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Original Research Article

Effects of Photoperiodism, Eyestalkablation on the Ovarian Development and Electrolyte Changes in a Freshwater Crab, *Oziotelphusa senex senex*

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Abstract

The effect of unilateral, bilateral eyestalk ablation and photoperiodism effect in freshwater crab, *Oziotelphusa senex senex* was investigated in the laboratory for 2 weeks. After eyestalks ablated the crabs were allowed to withstand for 2 weeks. After 2 weeks one group of crabs was dissected, some tissues and haemolymph were used for biochemical assays. The level of electrolytes in the all tissues significantly decreased at the end of the second week following eyestalk removal and also in the light treated compared to the control groups with intact eyestalk (p<0.05). Eyestalk ablated animals show enlarged oocytes, than their controls. The light treated crabs show better results than the ablated crabs. The study indicated a positive effect, incorporating continuous photoperiodism is sufficient to induce ovarian maturation in *O. senex senex.*

Keywords

Chloride
Freshwater crabs
Oziotelphusa senex senex
Potassium
Sodium

Introduction

Decapod crustaceans, which include shrimps, prawns, crabs, crayfish and lobsters, represent a large, diverse biological group with significant potential as an aquacultural resource. The crabs rank third after shrimps and lobsters for their esteemed seafood delicacy and also the value of fishery they support (Mohammed Savad and Rajeev Ragavan, 2001). Shell fish is one of the most important sources of proteins provided from sea and blue crab is one of the most important among them (Enzenross et al., 1997). The crab meat contains many nutrients like vitamins, carbohydrates, minerals and free amino acids many therapeutic properties are attributed to the crab meat and it is used to cure asthma and chronic fever (Raja, 1981).

Eyestalks are the endocrine center for regulating many physiological mechanisms, such as molting, metabolism, sugar balance, heart rate, pigments, and gonad maturation. Therefore, unilateral eyestalk ablation affects all aspects of shrimp physiology (Quackenbush, 1986). Predictable induced reproduction in captive penaeids without the use of eyestalk ablation was considered a long term goal for shrimp mariculture (Quackenbush, 1991).

Various alternatives to ablation have been evaluated, based on accumulated knowledge about environmental control and crustacean endocrinology. Photoperiod and temperature manipulations, based on seasonal natural variations of these parameters, have been successful in controlling maturation of unablated *P. japonicus* (Laubier-Bonichon,1978) , *P. stylirostris* (Chamberlain and Gervais, 1984), and *P. setiferus* (Chamberlain, 1988) however, photoperiod control seems to be more important for subtropical species for review: (Browdy, 1992) . Implantation of thoracic ganglia from a mature female into an immature female stimulates vitellogenesis (Otsu, 1963).

This principle was tested on *P. vannamei* by implanting thoracic ganglia from maturing *Homarus americanus* (Yano et al., 1988) the report indicates that the implantation technique generates maturation,but the experiment is based on a low number of replicates, without statistical analysis. Moreover, the current knowledge on tissue recognition between different crustacean species is limited, so there is no scientific evidence to support the assumption that tissue from a lobster would be recognized as self by a shrimp (Lackie, 1986).

This study was designed to determine the concentrations of the major biochemical constituents such as Sodium, Potassium and Chloride and total haemocytes, in the hemolymph of normal and ablated, light treated crabs. This will help to a better understanding of factors involved in crustacean reproductive and growth control and hemolymph biochemical components which are fundamental to successful aquaculture.

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 for 15 days. First batch of ten crabs were kept without any stress named as Control group- A. Second batch (group-B) of ten crabs was taken and their single eyestalks were cauterized, likewise, third batch (group-C) of ten crabs was 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 and bilateral eyestalk ablated crabs were dissected out. Tissues such as haemolymph, hepatopancreas, ovary, spermatheca, muscle and gills were taken for biochemical analyses and ovary were taken for histological Studies.

Sodium, potassium and chloride levels were determined by the method of optimized colorimetric standard kit method. Total haemocyte count was determined by the method of heamocytometer (Dacie and Lewis, 1968). Analysis of variance was performed using the General Linear Models procedure of Statistical Analysis System (SPSS version 17.0); Duncan's new multiple range test was used to obtain pairwise comparisons among sample means. Evaluations were based on a 5% significance level (p<0.05).

Results

In the present study, the level of sodium showed significant increase in hemolymph of the eyestalk ablated and also in the light treated groups. Whereas the concentration of sodium, potassium, and chloride shows significant decrease in hepatopancreas, ovary, spermatheca, muscle and gills of the eyestalk ablated and in the light treated groups (Table 1). The level of significance was p<0.001.

Histological sections of group-A (control) reveal that the ovary was covered by a thin connective tissue called ovarian epithelium and each oocyte was enclosed in a oogenetic pouch. The staining reaction was moderate in haemotoxylin and eosin. The oocytes were 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 were found to be less (Fig. 1).

The cross section of group-B (Unilateral ablation): The ovary reveals spherical oocytes and the nucleus stains darkly and the nucleolus was clearly seen. The oocytes were endowed with moderate amount of ooplasmic substances; many oocytes were seen surrounded by follicle cells. The developing oocyte showed distinct ovarian epithelium which was folded to form oogenetic pouches in which the oocytes were enclosed (Fig.1).

Fig. 1: Photomicrograph of cross section of ovary showing oocytes (Note: Oo – Oocytes, Op – Oogenitic pouch, Ow- Ovarian wall, Nu – Nucleus, Nl – Nucleolus, Od- Oil droplet , Ogr –Ovarian granules Stained in HE X 425).

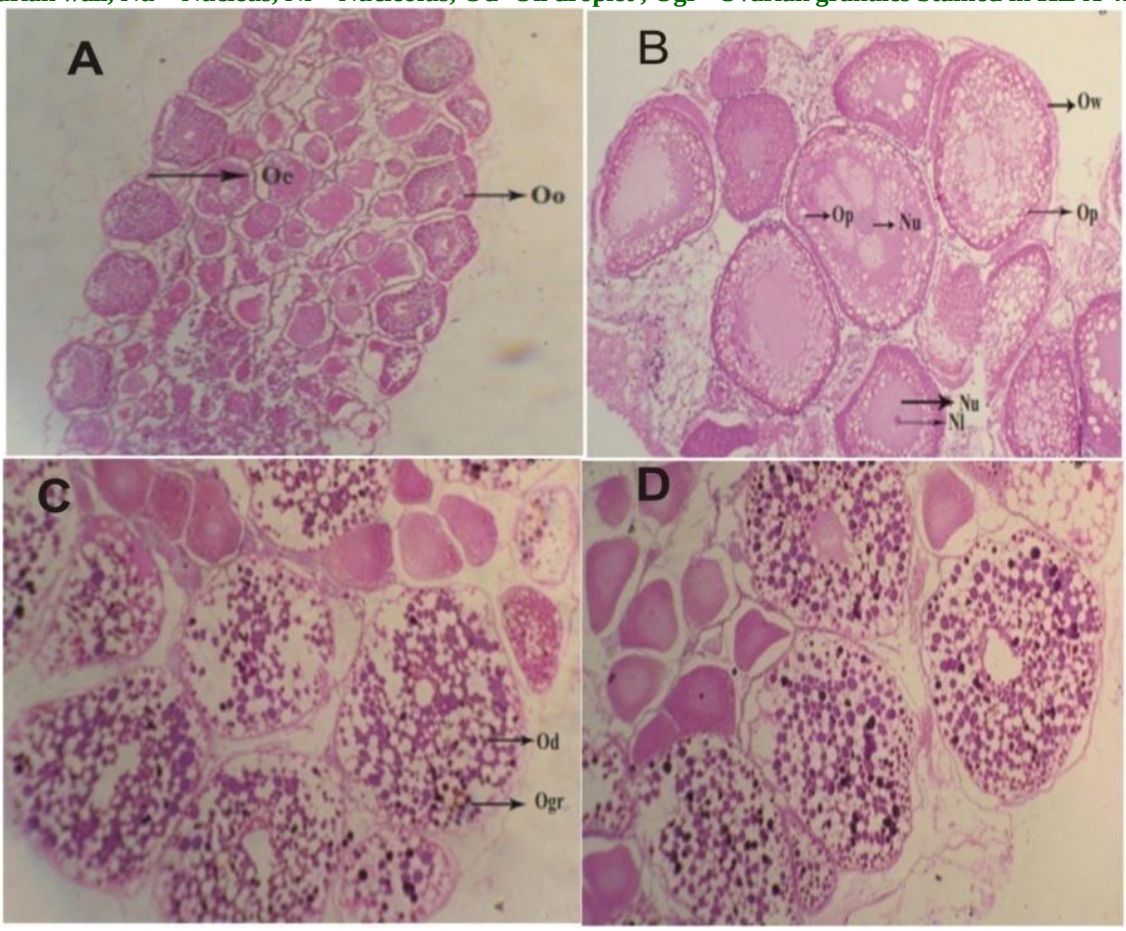

Table 1. Levels of Electrolytes in eyestalk ablated and light treated *O. senex senex*.

Parameters	Tissues	Control group-A	Unilateral group-B	Bilateral group-C	Light group- D
Sodium mmol/L	Haemolymph	188.2 ± 0.766	*198.7 ± 0.916	*204.8 ± 0.886	*189.8 ± 0.813
	Hepatopancreas	87.78 ± 0.860	*15.37 ± 0.738	*63.81 ± 0.885	*32.25 ± 0.879
	Ovary	101.2 ± 1.074	*7.18 ± 0.601	*15.37 ± 0.738	*39.3 ± 0.737
	Spermatheca	81.6 ± 0.958	*7.18 ± 0.601	*32.25 ± 0.879	*15.77 ± 0.624
	Muscle	87.78 ± 0.860	*38.4 ± 1.068	*15.37 ± 0.738	*48.13 ± 0.611
	Gills	54.8 ± 0.843	*21.3 ± 1.133	*32.25 ± 0.879	*15.37 ± 0.738
Potassium mmol/L	Haemolymph	7.38 ± 0.125	*3.5 ± 0.178	**4.55 ± 0.253	***4.49 ± 0.248
	Hepatopancreas	4.51 ± 0.244	*1.38 ± 0.235	**2.76 ± 0.465	*0.80 ± 0.052
	Ovary	3.62 ± 0.181	*1.25 ± 0.211	**0.6 ± 0.072	***0.60 ± 0.072
	Spermatheca	1.28 ± 0.226	*3.27 ± 0.212	*0.33 ± 0.081	***0.28 ± 0.078
	Muscle	2.61 ± 0.236	*9.27 ± 0.423	*0.45 ± 0.106	***0.45 ± 0.106
	Gills	1.44 ± 0.203	*6.1 ± 0.444	*0.75 ± 0.093	**0.45 ± 0.106
Chloride mmol/L	Haemolymph	120.8 ± 0.857	*108.9 ± 0.867	***110.2 ± 0.766	*124.6 ± 0.688
	Hepatopancreas	59.03 ± 0.603	*44.88 ± 0.652	*55.0 ± 0.608	*59.1 ± 0.776
	Ovary	28.7 ± 0.916	*15.96 ± 0.678	***15.05 ± 0.600	**16.1 ± 0.554
	Spermatheca	15.83 ± 0.506	*10.24 ± 0.766	***8.53 ± 0.475	** 10.2 ± 0.562
	Muscle	21.78 ± 0.787	*12.05 ± 0.862	*6.82 ± 0.601	*10.2 ± 0.562
	Gills	22.91 ± 0.915	*12.05 ± 0.862	***12.05 ± 0.862	**10.2 ± 0.562

Each value is mean ± SEM of 10 samples, expressed as mmol/L of wet tissue and mmol/L of haemolymph; Group-A Vs B, B Vs C, C Vs D, * Indicates significance *P<0.001 , **P<0.05 , *** Insignificant.

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.1).

Group D: The ovary showed the presence of yolk laden vitellogenic oocytes of enlarged size, nucleus and nucleolus were invisible; the follicle cells were 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 showed the presence of oocytes with the cytoplasm heavily laden with yolk globules and granules. The ovarian epithelium was invaginated and evaginated to form a number of oogenetic pouches. The ovarian sheath was found to be thick (Fig. 1).

Discussion

Crustaceans are characterized by a wide range of osmoregulatory powers which are developed based on the environment to which the animals are adapted. The most important ions involved in the maintenance of osmotic balance are the sodium and chloride. The exchange of these ions results from passive diffusion in an isosmotic medium as has been shown in *Carcinus maenas* (Zanders, 1980), *Hemigrapsus* sp. and *Pachygrapsus crassipes* (Rudy, 1966).

In the present study the macro minerals /electrolytes were quantitatively estimated and showed a significant increase in the mineral content in the bilateral eyestalk ablated group of crabs when compared with control group; whereas there was a significant decrease seen the light treated group when compared to the eyestalk ablated groups and the variation in the mineral content in the tissues of the light treated group is insignificant when compared with the control crabs. Nan et al. (2004) reported that osmolarity, potassium and calcium concentration were high in bilaterally ablated shrimps. In shrimps Cl-, Na+ and K+ levels were lower during the postmoult and were higher during intermoult and early premoult stages is considered to be associated with water uptake at the time of molting (Cheng et al., 2002).

The ionic regulation in the marine crustaceans under hormonal influence, however, revealed different from that of the freshwater forms. In crustaceans dopamine is also involved in osmoregulatory process as reviewed by Morris (2001) and Tierney et al. (2003). The same mechanism has been observed in the present study with high ionic influx in the case of eyestalk extract injected crab when compared to control crab. At the body surfaces it would decrease the outward permeability of the epithelial cells while at the same time promoting the active uptake of sodium across the gills by stimulating a Na+, K+ activated ATPase such as was found in the gills of the terrestrial crab, *Cardiosoma quanhumi* (Quinn and Lane, 1966).

Eckhardt et al. (1995) have observed that CHH may be an important osmoregulatory neuropeptide, a view which is reinforced considering previous studies that have shown that perfusion of gills of *P. marmoratus* sinus gland extracts rapidly increase Na+ influx and TEP. At the body surfaces it would decrease the outward permeability of the epithelial cells while at the same time promoting the active uptake of sodium across the gills by stimulating a Na+ -K+ activated ATPase such as was found in the gills of the terrestrial crab, *Cardiosoma quanhumi* (Quinn and Lane, 1966). Moreover, the DPhe3 isoform of *Astacus leptodactylus* CHH increases Na+ content of the hemolymph in postmoult crayfish, that had previously been eyestalk ablated (Serrano et al., 2003). This present report is in concurrence with (Eckhardt et al., 1995; Spanings-Pierrot et al., 2000; Devakumar et al., 2014).

In this present investigation the histological studies on the ovaries provide detailed information 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 substantiate 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 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 (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. 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 tool for enhanced gonadal maturation in crustaceans.

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