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Histological Changes in Some Tissues of a Light Treated and in a Eyestalk Ablated Fresh Water Crab, *Oziotelphusa Senex Senex*

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Abstract: *The effect of unilateral, bilateral eyestalk ablation and photoperiodism in freshwater crabs O.senex senex was investigated in the laboratory for 2 weeks. After eyestalks ablation the crabs were allowed to withstand for 2 weeks. After 2 weeks one group of crabs was dissected, some tissues (brain, ovary, gills) were used for histological study. The neurosecretory cells of the brain of eyestalk ablated animals show more neurosecretory materials, enlarged oocytes, the gill lamellae of the eyestalk ablated crabs showed notable alterations. The Haemocytes are accumulated more in the Haemocoelic space and gill lamellae. The interlamellar space has increased; necrosis is seen in most of the gill rachis. The epithelial lifting and disrupted pillar cells are very common. Most of the gill lamellae show hyperplasia with more Haemocyte, The light treated crabs, showed normal gill lamellae with even interlamellar space, less Haemocytes and are without necrosis and enlarged oocytes, further the light treated crabs show better results than the ablated crabs.*

Keywords: *Neurosecretory cells ,Oocytes , Epithelium lifting , Necrosis, O.senex senex., Fresh water crabs.*

1. INTRODUCTION

The decline in commercial crustacean fisheries around the world is widely known. Major factors contributing to the steady decline in crustacean population number include inadequate legislation providing protection for these species, increase in the harvest rate and decrease in the size of the crustaceans and increase in worldwide consumption. One way to maintain sustainable crustacean populations for consumption is by manipulating the crustacean's endocrine system in order to speed up reproductive development and thus reduce the overall maturation time.

The crustacean reproduction is influenced by both exogenous and endogenous factors. The exogenous factors include various environmental factors such as rainfall, temperature, salinity, photoperiod, dissolved oxygen, turbidity and abundance of food which are known to regulate the breeding cycle, development of egg, dispersal of larval and young ones of crustaceans (Adiyodi and Adiyodi, 1974; Fingerman, 1987; Charles, 1997).

The influence of temperature on the reproductive cycle has been examined in a large number of cases. In some cases the developmental history is influenced by temperature and related factors like humidity. In certain cases it may also influence the triggering off reproduction and subsequent processes like maturation, arrest of reproduction, mating and oviposition or spawning. Low humidity depletes water and is, therefore, generally antagonistic to egg production (Nayar, 1977).

Environmental control of photoperiod and temperature were found to result in successful reproduction in *Penaeus stylirostris* and *Penaeus japonicus* (Chamberlain and Lawrence, 1981). Daniel *et al.*, (1994) on the basis of field data and laboratory results collectively suggest that in red swamp Cray fish, *Procambarus clarkii*, temperature controls the onset of ovarian development, which is accelerated by increasing day length. So in the present study an attempt has been made to know the effect of unilateral ,bilateral eyestalk ablation and photoperiodism on the histological changes of *O.senex senex* tissues such as brain, ovary and gills.

2. MATERIALS AND METHODS

The female crabs, *Oziotelphusa senex senex* collected from Puzhal Lake were brought to the laboratory and maintained in plastic tubs. Acclimation condition was maintained for four sets of ten

crabs each for 15 days. First set of ten crabs were kept without any stress named as Control group- A is the initial control and was sacrificed on the 1st day of the experiment and the tissues such as brain, ovary and gills were taken for histological study. Second batch (group-B) of ten crabs were taken and their single eyestalks were cauterized, likewise, third batch (group-C) of ten crabs were taken and both their eyestalks were cauterized. Finally fourth batch (group-D) of ten crabs were exposed to measured light (1500 Lux) continuously for 15 days. The crabs were maintained for 15 days by feeding beef mutton *ad libitum* and the water was changed daily and was acclimatized in the prevailing room temperature.

The crabs were released back to the rearing system immediately after ablation. On the 15th day of the experiment both unilateral eyestalk, bilateral eyestalk ablated and light treated crabs were dissected out. Tissues such as brain, ovary and gills were taken for histological studies.

3. RESULTS

In the present histological studies on the brain of control crabs (group A) Neurosecretory cells (NSCs) are more in number and distributed along the periphery and are comparatively larger in size than the crabs. More NSM (Neurosecretory Materials) are seen in the NSCs (Fig.1). Unilateral ablated group B crabs show more number of NSCs located on the periphery of the antero–dorsal region of the brain. The cell boundary is indistinct and they are oval to round in shape. Nucleus is invisible and is with more NSM and show intense staining (Fig.1). In the histological sections of group C,(Bilateral ablation) the NSCs show intense staining reaction. The NSCs are found distributed in the mid dorsal region of the brain and are more with NSM and the cells are indistinguishable (Fig.1) Light treated crabs of group D the NSCs show intense staining reaction, NSCs are seen which are found distributed along the mid – dorsal region of the brain. NSCs have more amount of NSM and the nucleus is invisible and are comparatively more in number compared with the Group C crabs (Fig.1)

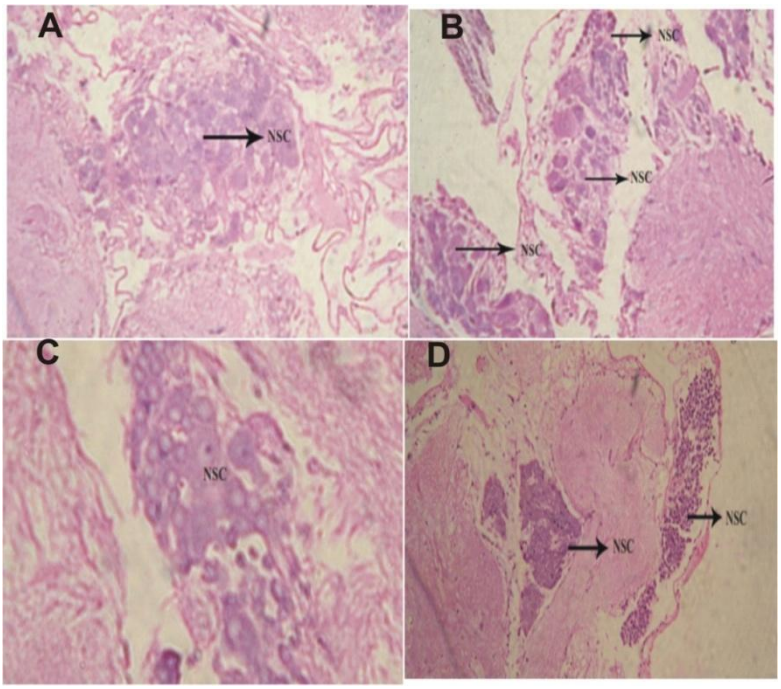

Figure1. *Photomicrograph of Cross Section of Brain Showing Neurosecretory Cells*

NSC – Neurosecretory cells, Stained in HE X 425

Histological sections of group-A (control) reveal that the ovary is covered by a thin connective tissue called ovarian epithelium and each Oocyte is enclosed in a oogenetic pouch. The staining reaction is moderate in haemotoxylin and eosin. The oocytes are of different sizes and shapes. Mostly the oocytes are polygonal in shape and the nucleus occupies the major portion of the cytoplasm. The nucleus shows distinct nucleolus placed towards the corner of the nucleus. The ooplasmic substances are less. (Fig.2) The cross section of group-B (Unilateral ablation) the ovary reveals spherical oocytes and the nucleus stains darkly, nucleolus is clearly seen. The oocytes are endowed with moderate amount of ooplasmic substances, many oocytes are seen surrounded by follicle cells. The developing oocyte show distinct ovarian epithelium which is folded to form oogenetic pouches in which the oocytes are enclosed (Fig.2).

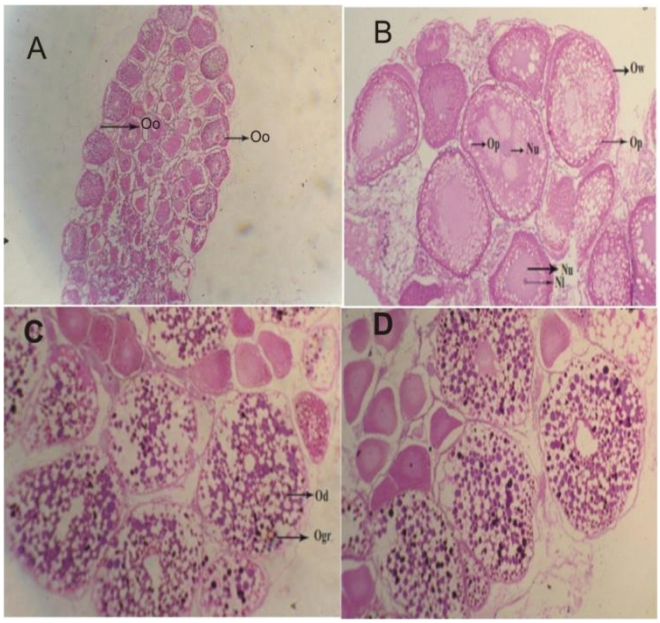

Figure2. *Photomicrograph of Cross Section of Ovary Showing Oocytes.*

Oo – Oocytes , Op – Oogenitic pouch, Ow-Ovarian wall, Nu – Nucleus, Nl – Nucleolus, Od- Oil droplet , Ogr – Ovarian granules Stained in HE X 425

Bilateral ablation (Group-C) The oocytes are polygonal in shape due to increased ooplasmic contents and the nucleus is invisible in all the oocytes. The ovarian epithelium is distinct and invaginated to form oogenetic pouches of various sizes. In the well developed oogenetic pouches the ovarian epithelium surrounds the oocyte. Follicle cells are comparatively smaller in size, cuboidal in shape and are found along the ovarian epithelium. The ooplasm shows more granular substances and yolk globules. Most of the oocytes show the presence of vacuoles and the nucleus and chromatin granules are invisible showing the advancement of ovarian development,oil droplets and ovarian granules are more in the oocytes (Fig.2).

In the group D, the ovary shows the presence of yolk laden vitellogenic oocytes of enlarged size, nucleus and nucleolus are invisible, the follicle cells are thin and few in number, the oocytes contain large number of yolk globules and yolk granules, the oocytes show high staining intensity .The histological sections show the presence of oocytes with the cytoplasm heavily laden with yolk globules and granules. The ovarian epithelium is invaginated and evaginated to form number of oogenetic pouches. The ovarian sheath is thick (Fig.2).

The histological sections of the group A crabs shows that the Gill rachis, filament and the tips are intact.The gill filament are with intact pillar cells.The haemocytes are moderate (Fig.3)

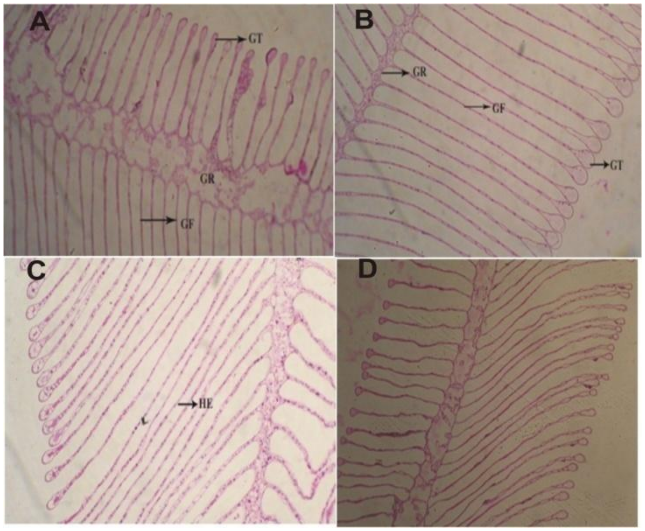

Figure3. *Photomicrograph of Cross Section of Gills . GT – Gill Tips; GR – Gill rachis; HE – Haemocytes; GF- Gill filaments Stained in HE X 425*

The cross section of group-B (Unilateral ablation) the haemocytes are moderate in the haemocoelic space and in the gill rachis (Fig.3). The interlamellar space is normal, gill tips are intact.

In the histological sections of group C,(Bilateral ablation) the haemocytes are fully packed in the gill rachis and epithelial lifting is seen in some of the gill filaments with more interlamellar spae (Fig.3).

In the group D the haemocytes are moderate in the haemocoelic space and in the gill rachis.The gill filaments are intact and gill tips are in normal shape and size.The pillar cells are intact (Fig.3).

4. DISCUSSION

The present investigation shows a gradual increase in the size of Neurosecretory cells (NSCs) and Neurosecretory materials (NSM) in the brain. The size of the NSCs and NSM has been found to be increased in the brain of the eyestalk ablated crabs, when compared to control crabs, likewise, the size of the NSCs and NSM has further significantly increased in the brain of light treated crabs when compared to the control and also with the eyestalk ablated crabs.

The histological studies on the ovaries provide detailed informations on the changes occurred due to eyestalk ablation compared to its control. In the normal crabs the oocytes of the ovary shows that the nucleus is occupying the major portion of the oocytes. In the histological sections of the unilateral and bilateral eyestalk ablated crabs the oocytes shows significant increase in size when compared to their controls. Further the ovaries of the light treated crabs shows well developed ovaries with oocytes laden with full of yolk globules and oil droplets.

Many earlier workers have reported on the effects of eyestalk ablation and light on the gonadal development in various crustaceans. The following reports substantiates the present findings. Ragunathan and Arivazhagan (1999) have reported the polymorphic oocytes in the stage – I ovaries, much enlarged oocytes in the 5-HT treated crabs and increased gonadal indices in eyestalk ablated 5-HT treated female *P. hydrodromous.* Aktas and Kumlu (2005) have suggested that maturation and spawning of penaeid shrimps including *Penaeus semisulcatus* can be successfully induced by serotonin injection in captivity. However, eyestalk ablation gives the highest and more predictable maturation and spawning in penaeid shrimps.

Meera (2005) has reported that eyestalk-ablation and Fluoxetine treatment and light plus Fluoxetine treatment show an enhanced growth of the Ovary and spermatheca than the non - ablated crabs.Pervaiz *et al*., (2011) have reported that the removal of the eyestalk has accelerated the gonad development. In males they have observed an increase in the testicular index in *Macrobrachium dayanum* after the eye-stalk ablation, increase in the size of Testis, follicle diameter and the number of the follicles and the mature follicles. In female animals also there is an increase in the ovarian index in eyestalk ablated prawns as compared to normal animals.

The use of light in the promotion of enhanced gonadal maturation results in stress free animals, a better effect due to light than the effect produced in eyestalk ablation can be achieved, the death of animals during eyestalk ablation can be avoided .The results on the histology of studied tissues proves light as an alternate tool for the enhanced gonadal maturation in crustaceans and the influence of light on acceleration of the gonadal development has support from the reports earlier workers (Aiken and Waddy, 1985; Nadarajalingam and Subramoniam,1987). In *Penaeus setiferus*, Wurts and Stickney (1984) have suggested the need for light of blue or green colour for their role as a promoter of maturation and spawning and to eliminate the need for eyestalk ablation in captivity spawned penaeid shrimp.

In aquatic organisms, the gills represent a vital organ, since they play an important role in the transport of respiratory gases and regulate the osmotic and ionic balance. Any stress may cause damage to gill tissues, thereby reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms (Ghate and Mulherkar, 1979). In the present study, unilateral eyestalk ablation and further the bilateral eyestalk ablation in both male and female crabs has resulted in notable structural alterations of the gill lamellae including accumulation of Haemocytes (HE) in the haemocoelic space, epithelium lifting (EL), necrosis (NCR), disrupted pillar cells (DPC). Similar lesions have been reported to occur in the prawns, *M. kistensis* (Ghate and Mulhekar, 1979) and *M. idae* (Victor *et al*., 1990) following exposure to heavy metals, Devakumar *et al*.,2013 (*O.senex senex*)

In the recent past several efforts have been made to evolve methods for the induction of maturation and spawning of commercially important crustaceans in captive conditions, the use of eyestalk

ablation procedure along with a pharmacological drug of gonadal stimulatory nature or photoperiod can be used as a better alternative and the use of neurohormones or neurotransmitters as suggested by Fingerman and Nagabhushanam(1992), or the use of Terpenoids as suggested by Laufer *et al.*,(1987) or extracts from the brain, thoracic ganglia (Gomez and Nayar, 1965) or MF containing reproductive hormones can be used (Laufer *et al.*,1987) and according to Kulkarni (2007) though many pharmacological drugs stimulates gonadal maturation field trials and cost effective drugs and methods have to be found out for the promotion of aquaculture.

Whereas the use of light in the promotion of enhanced gonadal maturation results in stress free animals, a better effect due light than the effect produced in eyestalk ablation can be achieved, of animals during eyestalk ablation can be avoided.The results on the biochemical analysis and also on the histology proves light as an alternate tool for the enhanced gonadal maturation in crustaceans and the influence of light on acceleration of the gonadal development is supported by many earlier workers (Wurts and Stickney, 1984; Aiken and Waddy, 1985; Nadarajalingam and Subramoniam,1987). Thus, in this present investigation the gonadal development in *O.senex senex* would have been brought about by the mechanisms as reported above and also gains support from the reports of earlier workers as reported in the above sections, further the present investigation highlights the light as a better to tool for enhanced gonadal maturation in crustaceans.

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