International Journal of Pure and Applied Zoology
Volume 6, Issue 3, pp: 30-34, 2018
http://www.ijpaz.com

ISSN (Print) : 2320-9577
ISSN (Online): 2320-9585

Research Article

EYE STALK ABLATION OF FRESHWATER CRAB, *BARYTELPHUSA LUGUBRIS*: AN ALTERNATIVE APPROACH OF HORMONAL INDUCED BREEDING

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Article History: Received 14th June, 2018; Accepted 22nd August, 2018; Published 10th September, 2018

ABSTRACT

Eyestalk ablation now a days is considered as the most effective method to facilitate molting and maturation, but its physiological responses is still not clearly studied in *Barytelphusa lugubris*. This study was aimed to know the success of eye stalk ablation to ripen ovary in spent female. Berried freshwater female crabs (*Barytelphusa lugubris*) were collected from Sangla Kunchi Pwakal Village Development Committee, Kathmandu Nepal in June, 2008 and were reared in the aquarium till all the eggs hatched into juveniles and left mother's brood pouch. Nine spent females of 50.43- 58.66 mm carapace width were chosen for induced breeding. Three experimental sets were made-each containing three crabs, as A, B and C groups. Group A was kept as controlled without extirpation. Remaining females were first narcotized in 20°C ice-cold water for 15-20 min. and single eyestalk was extirpated with fine scissors in-group B, and both eyestalks in group C. Then ablated eyes stumps were cauterized by hot scalpel and kept in 20c, ice-cold water for two minute. Each group was kept in separate glass aquarium of 85 cm length, 40 cm width and 50 cm height, containing little water with sand, pebbles and 4" PVC pipe as hide out for crabs. The experiments were terminated after two months. This study revealed that all crabs except controlled, moulted within 20 and 40 days, with large dark yellow colored ovaries average 0.16g, dark creamy ovaries average 0.08 g and average 0.04 g in bilaterally, unilaterally ablated and controlled sets respectively. This alteration in induced breeding for rapid ripening of ovaries of spent females resulted as a suitable technique to enhance meat yield in aquaculture of this crab.

Keywords: *Barytelphusa lugubris*; Eyestalk; Extirpation; Spent females

INTRODUCTION

The biological process of all the living organisms to produce offspring from their parents for the continuation of generation is known as reproduction. In other word it is a fundamental feature of all the living beings or the process of pro-creation or breeding biology by which new individuals are produced. For the commercial purpose normal process of reproduction may take long period, thus to enhance early maturation of gonad hormonal induction is most appropriate technique. The cheapest way to enhance maturation of gonads is through eyestalk ablation on crustaceans. Eyestalk ablation is now a day considered as the most effective method to facilitate molting and maturation (Lee et al., 2017). Eyestalk ablation is usually used for quick ovarian development and maturation in captive economic crustaceans (Guan et al., 2017) culture. Due to an increasing attention in invertebrate for the commercial applications in aquaculture business, crustacean reproductive physiology is drawing more excellence (Samyappan et al., 2015). Moulting and reproduction of crustaceans are physiological process strongly regulated by neurohormones secreted by the X-organ/sinus gland complex found in eyestalk of the species (Pervaiz and Sikdar, 2014; Fingerman, 1987). Eyestalk ablation was traditionally carried out to shorten the duration of molt cycle and to influence growth and reproduction along with other metabolic activities of crustaceans, (Chang 1995). They exert an inhibitory effect on vitellogenesis during the phase of eggs development (Bomirski and Klek, 1974). Eyestalk ablation reduces the titre of gonad inhibitory hormone in females accelerating ovarian growth (Aktas et al., 2003). Thus, endocrine manipulation to induce gonadal maturation has so far been tantamount with unilateral eyestalk ablation and has far reaching impact on crustacean aquaculture. Unilateral eyestalk ablation has been employed to induce both ovarian maturation and laying with varying achievement in many species (Zaib-Un-Nisa, 2001) as well as to shorten the moult interval and to stimulate gonad development in shrimps (Sivasubramaniam and Angell, 1991). Beside eyestalk, mandibular organ also possesses various types of hormones, which have been studied by various scientists. Nagaraju et al., (2004) reported the relation of mandibular organ in relation to body weight, sex, molt and reproduction in the crab *Oziotelphusa senex senex Fabricius* (1791). Wen et al., (2015) recorded earliest spawning time after eyestalk ablation in *Penaeus monodon*. Numerous experimental works have been done to show various activities of crustacean, decapods, regarding biochemical changes, hormonal activities, development of gonads and moult, (Nagabhushanam and Kulkarni, 1980; Nagaraju et al., 2006; 2004; Reddy and Reddy 2006; Reddy et al., 2006; 2006: 2004; Okumura and Aida, 2001; 2000). Bihormonal control of

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sexual cycle was studied in both sexes of the freshwater crab *Potamon dehaani* from eyestalk by Otsu, (1963). Ecdysones in the maturational moult of juvenile females of the spider crab, *Libinia emarginata* Leach, 1815 was reported by Rotllant and Takac, (1999). Pillay and Nair, (1973) observed biochemical changes in gonads and other organs of *Uca annulipes*, *Portunus pelagicus* and *Metapenaeus affinis* during the reproductive cycle. Methyl farnesoate stimulates testes in freshwater crab, *Oziotelphusa senex senex* resulting in the increase of the weight of testes, testicular index and testicular follicle diameter, (Kalavathi et al., 1999). In the same way eyestalk extirpation has also been used regularly to stimulate different metabolic activities, which in turn help in optimizing the production. McNamara et al., (1990) studied the effect of eyestalk extirpation in hemolymph osmotic and ionic concentrations during acute salinity exposure in the freshwater shrimp *Macrobrachium olfersii*. Reddy and Sainath (2006) described hyperglycemic hormone in freshwater prawn *Macrobrachium rosenbergii* and its purification from eyestalk nervous tissue and quantification by ELISA hemolymph following various stresses. Okumura and Sakiyama (2004) studied the hemolymph levels of vertebrate type steroid hormones in female kuruma prawn *Litopenaeus vannamei* during natural reproductive cycle and induced ovarian development. Bilateral eyestalk ablation possesses significant effects on the ion signal pathway of Litopenaeus vannamei (Li et al., 2017). Removal of the eyestalk in blue swimmer crab (*Portunus pelagicus*) resulted in the control of hormone, sex differentiation, growth and behavior of male crab (Sroyraya et al, 2010). Crude as well as partially purified eyestalk extract inhibited *in vitro* vitellogen and protein synthesis in ovaries of both intact and eyestalk-ablated crabs (Quackenbush and Keely, 1988). Eyestalk ablation techniques are now being employed extensively at commercial scale in many parts of the world to induce gonadal development, spawning and growth that involve removal of the sources of gonad inhibiting and/or moult inhibiting hormones.

Crab is now only second to shrimp in terms of earnings in shellfish products export line-up. Crab farmers now depend completely on wild seed, which limits the expansion of crab culture (Sroyraya et al., 2010).The continuous collection of wild seeds for culture /fattening has threatened the natural stock (Hoq et al., 2015). Production of seed from the commercially important crab species has the potential to be an effective tool to support the demand of crab due depleted seed stock from the wild (Nascimento et al., 2017).

METHODOLOGY

Ovigerous freshwater female crabs (*Barytelphusa lugubris*) were collected from Sangla Kunchi Pwakal Village Development Committee, Kathmandu Nepal in June, 2008. The collected female crabs were reared indifferent aquariums till all the eggs hatched into juveniles and left their mother's brood pouch. Chicken liver thawed in water were provided to feed them. Amongst, nine spent females of 50.43- 58.66 mm carapace width were chosen for the eyestalk ablation experiments. Three groups A, B and C were made containing three spent female crabs in each. Three female crabs were kept without ablation as controlled in group A. Remaining females were first narcotized in 20c ice-cold water for 15-20 min. depending individually on the responses of the crab as described by Miranda, et al., (2003). Single eyestalk was cut with fine scissors in-group B, and both eyestalks were extirpated in-group C. Ablated eye stumps were cauterized by hot scalpel then further kept in 20c, ice-cold water for two minute each to prevent excessive loss of hemolymph (Miranda, et al., 2003). Each group was kept in separate glass aquarium of 85 cm L and 40 cm width and 50 cm in height, containing little water with sand, pebbles and 4"diameter pvc pipes of 5" length (three in each group) as hide outs for crabs. Chicken liver thawed in water were provided as food in every alternate day. Some water, leftover food and garbage was changed and cleaned on the same day. The experiment was terminated after two months.

RESULTS AND DISCUSSION

The ovaries were large dark yellow in color with average 0.16 g in double eyestalk extirpated crabs and in the single eyestalk ablated crabs ovaries were dark creamy with average 0.08 g but in the controlled sets the ovaries were watery white in color average 0.04 g only. Destalked spent females of *B. lugubris* showed positive results in commencement of vitellogenesis, deposition of yolk in oocytes and increased weight of ovary. Single eyestalk ablation resulted molting within 40 days whereas the double eyestalk extirpated shortened the molting period to half the days than that of single eyestalk ablation thus, helping to shorten ovarian growth.

John and Sivadas (1978) reported that eyestalk ablation in *Scylla serrata* (Forskal) helped to speed up the maturation of the ovary corroborate with present finding. Padmanabhan and Raghayan (2016) recorded, bilateral eyestalk ablation of freshwater crab, *Travancoriana schirnerae* had induced hypertrophy and hyperplasia of the androgen gland. Similarly, bilateral eyestalk extirpation of *Uca triangularis* resulted in precocious acceleration of both molting and reproductions, irrespective of the season concede with present finding, Supriya et al., (2017). Sivasubramaniam and Angell (1991) delivered that eye-stalk ablation in mudcrabs dramatically shortened spawning interval to ten days. Unilateral eyestalk ablation of berried crab, *Scylla serrata* started spawning after 10-15 days of acclimation (Hoq et al., 2015). Unilateral eyestalk ablation in freshwater female crabs *Oziotelphusa sene xsenex* after 25 days showed marked decrease in lipid classes (cholesterol, phospholipid, triglycerides and free fatty acids) in the hepatopancreas but significant increase in ovarian tissue (Samyappan et al., 2015). All the above results substantiate present findings. Eyestalk ablation of mature female *Libinia* resulted extended periods or initiation of the ovigerous state but did not initiate molting, whereas in case of *B. lugubris* both ovarian development and moulting were accelerated. Breeding behavior and reproductive cycles seem unaltered by destalking but, in immature female *Libinia* which

have had their eyestalks removed moulted precociously and rarely reached to maturity (Hinsch, 1972), this corroborates with present results.

Lipids play important roles in the biochemistry, metabolism and reproduction of decapods crustaceans (Teshima et al, 1986; Xu et al., 1994; Soudant et al., 1996; Pillay and Nair, 1973; Palacios et al., 2000). Eyestalk-ablation of male Pacific white shrimp *Litopenaeus vannamei* during the molt cycle resulted hypertrophy and hyperactivity in tissue of the androgenic gland (Vázquez-Islas et al., 2015). Guan et al., (2017) revealed that the progesterone-mediated oocyte maturation pathway subsist in the ovary by eyestalk ablation. Eyestalk extirpation accelerated ovarian maturation in *Macrobrachium lamarrei lamarrei* (Hussain et al., 2017) substantiate with present findings. Eyestalk contained gonad inhibiting hormones, the removal of which resulted successful ovulation in *Litopenaeus vannamei* (Kang et al., 2014) concede with present findings. Unilateral eyestalk ablation in captive Po sugpo (*Penaeus monodon Fabricius*) females from coastal fry resulted viable Naupli after 22 days (Primavera 1978). Dan et al., (2014) delivered that eyestalk ablation in swimming crab *Portunus trituberculatus* regulated larval morphogenesis during metamorphosis in 2 ways: the morphogenesis of body parts that were enlarged were continuously controlled throughout the zoeal stages, whereas the resorption of body parts was controlled instantaneously at a critical point during the premoult of the third zoeal stage. Unilateral eyestalk ablation induced a rise in protein, oligosaccharide, polysaccharide, total free amino acid, total lipid, and cholesterol except moisture, in the muscle of the freshwater crab *Travancorianas chirnerae* (Padmanabhan and Raghayan, 2016). The alteration induced as an impact of unilateral destalkation in the biochemical parameters decided the suitability of this technique to enhance meat quantity and quality as practiced in aquaculture farms of marine decapods but, bilateral ablation may cause high mortality during culture.

Since the conventional food resources alone cannot fulfill the existing demands, aquaculture may surface up as the best way to increase food production without damaging and destroying the biological population and natural balance of that habitat. Therefore, efforts are being made for sustainable utilization of the inland aqua-treasure with a view to increase food production in general through eyestalk unilateral extirpation.

ACKNOWLEDGEMENTS

I would like to thank Professor Dr. Ramesh Shrestha (PhD) for his valuable suggestions and information to publish and submit this article. Thanks are due to Mr. Jay Raj Binadi for his help during manuscript preparation.

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