Insight Endocrinology and Metabolism 1 (1): 7-14, 2011
ISSN 2044-8805 / DOI: 10.5567/IEM-IK.2011.7.14

Influence of Sinus and Mandibular Glands on the Growth and Behaviuor of *Ocypode macrocera*

Sartaj Ahmad Allayie, S. Ravichandran and Bilal Ahmad Bhat
Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608502, India

Abstract: Background: The influence of sinus and mandibular glands on the growth and behaviour of the *Ocypode macrocera* was studied. Survivals of control and injected (mandibular and cheliped injected) crabs of both sexes were 100% and those of ablated, the survival rate was 60%.While the survival of eyestalk injected crabs of both sexes was 100% and those of ablated males were 80 and 60% in ablated females. **Result:** Both eyestalk ablation and Injection of mandibular gland extracts increased the food consumption of both the sexes of *O. macrocera.* Among the three groups used, the maximum weight gain in both the sexes was noticed in eyestalk ablated and mandibular extract injected crabs fed with bivalves and lowest range of weight gain was noticed in crabs fed with crab muscles. The result also showed that females grew faster than males throughout the experiment. Similarly eyestalk ablated crabs had higher indices of heart, mandibular glands and ovary than the control and cheliped injected crabs. Eyestalk ablation has altered the various behaviours of this crab. The feed intake, water intake, forward movements, number of burrows made, escaping movements by sound and escaping movements by touch of these crab between control and ablated differed significantly ($p \leq 0.05$). However, behavior like Air bowls through gills between control and ablated crabs differed insignificantly ($p \leq 0.08$). **Conclusion:** Thus, ablation experiments demonstrated that in several crustacean groups, the proximal eyestalk ganglia are important in a variety of behavior patterns.

Key words: Food consumption, weight gain, bivalves, heart, ovary and behaviours

INTRODUCTION

Crustaceans use a wide variety of hormones to regulate their growth, development, metabolism and other physiological processes, similar to vertebrates. Aspects of crustacean growth and postembryonic development, including moulting and regeneration, are controlled by C-27 steroid hormones termed ecdysteroids (Skinner, 1985). During the growing period, exuviation of the old exoskeleton followed by replacement with a new one is a common metabolic procedure for crustaceans. The periodic shedding of the old exoskeleton is accomplished by moulting, the procedures of which consist of numerous biochemical, physiological and morphological changes that temporally occupy much of the preceding molt cycle. Eyestalk removal has been shown to increase hemolymph MF titers in *Libinia emargianata* (Laufer *et al*., 1987a), *Homarus americanus* and *Orconectes virilis* (Tsukimura *et al*., 1989). Nagaraju *et al.* (2004) has reported that the weight of the mandibular organs increased significantly after eyestalk ablation in the crab *Oziotelphusa senex senex*. Eyestalk possesses both moult-inhibiting hormone and mandibular organ-inhibiting hormone (Reddy and Ramamurthi, 1999; Diwan, 2005; Chen *et al*., 2005). Castell *et al.* (1976) reported that eyestalk ablation may enhance the growth rate of *Homarus americunus.* Koshio *et al.* (1992) also demonstrated that growth of the freshwater prawn, *Macrobrachium rosenbergii* may be accelerated by unilateral eyestalk ablation. Ecdysteroids are steroidal moulting hormones that are responsible for the growth, development and reproduction of arthropods (Spaziani *et al*., 1989). Eyestalk ablation has been used since 1970 to improve the aquaculture production of *Penaeus* spp. larvae (Bray and Lawrence, 1992). Besides improving reproductive performance, there is evidence of other metabolic consequences that are not fully understood.

It was evident by the beginning of 20th century, that peripheral and Central Nervous System (CNS) tissue contained cells that were electrically excitable. These neurons, were believed to form the sole basis for integration of information within the brain by communicating at synapses (Sherrington, 1906). It is believed that they were the functional units of the brain, the integrative powers of which lead to behavior and ultimately consciousness.

Corresponding Author: Sartaj Ahmad Allayie, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608502, India

Studies on endocrinology aspects in crustaceans are studied in details from all over the world but such studies on *O. macrocera* crabs are few and are not carried out in detail hence the present investigation in this experiment was aimed at better understanding of endocrine roles of X-organ sinus gland complex and mandibular glands/organs in growth and behaviour in *O. macrocera.*

MATERIALS AND METHODS

Location: The crabs for the current study were captured from Pudupettai sandy shores adjacent to Department of CAS in Marine Biology, Annamalai University.

Captured method: Crabs are generally easy to collect and most often hand picking is very effective in intertidal and subtidal zones. The crabs for the current study were captured by digging the burrows at noon and in evening. Upon capturing the crabs, mature males, females and small crabs were placed in separate buckets. Little of wet sand was put in the buckets to offer substratum for crabs.

Acclimation: Upon arrival at unit all buckets were picked down, crabs were transferred to tubs containing sand in the form of heap and little amount of water at sides. The thickness of sand in the center was kept 10 and 5cm on sides. The crabs were stocked at a density of 5 crabs/tub. The optimum environmental parameters were maintained during experimental period (Salinity 10-34 ppt; Dissolved oxygen 4.2-5.8 mL L^{-1}; Temperature 22-30°C and pH 8.0-8.5).

Feeding: Crabs were fed with fresh fish, bivalves and crab muscles (Calappa) twice a day. The water was exchanged every two days in morning hours and left over feed and faecal matter was removed.

Ablation: Eyestalks were isolated from crabs by first cold anaesthetizing them for 30 min at -20°C. Bilateral eyestalk ablation was carried out by cutting the both eyestalks of experimental crabs below the eye using presterilized scissors. The wound was cauterized with a hot blunt forceps to prevent the loss of hemolymph.

Eyestalk extracts preparation: The exoskeleton of eyestalks was removed with dissecting instruments. The soft tissues isolated were homogenized and centrifuged at 15,000 rpm for 10 min at -4°C. The supernatant was collected in a pre-chilled microcentrifuge tube and homogenate re-extracted as before. The final supernatant containing the Eyestalk Extract (ESE) was aliquoted into cold microcentrifuge tubes and stored at -20°C until required.

Mandibular gland collection: Crabs selected for mandibular glands were first euthanized by submergence into salt water containing ice. The specimens were washed extensively with running water in order to remove the debris from it. Then the crabs were placed on a dissecting board one after another dorso-ventrally for the dissection. The hard cuticle region was cut open right from cephalo-thorax region using the fine scissor. The unwanted visceral parts and flesh were removed carefully to avoid damage of any mandibular glands. The animals were dissected in such a way to expose their mandibular glands out. Each gland was then cut and transferred by sterilized forceps into the pre-freezed centrifuged tube and stored at -20°C until use.

Mandibular gland extractions preparation: The isolated mandibular glands were homogenized and centrifuged at 15,000 rpm for 10 min at -4°C. The supernatant was collected in a pre-chilled microcentrifuge tube and homogenate re-extracted as before. The final supernatant containing the Mandibular Gland Extractions (MGE) was aliquoted into cold microcentrifuge tubes and stored at -20°C until required.

Injection: To observe the physiological effect of extractions (Eyestalk and Mandibular gland extracts), a single dose of gland extracts (equivalent to two glands) of the isolated supernatants were injected into each test crab. Injections were made through the arthrodial membrane at the base of the coxa of the third pair of walking legs.

Weight measurements: Weights were taken using electronic balance after blotting the animals on a filter paper. Wet weights of the live individuals in each experimental group were measured and recorded.

Behavior: For behavioural observations, the animals were grouped into control and ablated and were held in two troughs each filled with sand and seawater. Each behavioural activity was observed for four days. Observations were made daily for three hours (morning, midday and afternoon) throughout the duration of the experiment.

Indices measurement: Indices were determined using the standard formula:

$$\text{Index} = \frac{\text{Wet weight of the organ}}{\text{Wet weight of the crab}} \times 100$$

Data analysis: Data were treated statistically by one way Analysis of Variance (ANOVA) and t-Test (Two-Sample Assuming Unequal Variances) to test the significance. Results were considered significant if $p \leq 0.05$.

RESULTS

Growth due to Mandibular extraction: The results of 35 days growth trial are presented in Table 1 and 2. Survivals of control and injected crabs of both sexes were 100% and those of ablated, the survival was 60%. Injection of mandibular gland extracts increased the food consumption of both the sexes of *O. macrocera*. Among the three groups used, the maximum weight gain in both f the sexes was noticed in mandibular extract injected crabs ed with bivalves and lowest range of weight gain was noticed in crabs fed with crab muscles (Table 1 and 2). The result also showed that females grew faster than males throughout the experiment. The Percentage of Growth (PG) and Average Daily Growth (ADG) of *O. macrocera* were also more in mandibular extract injected females and male crabs fed with bivalves. To investigate the growth rate of crabs after injection, a comparison between control, mandibular gland extract injected and cheliped injected animals was made. One way analysis of variance done showed that growth rates among control, mandibular gland extract injected and cheliped injected of both sexes (Table 3, 4) differed

Table 1: Effect of mandibular gland extraction on growth of male *O. macrocera* fed with three different diets (Weight in grams)

Feed	Factors	Control	M-Injected	C-Injected
Fish	IW	20.13±0.03	20.11±0.02	20.12±0.02
	FW	22.51±0.34	23.40±0.10	22.28±0.38
	PG	10.54±1.37	14.02±0.26	10.84±1.51
	ADG	0.28±0.02	0.39±0.005	0.29±0.02
Bivalves	IW	20.40±0.34	20.14±0.03	20.50±0.34
	FW	23.27±0.48	23.90±0.20	23.20±0.58
	PG	12.27±3.20	15.72±0.08	11.68±3.63
	ADG	0.34±0.09	0.44±0.02	0.33±0.10
Crab muscles	IW	20.42±0.31	20.11±0.02	20.33±0.31
	FW	21.53±0.11	23.20±0.20	21.55±0.13
	PG	5.16±1.72	13.40±0.69	4.30±1.54
	ADG	0.14±0.05	0.38±0.01	0.15±0.05

IW: Initial weight, FW: Final weight, PG: Percentage growth, ADG: Average daily growth, M: Mandibular gland extract, C: Cheliped extract

Table 2: Effect of mandibular gland extraction on growth of female *O. macrocera* fed with three different diets (Weight in grams)

Feed	Factor	Control	M-Injected	C-Injected
Fish	IW	20.26±0.23	20.23±0.09	20.30±0.34
	FW	23.15±0.23	23.30±0.46	23.06±0.20
	PG	12.45±1.98	14.59±1.87	11.97±2.13
	ADG	0.35±0.05	0.41±0.05	0.34±0.06
Bivalves	IW	20.28±0.08	20.22±0.13	20.28±0.10
	FW	24.07±0.28	24.22±0.51	24.06±0.28
	PG	15.65±1.10	16.47±1.91	15.70±1.19
	ADG	0.44±0.02	0.46±0.05	0.44±0.03
Crab muscle	IW	20.19±0.08	20.30±0.27	20.21±0.09
	FW	22.80±0.49	23.30±0.40	22.60±0.36
	PG	11.36±2.23	12.48±2.37	10.53±1.81
	ADG	0.32±0.06	0.37±0.04	0.30±0.05

IW: Initial weight, FW: Final weight, PG: Percentage growth, ADG: Average daily growth, M: Mandibular gland extract, C: Cheliped extract

Table 3: Analysis of variance (Anova) for difference in growth among control, mandibular gland extract injected and cheliped injected male *O. macrocra* fed with three different feeds

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	2864.474	11	260.4067	88.71177	1.13E-16	2.216309
Within groups	70.4502	24	2.935425			
Total	2934.924	35				

Table 4: Analysis of variance for difference in growth among control, mandibular gland extract injected and cheliped injected female *O. macrocra* fed with three different feeds

Source of variation	SS	df	MS	F	P-value	F crit
Between Groups	2846.68	11	258.79	947.908	7.28E-29	2.21631
Within Groups	6.55227	24	0.27301			
Total	2853.24	35				

Table 5: Effect of eyestalk ablation and eyestalk extraction (ESE) on growth of male *O. macrocera* fed with three different diets (Weight in grams)

Feed	Factor	Control	Ablated	ESE-Injected	C-Injected
Fish	IW	10.40±0.29	10.30±0.22	10.70±0.32	10.90±0.15
	FW	11.70±0.22	12.70±1.24	11.80±0.01	12.30±0.05
	PG	11.60±2.10	18.70±6.30	9.20±3.03	11.36±0.86
	ADG	0.32±0.06	0.53±0.17	0.24±0.001	0.30±0.25
Bivalves	IW	10.50±0.15	10.40±0.29	10.50±0.78	10.53±1.03
	FW	12.70±0.58	14.10±0.73	11.80±0.27	13.78±0.58
	PG	16.80±4.01	26.30±4.48	11.20±0.34	23.80±2.59
	ADG	0.47±0.13	0.74±0.12	0.33±3.05	0.67±0.05
Crab muscles	IW	10.50±0.17	10.30±0.23	10.50±0.01	10.55±0.82
	FW	11.00±0.38	11.30±0.37	10.90±0.52	11.05±0.01
	PG	4.01±3.23	5.53±4.42	3.05±0.92	4.38±0.86
	ADG	0.11±0.09	0.23±0.09	0.07±0.05	0.16±0.04

IW: Initial weight, FW: Final weight, PG: Percentage growth, ADG: Average daily growth, M: Mandibular gland extract, C: Cheliped extract.

Table 6: Effect of eyestalk ablation and eyestalk extraction (ESE) on growth of female *O. macrocera* fed with three different diets (Weight in grams)

Feed	Factor	Control	Ablated	ESE-Injected	C-Injected
Fish	IW	10.53±0.15	10.55±0.21	10.53±0.26	10.50±4.02
	FW	11.96±0.30	12.70±0.13	11.20±0.62	12.01±7.50
	PG	11.92±3.04	16.52±9.10	6.58±1.81	13.52±5.09
	ADG	0.33±0.08	0.46±0.26	0.20±2.67	0.38±3.58
Bivalves	IW	10.43±0.05	10.45±0.07	10.50±0.62	23.94±0.28
	FW	13.40±0.52	14.13±1.13	12.98±3.34	24.96±0.45
	PG	22.05±3.17	26.67±6.29	19.35±3.61	3.83±1.13
	ADG	0.62±0.08	0.75±0.17	0.59±0.76	0.10±0.02
Crab muscles	IW	10.46±0.05	10.40±0.01	10.42±0.05	10.48±0.05
	FW	11.43±0.32	12.05±1.06	11.37±1.53	11.90±2.34
	PG	8.40±2.41	13.35±7.62	7.82±0.02	12.25±1.87
	ADG	0.23±0.07	0.37±0.21	0.22±1.32	0.34±3.02

IW: Initial weight, FW: Final weight, PG: Percentage growth, ADG: Average daily growth, M: Mandibular gland extract, C: Cheliped extract

Table 7: Analysis of variance for difference in growth among control, eyestalk extraction (ESE) injected and cheliped injected male *O. macrocra* fed with three different feeds

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	1635.611	11	148.6919	26.79092	4.52E-14	2.066608
Within groups	199.803	36	5.550084			
Total	1835.414	47				

Table 8: Analysis of variance for difference in growth among control, eyestalk extraction (ESE) injected and cheliped injected female *O. macrocra* fed with three different feeds

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	1629.03	11	148.094	8.79513	2.65E-07	2.06661
Within groups	606.173	36	16.8381			
Total	2235.2	47				

significantly ($p<0.005$). The results showed that the mandibular gland extract injected crabs had higher mean wet body weight than the control and injected animals throughout the experimental period. Feed efficiency also showed a significant trend in control, mandibular gland extract injected and cheliped injected crabs in both sexes.

Growth due to eyestalk factors: The results of 35 days growth trial are presented in Table 5 and 6. Eye stalk ablation had a significant effect on weight gain in *O. macrocera* fed with three different feeds. In both sexes weight gain of eye stalk ablated *O.macrocera* fed with bivalves was significantly better than the ablated crabs fed with crab muscles and fishes. Survival of injected crabs of both sexes was 100% and those of ablated males were 80 and 60% in ablated females. Injection of eye stalk extracts significantly decreased the food consumption rate of crabs and was found to have a negative effect on growth of these animals. To investigate the growth rate of crabs after ablation and injection, a comparison between injected, control and ablated animals was made. One way analysis of variance done showed that growth rates among control, ablated and injected (Table 7, 8) crabs differed significantly ($p<0.005$). The results showed that the ablated crabs had higher mean wet body weight than the control and injected animals throughout the experimental period. The Percentage of Growth (PG) and Average Daily Growth (ADG) of *O. macrocera* were also more in ablated females and male crabs fed with bivalves. It is also clear from Table 7 and 8 that injected animals had

Table 9: Analysis of variance for difference in indices of heart, mandibular gland and ovary of *O. macrocera*

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	1.77627	2	0.88813	88.9864	1.18E-06	4.2565
Within groups	0.08983	9	0.00998			
Total	1.86609	11				

Table 10: Effect of eyestalk ablation on some of the behavioral changes in *O. macrocera*

	Control (days)				Eyestalk ablated (day)				
Parameters	1day	2day	3day	4day	1day	2day	3day	4day	P-value
Feed intake (g)	2±0.03	1.5±0.21	1.8±0.001	1.5±0.02	3.5±0.71	2±0.002	2.3±0.01	2.3±0.001	p<0.05
Water intake (mL)	5±0.06	4±1.03	5±0.08	5.3±0.68	8±1.51	7.5±0.05	6±0.0003	4.5±0.80	p = 0.05
Forward movements	0±0	0±0	0±0	0±0	5±1.32	3±0.57	2±0.002	2±0.11	p<0.05
Number of burrows made	1±0.31	1±0.48	0±0	1±0.60	0±0	0±0	0±0	0±0	p<0.05
Air bowls through gills	150±1.24	130±0.001	140±0.85	156±1.85	200±0.01	195±0.12	150±1.54	140±0.12	p = 0.08
Escape movement by sound	15±0.1	13±0.82	10±0.69	7±0.4	0±0	0±0	0±0	0±0	p<0.05
Escape movement by touch	15±0.43	15±0.02	15±0.001	10±1.25	0±0	0±0	0±0	0±0	p<0.05

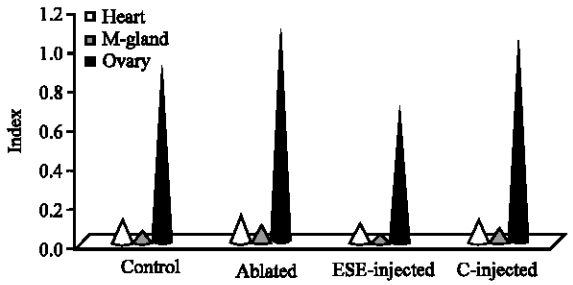

Fig. 1: Showing the heart, ovary and mandibular gland indices of control, ablated, ESE-injected and C-injected of *O. macrocera*

lowest mean wet body weight than the control and ablated animals throughout the experimental period. The wet weight of initial and final weight gain, percentage growth and average daily growth of crabs are shown in Table 5 and 6. Among the crabs, the maximum growth in both the sexes was noticed in ablated female crabs. Feed efficiency also showed a significant trend in control, ablated, eye stalk injected and cheliped injected crabs in both sexes.

Heart, mandibular glands and ovary indices: The weight of the heart, mandibular glands and ovary increased with increasing body weight of crabs. The results clearly showed that bilateral eyestalk ablation significantly increased heart, mandibular glands and ovarian indices in *O. macrocera* crabs. For this experiment, crabs in the weight range of 33.45±0.32 g were used. The mean weight

of heart, mandibular glands and ovaries increased progressively as the animal approached to ecdyces, whereas the weight of heart, mandibular glands and ovary between ablated and cheliped injected crabs showed no significant variations (Fig. 1). It is clear from the data that the injected and control crabs had low heart, mandibular gland and ovarian indices than the ablated animals throughout the experimental period. It is also clear that eyestalk ablation had highest effect on ovarian growth than M-organs and heart. One way analysis of variance done showed that growth indices of heart, mandibular glands and ovaries between control, ablated and cheliped injected crabs (Table 9) differ significantly ($p<0.05$).

Behaviour changes due to eyestalk ablation: The various behavioural changes of *O. macrocera* are presented in Table 10. The eyestalk ablation has altered the various behaviours of this crab. Student t- test done showed that feed intake, water intake, forward movements, number of burrows made, escaping movements by sound and escaping movements by touch of these crab between control and ablated (Table 10) differed significantly ($p=0.05$). However, behavior like Air bowls through gills between control and ablated crabs differed insignificantly ($p=0.08$).

DISCUSSION

The present study demonstrated that eyestalk ablation and injection of mandibular gland extraction accelerated the growth of *O. macrocera* and also improved their feed utilization efficiency. Recently growth and feed efficiency were already reported to be improved in many ablated and M-injected animals (Koshio *et al*., 1992; Tamone and Chang, 1993; Chang *et al*., 1993; Riddiford, 1994; Soundarapandian *et al*., 1995; Wyatt and Davey, 1996; Homola and Chang, 1997; Murugadass *et al*., 1988; Allayie *et al*., 2010a; b). But the growth of the mud crab *Rhithropanopeus harrisii* and the red king crab *Paralithodes camtchatica* was not enhanced due to eyestalk ablation (Costlow, 1966; Molyneaux and Shirley, 1988). The present study confirms the influence of eyestalk and mandibular gland crude extraction and protein rich diet on the growth of *O. macrocera.*

The greater weight gains of eyestalk ablated and M-injected crabs fed with three different feeds are in agreement with the findings of Sanjeevraj *et al.* (1996), Molyneaux and Shirley (1988), Koshio *et al.* (1992), Riddiford (1994), Wyatt and Davey (1996), Homola and Chang (1997) and Allayie *et al.* (2010a). Bivalves fed ablated and M-injected female crabs grow faster than those fed with fish and crab muscles. The weight gain of eyestalk-ablated and M-injected crabs in this study may be attributed to the reduced production of MIH (moult inhibiting hormone) factors from the eyestalk and increased production of Ecdysone (E) by Y-organs. The moult inhibiting hormone is produced in the eyestalk and stored in the sinus gland and the molting hormone is produced in Y organ. When the eyestalks along with sinus glands are ablated and mandibular gland extractions are injected, the moult inhibiting hormonal level decreases while as moulting hormonal level increases in haemolymph (Allayie *et al*., 2010a, b). The weight gain does not appear to be due to mere accumulation of water but subsequent tissue synthesis which replaces the water, absorbed during ecdysis (Vijayakumaran and Radhakrishnan, 1984). In the present study, eyestalk ablated and M-injected animals consumed more food than intact control and C-injected animals. The high food requirements of the ablated and M-injected crabs might be due increase demand of energy after ablation.

In this study, eyestalk ablation had a significant effect on growth of heart, mandibular organs and ovaries of *O. macrocera*. Sanjeevraj *et al.* (1996) in a study showed that moulting and growth rates in eyestalk-ablated prawns were higher than that of nonablated ones. Hinsch (1977) and Le Roux (1980, 1983) also observed hypertrophy of the MO in response to eyestalk ablation in *Libinia emarginata*, *Palaemonetes varians* and *Pisidia logicornis*. Eyestalk removal significantly increased the levels of MF in the hemolymph of spider crab, *L. emarginata* (Laufer *et al*., 1987a), American lobster *Homarus americanus* (Tsukimura and Borst, 1992) and *Orconectes virilis* (Tsukimura *et al*., 1989). A Mandibular Organ Inhibiting Hormone (MOIH) has been isolated from the sinus gland (Liu and Laufer, 1996), sequenced (Liu *et al*., 1997a) and cloned (Liu *et al*., 1997b; Wainwright *et al*., 1996). MOIH is a member of the crustacean hyperglycemic hormone family and it shares 50-60% sequence similarity with the members of this group, particularly with gonad inhibiting hormone and moult inhibiting hormone (Wainwright *et al*., 1996). MO weight increased significantly during reproduction in the crab *O. senex senex*. Hinsch (1980) observed that the increase in gonad size is stimulated by MO implantation in *L. emarginata*. Using *P. clarkii*, Laufer *et al.* (1998) demonstrated that MF promotes ovarian maturation. Coincubation of MO with Y-organs stimulated ecdysteroid secretion in Cancer magister *in vitro* (Tamone and Chang, 1993) also indicates that the MO was responsible for the stimulation of a precocious moult, via an invivo stimulation of secretion of ecdysteroids from Y-organs. In the crab *O. senex senex* also, the weight of the MO increased as the crab approached ecdysis. Similar results were observed for the spider crab *L. emarginata* (Laufer *et al*., 1987b), crayfish *P. clarkia* (Landau *et al*., 1989) and fresh water prawn *M. rosenbergii* (Sagi *et al*., 1991). Thus the findings of the present study corroborate the above works in the sense that eyestalk factors and hormones of Y-organs are responsible for growth of heart, mandibular organs and ovaries in *O. macrocera*. Eyestalk ablation removes the source of inhibition of the MO, heart and ovaries, resulting in increased MF and ecdysteroid levels in the hemolymph. Tamone and Chang (1993) reported that ecdysteroid secretion was stimulated by MF in Cancer magister. Thus, ablation of the eyestalks leads to enhanced ecdysteroid secretion by Y-organs, an increase in the ecdysteroid titer and precocious molting while injection of eyestalk extract or synthetic MIH into eyestalk-ablated animals lowers the ecdysteroid titer and delays molting and growth (Tamone *et al*., 2005; Allayie *et al*., 2010a).

The removal of eyestalks caused a decreased secretion of moulting inhibiting hormone and increases the secretion of ecdysone which may be responsible for growth of heart, Mandibular Organs (MO) in *O. macrocera*. When the extraction of moulting inhibiting hormone was injected in to the E-injected animals; it inhibited the secretion of ecdysone by Y-organ and MF by MO, lengthened their molting process and decreases their growth (Rangarao, 1965; Allayie *et al*., 2010a). Whether MF is directly involved in the regulation of moulting or acts by stimulating the ecdysteroid production by the Y-organs in the crab *O. macrocera* needs further study.

Taken together with what now appears to be a multi-functional role for MF, the present study suggest that like many other crustacean hormones, MF has many functions-involved in the regulation of moulting, metabolism and reproduction, etc. Further studies are needed to confirm the other physiological roles of MF in crustaceans. Since it is known that relatively large MOs are present during moulting and reproduction (Hinsch, 1977; Yudin *et al*., 1980), it is possible that MOs also control related processes such as regulation of salt and water balance, mineral metabolism, vitellogenin synthesis, etc.

Further, observation recorded in the present study clearly demonstrated that eyestalk ablation induces behavioural changes in *O. macrocera*. One of these changes is hyperactive state of the crab after eyestalk ablation that may be due to removal of X-organ-sinus gland complex and in hyperstate, crab needs more oxygen and food, Hence the crab takes more water and food to meet extra demand of oxygen and energy. Other parameters also show that these behavioural changes probably may occur due to the deficiency of hormonal supply from X-organ sinus gland complex.

The results also suggested that the eyestalks are sites for the production and/or release of a hormonal factor that modulates the activity of CNS pathways controlling various behaviours. However, since the ligation/ablation procedure also removes a substantial portion of the crab's nervous system (i.e., ganglia located in the eyestalks). Thus, ablation experiments demonstrated that in several crustacean groups, the proximal eyestalk ganglia are important in a variety of behavior patterns.

This is an exciting time for research on crustacean endocrinology. There is increased interest in the topic due to aquaculture applications and the focus on crustacean as keystone species in aquatic environments. Shell fish endocrinology has been development without sufficient understanding of basic physiology of species of interest. The more understanding of the hormonal system processes that under like crop performance leads to the improvement and optimization of aquaculture production.

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