Concurrent Effects of Eyestalk Ablation and Fluoxetine on the Nutrient Depositon During Ovarian Development in a Fresh Water Prawn, *Machrobrachium lamarrei lamarrei*



R. Eswaralakshmi, J. Jayanthi and M.G. Ragunathan Department of Advanced Zoology and Biotechnology, Guru Nanak College, Chennai - 600 042, INDIA

Abstract : The organic compounds like protein, carbohydrate and lipid have been quantitatively estimated in tissues such as haemolymph, hepatopancreas, muscle and ovary after injecting fluoxetine to an eyestalk ablated female fresh water prawn, *Macrobrachium lamarrei lamarrei* and all the tissues show fluctuations in their contents due to fluoxetine treatment. The results show that the more deposition of organic contents in the ovary of the fluoxetine treated prawn due to stimulatory action of fluoxetine.

Key words : Bilateral eyestalk ablation, prawns, hepatopancreas, muscle, haemolymph, ovary, Biochemical contents

Introduction

Reproductive activity is the cyclical process, in which biochemical composition of the animals occurs. The hepatopancreas is an important site of intermediary metabolism in crustaceans and may also be the major source of vitellogenin precursors. There is an evidence which shows that the eyestalk hormones in some crustaceans may regulate the accumulation and subsequent liberation of organic reserves in the hepatopancreas. An eyestalk-ablation study further reveals the depletion of reserve substance and degeneration of the hepatopancreas (Kulkarni and Nagabhushanam, 1979). Meera et al., 2006(b) have reported the gonadal maturation and biochemical deposition due to eyestalk ablation and fluoxetine treatment in a brackishwater crab, Uca lactea annulipes. From the literature survey it is clear that mobilization of organic reserves during

enhanced and ovarian development due to fluoxetine has not done in an eyestalk ablated fresh water prawn; hence the present investigation has been undertaken.

Materials and Methods

The stage I intermoult females Macrobrachium lamarrei lamarrei in the present study were collected from a lake in Vattampakam, which is 40 kms away from the Chennai city. After acclimatization 60 prawns were selected and their eyestalks were ablated and were divided into three Groups (A, B and C) of twenty each. Group A served as the initial control and was sacrificed and tissues were analyzed for biochemical content on the first day itself. Group B was an additional control ran concurrently with Group C. Group B received physiological saline alone at a dose of 25 µl and the Group C received fluoxetine of 25 µl/crab at 1 mM conc. Injections to the two

* **Corresponding Author :** M.G. Ragunathan, Department of Advanced Zoology and Biotechnology, Guru Nanak College, Chennai - 600 042 (India); E-mail : *mgragunathan@yahoo.co.in* Groups namely B and C were administered on the 1st, 5th and 10th days of the experiment. Fluoxetine is a 5-Hydroxytrypamine reuptake inhibitor.

On the 15th day the prawns were sacrificed and the tissues such as haemolymph, hepatopancreas, muscle and ovary were taken for biochemical analysis. The different tissues were stored in icebox until further biochemical analysis of protein carbohydrates and lipids were estimated by following standard methods for protein (Lowry *et al.*, 1951), Carbohydrate (Roe, 1955) and lipids (Barnes and Blackstock, 1973). The results obtained from the biochemical analysis were statistically analysed.

Results

The results were statistically analyzed, it shows that protein levels in the haemolymph and ovary shows significant (P<0.01) increase in the group B when compared with the group A then there is a significant (P<0.01) increase in the group C when compared with the group B. The protein levels in the hepatopancreas and muscle shows significant (P<0.01) decrease in the group B when compared with the group A and further significant (P<0.01) decrease in the group C when compared with the group B (Table 1).

When the results were statistically analyzed, the hepatopancreas and muscle shows significant (P<0.01) decrease in carbohydrate level in the group B when compared with the group A further there is a significant (P<0.01) decrease in the group C when compared with the group B. The amount of carbohydrate content in the haemolymph and the ovary of the group B shows significant (P<0.01) increase in the carbohydrate content when compared with the group A then there is a significant (P<0.01) increase in the group C when compared with the group B (Table 2).

The lipid levels in the haemolymph and ovary shows significant (P<0.01) increase in the group B when compared with the group A then there is a significant increase in the group C when compared with the group B. The lipid levels in the hepatopancreas and muscle shows significant (P<0.01) decrease in the group B when compared with the group A, further, there is a significant (P<0.01) decrease in the group C when compared with the group B (Table 3).

Tissues	Group A (Untreated)	Group B (Saline)	Group C (Fluoxetine)		
Haemolymph	11.9 ± 0.229	$*20.56 \pm 0.554$	$*24.85 \pm 0.237$		
Hepatopancreas	21.24 ± 0.335	$*17.11 \pm 0.202$	$*10.04 \pm 0.977$		
Muscle	15.40 ± 0.314	$*12.32 \pm 0.499$	$*10.25 \pm 0.417$		
Ovary	31.9 ± 0.371	$*35.29 \pm 0.404$	$*42.57 \pm 0.300$		

 Table 1 : Total Protein Levels in the Eyestalk Ablated – Fluoxetine treated

 Macrobrachium lamarrei lamarrei

Each value is mean \pm SEM of 10 samples, expressed as mg/gm wet tissue and mg/ml haemolymph. * P < 0.01 Group A vs B vs C

Tissues	Group A (Untreated)	Group B (Saline)	Group C (Fluoxetine)		
Haemolymph	2.574 ± 0.506	$*4.47\pm0.225$	$*7.06 \pm 0.220$		
Hepatopancreas	7.295 ± 0.463	$*5.56\pm0.264$	$*2.86\pm0.240$		
Muscle	6.15 ± 0.405	$*4.23\pm0.208$	$*2.96\pm0.351$		
Ovary	2.797 ± 0.233	$*4.7\pm0.372$	$*6.354 \pm 0.377$		

 Table 2 : Total Carbohydrate Levels in the Eyestalk Ablated - Fluoxetine treated

 Macrobrachium lamarrei lamarrei

Each value is mean \pm SEM of 10 samples, expressed as mg/gm wet tissue and mg/ml haemolymph. * P < 0.01 Group A vs B vs C

 Table 3 : Total Lipid Levels in the Eyestalk Ablated - Fluoxetine Treated

 Macrobrachium lamarrei lamarrei

Tissues	Group A (Untreated)	Group B (Saline)	Group C (Fluoxetine)
Haemolymph	9.16 ± 0.503	$*13.61 \pm 0.339$	$*19.85 \pm 0.275$
Hepatopancreas	17.81 ± 0.346	$*14.98\pm0.315$	$*12.34 \pm 0.378$
Muscle	13.67 ± 0.399	$*10.12 \pm 0.525$	$*6.48\pm0.186$
Ovary	20.38 ± 0.639	$*24.59\pm0.714$	$*30.17 \pm 0.598$

Each value is mean \pm SEM of 10 samples, expressed as mg/gm wet tissue and mg/ml haemolymph. * P < 0.01 Group A vs B vs C

Discussion

In the present investigation the eyestalk ablated - Fluoxetine treated prawns shows significant decrease in the protein level of the hepatopancreas and muscle when compared with their experimental controls. Similarly ovary and haemolymph of the eyestalk ablated - Fluoxetine treated group shows significant increase in the protein level when compared with their experimental controls.

In this present investigation the hepatopancreas and muscle of eyestalk ablated - Fluoxetine treated prawns shows significant decrease in the carbohydrate levels when compared with their experimental controls. Similarly, ovary and haemolymph of the eyestalk ablated -Fluoxetine treated group shows significant increase in the carbohydrate levels when compared with their experimental controls.

It has been reported that eyestalk ablation accelerated the development of female gonads, in parallel with transportation of reserves from the hepatopancreas to the ovaries through the haemolymph (Wouters *et al.*, 2001; Arcos *et al.*, 2003; Meera *et al.*, 2006b; Meera *et al.*, 2007).

The potential importance of lipid as an essential reserve which facilitates a sustained activity of gametogenesis during breeding season in Macrobrachium lamarrei lamarrei was evident from the total amount of lipid stored in ovary and hepatopancreas (Kulkarni et al., 2002). In present investigation this the hepatopancreas and muscle of eyestalk ablated - Fluoxetine treated prawns shows significant decrease in the lipid level when compared with their experimental controls. Similarly, ovary and haemolymph of the eyestalk ablated - Fluoxetine treated group shows significant increase in the lipid level when compared with their experimental controls.

Meera *et al.* (2006a, b) and Ragunathan *et al.* (2007) have reported a significant increase in the organic contents from the hepatopancreas to ovary during gonadal development in the fiddler crab, *Uca lactea annulipes* due to Fluoxetine in nonablated, eyestalk ablated and also in light treated crabs.

In the present work the accumulation of organic substances in the ovary of fluoxetine treated prawns strongly reveals that the concurrent ovarian development due to eyestalk ablation and fluoxetine treatment and it is well documented with the earlier reports.

References

Arcos G.F., Ibarra A.M., Boucard C.V., Palacios E. and Racotta I.S. (2003): Haemolymph metabolic variables in relation to eyestalk ablation and gonad development of pacific white shrimp, Litopenaeus vannamei Buone. *Aquaculture Research*, **34**, 749-755.

- Barnes H. and Blackstock Z.J. (1973): Estimation of lipids in marine animals and tissues. Detailed investigation of the Phosphovanilin method for total lipids. J. Exp. Mar. Bid. Ecol., 12, 103 – 118.
- Kulkarni R.R., Sarojini R. and Nagabhushanam R. (2002): Transfer of energy from hepatopancreas to ovary during reproductive cycle of fresh water prawn, Macrobrachium lamarrei. J. Aqua. Biol., **17**(1), 40-42.
- Lowry O.H., Rosebrough N.K., Farr A.L. and Randall R.J. (1951). Protein measurement with Folinphenol reagent. J. Biol. Chem., 193, 265-275.
- Meera N., Jayanthi J. and Ragunathan M.G. (2006a): Stimulation of ovarian maturation due to fluoxetine in a brackish water crab, Uca lactea annulipes- A Biochemical study. J. Aqua. Biol., 21(2), 178-179.
- Meera N., Jayanthi J. and Ragunathan M.G. (2006b): Nutrient deposition in gonads during their maturation due to fluoxetine in an eyestalk ablated fiddler crab, Uca lactea annulipes. *J. Aqua. Biol.*, **21**(2), 180-181.
- Ragunathan M.G, Meera N., and Jayanthi J. (2007): Prolonged light and fluoxetine stimulates deposition of organic reserves in the gonads of a female brackish water crab, Uca lactea annulipes. *J. Aqua. Biol.*, **22** (1), 166-168.
- Roe J.R. (1955): The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol. Chem.*, **20**, 335-343.
- Wouters R., Molina C., Lavens P. and Calderon J. (2001): Lipid composition and vitamin content of the wild female Litopenaeus vannamei. Different stages of sexual maturation. *Aquaculture*, **198**, 307-323.