UNILATERAL EYESTALK ABLATION INDUCED GONADAL MATURATION IN SPIRALOTHELPHUSA HYDRODROMA

Article · May 2019

2 authors, including:

Anne Rebecca
PSG College of Arts and Science
17 PUBLICATIONS **50** CITATIONS

SEE PROFILE

UNILATERAL EYESTALK ABLATION INDUCED GONADAL MATURATION IN *SPIRALOTHELPHUSA HYDRODROMA*

ANNE REBECCA, A & DHIVYA, K
PG department of Zoology, PSG College of Arts and Science, Coimbatore-641014, Tamilnadu, India.

ABSTRACT

Unilateral eyestalk in *S. hydrodroma* has resulted in increased feed intake (0.72±0.04/ 0.62±0.84 and 0.39±0.07/ 0.29±0.1), daily growth rate (0.41±0.39/ 0.16±0.1 and 0.14±0.34/ 0.05±0.08), heart index (.37±0.66/ 3.14±0.1 and 3.04±0.35/ 2.95±0.43), testicular index (1.34±0.73/1.09±0.07) and ovarian index (0.33±0.1/0.15±0.03) in male and female ablated against their control and equated with the gonadal biochemical indices with higher protein (37.26±1.17/19.32±1.17 and 36.2±0.97/32.22±0.84), carbohydrate (322.19±1.23/92.89±0.93 and 159.19±1.00/91.09±1.12) and lipid (33.17±1.05/29.22±0.88 and 36.11±0.93/32.09±0.91) levels on UEA. A negative correlation with somatic biochemical indices with decreased levels of proteins (95.95±1.36/141.22±1.2 and 114.28±0.9/138.25±1.09), carbohydrates (4.67±0.77/5.58±1.17 and 3.56±0.8/4.43±1.2) and lipids (84.25±1.08/87.5±1.1 and 89.51±1.16/91.52±1.21) demonstrates the mobilization of such nutrients from somatic tissues to gonads for its growth and development with UEA.

Keywords: Freshwater aquaculture, *Spirothelphusa hydrodroma*, induced breeding, Eyestalk ablation.

INTRODUCTION

India has got a growing aquaculture sector, capable of global contribution (Mishra, 2017). Majority of which comes from the freshwater environment (Bondad-Reantaso *et al.,* 2005). Several edible crustaceans constitute one of the major sources of nutritive food materials for human beings and form one of the key points of food chain (Salam, 2014). Shell fish is one of the most important sources of proteins provided from sea and blue crab is one of the most important among them (Enzenross *et al*., 1997). Crabs too are favoured after shrimps or lobsters among crustaceans (Savad and Ragavan, 2001).

Spirothelphusa hydrodroma is an edible, significant freshwater crab, exant to the Indian subcontinent capable to be cultivated through artificial breeding techniques. In crustaceans, eyestalk hormones are attributed with control of a number of physiological process namely, somatic changes, blood glucose level, osmoregulation, moulting, reproduction and oxygen consumption (Fingerman, 1970;). Eyestalk ablation is one of the artificial induced breeding method. In crustaceans, the X-organ sinus gland (XO-SG) complex located in the eyestalks synthesis and release a number of neuropeptide hormones such as gonad inhibiting hormone (GIH), mandibular organ inhibiting hormone (MO-IH), crustacean hyperglycemic hormone (CHH) and moult inhibiting hormone (MHI) which play important roles in growth and reproduction. These hormones also play a role in the metabolism of proteins, lipids, nitrogen, calcium, carbohydrate and water balance (Chang, 1992). Sinus glands, which are located in the eyestalks, Y-organ and mandibular organs are reported to be important endocrine glands in crustaceans (Herp and Soyes, 1997).

However, such a physiology or the effect of Unilateral Eyestalk Ablation (UEA) in *S. hydrodroma* is not well documented. Therefore, the present study focuses on the growth, survival, reproductive and biochemical changes owing to UEA in the scarcely distributed species in an attempt to enhance the propagation.

MATERIALS AND METHODS

Collection, transportation and maintenance of crabs

Males of 4.37±0.80 cm in length and 28.8±4.51 gms in weight and females of 4.63±0.32 cm in length and 29.84±2.52 gms in weight were used for the study Crabs were collected from the Chinnaputhur lake, Tirupur district of Dharapuram, Tamil Nadu, India. The collected specimen were transported to the laboratory in perforated plastic containers. Mature male and female crabs with measured size is taken for the induced breeding. Females with triangular flap was avoided as they are immature. The selected crabs are acclimatized for about a week in glass aquaria with moist sand and gravel. Unfed feed and excreta were removed daily. The crabs were subjected to a pool of water daily to avoid dehydration.

Eyestalk Ablation

The experimental crabs were divided into four groups: control males, control females, ablated males and ablated females. An unilateral eyestalk ablation was performed in male and female experimental crabs by cutting the right eye at its base with a fine and clean scissor. The wound was cauterized using a hot blunt needle in order to prevent the loss of haemolymph and mortality. Eyestalk ablated and non-ablated crabs were introduced into plastic tubs and 1gm of commercial pelletized feed was given once a day as a feed for both ablated and non-ablated crabs individually. Analysis was carried out after 10 days of ablation. After which, the tissues such as: muscle, hepatopancreas, heart, testis and ovary were dissected out to study growth, survival, reproductive, biochemical and histological indices.

Growth and Survival Indices

a. Feed Intake (FI)

The ablated and non-ablated crabs were fed with 1gm of fresh commercial pelletized feed per day. The unfed was collected and measured after every 24 hours and the individual plastic tubs were cleaned after the collection of unfed. The feed consumption of ablated and non-ablated crabs was calculated by using the following formula:

Feed intake (g) = Total feed offered (g) – feed unfed (g)

b. **Survival Rate (SR)**

During the experimental period the mortality of the ablated and non-ablated crabs were noted. By using the following formula, the survival rate was calculated. The survival rate was denoted in percentage.

$$\text{Survival rate (\%)} = \frac{\text{Total number of crabs} - \text{total number of dead crabs}}{\text{Total number of crabs}} \times 100$$

c. Daily Growth Rate (DGR)

Before eyestalk ablation the crabs were weighed and the initial weights of the crabs were measured. After 10 days of ablation the weights of the crabs were measured and it is considered as final weight which gained by the crabs during the experimental period. The daily growth rate was calculated by using the following formula:

$$\text{Daily Growth Rate (g)} = \frac{\text{Final weight - initial weight}}{\text{No. of days}}$$

d. Hepatosomatic Index (HSI)

After 10 days of experimental setup both ablated and non-ablated male and female crabs were dissected out and hepatopancreas was removed and weighed. The hepatopancreatic index was determined using the following formula:

$$\text{HSI (\%)} = \frac{\text{Wet weight of the hepatopancreas}}{\text{Weight of the crab}} \times 100$$

e. Coagulation Time

The coagulation time was estimated by following the method of Mattson *et al*., (1985) and Eddy *et al*., (2007). 0.5 ml of haemolymph was withdrawn by puncture of the perio-arthrodal membrane at the base of the fourth walking legs. Haemolymph was placed on a clean glass slide and shaken slowly until jellification. The time between haemolymph withdrawal and jellification was considered the coagulation time in minutes.

f. **Heart Index (HI)**

The hearts were 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 the hearts were removed. Heart indices were determined by the standard formula:

$$\text{HI (\%)} = \frac{\text{Wet weight of the heart}}{\text{weight of the crab}} \times 100$$

g. **Moulting Frequency**

To register the moult events, the number of moults was recorded daily in control male and female crabs as well as in the ablated male and female crabs.

Reproductive Indices

Gonadosomatic Index (GSI)

After 10 days of experimental setup both the ablated and non-ablated male and female crabs were dissected out and gonad was removed and weighed. The GSI were calculated by using the following formula:

$$\text{GSI (\%)} = \frac{\text{Wet weight of the gonad}}{\text{Weight of the crab}} \times 100$$

Histological Indices

After an experimental period both eyestalk ablated and non-ablated male and female crabs were dissect out. Tissues such as ovary and testis were taken for histological studies. Cross section of testis and ovary was done by using a microtome. Sections were stained using differential stain (H&E). Stained slides were viewed under light microscope of 40X illumination and photomicrographs were taken for further investigations.

Biochemical Analysis

Gonadal tissues were examined for protein, carbohydrate and lipid quantitatively. Muscle tissues were examined for protein and carbohydrate and hepatopancreas for lipids. Estimations were done following the standard methods: Roe *et al.,* 1955 for carbohydrates, Lowry *et al.,* 1951 for proteins and Folch *et al.,* 1957 for lipids.

Statistical analysis

All Data obtained from growth, reproductive and biochemical indices were analyzed using SPSS statistical V-20 software. Data were obtained as mean ± standard deviation of triplicate analysis (Zar, 2009).

RESULTS

Table-1: Growth and survival indices in *S. hydrodroma*

Indices	Male		Female	
	Control	Ablated	Control	Ablated
Food intake(g)	0.62±0.84	0.72±0.04	0.29±0.1	0.39±0.07
Daily Growth Rate (g)	0.16±0.1	0.41±0.39*	0.05±0.08	0.14±0.34
Survival rate (%)	100±0.00	68±13.98	100±0.00	64±12.65
Hepatosomatic index (%)	13.77±3.22	10.62±2.23	10.22±0.14	8.93±0.37
Heart index (%)	3.14±0.1	3.37±0.66	2.95±0.43	3.04±0.35
Coagulation time (minutes)	8.96±0.81	32.53±2.54	12.84±1.39	41.29±1.08

Data obtained as a result of triplicate analysis expressed as Mean ± S.D. All values are significant at P≤0.05.

* -Value not significant at P≤0.05.

Table-2: Reproductive indices in *S. hydrodroma*

Indices	Control male	Ablated male
Testicular index (g)	1.09±0.07	1.34±0.73
Ovarian index (g)	Control female	Ablated female
	0.15±0.03	0.33±0.1

Data obtained as a result of triplicate analysis expressed as Mean ± S.D. All values are significant at P≤0.05.

Table-3:Somaatic biochemical indices in *S. hydrodroma*

Indices	Male		Female	
	Control	Ablated	Control	Ablated
Muscle carbohydrate (mg/g)	5.58±1.17	4.67±0.77	4.43±1.2	3.56±0.8

Muscle protein (mg/g)	141.22±1.2	95.95±1.36	138.25±1.09	114.28±0.9
Pancreatic lipid (mg/g)	87.5±1.1	84.25±1.08	91.52±1.21	89.51±1.16

Data obtained as a result of triplicate analysis expressed as Mean ± S.D. All values are significant at P≤0.05.

Table-4: Gonadal biochemical indices in *S. hydrodroma*

Indices	Male		Female	
	Control	Ablated	Control	Ablated
Carbohydrate (mg/g)	92.89±0.93	322.19±1.23	91.09±1.12	159.19±1
Protein (mg/g)	19.32±1.17	37.26±1.17	32.22±0.84	36.2±0.97
Lipid (mg/g)	29.22±0.88	33.17±1.05	32.09±0.91	36.11±0.93

Data obtained as a result of triplicate analysis expressed as Mean±S.D. All values are significant at P≤0.05.

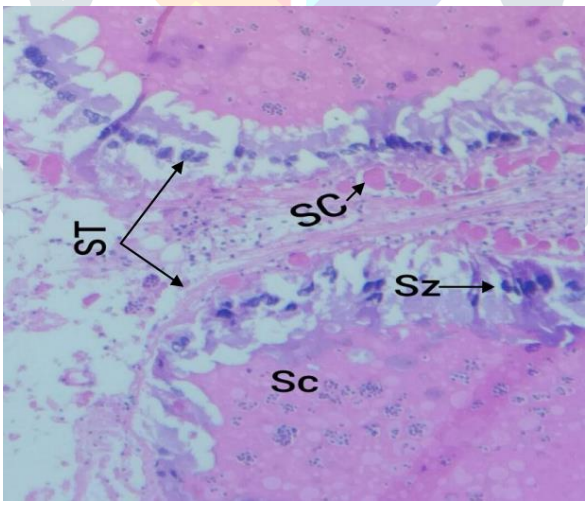

Fig-1: Photomicrograph on cross section of control male *S. hydrodroma* showning developing spermatozoa and immatured spermatocytes in seminiferous tubules (X540). H and E stain.

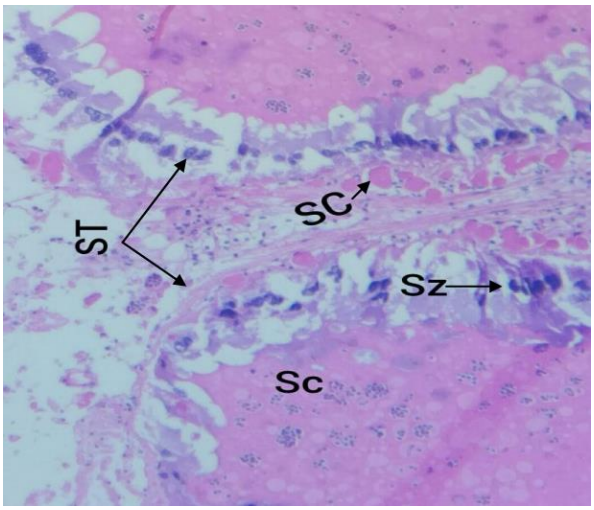

Fig-2: Photomicrograph on cross section of ablated male *S. hydrodroma* showing developed spermatozoa and matured spermatocytes in seminiferous tubules (X340). H and E stain.

(IC- Interstitial Cell, ST- Seminiferous Tubules, LN- Lumen, SC- Sertoli Cells, Sz- Spermatozoa, Sc- Spermatocytes).

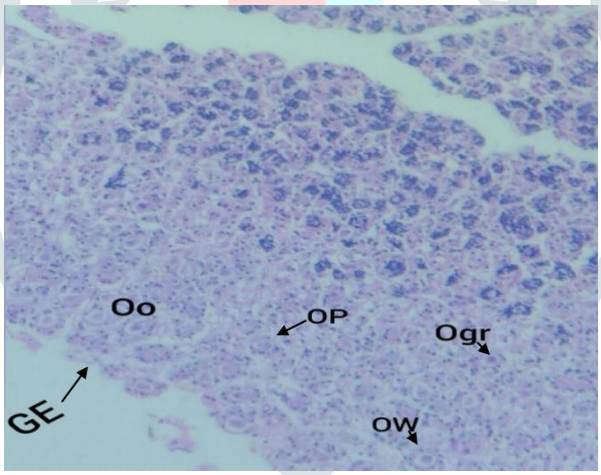

Fig-3: Photomicrograph on cross section of control female *S. hydrodroma* showing pre-vitellogenic (primary) oocytes (X340). H and E stain.

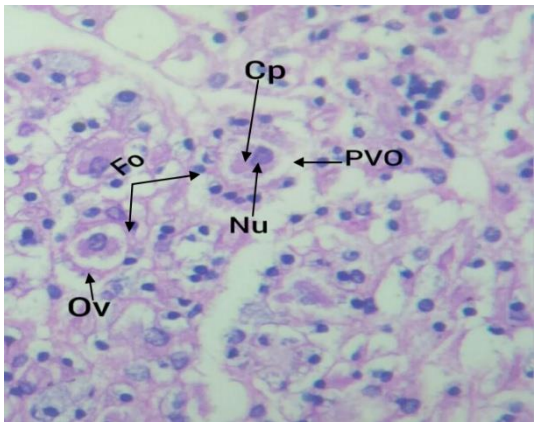

Fig-4: Photomicrograph on cross section of ablated female *S. hydrodroma* showing increased follicle diameter, follicle number and post-vitellogenic oocytes (X340). H and E stain.

(Oo-Oocyte, OP- Oogenetic Pouch, Ogr-Ovarian granules, OW- Ovarian Wall, GE- Germinal Epithelium, Fo-Follicles, Cp-Cytoplasm, PVO- Post-vitellogenic oocyte, Nu-Nucleus, Ov-Ovum).

Moulting Frequency

Through out the experimental trial of 10 days, control as well as ablated male and female crabs of *S. hydrodroma* were in interphase stage and no moulting stage was observed.

Histological Indices

The histological studies showed the increased follicular diameter, follicle number and post-vitellogenic oocytes in ablated female (fig-4). In control female, histological sectioning showed pre-vitellogenic oocytes (fig-3). Ablated male histological section showed developed spermatozoa and matured spermatocytes in seminiferous tubules (fig-2). In control male histological section showed developing spermatozoa and immatured spermatocytes in seminiferous tubules (fig-1).

DISCUSSION

The eyestalk of the crustacean influences the endocrinological control of growth and reproduction in crustaceans (Chandry and Kalwalkar, 1984). The biochemical compositions of crustacean tissues are very important in nutritive aspect. Eyestalk ablation induces hormonal effects on carbohydrate, protein and lipid

metabolism in crustaceans (Highnam and Hill, 1979). The present study was attempted to know the effect of unilateral eyestalk ablation on the growth, survival, reproductive and biochemical characters of the male and female *S. hydrodoma*.

FI increased with UEA. 0.72±0.034/0.62±0.84 and 0.389±0.07/0.29±0.1 in male and female/control. Jiang *et al*., (2017) stated that, "food consumption, increased food consumption energy (FCE) to 1.89% in *Penaeus monodon* and food was transformed into energy for gonad development more effectively. However, most of the energy was consumed for the maintenance of body metabolism (respiration and excretion). In contrast, Venkitraman *et* al., (2003) recorded decreased food consumption rate in ablated *Metapenaeus dobsoni*, when compared to their control animals due to smaller specimen size. FI is related to temperature, salinity, light and food species (Yu *et al*., 2007). Also, DGR (g) of ablated male (0.41±0.39) and female (0.14±0.34) crabs was higher than non-ablated male (0.16±0.1) and female (0.05±0.08) crabs. Therefore, eyestalk ablation had significantly affected the growth of crabs. Similar result was reported by Varalakshmi and Reddy, (2010) with UEA in *M. lanchesteri* showing high ovarian maturation

However, SR of intact male (100±0.00) and female (100±0.00) crabs was higher, compared to ablated male (68±13.98) and female (64±12.65) crabs. The males showed higher survival rate compared to females on ablation. These findings correlated with that of Varalakshmi *et al*., (2010) in *M. lanchesteri* on UEA. In shrimps and other crustaceans, eyestalk ablation is known to result in heavy mortality (Primavera *et al*., 1978: Radhakrishnan and Vijayakumaran, 1984: Koshio *et al*., 1992). SR can be improved by controlling environments such as stocking density, tank size and rearing methods. In contrast, Asusena *et al*., (2012) showed higher survival rate (100%) in unilateral eyestalk ablated female prawns compared to their control female (80%) prawns due to unsual passivity of the ablated *Macrobrachium americanum* demonstrating, aggressiveness as the main cause of mortality. The HSI dropped down significantly. 10.62±2.23/13.77±3.22 and 8.93±0.37/10.22±0.14 were the recorded HSI in male and female ablated/control correlating with HSI of ablated female crab, *Potamon persicum* (Khazraeenia and Khazraiinia, 2009). This may be indication of the utilization of tissue synthesis.

UEA significantly increased the HI in male (3.37±0.66) and female (3.04±0.35) against control male (3.14±0.1) and female (2.95±0.43) shows parallel results with the mud crab, *Syclla serrata* stating that, "'eyestalk factors and hormones of Y-organ were responsible for the growth of heart in mud crab. The removal of eyestalk caused decreased secretion of the MIH and increased the secretion of ecdysone, which may be responsible for growth of heart in *S. serrata*." (Allayie *et al*., 2011). The coagulation time on UEA increased. However, in *M. americanum* the clotting system in females was not found to be affected by UEA (Asusena *et al*., 2012). The clotting mechanism in *S. hydrodroma* is not yet documented. Khazraeenia, (2009) observed precocious moulting in destalked *Potamon persicum* crabs, which was probably related to the removal of MIH and consequent activation of the Y-organ, which produces the moulting hormone (MH). However, in the present study short span of experimental trial has not sufficiently resulted in moulting. Increase in experimental span of more than 10 days is assumed to demonstrate moulting phase in *S. hydrodroma.*

Ablation of the eyestalks accelerated gonads development in adult male and female crabs. GSI of ablated male (1.34±0.73) and female (0.33±0.1) crabs was increased than control male (1.09±0.07) and female (0.15±0.03) crabs. A similar trend was found in female *P. persicum* (Khazraeenia, 2009) and *P. hydrodromous* males and female (Banoo *et al*., 2018) against their controls. Gonad development in eyestalk ablated animal might indicate the presence of GIH in the eyestalk and Gonad Stimulating Hormone (GSH), which is another hormone that takes part in the reproductive process. GSH is secreted from the brain and thoracic ganglion (Laufer *et al*., 1993). Gonad stimulation is further evident from histological studies. Ablated males showed developed spermatozoa and matured spermatocytes in seminiferous tubules (Fig-2) against immature spermatocytes in control (Fig-1). Ablated females showed increased follicle diameter, follicle number and post-vitellogenic oocytes (Fig-4) against pre-vitellogenic oocytes in control (Fig-3). An investigation on *Oziotelphusa senex senex* by Kumaran, *et al*., (2015) showed a well developed ovaries with oocytes laden with full of yolk globules and oil droplets in eyestalk ablated and light treated crabs. Moreover, Pervaiz and Sikdar, (2013) observed similar result in *M. dayanam* with pronounced development of different cells as

compared to unablated prawns. The removal of gonad inhibiting factor present in the X-organ of the eyestalk, increased reproductive activity and the system in crustaceans (Banoo *et al*., 2018).

As with the present study, the somatic biochemical indices dropped down with UEA in male and female. Muscle protein were quantified to be 95.95±1.36/I141.22±1.2 and 114.28±0.9/ 138.25±1.09 for male and female agaist their control stating a parrel report with Sarojini *et al*., (2016) *S. hydrodroma*. Because, after the eyestalk ablation, the protein content in muscle was utilized for the maturation of gonads. In contrast, protein content of muscle was found to be higher in eyestalk ablated *Charybdis lucifera* crabs (68.97%) than control (41.64%) as reported by Murugesan *et al*., (2008). Soundarpandian and Ananthan, (2008) stated, not only eyestalk ablation, but also feed, dietary proteins in particular, play important roles in the proximate composition of the animals, when *M malcomsoni* fed with adult *Artemia*, earthworm, oyster and two different commercial feed. However, in the present study crabs were fed with commercial fish food throughout the study. Therefore the present study states that the changes in biochemical composition between control and ablated crabs are obviously due to hormonal influence on eyestalk ablation. Also, the muscle carbohydrate content was lower in eyestalk ablated male (4.67±0.77) and female (3.56±0.8) than control male (5.58±1.17) and female (4.43±1.2) crabs showing a similar result with *C. lucifera* crabs studied by Murugesan *et al*., (2008) due to direct removal of the eyestalk, which is a major source of Crustacean Hyperglycemic Hormone (CHH). CHH is responsible for glucose homeostasis. It acts in carbohydrate metabolism by stimulating the glycogenolysis in muscles and hepatopancreas which inhibits the synthesis of glycogen (Keller and Orth, 1990). Similarly the hepatopancreatic lipids dropped on UEA. 84.25±1.08/ 87.5±1.1and 89.51±1.16/91.52±1.21 were the recorded lipids levels (mg/g) in male and female against control.

A negative correlation is recorded for gonaldal biochemical indices against somatic biochemical indices. In other terms, UEA is found to decrease the nutrient levels in somatic cells and increase them in the gonadal cells. Protein levels in gonads were 37.26±1.17/19.32±1.17 and 36.2±0.97/32.22±0.84 for male and female/conrol. Kale, (2017) reported similar results *B. cunicularis* stating that "the eyestalk ablation stimulated the ovarian maturation in freshwater female crab, *B. cunicularis* by transporting the proteins from hepatopancreas to ovary." Also, 322.19±1.23/92.89±0.93 and 159.19±1/91.09±1.12 were the recorded

carbohydrate levels in ablated male and female against control allinged with findings of Varalakshmi and Reddy, (2010) in *M. lanchesteri*. Similarly, 33.17±1.05/29.22±0.88 and 36.11±0.93/32.09±0.91 (mg/g) level of lipids were recorded in the gonads of ablated male and female/control. Lipid level indicated inverse co-relatioship between hepatopancreas and ovary in eyestalk ablated animals. Decreased lipid content in hepatopancreas due to the flow and fluxes of energy to ovary from other storage organs like hepatopancreas when unilateral eyestalk ablation in female crabs is performed. These were verified with findings of Samyappan *et al*., (2015) in hepatopancreas and ovary of *O. senex senex* showing 2.12±0.88 and 3.86±0.25 (mg/g) level of lipids after extirpation.

CONCLUSION

UEA in *S. hydrodroma* sufficiently induced gonadal and growth development, which is evident from the increased GSI and daily growth rate attributed to the mobilization of protein, carbohydrates and lipid from muscle tissue to gonads concurrently, maintaining an increase in weight of the crabs. Therefore, it can be concluded that, unilateral eyestalk ablation is promising for the cultivation of *Spiralothelphusa hydrodroma*.

REFERENCES

Allayie, S.A., Ravichandran, S., Bhat, B.A., 2011. “Hormonal regulatory role of eyestalk factors on growth of heart in mud crab, *Scylla serrata*.” *Saudi Journal of Biological Sciences*. 18:283-286.

Asusena, A.J., Carlos, S.J., Arturo, F.J., Genaro, D., 2012. “The effects of eyestalk ablation on the reproductive and immune function of female *Macrobrachium americanum*.” *Journal of Aquaculture Research and Development*. 3(8).

Banoo, S., Rather, S.H., Waseem, M., 2018. “Effect of unilateral eyestalk ablation (ULEA) on gonadosomatic indices and gonad maturation of freshwater crab, *Paratelphusa hydrodromous* (Henderson 1893).” *International Journal of Zoology Studies.* 3(2):289-292.

Bondad-Reantaso, M.G., Subasinghe, R.P., Arthur, J.R., Ogawa, K., Chinabut, S., 2005. “Disease and health management in Asian aquaculture.” *Veterinary Parasitology*.249-272.

Chandry, J.P., and Kalwalkar, D.G., 1984. "Role of eyestalk hormone in energy metabolism of the crab *Charvbdis lucifer* (Fabricus)." *Comp. Physiol. Ecol.*

Chang, E.S., 1992. "Endocrinology." In: Marine shrimp culture principles and practices. Fast A.W., Laster, J. (Eds). *Amsterdam Elsevier*. .

Eddy, F., Powell, A., Gregory, S., Nunan, L.M., Lightner, D.V., Dyson, P., Rowley, A.F., Shilds, R.J. 2007. "A novel bacterial disease of the European shore crab, *Carcinus maenas* molecular pathology and epidemiology." *J. Microbiol*. 153:2839-2849.

Enzenross, R., Enzenross, L., Bringel, F., 1997. "Occurrence of blue crab, *Callinectes sapidus* (Rathbun, 1896) (Crustaceae, Brachyura) on the Turkish Mediterranean Adjent Aegean Coast and its size distribution in the Bay of Iskenderun." *Turk. J.zool*.21:113-122.

Fingerman, M., 1970. "Perspectives in crustacean endocrinology." *Scientia*, 105:1-23

Folch, J., Lesd, M.D., Sloane-Stainely, G.H., 1957. "Simple method of isolation and purification of total lipids from animal tissues." *J. Biochem*. 226:297-308.

Herp V.F., Soyes, D., 1997. "Arthropoda-Crustacea." In Reproductive Biology of Invertebrates. Volume 8. Edited by K.G and R.G Adiyodi. Wiley-Interscience publication. Pp:247-275.

Highnam, H.C., and Hill, L., 1979. "The comparative endocrinology of the invertebrates." *Edward Arnold Publishers*. Pp:209.

Jiang, S., Zhou, F., Huang, J., Yang, Q., Yang, L., 2017. "Effects of eyestalk ablation on feeding, gonad development and energy conversion of *Penaeus monodon* broodstock." *Pakistan J.zool*. 50(4):1217-1223.

Kale, R.S., 2017. "Influence of beta-estradiol hormone and eyestalk ablation on protein metabolism in freshwater crab, *Barytelphusa cunicularis*." *Bioline discovery*. 8(3):602-607.

Keller, R., Orth, H.P., 1990. Hyperglycemic neuropeptides in crustaceans. In Comparative Endocrinology. Edited by Epple, A., Scanes, C.G., Stetson, M.H., Willey-Liss, New York, USA. Pp:265-271.

Khazraeenia, S., and Khazraiinia, P., 2009. "Effects of bilateral eyestalk ablation on gonadal maturity, moulting and biochemical changes in the hemolymph of female *Potamon persicum* crabs (Decapoda, Brachyura, Potamidae)." *Int.J.Vet.Res.* 3(2):143-150.

Koshio, S., Teshima, S., Kanazawa, A., 1992. "Effects of unilateral eyestalk ablation and feeding frequencies on growth, survival and body compositions of the juvenile freshwater prawn, *Macrobrachium rosenbergii*." *Nippon Suisan Gakkaishi*. 58(8):1419-1425.

Kumaran, T., Devakumar, D., Jayanthi, J., Ragunathan, M.G., 2015. "Effects of photoperiodism, eyestalk ablation on the ovarian development and electrolyte changes in a freshwater crab, *Oziotelphusa senex senex*." *Int. J. Curr. Res. Biosci. Plant Biol*. 2(8):130-135.

Laufer, H., Jonna, S.B., Sagi, A., 1993. "The role of juvenile hormones in crustaceans reproduction." *Americ.Zool*. 33:365-374.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. "Protein measurement with folinphenol reagent." *J.Biol.Chem.,* 193:265-275.

Mattson, M.P., and Spaziani, E., 1985. "5-hydroxytryptamine mediates release of moult-inhibiting hormone activity from isolated crab eyestalk ganglia."*Biol.Bull.* 169:246-255.

Mishra, S.S., 2017. "Prevalence of Fish and Shrimp Diseases and Use of Various Drugs and Chemicals in Indian Aquaculture for Disease Management." *Journal of Fisheries and Aquaculture Development.*

Murugesan, R., Soundarapandian, P., Manivannan, K., 2008. "Effect of Unilateral Eyestalk Ablation on the Biochemical Changes of Edible Portunid Crab *Charybdis lucifera* (Fabricius)." *Journal of Fisheries and Aquatic Science*. 3(1):82.

Pervaiz, A.P., and Sikdar, M., 2013. "Influence of bilateral eyestalk ablation on gonads of freshwater prawn, *Macrobrachium dayanam*." *Journal of Environmental Biology*. 35:821-825.

Pervaiz, P.A., John, S.M., Sikdar-Bar, S., Khan, H,A., Wani, A.A., 2011. "Studies on the effect of unilateral eyestalk ablation in maturation of gonads of a freshwater prawn *Macrobrachium dayanam*." *World Journal of Zoology.* 6(2):159-163.

Primavera, J.H., Borlongan, E., Posadas, R.A., 1978. "Mass production in concrete tanks of surgo *Penaeus monodon* fabricius spawners by eyestalk ablation." *Fish. Res. J. Phil*. Vol.3, Pp:1-12.

Radhakrishnan, E.V., Vijayakumaran, M., 1984. "Effect of eyestalk ablation in spiny lobster *Panulirus homarus* (Linnaeus) 1.Moulting and Growth." *Indian Journal of fisheries*. Pp:130-147.

Roe, J.H., 1995. "The determination of sugar in blood and spinal fluid with androne reagent." *J. Biol. Chem.* 153:373-380.

Salam, H.A., 2014. "Protein and electrophoretic analysis of edible muscle of commercially important crustaceans and mollusks species from Egyptian and Saudi coasts." *Science PG, Animal and Veterinary Science.* 2(4):109-117.

Samyappan, K., Saravanan, R., Vijayakanth, T., Prabakaran, D., 2015. "Impact pf unilateral eyestalk ablation on lipid profiles in freshwater female crab *Oziotelphusa senex senex*." *Asian Journal of Science and Technology*. 6(5):1474-1478.

Sarojini, N., Bharathi, A., Padmaja, M., 2016. "Impact of unilateral eyestalk ablation on protein content in freshwater crab *Spiralothelphusa hydrodoma*." *Global journal of bio-science and biotechnology*. 5(1):31-34.

Savad, M.A., and Ragavan, R.P., 2001. "Mud crab-culture and fattening techniques, status and prospects." *Seafood Export J*. 32(11):25-29.

Soundarpandian, P., Ananthan, G., 2008. "Effect of unilateral eyestalk ablation on the biochemical composition of commercially important juveniles of *Macrobrachium malcolmsonii*." *International Journal of Zoological Research*. 4(2):106-112.

Varalakshmi, K.N., Reddy, R., 2010. "Effects of eyestalk ablation on growth and ovarian maturation of the freshwater prawn *Macrobrachium lanchesteri* (de man)." *Turkish Journal of Fisheries and Aquatic Sciences*. 10:403-410.

Venkitraman, P.R., Jayalakshmy, K.V., Balasubramanium, T., Maheshwari, N., Nair, K.K.C., 2003. "Effects of eyestalk ablation on molting and growth of penaid prawn *Metapenaeus dobsoni* (de man), Indian." *Journal of Experimental Biology,* 42:403-412.

Yu, H.N., Lin, X.T., Zhou, X.Z., Xu, Z.N., Li, H., 2007. "Behavioral studies of *Litopenaeus vannamei* under abrupt temperature and salinity changes." *J.trop. Oceanogr*. 26:38-43.

Zar, H.J., 2009. "Biostatistical analysis." (5th Edition). *Prentice hall*.