricarinatus* that unilateral eyestalk ablation did not cause significant differences in the male or the female components of the reproductive system compared with the control group.

In the present study prawns being subjected to eyestalk ablation is similar to the complete darkness, as in both the case the main aim is to keep the optic ganglion from being exposed to light. Therefore, the observation made in both the case should have similar effects.

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