



Metabolic effects of sinus and mandibular glands of the ghost crab, *Ocypode macrocera*

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Abstract: The influence of sinus and mandibular glands on the metabolism of the *Ocypode macrocera* was studied. Carbohydrate content of heart, hepatopancreas, muscles and hemolymph of eyestalk extract injected crabs was significantly higher than that of control, ablated and cheliped injected crabs ($P < 0.05$). Protein content of heart, gill, muscles and hemolymph of ablated crabs was also significantly higher than that of control, eyestalk extract injected and cheliped injected crabs ($P < 0.05$). The result showed that the lipid content in the hepatopancreas (2.46 mg/g) and ovary (3.24 mg/g) of ablated animals was significantly higher when compared to control, eyestalk extract injected and cheliped injected crabs. Similarly, carbohydrate content of gill, hepatopancreas and muscles of mandibular injected crabs was significantly higher than that of control and cheliped injected crabs ($P < 0.05$). Protein content of heart, gill, hepatopancreas, muscles and hemolymph of mandibular injected crabs was significantly higher than that of control and cheliped injected crabs. The result showed that mandibular extract injection decreased the lipid content of hepatopancreas and muscles.

Key words: Sinus glands; mandibular glands; metabolism; carbohydrate; Protein and lipid

Introduction

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 moult cycle. The eyestalk hormones have regulative (positive) influence on carbohydrate, nitrogen and lipid metabolism in crustaceans (Highnam and Hill, 1979) which were found to vary with times and species (Vernberg and Vernberg, 1974; Madyasthan and Rengnekhar, 1976; Soundarapandian, 1996; Murugesan *et al.*, 2008). Crustacean hyperglycemic hormone (CHH) is a peptide that regulates blood sugar, particularly glucose, in crustaceans (Quackenbush, 1986). The hormone is unique among crustaceans in that it is taxon-specific.

The X-organ sinus gland complex, located in the eyestalks, is the principal neuroendocrine gland in crustaceans (Beltz, 1988; Chang, 1992). In this gland, hormones are synthesized, stored and secreted to the hemolymph to regulate several metabolic processes (Chang, 1992). The most studied processes are vitellogenesis (Fingerman, 1995; Palacios *et al.*, 1999), food intake, digestion,

and nutrient transport (Rosas *et al.*, 1995), moulting (Chang and O'Connor, 1988), metabolism of lipids (Teshima *et al.*, 1988; Santos *et al.*, 1997), regulation of glucose and proteins in hemolymph (Santos and Keller, 1993a,b; Teshima *et al.*, 1988), hydromineral balance, regeneration and pigment production (Keller and Sedlmeier, 1988). Other roles for CHH have been identified and include: amylase secretion (Sedlmeier, 1988), osmoregulation (Charmantier-Daures *et al.*, 1994; Spanings-Pierrot *et al.*, 2000), moult regulation (Liu *et al.*, 1997; Chang *et al.*, 1999), immune response (Lorenzon *et al.*, 1997) and vitellogenesis (De Kleijn *et al.*, 1998; Khayat *et al.*, 1998). The mandibular glands synthesize and secrete methyl farnesoate (MF). MF plays important role in the regulation of crustacean morphogenesis, metamorphosis, reproduction and moulting.

Studies on endocrinological aspects in crustaceans are studied in details from all over the world but such studies on *O. macrocera* are few and are not carried out in detail hence after reviewing the extensive body of work related to animal endocrinology, research in this experiment was directed to understand better the endocrine roles of X-organ sinus gland complex and mandibular glands in metabolism of *O. macrocera*.

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Materials and Methods

Location

The crabs for the current study were captured from Pudupettai sandy shores (Fig. 1), adjacent to Department of CAS in Marine Biology, Annamalai University.



Fig. 1: Showing Pudupettai sandy shore.

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 10cm 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; Temperature 22- 30°C and pH 8.0-8.5).

Feeding

Crabs were fed with fresh fish 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 minutes at -20°C. Bilateral eyestalk ablation was carried out by cutting the both eyestalks of experimental crabs by using presterilized scissors. The isolated eyestalks, exoskeleton intact, were stored at -20°C. The wound was cauterized with some 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 4,000 rpm

for 10 minutes 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 micro centrifuge 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 gland. 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-frozen 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 minutes 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 extractions), 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 arthroal membrane of the walking legs.

Tissue collection

Crabs selected for extraction of tissues were first euthanized by submergence into salt water containing ice. The tissues were then removed from the crabs by cutting along the dorsal surface just below the cuticle to ensure no perforation of tissue occurred. The incision was then carefully opened and muscle tissues, gill, heart, ovary and hepatopancreas tissues were removed. After extraction tissues were placed in labeled Petri dishes and transferred to a freezer until required for analysis.

Hemolymph collection

Hemolymph was collected from the crabs through the arthroal membrane covering the articulated base of the walking legs. The area was swabbed or flushed with 70% ethanol to sterilise the surface and a 25-gauge sterile hypodermic needle and syringe was used to extract around 1 ml of

hemolymph from each crab. The hemolymph was collected in sterilized pre-chilled test tubes contain anticoagulants and stored at -20°C until required.

Determination of total protein, carbohydrate and lipid contents

The protein, carbohydrate and lipid contents were estimated by adopting the standard methods of the Biuret method of Raymont *et al.*, (1964), Dubois *et al.*, (1956) and Folch *et al.*, (1956), respectively.

Weight measurements

Weights were taken using electronic balance after blotting the animal and tissues on a filter paper. Wet weights of body tissues, organs and live individuals in each experimental group were measured and recorded.

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

Influence of sinus glands on biochemical studies.

Carbohydrate

Carbohydrate content of heart, hepatopancreas, muscles and hemolymph of eyestalk extract injected crabs was significantly higher than that of control, ablated and cheliped injected crabs (Fig.2). The result also showed that eyestalk ablation decreased the carbohydrate content of these crabs. One way analysis of variance done showed that carbohydrate content among control, ablated, eyestalk extract injected and cheliped injected crabs ($P < 0.05$, Table 1) differed significantly.

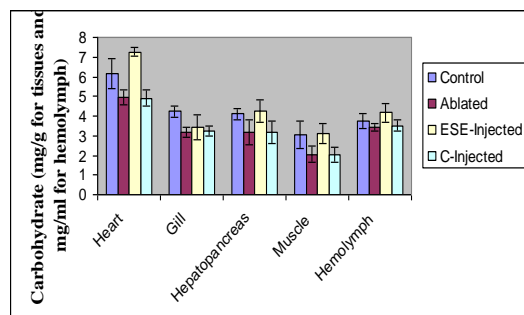


Fig. 2: Showing the carbohydrate content of heart, gill, hepato-pancreas, muscle and hemolymph of control, ablated, ESE-injected and C-injected of *O. macrocera*

Table 1: Analysis of variance for difference in carbohydrate content among control, ablated, eyestalk extract injected and cheliped injected *O. macrocera*.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	22.8941	4	5.72353	12.4603	0.00012	3.05557
Within Groups	6.8901	15	0.45934			
Total	29.7842	19				

Protein

Protein content of heart, gill, muscles and hemolymph of ablated crabs was significantly higher than that of control, eyestalk extract injected and cheliped injected crabs. The result showed that eyestalk extract injection had decreased the protein content of these tissue samples (Fig. 3). One way analysis of variance done showed that protein content among control, ablated, eyestalk extract injected and cheliped injected crabs (Table 2) differed significantly ($P < 0.05$).

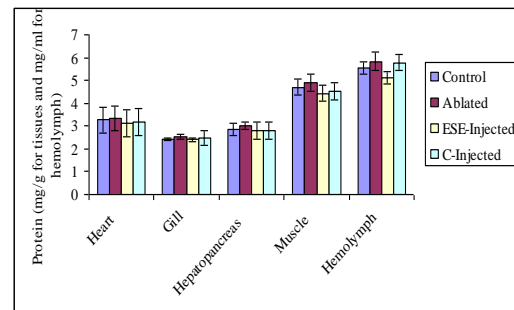


Fig. 3: Showing the protein content of heart, gill, hepatopancreas, muscle and hemolymph of control, ablated, ESE-injected and C-injected of *O. macrocera*

Table 2: Analysis of variance for difference in protein content among control, ablated, eyestalk extract injected and cheliped injected *O. macrocera*.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	27.2205	4	6.80513	201.375	7.72E-13	3.05557
Within Groups	0.5069	15	0.03379			
Total	27.7274	19				

Lipid

The result showed that the lipid content in the hepatopancreas (2.46 mg/g) and ovary (3.24 mg/g) of ablated animals was significantly higher when compared to control, eyestalk extract injected and cheliped injected crabs (Fig. 4). Whereas the lipid content of heart, muscles and hemolymph of eyestalk extract injected crabs was significantly higher than that of control, ablated and cheliped injected crabs. The result also showed that eyestalk ablation decreased the lipid content of these crabs except in hepatopancreas and ovary. One way analysis of variance done showed that lipid content among control, ablated, eyestalk extract injected and cheliped injected crabs (Table 3) differed significantly ($P < 0.05$).

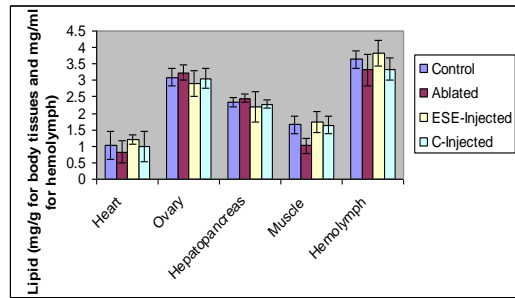


Fig. 4: Showing the lipid content of heart, ovary, hepato-pancreas, muscle and hemolymph of control, ablated, ESE-injected and C-injected of *O. macrocera*.

Table 3: Analysis of variance for difference in lipid content among control, ablated, eyestalk extract injected and cheliped injected *O. macrocera*

Source of variation	SS	Df	MS	F	P-value	F crit
Between Groups	17.575	4	4.39375	96.2697	1.66E-10	3.05557
Within Groups	0.6846	15	0.04564			
Total	18.2596	19				

Influence of mandibular glands on biochemical studies.

Carbohydrates: Carbohydrate content of gill, hepatopancreas and muscles of M-injected crabs was significantly higher than that of control and cheliped injected crabs (Fig. 5). The result also showed that there is insignificant variation in the carbohydrate content of heart and hemolymph of M-injected and cheliped injected of these crabs. One way analysis of variance done showed that carbohydrate content among control, M-injected and cheliped injected crabs (Table 4) differed significantly.

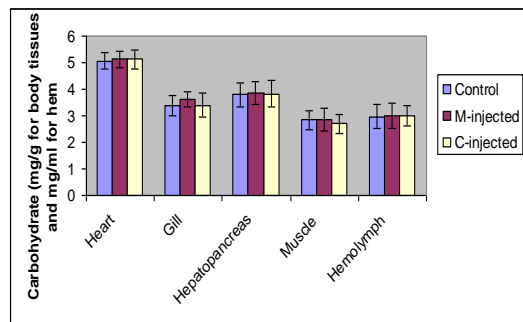


Fig. 5: Showing the carbohydrate content of heart, gill, hepatopancreas, muscle and hemolymph among control, mandibular gland extract injected and cheliped injected *O. macrocera*.

Table 4: Analysis of variance for difference in carbohydrate content among control, mandibular gland extract injected and cheliped injected *O. macrocera*.

Source of Variator	SS	Df	MS	F	P-value	F crit
Between Groups	9.97629	4	2.49407	479.015	2.25E-11	3.47805
Within Groups	0.05207	10	0.00521			
Total	10.0284	14				

Protein

Protein content of heart, gill, hepatopancreas, muscles and hemolymph of M-injected crabs was significantly higher than that of control and C-injected crabs (Fig. 6). One way analysis of variance done showed that protein content among control, M-injected and C-injected crabs (Table 5) crabs differed significantly.

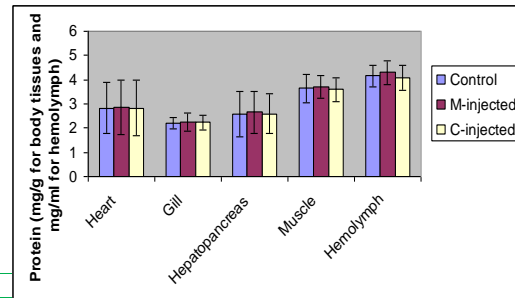


Fig. 6: Showing the protein content of heart, gill, hepato-pancreas, muscle and hemolymph among control, mandibular gland extract injected and cheliped injected *O. macrocera*.

Table 5: Analysis of variance for difference in protein content among control, mandibular gland extract injected and cheliped injected *O. macrocera*.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7.54944	4	1.88736	516.613	1.55E-11	3.47805
Within Groups	0.03653	10	0.00365			
Total	7.58597	14				

Lipid: The result showed that the lipid content of heart, ovary, and hemolymph of M-injected crabs was significantly higher than that of control and cheliped injected crabs (Fig. 7). It is also clear that mandibular extract injection decreased the lipid content of hepatopancreas and muscles. One way analysis of variance done showed that lipid content among control, M-injected and C-injected crabs (Table 6) differed significantly.

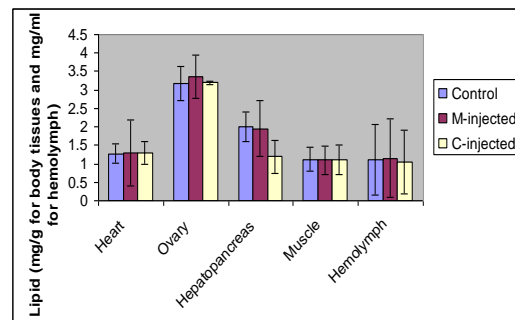


Fig. 7: Showing the lipid content of heart, ovary, hepato-pancreas, muscle and hemolymph among control, mandibular gland extract injected and cheliped injected *O. macrocera*.

Table 6: analysis of variance for difference in lipid content among control, mandibular gland extract injected and cheliped injected *O. macrocera*.

Source of Variation	SS	Df	MS	F	P-value
Between Groups	9.76229	4	2.44057	55.9252	8.30E-07
Within Groups	0.4364	10	0.04364		
Total	10.1987	14			

Discussion

Biochemical constituents in animals are known to vary with season, size of the animal, stage of maturity, temperature and availability of food etc. The results reported in the present study indicate that the eyestalk extraction and mandibular extraction plays a major role in regard to controlling of carbohydrate, protein and lipid in the *O. macrocera* during the moult cycle. The eyestalk extraction seems to influence the various body tissues and organs. The results of these investigations are more or less in agreement with earlier studies of Winget *et al.*, 1977; Rao *et al.*, 1991; Smullen and Bentley, 1994 and Sudhakar *et al.*, 2009. In most of the above, hypoglycemia occurred as a result of eyestalk ablation. Hence, the investigators postulated the presence of a hyperglycemic factor in the eyestalk. The results of the present study on *O. macrocera* support this view, showing that the eyestalk extractions contain CHH that plays a prominent role in controlling carbohydrate metabolism in the crab. In the same way carbohydrate content of these tissues were also increased by mandibular injection. This increase in carbohydrate content may be due to positive stimulus of methyl farsonate present in mandibular glands that might have increased the CHH level in *O. macrocera*.

Protein content was higher in the ablated and mandibular gland extract injected *O. macrocera* than those of other crabs of this experiment. These differences in the protein contents are statistically highly significant. Thus, the protein content in the present study is in agreement with other studies (Radhakrishnan and Natarajan, 1979; Paulson and Skinner, 1988; Sheen and Abramo, 1991; Soroka *et al.*, 1993; Balasubramanian and Suseelan, 2001; Murugesan *et al.*, 2008). Chang and O'Connor (1983) reported that it is 20-hydroxysone that is involved in the regulation of protein in the presence of other factors. Thus, the findings of this experiment are also in this agreement.

In the present study, lipid content of the ovary and hepatopancreas of ablated *O. macrocera* crabs was higher than control, E-injected and C-injected crabs. This was consistent with the findings of Palacios *et al.*, (1999) who showed ablation caused an increase in the number of lipid droplets in the ovaries of *P. vannamei*. This increase in lipid content in ovaries and hepatopancreas may be due to vitellogenesis inhibiting hormone (VIG), one of

the eyestalk inhibiting hormone which is known to regulate synthesis and accumulation of egg yolk (vitellin) and its precursors (vitellogenin) in both the ovary and hepatopancreas respectively (Thurn and Hall, 1999; Okumura, 2004; Tsutsui *et al.*, 2005; Coman *et al.*, 2006). Similarly, the mandibular gland extraction has also increased the lipid content of heart, ovary and hemolymph. This increase may be due to the increase in MF along with some other hormone that decreased the lipid content in hepatopancreas. To find and isolated this hormone needs further study.

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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