

Effect of Eyestalk Ablation and Eyestalk Extract Injection on Oxygen Consumption in the Crab, *Ocypode macrocera* (H. Milne-Edwards 1852)

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Abstract: The effects of eyestalk ablation and eyestalk extract injection on oxygen consumption of *Ocypode macrocera* were studied on 30 specimens over a period of 10-days. Statistical analysis of the results show significant differences between stalked and destalked animals as well as stalked and eyestalk extract injected ones. Oxygen consumed in destalked animals was higher than in stalked ones, whereas oxygen consumed by eyestalk extract injected animals was lesser than stalked ones. The differences were discussed on the hormonal basis.

Key words: Eyestalk ablation, eyestalk extracts injection, hormones, ocypode macrocera, oxygen consumption

INTRODUCTION

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. In crustaceans, eyestalk hormones are attributed with control of a number of physiological processes namely, somatic changes, blood glucose level, osmoregulation, moulting, reproduction and oxygen consumption (Fingerman, 1970). As we know that oxygen consumption is the amount of oxygen in milliliters per minute required by the body for normal aerobic metabolism; normally about 250 mL/min. (Scudamore, 1947) recorded that normal oxygen consumption is strongly increased from one week before the moult until one week after the moult. (Bliss, 1953a, b) found that 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 CO₂ released to O₂ 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). Some times marine organisms are feeling oxygen deficiency so the "episodes of hypoxia" are common in the marine environment, particularly associated to upwelling events (Grantham *et al.*, 2004), as

well as in shallow coastal zones. Coastal hypoxia generally follows seasonal patterns, some of them directly related to the influx of freshwater and anthropogenic eutrophication (Turner *et al.*, 2008). Hypoxic zones are becoming more widespread and are one of the most deleterious human-induced impacts on benthic communities (Turner *et al.*, 2008).

Extract from cold-acclimatized crabs increases oxygen consumption when injected into warm-acclimatized crabs, and extract from warm-acclimatized crabs decreases oxygen consumption when injected into cold-acclimatized crabs. Two distinct eyestalk hormones might then affect respiratory metabolism, one reducing the metabolic rate of crabs under high temperature stress and the other increasing the metabolic rate of crabs exposed to cold stress. These hormones would thus be adaptive in enabling the poikilothermic crab a greater metabolic independence of the ambient temperature (Highnam and Hill, 1978). Passano (1953, 1960) also recorded that if the whole eyestalk neurosecretory system were implanted into eyestalkless animals it showed that accelerated onset of proecdysis was delayed. Since there has been no such study on the crab, *Ocypode macrocera*, which is present in abundance near the banks of the Vellar estuary Anankovil (Parangipettai) one of the bigger landing, center in south east coast of India. Therefore it was thought to be worthwhile to investigate this problem in this convenient species.

MATERIALS AND METHODS

The crabs, *Ocypode macrocera*, used in the present investigations were collected during the period August-

September 2010 from the shore of Velar estuary Anankovil (Parangipettai) one of the major landing centre of the south east coast (Lat 11°29'N; 79°46' E) of the India. Crabs were almost of the same weight (29±2 g) so as to eliminate any size effect. After collection, all the crabs were brought to the laboratory where the experiment was held in Centre of Advanced Study in Marine Biology Annamalai University. The crabs were transferred to the troughs already prearranged in the laboratory at the room temperature (24±2°C). The troughs were filled with sand with little saline water sprayed to keep moisture and salinity. The crabs were fed with the bivalves, fish meat and dull crab meat. Saline water was sprayed in every after one day to keep the moisture and salinity in the sand and excessive water was being drained out. After six days of acclimation, the crabs were divided into three troughs each containing 10 crabs with the sex ratio 5:5. The troughs were marked as control, eyestalk ablated and eyestalk extract injected. After acclimation the experiment was started, to measure the oxygen consumption Winkler's bottle method was used and the experiment has been repeated three times to get concordant values in ten days period. Since the mortality rate was high beyond the 10th day, the estimations were concluded to the first 8 days after eyestalk removal.

Determination of oxygen consumption: For determining the oxygen consumption the Winkler's bottle method were used. In this titration method, the titration of the water sample was done against sodium thiosulphate in the burette (James, 1965).

Initially the oxygen content of ordinary water was determined before starting the experiment and then oxygen consumption were measured after placing the animals in to the jars containing water of vol. 1.5 L for half an hour. After ½ h the animals were transferred to their alternative troughs and the water sample were used to measure the oxygen content present in the water, the result will be the difference between initial content of the oxygen in the water already measured and the final content of the oxygen present in the water after putting the animal inside the jar and the result were expressed as ml O₂/1/2 h animal (Yousif, 1981).

Eyestalk Ablation and extract preparation: Bilateral extirpation was carried out by cutting the both eyestalks of experimental crabs below the eye stalk using presterilized scissors and forceps. The wound was cauterized with a hot blunt needle to prevent the loss of hemolymph. After anesthetizing the crabs eyestalks were removed by cutting through the arthrodiol membrane at the base of each eyestalk using clean dissecting scissors and forceps. 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 minutes 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 arthrodiol membrane at the base of the coxa of the third pair of walking legs to observe some of the physiological changes.

RESULTS AND DISCUSSION

Comparison of the control crabs with the eyestalk ablated crabs (Table 1) showed that oxygen consumed by the eyestalk ablated group increased significantly after removal of the eyestalk. The increase started from the first day until the 8th day, where the difference is almost insignificant. This phenomenon is likely to have a hormonal basis

The removal of eyestalks or both sinus glands causes a considerable shortening of the normal intermoult period and thus induces preparation for moult (Scudamore, 1947; Edwards, 1950; Bliss 1953a, b; Jegla *et al.*, 1983). 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 (Bliss 1953a, b; Scheer and Scheer 1954; Scudamore, 1947). Silverthorne (1973) correlates the respiration in the eyestalkless *Uca* with temperature. He found that the removal of eyestalks increases the oxygen consumption of warm-acclimatized crabs by 117% and of cold-acclimatized crabs by 47% since cold-acclimatized crabs do not moult. It is assumed that crustecdysone is not produced even though the source of moult-inhibiting hormone is removed. That the oxygen consumption still increases in these animals suggest the existence of eyestalk hormones that regulate oxygen consumption independently, and this is confirmed by the effect of sinus gland extract on oxygen consumption. It is thus apparent that there is a strong relationship between the hormones present in the eyestalk and the respiration rate of the animal.

In *Maja* at least the amount of respiratory pigment in the hemolymph reaches a maximum at this time (Zukerkandle, 1956). It was also indicated by (Sinha and Mooswi, 1978) that the concentration of ascorbic acid in the muscle and hepatopancreas of the crab *O. macrocera* increases significantly after the removal of eyestalks, since ascorbic acid is involved in oxidation-reduction reactions, its higher level 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 of the eyestalk-extract-injected animals, the oxygen consumed by this group is

Table 1: Comparison between oxygen consumption in control and ablated *O. macrocera*

Duration	Sex	N	Control Mean±S.D	Eyestalk ablated Mean±S.D	p-value
1 st day	M	5	2.90±0.025	3.24±0.035	<0.05
	F	5	4.24±0.036	5.94±0.030	<0.05
4 th day	M	5	2.70±0.025	2.90±0.02	<0.05
	F	5	2.83±0.025	4.74 ±0.03	<0.05
8 th day	M	5	2.20±0.02	2.25±0.02	<0.05
	F	5	2.44±0.02	4.53±0.02	<0.05

N: number of animals; M: the mean values; S.D.: Standard deviation; P: level of significance; N.S.: not significant

Table 2: Comparison between oxygen consumption in control and injected *O. macrocera*

Duration	Sex	N	Control Mean±S.D	Injected Mean±S.D	p-value
1 st day	M	5	2.90±0.025	2.50±0.025	N.S
	F	5	4.24±0.036	4.10±0.04	<0.05
4 th day	M	5	2.70±0.025	1.43±0.025	<0.05
	F	5	2.83±0.025	2.60±0.03	<0.05
8 th day	M	5	2.20±0.02	1.24±0.03	N.S
	F	5	2.44±0.02	2.40±0.025	<0.05

N: number of animals; M: the mean values; S. D.: Standard deviation; P: level of significance; N.S.: not significant

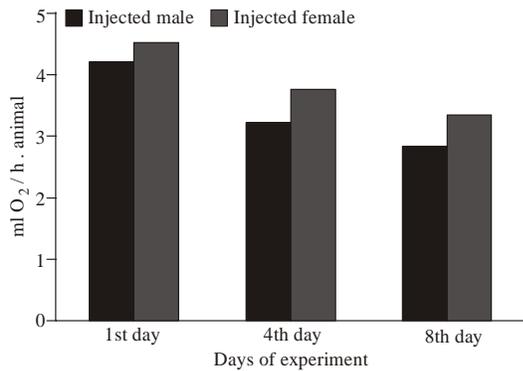


Fig. 1: Oxygen consumption in male and female *O. macrocera* after eyestalk extract injection

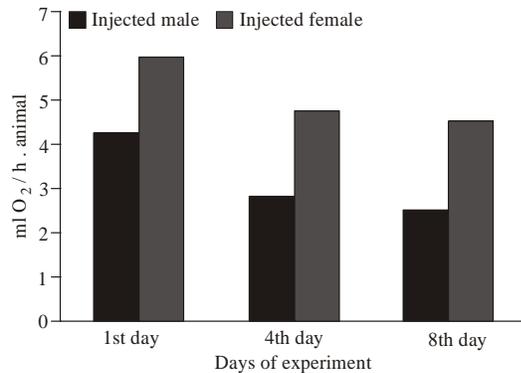


Fig. 2: Oxygen consumption in male and female *O. macrocera* after extirpation of eyestalk

significantly less than the control as shown in the (Table 2).

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, 1953, 1960).

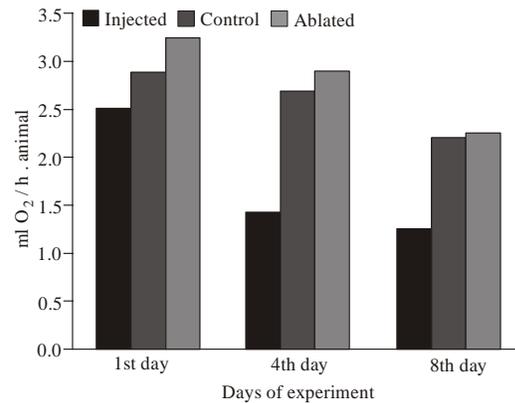


Fig. 3: Comparative oxygen consumption in control, eyestalk ablated: Eyestalk injected male crabs

One more aspect which has been observed is that respiratory rate of the female crabs were more as compared to the male crabs as shown in the Fig. 1 and 2 in both the cases eyestalk ablated and eyestalk injected as shown below in the graphs,

Oxygen consumed by the female crab is higher than that of the male, and this could be explained by higher respiratory rate in the ovaries as indicated by (Yousif, 1981). Figure 3 and 4, shows about the comparative oxygen consumption by three groups Control: Eyestalk ablated: Eyestalk injected in both males and females.

The overall changes we have observed in all these graphs above is because of the hormonal regulation because Hormonal regulation of physiological processes is common to all animals but some of these processes, such as molting, are unique to specific groups of invertebrates such as crabs. A number of endocrine-regulated processes are unique to invertebrates, or more specifically to Ecdysozoans (animals that molt). These processes provide ways of evaluating potential endocrine disruption that is unique to the invertebrates. The molt cycle, i.e. the period between two subsequent ecdyses or

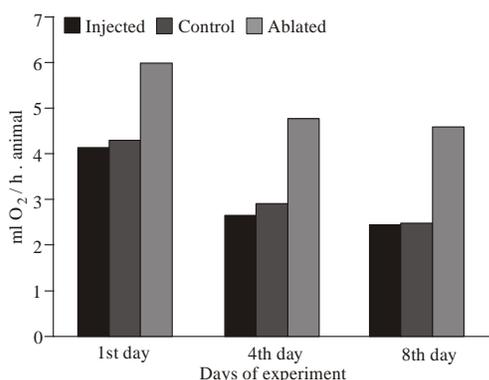


Fig. 4: Comparative oxygen consumption in control: eyestalk ablated: Eyestalk injected female crabs

molts is generally divided into four major phases: postmolt, intermolt, premolt and ecdysis. (Elena, 2002). It is understood that hormones are important in controlling physiological processes in invertebrates (Lafont, 2000; Oehlmann and Schulte-Oehlmann, 2003). Grantham *et al.* (2004) also defined oxygen consumption in terms of “Episodes of hypoxia” which is common in marine environment particularly associated to upwelling events by which marine organisms often oxygen deficiency. Portner (2001, 2002), has reported that oxygen supply does limit thermal tolerance in marine animals.

CONCLUSION

From the current investigation it is determined that hormonal factors greatly influence the body physiology in terms of oxygen intake and their basal metabolism. As the hormonal influence specificity is unique to both sexes, the effect of eyestalk ablation increases the oxygen uptake significantly in case of female crab than in males and eyestalk extract decreases the oxygen consumption in females as well male crabs. From the present findings it is highly approved that neuronal hormones has considerable role in regulating the body physiology of crustaceans.

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REFERENCES

Bliss, D.E., 1953a. Neurosecretion and crab metabolism. *Anat. Record*, 177: 599.

- Bliss, D.E., 1953b. Endocrine control of metabolism in the land crab, *Gecarcinus lateralis* Freminville. 1. Differences in the respiratory metabolism of sinus glandless and eyestalkless crabs. *Biol. Bull.*, 104: 272-296.
- Edwards, G.A., 1950. The influence of eyestalk removal on the metabolism of the fiddler crab. *Physiol. Comarataet Oecol.*, 2: 34-50.
- Fingerman, M., 1970. Perspectives in crustacean endocrinology. *Scientia*, 105: 1-23.
- Elena, G., 2002. Moults cycle and its chronology in *Mysis mixta* and *Neomysis integer* (Crustacea, Mysidacea): Implications for growth assessment. *J. Exp. Mar. Biol. Ecol.*, 278(2): 179-194.
- Grantham, B.A., F. Chan, K.J. Nielsen, D.S. J.A. Fox, Barth, A. Huyer, J. Lubchenco and B.A. Menge, 2004. Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast Pacific. *Nature*, 429: 749-754.
- Highnam, K.C. and L. Hill, 1978. Endocrine Mechanism in Crustacea. In: *The Comparative Endocrinology of Invertebrates*. 2nd Edn., Edward Arnold Publishers, Ltd., pp: 91-96.
- James, H.C., 1965. The accuracy of the Winkler method for dissolved oxygen analysis. Department of Oceanography, The Johns Hopkins University, Baltimore, Maryland, 1: 135-140.
- Jegla, T.C., C. Runald, C. Kegel and R. Keller, 1983. The role of the Y-organ and cephalic gland in ecdysteroid production and the control of moulting in the crayfish, *Orconectes limosus*. *J. Comp. Physiol. B. Biochem. Syst. Environ. Physiol.*, 152: 91-96.
- Lafont, R., 2000. The endocrinology of invertebrates. *Ecotoxicology*, 9(1-2): 41-57.
- Oehlmann, J. and U. Schulte-Oehlmann, 2003. Endocrine disruption in invertebrates. *Pure. Appl. Chem.*, 75(11-12): 2207-2218.
- Passano, L.M., 1953. Neurosecretory control of moulting in crabs by X-organ sinus gland complex. *Physiologia. Comp. Oecol.*, 3: 155-189.
- Passano, L.M., 1960. Moulting and its control in the physiology of Crustacea. In: Waterman, T.H. (Ed.), *The Physiology of Crustacea*. Vol. 1, Academic Press, London and New York, 15: 443.
- Portner, H.O., 2001. Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. *Naturwissenschaften*. 88: 137-146.
- Portner, H.O., 2002. Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. *Comp. Biochem. Physiol.*, 132 A: 739-761.
- Scheer, B.T. and M.A.R. Scheer, 1954. The hormonal control of metabolism in crustaceans. VIII. Oxygen consumption in *Lenander serratus*. *PbbI. Staz. Zool. Napoli*, 25: 419-426.

- Scudamore, H.H., 1947. The influence of sinus glands upon moulting and associated change in the crayfish. *Physiol. Zool.*, 20: 187-208.
- Silverthorne, S.U., 1973. Respiration in eyestalkless *Uca* (Crustacea: Decapoda) acclimated to temperatures. *Comp. Bioch. Physiol.*, 45(a): 417-420.
- Sinha, R.C. and H.K. Mooswi, 1978. Effect of eyestalk removal on the different biochemical constituents of muscle and hepatopancreas of the crab *Sesarma bouleengeri* Calman. *Comp. Physiol. Ecol.*, 3(1): 49-51.
- Turner, R.E., N.N. Rabalais and D. Justic, 2008. Gulf of Mexico hypoxia: Alternate states and a legacy. *Environ. Sci. Technol.*, 42: 2323-2327.
- Vonk, H.J., 1960. Digestion and metabolism. In: Waterman, T.H. (Ed.), *The Physiology of Crustacea*, Vol. 1, Academic Press, London and New York.
- Yousif, Y.Y., 1981. Studies on the seasonal variation in component indices, chemical composition and oxygen uptake in the crab *Sesarma bouleengeri* Calman. M.Sc. Thesis, Basrah University, Basrah, Iraq.
- Zukerkandle, E., 1956. The change in the cycle dintermue protein fractions of hemolymph *Maja squinado* adorned by *electrophoresis*. *Rend. Soc. Biol.*, 150: 39-41.

AUTHOR'S CONTRIBUTION

Bilal Ahmad Bhat is the theme researcher of this study who carried out all this investigation. Ravichandran, S. is the research mentor to guide this study. Sartaj Ahmad Allayie is the person to assist in the current research study.