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Neuroendocrine Role of Eyestalks in Respiratory Regulation of Swimming Crab *Portunus pelagicus* (Lineaus, 1857)

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Abstract: Purpose: The effects of eyestalk ablation and eyestalk extract injection on oxygen consumption and hemocytes count of *Portunus pelagicus* were studied over the 30 animals. Oxygen consumption in destalked animals was higher than in normal ones, whereas oxygen consumed by eyestalk extract injected animals was lesser than normal ones. **Results:** The oxygen consumption in both the sexe' s increased significantly after eyestalk ablation and decreased after eyestalk extract injection vigorously. Statistical analysis of the results showed significant differences in both sexes between stalked and destalked animals as well as stalked and eyestalk extract inject ones ($p<0.05$). The total hemocyte count was found to be high in injected females (1.493×10^7 cells mL^{-1}) while as in males, the total hemocyte count was found to be high in normal crabs (1.073×10^7 cells mL^{-1}). **Conclusion:** The mean number of circulating hemocytes in female *P. pelagicus* was found to increase significantly due to eyestalk injection. Thus clears that the ablation decreases the total hemocytes count in these crabs.

Key words: Oxygen consumption, eyestalk hormones, hemocytes, *Portunus pelagicus*

INTRODUCTION

In decapod crustaceans, the sinus gland comprises an endocrine structure of primary importance and is located in the eyestalk. It is a classical neurohemal organ, composed of axon terminals from cell bodies located within medulla terminal X-organ (MTXO) which is supported by muscle and connective tissues (Chaigneau, 1983; Fingerman, 1987; Stuenkel and Cooke, 1988). The various processes of crustacean metabolism are inadequately known and for this reason, it is difficult to assess the effects of hormones on crustacean metabolism. However, most investigators agree on the relationship between eyestalk ablation and oxygen consumption.

The oxygen uptake is influenced by many extrinsic and intrinsic factors. All these factors individually and collectively affect the respiratory metabolism in animals (Diwan and Nagabhushanam, 1972) and are under neuroendocrine control (Nagabhushanam and Kulkarai, 1978; Sangvikar and Nagabhushanam, 1981). Scudamore (1947) recorded that normal oxygen consumption is strongly increased from one week before the moult until one week after the moult. If the eyestalks of the land crab *Gecarcinus* are removed, throwing the animal precipitously into proecdysis, the respiratory quotient or R.Q. (the ratio of CO_2 released to O_2 consumed) falls from 0.77 to 0.69. This shift is suggestive of a change in the metabolic pattern of the animal towards greater lipid utilization. Vonk (1960) indicated that bilateral extirpation of the eyestalks (and less so of the sinus glands) increase the normal oxygen consumption as much as 60%. This effect may last until the next moult although its duration and intensity differ somewhat according to species. The increased oxygen uptake which occurs normally during moult, is further enhanced if the eyestalks have been previously removed (Edwards, 1950; Scudamore, 1947). Passano (1960) also recorded that if the whole eyestalk neurosecretory system were implanted into eyestalkless animals it showed that accelerated onset of proecdysis was delayed.

The intra specific variations in hemocytes population cannot be entirely explained by the differences in techniques used in the counting procedures. In *Eriocheir sinensis*, Bauchau and Plaquet (1973) have shown variations in total hemocytes count in relation to different physiological conditions of the animal. Matozzo and Marin (2010) have studied the role of hemocytes from the crab *Carcinus aestuarii* in immune responses.

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It is known that the X-organ plays a major role in the inhibition of the moulting process for decapods crustaceans (Chang and O'Connor, 1987). Ablation of the eyestalk may remove or destroy the function of X-organ si`nus gland complex and may accelerate moult or growth of the crustacean. However, studies with American lobster (*Homarus americanus*) and western Atlantic spiny lobster (*Panulirus argus*) eyestalk ablation did not result in an acceleration of their moulting cycle (Sochasky *et al*., 1973). In addition to metabolic processes, the operation of eyestalk ablation may also result in a decrease of blood glucose (Keller *et al*., 1985), promotion of vitellogenesis (Adiyodi, 1985) accumulation of water (Jackson *et al*., 1987) increase in respiratory rate (Rosas *et al*., 1991). Sroyraya *et al*. (2010) have studied the bilateral eyestalk ablation of the blue swimmer crab, *Portunus pelagicus*, produces hypertrophy of the androgenic gland and an increase of cells producing insulin-like androgenic gland hormone.

Environmental factors may also influence the hemocytes population in crustaceans. Although these studies suggest that variations in the circulating hemocytes population may result from a number of variables including techniques, physiological factors and environment, the mechanism underlying such variations has not been elucidated in the *P. pelagicus* crabs. Hence, the present study attempts were made to investigate the neuroendocrine role of eyestalk hormones on the respiration and hemocytes population count.

MATERIALS AND METHODS

Collection and culture: The crabs for the present study were collected from neritic zone of Vellar estuary (Lat. 11°29" N and Long. 79°46" E). After collected from the net the inter-moult crabs were taken for the experiment. The crabs were segregated as male and female in different tubs at the laboratory.

The crabs were stocked at a density of 10 crabs/tub with the sex ratio 5 in each. The optimum environmental parameters were maintained during experimental period (Salinity 33-35 ppt; pH 7.5-8.0 and Temperature 28-31°C).

Crabs were fed with fresh fish meat, bivalves and some of the crustacean like squilla and hermit crab muscles twice a day. The water were exchanged every after two days in morning hours and left over feed and fecal matters were removed.

Eyestalk ablation and extract preparation: Bilateral extirpation was carried out by cutting the both eyestalks of experimental crabs below the eyestalks using presterilized scissors and forceps. The wound was cauterized with a hot blunt needle to prevent the loss of hemolymph. The isolated eyestalks, exoskeleton intact, were stored at 20°C. The exoskeletons of eyestalks were removed with dissecting instruments. The soft tissues isolated were homogenized and centrifuged at 15,000 rpm for about 10 min at 2°C. The supernatant was collected in a pre-chilled micro centrifuge tube and homogenate re-extracted as before. The final supernatant containing the eyestalk extract was transferred into cold micro centrifuge tubes and stored at-20°C until required. A single dose of gland extracts of the isolated supernatants were injected into the animal body through the arthrodial membrane at the base of the coxa of the third pair of walking legs to observe some of the physiological changes.

Determination of oxygen consumption: The experiment has been carried out over the period of 12 days for oxygen consumption after the eyestalk ablation and eyestalk extract injection. For measuring the oxygen consumption the Winkler's bottle method were used by which the titration has been done against sodium thiosulphate in the burette (Carpenter, 1965). The result was expressed as ml O_2/h/animal. Data were treated statistically by one-way analysis of variance (ANOVA) to test the significance.

Total hemocytes count: Hemolymph from three groups of crabs, control, ablated and injected was taken for the hemocytes count. Hemolymph was collected from the crabs with the help of 1 mL syringe (25 gauges) and diluted with anticoagulant (1:5) ratio. 1 mL of diluted hemolymph was placed in the Neubauer chamber and cells were counted from 4 squares.

The total hemocytes count was calculated as cells mL^{-1} by the formula given below:

$$\text{Cell count} = N \times \frac{D}{A} \times 10 \times 10^3 \text{ cells mL}^{-1}$$

RESULTS

In the present study, the effect of eyestalk extract injection in the swimming crab *P. pelagicus* and its physiological response over it is well monitored.

Oxygen consumption: The present study clearly showed that after the ablation, oxygen consumed by the eyestalk

Table 1: Comparison of oxygen consumption between control and ablated

		Control		Ablated		
Duration	Sex	N	M±SD	N	M±S.D	p
4th day	M	5	11.02±0.01	5	12.42±0.09	<0.05
	F	5	12.92±0.19	5	14.17±0.39	<0.05
8th day	M	5	08.45±0.03	5	9.30±0.56	<0.05
	F	5	09.68±0.23	5	11.09±0.24	<0.05
12th day	M	5	08.28±0.01	5	008.90±0.48	NS
	F	5	09.02±0.24	5	10.01±0.01	<0.05

N: No. of specimens used. M: Mean values. SD: Standard deviation. p: Level of significance. NS: Not significant

Table 2: Comparison of oxygen consumption between control and injected crabs

		Control		Eyestalk injected		
Duration	Sex	N	M±SD	N	M±SD	p
4th day	M	5	11.02±0.01	5	009.8±0.36	<0.05
	F	5	012.92±0.19	5	10.13±0.26	<0.05
8th day	M	5	008.45±0.03	5	008.1±0.07	<0.05
	F	5	009.68±0.23	5	08.43±0.17	<0.05
12th day	M	5	008.28±0.01	5	08.05±0.01	<0.05
	F	5	009.02±0.24	5	08.58±0.02	<0.05

N: No. of specimens used. M: Mean values. SD: Standard deviation. p: Level of significance

Table 3: Comparison of oxygen consumption between ablated and injected crabs

		Ablated		Eyestalk injected		
Duration	Sex	N	M±S.D	N	M±S.D	p
4th day	M	5	12.42±0.09	5	9.80±0.36	<0.05
	F	5	14.19±0.39	5	10.13±0.26	<0.05
8th day	M	5	9.30±0.56	5	08.10±0.07	<0.05
	F	5	11.09±0.24	5	08.43±0.17	<0.05
12th day	M	5	8.90±0.48	5	08.05±0.01	>0.05
	F	5	10.01±0.01	5	08.58±0.02	<0.05

N: No. of specimens used. M: Mean values. SD: Standard deviation. p: Level of significance

ablated male group increased significantly ($p<0.05$) up to 8th day and started decreasing from 8th day onwards ($p>0.05$). However, in eyestalk ablated females oxygen consumption increased significantly beyond the 8th day also ($p<0.05$) (Table 1).

It was also observed that the oxygen consumption increases significantly after eyestalk ablation and decreases after eyestalk extract injection vigorously. Similarly, data for the oxygen consumption between normal and eyestalk injection were given in Table 2. The result clearly showed that eyestalk injection decreased oxygen consumption significantly ($p<0.05$) in both male and female. The data clearly showed that ablation significantly increase the oxygen consumption in *P. pelagicus* (Table 3).

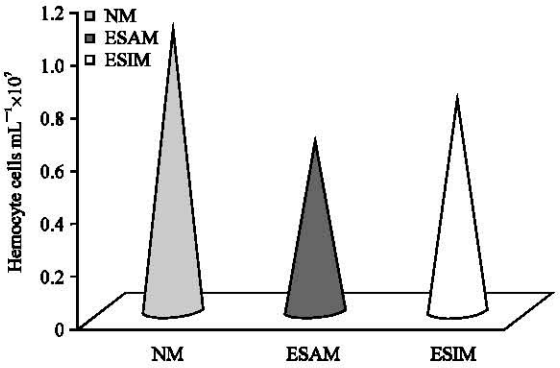

Fig. 1: Showing the difference in number of the hemocytes in (Normal, Eyestalk ablated and Eyestalk injected) male crabs

Total hemocyte count: After 12 days experiment the variation in the total hemocyte count in ablated, control and injected crabs. The total hemocyte count was found to be high in injected females (1.493×10^7 cells mL^{-1}) while as in males, the total hemocyte count was found to be high in normal crabs (1.073×10^7 cells mL^{-1}). The present study showed that eyestalk injection increases the hemocyte count in females; however, in males both ablation and eyestalk injection decreased the hemocytes count (Fig. 1-3).

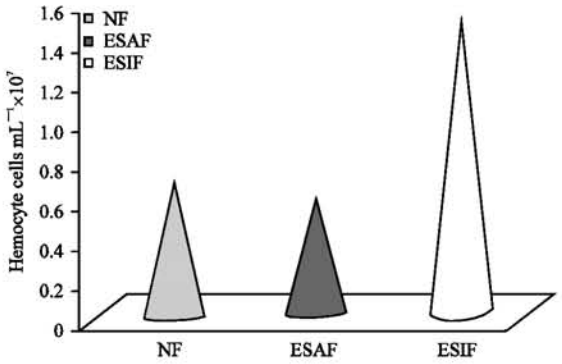

Fig. 2: Showing the difference in number of the hemocytes in (Normal, Eyestalk ablated and Eyestalk injected) female crabs

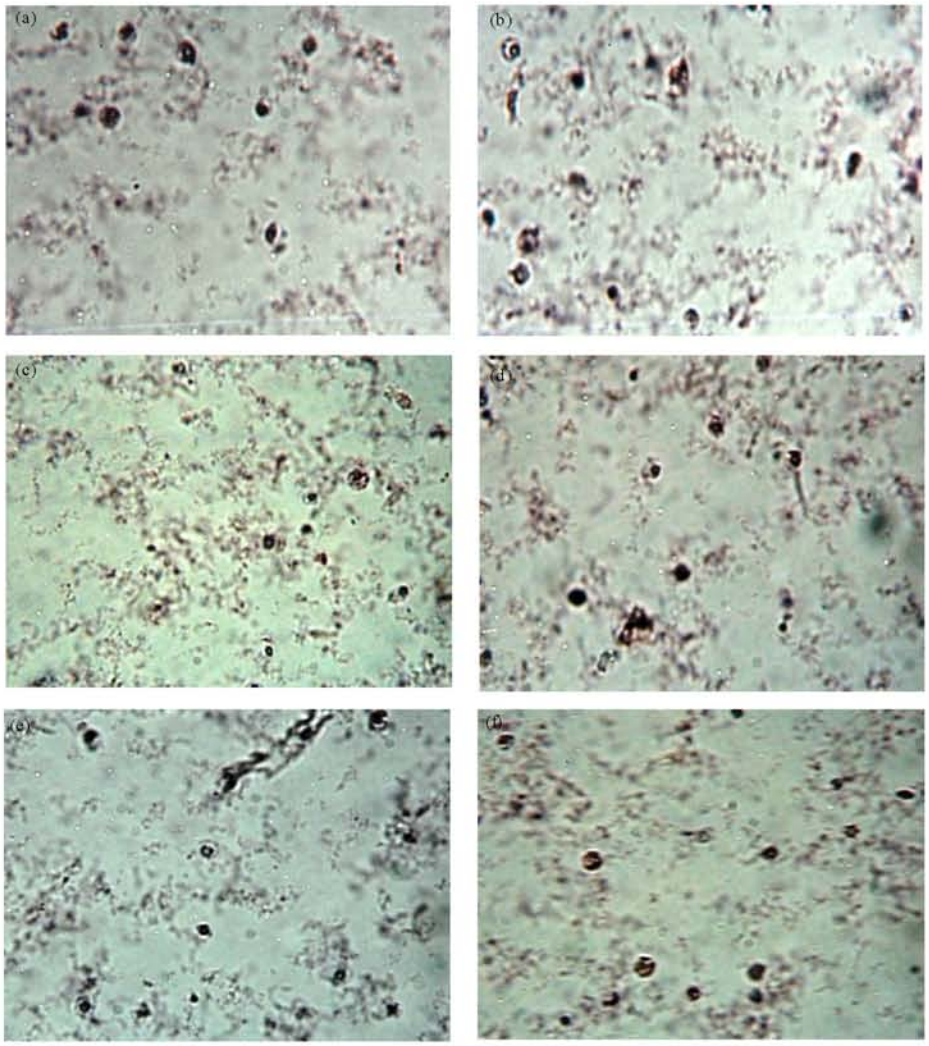

Fig. 3: The hemocytes density of (a) NM, (b) ESIM, (c) NF, (d) ESIF, (e) ESAM and (f) ESAF

DISCUSSION

In the present study it clearly shows that eyestalk ablation increase the oxygen consumption of both male as well female crabs. This means that the crab is thrown directly into the proecdysis stage where the major physiological events of decapods occur. Among these events, oxygen uptake increases markedly just before exuviation (Scudamore, 1947; Maciel *et al*., 2010).

The increase in oxygen in ablated crabs may be due to increased demand of the tissues for the oxidation-reduction processes to meet the increased rate of oxygen uptake.

In the reciprocal experiment (injected animals) of the eyestalk-extract-injected animals, the oxygen consumed by this group is significantly less than the control. This could be due to the reason that eyestalk extract injection which may contain moulting inhibiting hormone, delayed the accelerated onset of proecdysis and this consequently led to decreased oxygen consumption (Passano, 1960). Hence, the results of this study are in agreement with the earlier works.

In the present study, the mean number of circulating hemocytes in female *P. pelagicus* was found to increase significantly due to eyestalk injection, whereas in males it was higher in normal crabs. Thus clears that the ablation decreases the total hemocyte count. This also shows that in the eyestalk of these crabs; there may be some hormones that are responsible for the decreasing of hemocytes. Further proper functioning of the hemocytes is highly essential for correct defense against infection by pathogens and parasites. *P. pelagicus* is a swimming crab which is found mainly in the backwaters and estuaries, hence more chances of infection. In crustaceans, hemopoietic activity is under hormonal control. The stimulatory effects of the x-organ sinus gland complex which is located in the eyestalks and the inhibitory effects of the y-organ on crustacean haematopoiesis have been discussed (Ghiretti-Magaldi, 1977; Johansson *et al*., 2000). In each nodule of hemopoietic organ, stem-cells or hemoblasts undergo regular mitosis to produce various kinds of hemocytes (Bauchau, 1981; Johansson *et al*., 2000; Jiravanichpaisal *et al*., 2006). Removal of the sinus glands in the eyestalks might have suppressed a marked increase in mitosis. This is probably under the influence of the y-organ, once it has been freed from the inhibition by the sinus gland.

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