Eye Stalk Ablation

C. Ganesh[1*], Ashish R. Urkude[2], and Vedant V. Kharabe[3]

[1]M.F.Sc. Department of Fisheries Resource Management,
[2]M.F.Sc. Department of Aquatic Animal Health Management,
[3]M.F.Sc. Department of Aquaculture,
Sher-e-Kashmir University of Agricultural Science and Technology of Kashmir (SKUAST-K),
Faculty of Fisheries, Rangil, Ganderbal, Jammu & Kashmir, India -190006

Corresponding Author
C. Ganesh
Email: chiranjiganesh@gmail.com

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ABSTRACT

India ranks second in world aquaculture production. The production of animal protein, jobs, and foreign exchange are all major economic benefits of the rapidly growing field of crustacean cultivation, which includes species from the order Decapoda like crayfish, shrimp, crabs, and lobsters. Decapod reproductive efficiency can only be increased and selective breeding made easier with a thorough understanding of sexual differentiation and reproductive biology. This review investigates the function of eyestalks in reproduction as well as the effects of sexual differentiation. Decapods eyestalk hormones, such as Gonad Inhibiting Hormone (GIH) and Gonad Stimulating Hormone (GSH), as well as insulin-like androgenic gland hormone (IAG), determine their sexual differentiation. To promote ovarian maturation and enhance breeding results in crab aquaculture, eyestalk ablation which entails the removal of one or both eyestalks is a widely employed procedure. Using AG implantation and IAG silencing to produce neofemales and neomales. Eyestalk ablation techniques including enucleation, ligation, and electrocauterization are reviewed with a focus on their uses, benefits, and drawbacks. To increase knowledge of sexual differentiation mechanisms and enhance decapod crustacean sexual manipulation techniques, more study into sex-related genes and non-coding RNAs is recommended.

INTRODUCTION

Crustacean culture is a rapidly developing industry, due to the high economic value as source of animal protein, employment, and foreign exchange gains. The order Decapoda includes approximately 17,000 species of crayfish, shrimps, crabs, and lobsters (De Grave *et al*., 2009). The order Decapoda comes under Kingdom: Animalia; Phylum: Arthropoda; Subphylum: Crustacea, Class: Malacostraca. The subphylum crustacea include six classes Branchiopoda (Brine shrimp), Remipedia (Blind crustaceans), Chephlocarida (Horseshoe shrimp), Maxillopoda (Barnacles), Ostracoda (Seed shrimps), and Malacostraca (Crabs, lobsters, shrimps). Crustaceans include 23% of total aquatic animal trade in 2022 (FAO, 2024) with 24.6% increase compared to 2020 in global production. Understanding the reproductive biology and mechanism of sexual differentiation are key issues for sexual manipulation and improving the reproductive efficiency of decapods.

Male and female decapods have different growth performances at different growth stages, which is important in artificial selective breeding and farming (Shi X. *et al*., 2019). For example, in crayfish and prawns (freshwater Palaemonidae), males grow larger and faster, and as such, all-male individual rearing is desired. In contrast, females grow larger and faster than males in shrimps (marine Penaeidae) and crabs (Ventura and Sagi, 2012). In hatcheries, females are considered more valuable than males for increasing the size of the population since males can copulate with more females with no negative effect on the percentage of berried females (Harlioglu and Farhadi, 2017a). Studies showed that male and female decapods have different biochemical composition, nutritional value, and flesh quality (Wu *et al*., 2019). Another benefit of sexual manipulation is the ecological application of non-reproductive and mono-sex crustaceans in controlling invasive species.

SEXUAL DIFFERENTIATION AND MATURATION

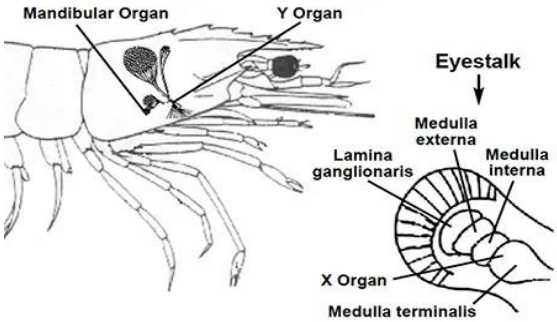
The complex process of sexual differentiation results in sex-specific phenotypic development. Eyestalk is the major neuroendocrine control center and eyestalk ablation influences gonadal development in crustaceans (Fingerman, 1987). The CHHs (crustacean hyperglyceamic hormones) secreted from X-organ have been classified as subfamily I peptides containing a CHH precursor related peptide (CPRP) and the subfamily II peptides lack the CPRP (Jia *et al*., 2012). The subfamily II peptides including moult inhibiting hormone (MIH), gonad/vitellogenesis-inhibiting hormone (GIH or MIH), and mandibular organ-inhibiting hormone (MOIH) control the moulting and gonadal maturation (Lacombe *et al*., 1999: Bocking *et al*., 2002). Serotonin (5-HT), dopamine, and gonadotropin-releasing hormones (1-GnRH-III) are other important factors affecting the sexual development pathway of decapods (Tinikul *et al*., 2008, 2011; Siangcham *et al*., 2013). The insulin like androgenic gland hormone (IAG) secreted from androgenic gland (AG) is a key regulator in sexual differentiation.

ROLE OF EYESTALK IN REPRODUCTION

In prawns, the neurosecretory centres located in the ganglia of eyestalk (X organ sinus gland complex, XO-SG), brain and thoracic ganglia mainly influence the egg production (vitellogenesis). While the X organ sinus gland complex of the eyestalk inhibits vitellogenesis under the influence of its hormone, viz., Gonad Inhibiting Hormone (GIH), The brain

and thoracic ganglia promote vitellogenesis by their hormone viz., Gonad Stimulating Hormone (GSH). Under natural conditions, when the physiological parameters of the prawns and the environmental factors are favourable, the GSH promotes vitellogenesis. On the basis of this principle, the ovarian development and maturation of gonads are obtained in prawns through the unilateral eyestalk ablation (removal of one eyestalk).

It is also worth mentioning here, that the removal of both the eyestalks though lead to rapid ovarian growth, spawning does not result due to physiological stress. It has been observed that the ova are reabsorbed in the ovary. The eye stalks apart from GIH, produce other neurosecretory hormones which regulate lipid metabolism and protein synthesis in hepatopancreas; induce hyperglycaemia in blood to combat stress; regulate calcium metabolism during cuticle formation; effect water balance during moulting; inhibit production of moulting hormone by 'Y' organ and influence movement of pigments in chromatophores. The behavioural pattern of the eyestalk ablated females especially their feeding and mating are also not affected.



Sochasky *et al*., (1972)

SEXUAL MANIPULATION IN DECAPOD CRUSTACEANS

Androgenic gland is the key regulator of primary and secondary sexual characters in male decapods. It controls behavior, spermatogenesis, and sexual differentiation by secretion of IAG. AG is associated with the subterminal part of the sperm duct and attached to the posterior vas deferens in male decapods. IAG silencing had adverse effects on sexual development (i.e., spermatogenesis) in male *Macrobrachium rosenbergii* (Ventura *et al*., 2009).

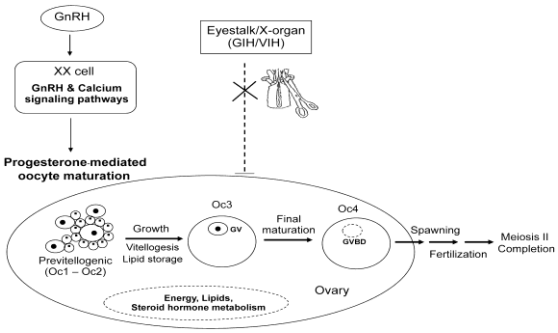
Such as AG ablation and IAG suppression in males, which produce neofemales (phenotypically female but genetically male) in *M. rosenbergii* (Aflalo *et al*., 2006; Ventura *et al*., 2009). Neofemale *M. rosenbergii* (complete and functional sex reversal) was obtained by long-term IAG silencing at PL30 stage (Ventura *et al*., 2012). AG implantation into females induced sex reversal in females, called neomales (phenotypically male but genetically female) (Aflalo *et al*., 2006; Ventura *et al*., 2009). Until now, the only successful AG manipulation that led to complete and functional sex reversal has been recorded in *M. rosenbergii* (Aflalo *et al*., 2006; Ventura and Sagi, 2012). In *Macrobrachium sp.*, IAG is expressed exclusively and abundantly in the AG rather than in the testis or other tissues (Ventura and Sagi, 2012; Ventura *et al*., 2012; Ma *et al*., 2013). However, studies showed that in other decapod groups such as shrimps (Mareddy *et al*., 2011; Li *et al*., 2012b), crayfish (Shi X. *et al*., 2019), and crabs (Zhang *et al*., 2014; Jiang *et al*., 2020a) the IAG is expressed not only in the AG, but also in other tissues. The AG-specific expression pattern of IAG could explain why AG manipulation led to complete sex reversal only in *M. rosenbergii*. The broad tissue expression pattern of IAGs in other decapods indicates that IAGs may have some additional functions.

Recently, several sex-related genes and non-coding RNAs have been identified in decapods, suggesting that sexual differentiation mechanisms in decapods are more complicated than previously expected (Chandler *et al*., 2018). Further studies are required to identify more sex-related factors such as genes, non-coding RNAs and IAG

hormones, which could be involved in the sexual development of decapods. Understating the function of these genetic factors may provide novel insights into the development of new sexual manipulation techniques.

EYESTALK ABLATION IN CRUSTACEANS

It was earlier understood that the endocrine gland present in the eyestalk of the crustaceans is responsible for the blocking of certain activities. It has been named as X organ sinus gland and sinus complex, that secretes Moulting Inhibiting Hormone (MIH). As it generally inhibits growth, it is also known as Growth Inhibiting Hormone (GIH). Therefore, removal or blocking of this hormone from its activity is believed to help in the speed maturation of the animals.



Scheme to induce ovarian maturation by eyestalk ablation

Uawisetwatthana *et al.,* (2011)

Panouse (1943) observed that removal of eyestalk of shrimp *Palaemon serratus* led to ovarion development. Ever since this discovery several workers have tried to induce gonadal maturation in prawn through ablation of eyestalk and also the breeding of different crustacean. Ablation or removal of the eyestalk for induction of maturation in crustacean is an approval practice in the crustacean seed production as an essential prerequisite for the maturation of the shrimps in captivity.

Eyestalk ablation is of two types:

- **Unilateral**: Only one eye will be removed and the animal will be allowed to live one eyed. This method is most preferrable method and has been widely used in commercial hatcheries.
- **Bilateral**: Both the eyes will be removed with the stalks of the eye. Although the bilateral ablation results in faster maturation and yielding desirable results, there are some demerits in this method.

ABLATION PROCESS

It is more crucial than ever to carry out the ablation at the right time to prevent mother shrimps from dying. It states that ablation should only be carried out on inter-molt shrimp. It was observed that ablation under taken between 8-20 days post-moult resulted in significantly greater egg production then that of ablation at 13-15 days post-moult indicating the inter moult period is the best one for ablation process. Post-moult(stage) female shrimps are not recommended for ablation, due to increased risk of handling mortality associated with softened exoskeleton and weakness of the animal Pre-moult (stage IV) individuals are also not recommended since there may be immediate moulting during recover from the ablation process leading to loss or delay in spawning.

METHODS OF EYESTALK ABLATION
ENUCLEATION

- Squeeze hard and roll the thumb and finger outwards away from the body, thus crushing the eyestalk and squeezing out the contents of the eye.
- The objective is to squeeze the contents outwards and not let them follow the eyestalk back in the head region.
- Enucleation has the advantages of simplicity and rapid clotting of haemolymph with the empty eyestalk.

- Grasp the eyestalk just behind the eyeball using the thumb and index finger.

LIGATION

- A string is tied around the base of the eyestalk as close the carapace as possible.
- The string should be drawn fairly tight causing the eyestalk to fall off in a few days.
- The procedure does not leave the shrimp with open wound.
- However, successful ablation is often unpredictable and this process will give the result after two or three days.

ELECTROCAUTERIZATION

- This method is the severing eyestalk followed by sealing of the wound through the process called 'electrocauterization'.
- Heated forceps, or the application of a silver nitrate bar on the wound will prevent the secondary infection at the wounded site.
- Pinching method of ablation required one person and it is without application of antibiotics. Ligation requires two.
- Cautery requires either a cauteriser (or) silver nitrate bar.

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