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Research Article

Endocrine involvement in osmoregulation in a Freshwater Crab B*arytelphusa guerini*

Vaishali Gangotri and Madhav Gangotri [*]
Department of Zoology, Shri Dhokeshwar Mahavidyalaya, Takali Dhokeshwar, District Ahmednagar 414304 Maharastra, India
[*] Department of Zoology, Padmashree Manibhai Desai Mahavidyalaya, Uruli Kanchan, Pune 412202 Maharastra, India
*Corresponding author, Email: mgangotri@gmail.com

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Abstract: The effect of eyestalk ablation on osmotic and ionic regulation, the total *ATP-ase* and Na+ / K+ATP-ase activity of the freshwater crab, *Barytelphusa guerini* (H. Milne Edwards) (Decapoda, Potamidea) investigated in a period of 30 days. The survival rates of normal and eyestalk ablated animals were 75% and 50% after 30 days of treatment, respectively. Bilateral eyestalk extirpation showed the increase in weight of the animal after 30 days. Hence, suggests the retention of water in tissues after eyestalk ablation. Eyestalk ablation resulted in a decrease in the haemolymph sodium concentration and an increase in the potassium and calcium concentration of the crabs. The osmolarity of haemolymph and total protein concentration of eyestalk ablated animal were demonstrated to be higher than those of the normal intact animal. The bilaterally eyestalk ablated crabs had higher total ATP-ase and Na+ / K+ATP-ase activities in the gills than those of normal intact animals. It is concluded that the eyestalk ablation of crabs causes significant changes in the osmotic and ionic regulation and also affects the total ATP-ase and Na+ / K+ATP-ase activity of the gills.

Keywords: Osmoregulation, ATP-ase activity, EyestalkHormone, Freshwater Crab, *Barytelphusa guerini*

INTRODUCTION

Osmoregulation can be understood in a better manner by studying the neuroendocrine control of chloride regulation (Venkatachari et al., 1979) working on *Barytelphusa guerini* demonstrated that the eyestalk removal decreased blood chloride content, while it was recovered to normal level after eyestalk extract injection. Scudamore (1947) demonstrated that the disturbed ecdysial water metabolism of eyestalkless crayfish was due to absence of sinus gland (Kamemoto et al., 1966) have given evidence for neuroendocrine regulation of

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salt water homeostasis in *Procambarus clarkii* and *Metapograpsus messor*. Operation of eyestalk ablation in various crustaceans may result accumulation of water (Jackson et al., 1987), increase in respiratory rate (Rosas et al.,1991) and lowering of sodium concentration in haemolymph. (McNamara et al., 1990)

The mechanisms of osmotic and ionic regulation have been demonstrated to be under neuroendocrine control (Kamemoto and Tullis, 1972; Kleinholtz, 1976; Mantel and farmer, 1989). Kamemoto and Tullis (1972) injected a cerebral ganglion extract derived from *Metapograpsus rosenbergii*, which resulted in an increase influx in the freshwater crayfish, *Procambarus clarkii*. The importance of the eyestalk ganglionic system in the regulation of water and ion movement has also been demonstrated in a variety of crustaceans (Heit and Fingerman, 1975; Davis and Hagadorn, 1982).

Moreover, Charmantier et al., (1984), reported that eyestalk neurohaemal factor may regulate haemolymph osmosis, sodium and chloride concentration in *Homarus americanus*. It was also concluded that eyestalk removal may initiate a lowering of haemolymph sodium (Heit and Fingerman, 1975) due to increased sodium efflux and result in an increase of sodium permeability in crustaceans (Davis and Hagadorn, 1982). Bonga (1972) studied the neuroendocrine involvement in osmoregulation in the freshwater mollusc *Lymnaea stagnalis* and suggested that one type of cells activates water elimination, while the other type stimulates ion-uptake mechanism.

The active transport of Na+ / K+ requires a specific carrier enzyme, Na+, K+ ATP-ase. (Towle et al., 1976). Na+, K+ ATP-ase, a ubiquitous component from cell membrane of animals (Towle, 1981) is a prime mediator of ion transport across cellular membranes (Neufeld et al., 1980) which may regulate ion balance at the organ or cell level for marine animals (Towle, 1981). Osmoregulation and Na+, K+ ATP-ase expression in osmoregulatory organs of *Scylla paramamosain* was studied by Kuei-Fang Chung and Hui-Chen Lin (2006) and concluded that, the posterior gill play the most important role in osmoregulation in dilute sea water and the increase in Na+, K+ ATP-ase activity on the 7th day may be due to its gene transcription and / or mRNA translation. The effect of eyestalk ablation on oxygen consumption and ammonia excretion of *Penaeus japonicus* was studied and it was concluded that eyestalk ablation significantly affects nitrogen metabolism (Jiann-Chu Chen and Peng-Gek Chia, 1995). Bilateral eyestalk ablation led to decrease in haemolymph sodium concentration and increase in potassium and calcium concentration and also possess higher total ATP-ese and Na+, K+ ATP-ase activities in the gill of *Penaeus manodon* (Fan-Hua, Nan, et al., 1993) and *Marsupenaeus japonicas* (Nann et al., 2004). ATP reservoirs are reduced by increasing energy demand in response to physiologically stressful condition (Chen and Nan, 1992). Therefore, examination of ATP-ase activity could be an indicator for the physiological well being of an animal.

A very little information is available regarding the neurondocrine mediation of haemolymph composition of freshwater crab. The present study therefore, attempts to examine the effects of eyestalk ablation on the haemolymph composition and Na+, K+ ATP-ase activity in the gills of the freshwater crab. As the freshwater Crab, *Barytelphusa guerini* is a good osmoregulator, neuroendocrine control of osmoregulation was already studied in this animal with special reference to chloride ion

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regulation (Venkatachari et al., 1979).

MATERIALS AND METHODS

The collection and maintenance of the freshwater crab, *Barytelphusa guerini* was carried out and the following set of experiments was conducted.

Collected specimens maintained in the laboratory for 24 to 48 hours before starting of the experiment. The animals were selected of intermoult stage have the same size. The animals were divided into two groups. Each group is of 50 animals.

First group will serve as intact control. To observe the physiological effect of eyestalk ablation, the eyestalks of experimental crabs were ablated using iridectomy scissors under tap water. The crabs were fed with liver of goat. Wet weight of the live individuals in each experimental group were measured and recorded at 30 days period.

At the end of the experiment (30 days after eyestalk ablation) only 10 animals of the same size were selected. The haemolymph were sampled and centrifuged at 15,000 rpm for 5 minutes and collected samples were then used for osmotic, ionic and total protein analysis. osmotic concentration was determined by the freezing point depression method as described by Salomao (1980). The ionic concentrations were determined by flame spectrophotometry and the total proteins were measured by Bradford (1976).

The gills were excised at the end of experiment (at 30 days after eyestalk ablation from 10 animals), transferred to an ice bath and homogenized. Total ATP-ase and Na+, K+ ATP-ase activities were determined following the method described by Tucker (1979). The amount of inorganic phosphate (Pi) liberated in the reaction of ATP --> ADP + Pi was expressed as m mol pi mg-1 protein hr-1 (Bartlett, 1958). The protein content of the homogenate was determined by (Lowry et al., 1951) The detection limit for the total ATP-ase and Na+, K+ ATP-ase was 0.02.

All data were subjected to statistical analysis keeping the level of significance $p < 0.05$ (Seetharaman, 1958).

RESULTS

The results obtained in the present study showed that the eyestalk ablated crabs had higher mean body weight than the normal intact animals at the end of 30 days. The mean wet weight of normal intact animals was 45.56 ± 7.2 g which raised upto 65.21 ± 7.35 g after the 30 days of eyestalk removal the values obtained in the experimental animals were significantly higher ($P < 0.05$), when compared with normal intact animals. However, the survival of normal intact animals was higher than that of eyestalk ablated crabs. The survival value in normal intact animals was 75%, where-as in ablated animals it was 50% (Table 1).

Table: 1. Mean (Standard deviation) weight gain and survival of eyestalk ablated and eyestalk intact freshwater crab *Barytelphusa guerini* reared for One month.

S. No.	Treatment	Body Weight (gram)		Weight Gain	Survival (%)
		Initial	Final		
I	Normal Intact Animals	45.56 ± 7.21	48.36 ± 8.52	2.80 ± 0.75	75 %

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	Percentage Change	--	--	+ 6.66	--
	Students ‘ t ’ Test	--	--	0.7356	--
	Level of Significance	--	--	P > 0.1	--
	Inference	--	--	Insignificant	--
II	**Eyestalk Ablated Animals**	47.32 ± 6.25	65.21 ± 7.35	18.10 + 2.35	50 %
	Percentage Change	--	--	+ 36.30	--
	Students ‘ t ’ Test	--	--	3.389	--
	Level of Significance	--	--	P < 0.05	--
	Inference	--	--	Significant	--

The results after one month of eyestalk ablation on osmolarity, ion concentrations and total protein in the haemolymph of *Barytelphusa guerini* are shown in Table 2.

Table: 2. Mean (Standard deviation) haemolymph contents of eyestalk ablation and eyestalk intact in freshwater crab *Barytelphusa guerini* reared for one month.

S. No.	**Treatment**	**Heamolymph Contents**				**Protein**
		Osmolarity mosm/Kg	**Na +**	**K +**	**Ca^{2} +**	
			mEq / l	**mEq / l**	**mEq / l**	**□g**
I	Normal Intact Animals	777.86 ± 8.24	385.15 ± 6.05	9.76 ± 0.07	45.78 ± 2.27	185.15 ± 6.13
II	Eyestalk Ablated Animals	888.56 ± 10.25	335.25 ± 8.25	13.12 ± 0.08	60.25 ± 3.25	245.35 ± 7.25
III	Percentage Change	+ 14.41	- 13.01	+44.40	33.30	30.01
IV	Students ‘ t ’ Test	2.789	2.357	5.357	3.575	2.145
V	Level of Significance	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05
VI	Inference	Significant	Significant	Significant	Significant	Significant

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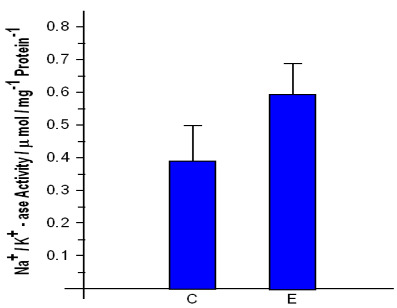
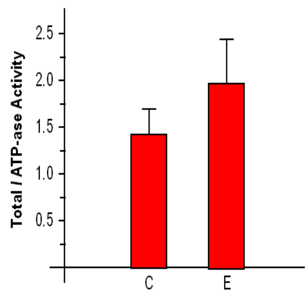
The total Osmolarity in normal intact animals was 777.86 ± 8.24 mosm / kg., Potassium 9.76 ± 0.07, Calcium 45.78 ± 2.27 and total Protein 185.12 ± 6.13 mg, which increased upto 888.57 ± 10.35 (Osmolarity), 13.12 ± 0.08 (K+ mg / l), 60.25 mEq / l (Ca2+) and 245.35 ± 7.25 mg (Protein).

The values obtained after eyestalk ablation were significantly higher ($P<0.05$) than those of normal intact animals. In comparison, the haemolymph sodium concentration in normal intact animals was 385.15 ± 6.05 (mEq / l), which decreased upto 335.25 ± 8.25 (mEq / l), which was significantly lower than that of the control animals. The analysis of variance suggested that eyestalk ablation significantly ($P<0.05$) affected the haemolymph composition in the experimental animals.

Total ATP-ase activity in normal intact animals was 1.5 ± 0.25, which increased upto 2.0 ± 0.5 and Na+ / K+-ase activity was 0.4 ± 0.1, which increased upto a 0.6 ± 0.15 after 30 days of eyestalk ablation. The eyestalk ablated carbs showed significantly higher ($P<0.05$) ATP-ase activity and Na+ / K+-ase activity in the gills than those obtained from normal intact animals (Figure).

Figure: Total ATP-ase activity and Na+, k+ -ATP-ase activity in the gill of normal intact (c) and eyestalk ablated (E) of crab after 30 days of eyestalk ablation





DISCUSSION

The present study demonstrated that bilateral eyestalk ablation led to accelerate the increase in weight of *Barytelphusa guerini*. It was observed that there was an increase in weight of eyestalkless crabs due to uptake of water. It is known that salt and water balance is controlled at least in part by hormones secreted in X-organ of eyestalk which is stored in sinus gland. Extirpating the source as well as storage site (i.e. eyestalk ablation) will affect the salt-water balance. There will be influx outflow of water of the body depending upon the medium in which the animals were placed. The results obtained in the present study supported by earlier workers. Scudamore (1947) demonstrated that the disturbed ecdysial water metabolism of eyestalkless crayfish was due to absence of sinus gland. Kamemoto et al., (1966) have given evidence of neuroendocrine regula-tion of salt water homeostasis in *Procambarus clarkii* and *Metapograpsus messor*.

It was also reported that high mortality of crustacean may be caused by eyestalk ablation (Mauviot and Castell, 1976; Nakatani and Otsu, 1979; 1981; Trider et al., 1979; Freeman et al., 1983). Similarly, the present study further confirmed by the eyestalk ablated crabs revealed higher mortality than the normal intact ones. The high mortality of eyestalk ablated crabs may be caused by cannibalism of animals that are

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confined in a limited environment with unsuitable stocking density or an incorrect rearing method and inferior food quality.

Physiologically, the present study also showed that *Barytelphusa guerini* treated by eyestalk ablation had a significantly lower ($P<0.05$) sodium concentration than that of the normal intact ones. This result is similar to those obtained by McNamara et al., (1980) in a study with the freshwater prawn, *Macrobrachium olfersii*. Ehrenfeld and Isaia, (1974) showed that the freshwater crayfish, *Astacus leptodactylus* diminished sodium influxes by 30% in eyestalk ablated animals, while effluxes remained unchanged. These results may suggest that reduction of sodium concentration in the haemolymph of crabs by eyestalk ablation could be initiated by an increase of sodium efflux. Furthermore, Heit and Fingerman, (1975) demonstrated that a neuro factor existing in the eyestalk of fiddler crabs (*Uca pugilator*) may play a major role in the prevention of sodium loss in hyposmotic media by promoting active transport of sodium. Venkatachari et. al., (1979), in *Barytelphusa guerini* demonstrated that the eyestalk removal decreased blood chloride content. The results obtained in the present study incorporated with the previous studies, therefore, suggest that eyestalk may play a major role in either regulating sodium uptake mechanism or increasing sodium permeability.

The main organ involved in crustacean osmoregulation is gill (Mantel and Farmer, 1983). The difference in diffusion distance implies that gaseous exchange takes place primarily in the anterior gills; whereas; the main function of the posterior gills in osmoregulation (Mantel and Farmer, 1983; pequeux, 1995).

Na+ / K+ ATP-ase provides the primary driving force for ion transport. The activity of Na+ / K+ATP-ase reflects the osmoregulation of crustaceans (reviewed by Lucu and Towle, 2003). Because of its role in regulation of osmotic pressure of the haemolymph, Na+ / K+ATP-ase activity is higher in the posterior gills than in the anterior gills of *Carcinus maenas* (Siebers et al., 1982; Henry et al., 2002), *Carcinus sapidus* (Neufeld et al., 1980), *Uca vocans* and *Uca lacteal* (Lin et al., 2002). A discrepancy in activity of the enzyme among the anterior, posterior gills and other osmo regulatory organs would suggest the relative importance of each organ in osmo regulation.

It was reported that the chloride cells of teleost gill contain a large amount of Na+ / K+ATP-ase (Sargent et al., 1980). The efflux of sodium ion out of the chloride cells requires energy which could be indirectly supplied by Na+ / K+ATP-ase. The increase in activity of gill Na+ / K+ATP-ase is, therefore, always accompanied by an efflux of Na+. Heit and Fingerman, (1975) indicated that a hormone present in eyestalks may inhibit the loss of haemolymph Na+ in hyposmotic sea water.

The results obtained in the present study demonstrated that following eyestalk ablation, the experimental crabs have a higher ATP-ase activity in the gills and lower haemolymph sodium ion than those of the normal intact animals. It is reasonable to assume that the intact crabs may secrete unknown substances to inhibit ATP-ase activity and prevent Na+ loss from haemolymph. On the other hand, ATP-ase activity in the eyestalkless crabs may initiate a removal of Na+ from the haemolymph. The physiological effects of neuroendocrine factor in the eyestalk of the crabs needs to be further investigated.

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International Journal of Global Science Research **ISSN: 2348-8344 (Online)**
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