EFFECTS OF EYESTALK ABLATION ON THE MOLTING, GROWTH AND SURVIVAL OF KADAL SHRIMP

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Roll No: 0122/01

Registration No: 1089

Session: 2021-2022

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Fish Biology and Biotechnology

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JUNE 2023

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made

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June 2023

ACKNOWLEDGEMENTS

All the praises and thanks to Allah, the Almighty, most gracious, most merciful, most benign who has enabled him to pursue the study in fisheries science successfully as well as to submit the thesis for the degree of Master of Science (MS) in Fish Biology and Biotechnology and also pay gratitude to the Almighty for enabling and giving strengths to complete research work as well as thesis within due course of time.

The author expresses his gratitude and indebtedness to Vice-Chancellor, Professor ***Dr. A.S.M Lutful Ahasan*** *and Dean, Faculty of Fisheries and Director, Institute of Coastal Biodiversity, Marine Fisheries and Wildlife Research Center Professor* ***Dr. Mohammed Nurul Absar Khan*** *from the bottom of his heart for their immense administrative support to complete his research work.*

The author expresses his deepest sense of gratitude and sincere appreciation to his honorable teacher and research supervisor, ***Dr. Md. Mahiuddin Zahangir,*** *Associate Professor and Head, Department of Fish Biology and Biotechnology, Chattogram Veterinary and Animal Sciences University, Chattogram for his unfailing support, authoritative guidance, constructive criticism, advice and continuous motivation.*

The author also sincerely expresses his gratitude to his co-supervisor, ***Dr. Md. Faisal,*** *Associate Professor, Department of Fishing and Post-harvest technology, Chattogram Veterinary and Animal Sciences University, Chattogram for his valuable guidance to teach him to be more confident person.*

The author expresses his thanks to ***Md. Moudud Islam****, Associate Professor,* ***Fatema Akhter,*** *Assistant Professor and* ***Md. Main Uddin Mamun,*** *Assistant Professor, Department of Fish Biology and Biotechnology, Chattogram Veterinary and Animal Sciences University, Chattogram for her valuable and constructive suggestions during the research work.*

It's his fortune to gratefully acknowledge the support of his friends Muhammed Erfanul Hoque, Azmaien Naziat, Joya Chakrabarty, Swapan Chakma, Tahmina Parvin Bristy and Md. Mizanur Rahman for their support and generous care throughout the research tenure also thanks all students and staff. Last but not least, I express my deepest sense of gratitude to my beloved parents and friends for their sacrifice, blessings and encouragement.

The Author

Table of contents

List of figures

Abstract

Due to the growing demand, marine crustacean species are becoming a significant part of the export economy of Bangladesh. Therefore, sustainable shrimp culture technologies are a prerequisite for better aquaculture and export output. Here, *Metapenaeus dobsoni*, known as yellow shrimp which is highly adapted to our coastal conditions can play a big role to increase overall shrimp production. Eyestalk ablation is frequently employed in decapod crustaceans to hasten molting, growth, ovarian maturation, and spawning. Therefore, this study's objective was to assess the effect of eyestalk ablation on growth, molting, and survival of *Metapenaeus dobsoni*, a commercially important marine shrimp species of Bangladesh. *M. dobsoni* was ablated unilaterally or bilaterally and reared in the aquarium conditions (17 ppt) for a period of 30 days. Results showed that unilateral eyestalk ablation has a higher molting rate (%) and molting events compared to bilateral ablation and no ablation. In addition, specific growth rate (SGR) and higher weight gains were also observed in unilaterally ablated shrimps. In contrast, bilaterally ablated shrimp had higher mortality (%) and feed conversion efficiency (FCE) compared to unilateral and no ablated shrimp. The findings of the present study showed that unilateral eyestalk ablation may be used to speed up growth in *M. dobsoni* farming in the coastal area of Bangladesh. Further studies with a greater number of samples and longer duration will better confirm the findings of the present study.

Keywords: Eyestalk ablation, Growth, Molting, Mortality, *Metapenaeus dobsoni*

INTRODUCTION

The Bay of Bengal, which is located in the northern Indian Ocean, is regarded as one of the largest marine ecosystems in the world due to its huge biological and ecological significance and nutrient-rich environment. This ecosystem, which has great production and distinct mangrove effects on the coastal and marine zone is also regarded as the richest in the entire globe (Islam, 2003). Mangroves, coral reefs, estuaries, and river canals are just a few of the diverse habitats found in the Bay of Bengal that are mostly used by the fish and shrimp used for their feeding, mating, and raising purposes. The marine fisheries sector of Bangladesh, which is believed to be a crucial part of the national economy, produces a huge variety of species that are important for local consumption and foreign commerce. Recently, Bangladesh regained its access to 1.10 million square kilometers of marine water in the Bay of Bengal by the International Tribunal for the Law of the Sea (ITLOS) (DoF, 2019) which has benefitted both coastal and marine fisheries as well as the country's natural diversity and economic development.

In the year 2019–20, the marine ecosystem of the country produced 64688 MT of shrimp (DoF, 2020). Due to the country's favorable climate, accessibility of resources like feed, seed, water, and a cheap labor force, shrimp aquaculture has grown significantly. (Islam, 2003). There are risks involved in shrimp farming, such as viruses, diseases and contamination of the culture area or Gher, natural disasters, and salinity intrusion, etc. Though there was a sharp fall in shrimp fry production fishery, it still has had a favorable effect on socioeconomic standing of the marginal fishing community (Islam, 2003). Therefore, sustainable shrimp production methods are so essential for the coastal community to enhance the general quality of life, boost export sales, and for the overall economy of Bangladesh.

In terms of the number of living species and the colonization of several habitats, the crustacean family has achieved outstanding success (Varalakshmi et al., 2010). The molt cycle is the most important and challenging phase of a crustacean's life cycle. Crustaceans like shrimp, crabs, and others naturally experience the phenomena of molting, which they perform in order to grow. Molting is referred to as a perpetually recurring crisis and at the same time, it is a dangerous and expensive process of decapod crustacean's life. Shrimp shed their old exoskeleton during the molting process, and as their bodies grow larger (15-20%) as a result of molting, the size of the exoskeleton remains constant. A new exoskeleton, including appendages, entirely replaces the old one during molting. As they become older, shrimp will molt, which is a fully normal process. If they are molting more frequently, it means they are growing properly and are in good health. If they are not growing properly and are having problems, it seems that they are not molting. In ideal circumstances, adult shrimp can molt once every three to four weeks, whereas baby shrimp molt more frequently than adults. (Daoud et al., 2010).

Due to the great diversity and complexity displayed by these intriguing animals, scientists have long been fascinated by crustacean physiology and behavior. There are two main barriers to the mass culture and growth of crustaceans, that are social restraints and cannibalism. Crustaceans raised in groups experience higher rates of cannibalism than those raised alone, which lowers their chance of survival. Social repression appears to highlight the different growth rates in the community culture of crustaceans. This often occurs because the development rates are a slowdown in smaller individuals in contrast to isolated controls (Stewart and Squires, 1968; Mc Leese, 1972; Van Olst et al., 1980; Cobb et al., 1982; Kendall et al., 1982; Lee and Fielder, 1983). Cannibalism and socially repressed development may be affected by other factors like the presence or absence of claws, which many use for predation and aggression in crustaceans. Therefore, sustainable and efficient technologies should develop to reduce cannibalism and social repression for commercial mass-scale shrimp farming.

Artificial ablation or autotomy may have a significant impact on how crustaceans shed their exoskeleton. Autotomy is the removal of an organ from a crustacean that enables regeneration. It is routinely practiced in almost every marine shrimp for the induction of growth, maturation, and reproduction facility in the world for both research and commercial purposes. It is known that crustaceans can molt after ablation to re-grow the same organ. Previous studies on the ablation of the eyestalk and claw in shrimp and crab showed higher growth and molting (Rahman et al., 2020). Previous studies also demonstrated that claw autotomy improved growth and output results in a variety of crab species (Venkitraman et al., 2004; Tamone et al., 2005; Majid et al; 2008, Rahman et al., 2020). Moreover, eyestalk ablation has been a viable strategy for boosting the output of

Penaeus spp. larvae in aquaculture (Bray and Lawrence, 1992; Subramoniam, 2011). Furthermore, *Litopenaeus vannamei*, *Macrobrachium rosenbergii*, *Farfantepenaeus aztecus* and *Farfantepenaeus dourarum* all exhibit improved reproductive success as a result of eyestalk ablation (AQUACOP, 1977 and Caillouet, 1972). It has been shown that eyestalk ablation affects limb growth, gonad inhibition, lipid metabolism, protein metabolism, and carbohydrate metabolism (Cook and Sullivan, 1985).

Additionally, the state of the water has a big impact on shrimp molting. By using appropriate technology, such as the nanobubble machine that speeds up the shrimp molting process and the water quality of ponds can be enhanced. With the aid of a contemporary aeration method termed nanobubble, air bubbles with sizes between 80 and 200 nm are produced that are effective in transferring gas. The high oxygen output of the nano-bubble machine can satisfy the oxygen needs, enabling quicker growth and greater health in shrimp. Nanobubbles can also increase the efficiency of feed, reduce the chance of bacterial and viral outbreaks during cultivation, and increase survival rates. Therefore, autotomy and nanobubble could be promising factors in the regulation of cannibalism and social repression in commercial mass-scale shrimp farming (Naser et al., 2022).

An obvious characteristic of crustaceans, the eyestalk is essential to their physiology and behavior. It functions as a sophisticated sensory organ that combines optical data with hormonal and neurological regulation. Understanding the effects of eyestalk ablation for crustaceans depends on understanding the complex roles of the eyestalk. It entails removing either one or both eyestalks from the shrimp, which causes the gonadotropin-releasing hormone (GnRH) to be released (Pervaiz et al., 2011). The shrimp becomes pre-matures as a result of GnRH stimulation in the ovaries or the testes and is intended to release eggs or sperm, respectively (Treerattrakool et al., 2014). The method is frequently applied in aquaculture to boost shrimp population growth and reproductive output, especially in adult shrimp. However, due to ethical worries about animal welfare and the potential impact on crustaceans' natural behavior, the practice of eyestalk ablation has been the subject of discussion. According to some studies, eyestalk ablation may cause the shrimp to experience more stress and have a worse chance of surviving (Palacios et al., 1999). Eyestalk ablation can have a negative impact on feeding and predator avoidance behaviors in shrimp. Furthermore, the stress brought on by the ablation may result in higher mortality rates. Concerns also exist regarding the moral ramifications of eyestalk ablation (Venkitraman et al., 2004). In contrast, it has been demonstrated that female shrimp with eyestalk ablation perform better in terms of growth and reproduction (Emerenciano et al., 2012). Due to greater feeding activity and better nutrient absorption following ablation, shrimp typically grow more quickly (Cuzon et al., 2000). Therefore, the evaluation of eyestalk ablation should be analyzed for sustainable coastal shrimp aquaculture.

Metapenaeus dobsoni is more readily available in the Cox's Bazar region of Bangladesh. This species is crucial to a huge number of coastal residents for their diets. It is a benthic species that lives in brackish water, with a depth range of 1 to 37 meters (Holthuis, 1980). According to Holthuis (1980), this species reaches sexual maturity at 6.4 cm in length and it is found in mangroves, muddy substrates, and amid shells and can withstand a wide range of salinity, from 3−43 ppt. The larvae, which go through 5 naupliar, 3 protozoal, and 3 myses stages, are found in inshore waters. In order to feed and develop until adult, post-larvae travel to brackish waters, after that they return to the sea where they reach maturity (Sukumaran et al., 1993). Pre-copulatory courtship rituals in *Metapenaeus dobsoni* are frequent (via olfactory and tactile signals), with indirect sperm transfer being the norm (Rupert et al., 2004).

Much of the research has been conducted on other shrimp species in Bangladesh. Very few studies have also been conducted on autotomy or eyestalk ablation in commercial crustacean species in Bangladesh. By understanding the implications of eyestalk ablation, we can gain deeper insights into the physiological and behavioral adaptations which ultimately advanced our understanding of crustacean biology and pave the way for sustainable management strategies and conservation efforts of commercially important species like *M. dobsoni*. Based on the aforementioned phenomenon, the current study proposed that eyestalk ablation of crustaceans would: (a) accelerate the molting process and thereby reduce the molting duration in farming systems; (b) decrease the tendency to fight, which would enable more people to be stocked in the current cultural systems; and (c) be a possible strategy to increase the production efficiency due to the shorter molting duration.

Objectives:

The study's aim is to ascertain the impact of eye stalk ablation of *Metapenaeus dobsoni* to improve production performances.

The specific objectives of the study are to:

- ✓ To determine the effects of eyestalk ablation on the weight gain, net yield, specific growth rate (SGR%/day), feeding efficiency (FE) and survival (%);
- ✓ To determine the effect of eyestalk ablation on molting events and molting number

LITERATURE REVIEW

This literature review examines the effect of eyestalk ablation on the molting, growth, and survival of *Metapenaeus dobsoni*, a commercially important shrimp species in Crustacea farming. The review seeks to offer insights into the physiological reactions, growth patterns, and survival rates of *M. dobsoni* after eyestalk ablation by summarizing pertinent investigations that have been completed recently. The findings discussed in this review contribute to our understanding of the implications of eyestalk ablation on *M. dobsoni* in commercial shrimp farming.

Eyestalk

In anatomy, an eyestalk, also known as an ommatophore or eye stalk, is a projection that extends an eye away from the body to provide it a wider field of view. It is a typical element of nature and regularly shows up in works of fiction (Allayie et al., 2011). Crustacean eyestalks contain neurosecretory cells that play a role in molting regulation (Meade and Watts, 2001). As in shrimp, lobsters, snails, and certain other crustaceans and mollusks, it is essentially a moveable stalk with a compound eye at the tip.

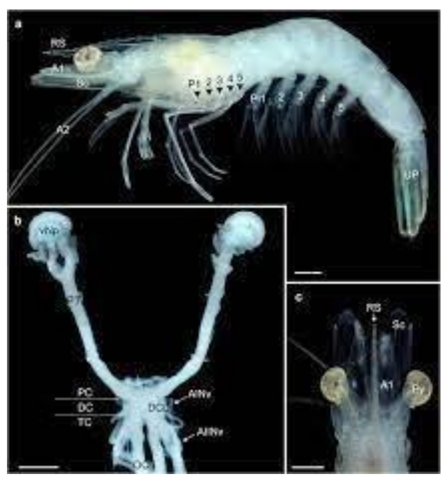

Figure 1: Eyestalk in shrimp

X organ-sinus gland complex

The medulla terminalis ganglionic X organ-sinus gland complex is an essential endocrine regulating area present in the eyestalks of the majority of stalk-eyed crustaceans (Reddy and Ramamurthi, 1999). The X-organ is a collection of neurosecretory somata. The X-organ/sinus gland complex is a tissue made up of a group of neurosecretory cells that is in charge of producing a variety of significant neuropeptides (Wilder et al., 2018). X-organ cell axons which congregate on a lateral hemolymph sinus to form the sinus gland with its dilated axon terminals are thought to make up the majority of the nerves in each eyestalk (Spaziani et al., 1994). The X-hormone organs are kept in reserve by the sinus gland for the release of hormones in response to stimuli. The X-organs produce vitellogenin inhibitory hormone (VIH), which is then released or retained in the hemolymph via the sinus gland of the eyestalks. In order to control metabolic activities such vitellogenesis, food intake, nutrient transport, digestion, metabolism of lipids, molting and regulation of glucose and proteins, hormones are generated, stored, and secreted to the hemolymph in this gland (Chang and O`Connor, 1988; Chang, 1992; Chen and Cheng, 1995; Fingerman, 1995; Ponnuchamy et al., 1980; Rosas et al., 1995; Santos and Keller, 1993a; Santos and keller, 1993b; Santos et al., 1997; Subramoniam, 2011; Taylor et al., 2004; Teshima et al., 1988).

Y-organ

The Y organs, also known as ecdysial glands, are endocrine structures derived from the ectoderm that are found in the cephalothorax. The Y-organs are located in the maxillary segment of decapods and are the source of molting hormones, or ecdysteroids, which encourage molting and interact with molt-inhibiting hormones from the X-organ. In different groups of Malacostraca, they are derived from the epidermis and either remain linked to it or develop into entirely autonomous organs (Bückmann, 1984). These structures are cytologically similar to the prothoracic glands of insects and regulate various aspects of crustacean reproduction and growth (Chang et al., 1993). The eyestalk system and the Y-organ (endocrine gland) regulate shrimp molting by producing the hormone ß-ecdysone, which is a precursor to 20-hydroxyecdysone, which triggers molting. According to Mykles (2011), it is a steroid hormone made in the Y-organ. As opposed to this, MIH inhibits the synthesis of 20E in the Y-organ, preventing ecdysis (Tamone et al., 2005; Nagai et al., 2011; Pamuru et al., 2012).

Eyestalk and major hormones in shrimp

Ecdysteroids are steroid hormones produced by arthropods that are primarily responsible for the development, reproduction, and molting (de Loof, 2006; Margam, 2006). Ecdysone, ecdysterone, turkesterone, and 2-deoxyecdysone are a few examples of ecdysteroids. Following metabolism by the Halloween family of cytochrome P450s, these substances are produced in arthropods from dietary cholesterol (Mykles, 2011). The Y-organs produce the molting hormone, which is secreted into the hemolymph as the precursor ecdysone, where it is transformed into the active hormone 20-OH-ecdysone by a 20-hydroxylase activity found in the epidermis and other organs and tissues. The primary circulating ecdysteroid in premolt animals is 20-OH-ecdysone, but the primary ecdysteroid produced in vitro by the Y-organ of the penaeid shrimp *P*. (*Litopenaeus*) *vannamei* is 3 dehydroecdysone. Parallel to the growth in ecdysteroid production, hemolymph ecdysteroid levels increased and peaked at the late pre-molt stage (Blais et al., 1994).

Molt-inhibiting hormone (MIH), vitellogenesis-inhibiting hormone (VIH), mandibular organ-inhibiting hormone (MOIH), and crustacean hyperglycemic hormone (CHH) is among the key peptide hormones produced by the sinus gland in the eyestalk. In addition to CHH-family peptides, the crab eyestalk also produces and secretes neurotransmitters including serotonin, melatonin, and dopamine, which are chromatophore-regulating peptides like pigment-dispersing hormone (PDH) and red pigment-concentrating hormone (RPCH) (Wainwright et al., 1996). Ovarian growth inhibitor was found in eyestalks in *Leander serratus* (Panouse, 1943). The process of vitellogenesis is essential for female crustacean reproduction. Vitellogenin (VG) (VT) is the building block of vitellin, which is the main protein in egg yolks. It is mostly formed at the hepatopancreas and ovary in decapods, where it is then deposited in the hemolymph and then built up in the growing oocytes. Gonadal- or vitellogenesis-inhibiting hormone (GIH/VIH), which is located in the eyestalk, is the major hormone that prevents conception. Due to the ablation of GIH, the eyestalk was damaged during sexual inactivity, which led to a constant increase in ovarian size and an early egg deposit. Several investigations that followed Panouse's study indicated that most crustaceans' ovarian development is hindered by VIH (De Kleijn et al., 1998; Edomi et al., 2002; Ohira et al., 2006; Ollivaux et al., 2006; Treerattrakool et al., 2008; Treerattrakool et al., 2011; Tsutsui et al., 2007).

The main effect of MIH is to inhibit Y-organ ecdysterone production. There aren't many reports on MIH affecting crustacean reproduction. It has been investigated how MIH prevents shrimps from developing vitellogenesis (Tiu and Chan, 2007). Chang (1985) claimed that the MIH antagonist functions as a bridge between neurological signals and the steroidal control of developmental processes including molting and embryogenesis. It does this by facilitating the Y organ's production of molt hormone.

Crustacean Hyperglycemic Hormone (CHH) in reproduction is identified in the crustacean's neuroendocrine system locates in the X Organ Sinus Gland. In decapod crustacean reproduction, the mobilization of glucose for energy metabolism, ecdysis, and osmotic pressure regulation are just a few of the crucial physiological processes supported by members of the CHH family (Bocking et al., 2002; Chan et al., 2003; Chang and Mykles, 2011; Chung et al., 2010; Webster et al., 2012). The bulk of the CHH- peptides are composed of 70 to 80 amino acid residues, and three intramolecular disulfide connections (Gorgels-Kallen and Vooter 1985; Mattson and Spanziani 1985). CHH not only regulates glucose metabolism but also encourages the start of vitellogenesis, which leads to reproduction. Van (1998) has also presented an explanation of how CHH promotes vitellogenesis. Methyl farnesoate (MF), which limits reproduction in crustaceans, is made by the mandibular organs. Female *Homarus americanus* lobsters had a larger mandibular organ during the maturation of their ovaries (Byard, 1975). Eyestalk neuropeptide mandibular organ-inhibiting hormone (MOIH) has a detrimental influence on the mandibular organ by blocking the synthesis of methyl farnesoate (Wainwright et al., 1996; Reddy et al., 2004).

Eyestalk ablation

Eyestalk ablation has been used as a technique to encourage ovarian development and spawning in crustaceans since the 1940s. This methodology is justified by the presence of an inhibitory substance known as vitellogenesis-inhibiting hormone (VIH), also known as gonad-inhibiting hormone (GIH) in the eyestalks. As a result, VIH activity negatively regulates vitellogenin production. The most efficient way to cause molting and maturation in decapod crustaceans is currently thought to be eyestalk ablation (Sainz-Hernández et al., 2008; Pervaiz et al., 2011; Wen et al., 2015).

Eyestalk ablation is a process where the eyestalk is tightly tied off with surgical or other thread by ligation. It is routinely used on female shrimp (or female prawns) in almost all marine shrimp maturation or reproductive facilities around the world, for both scientific and commercial purposes. Using the eyestalk ablation method, shrimp eyestalks can be reduced or removed entirely. Both unilateral ablation and bilateral ablation are possible; however, unilateral ablation is more common (Antony and Kumar 2022). In these conditions, the purpose of ablation is to encourage the development of mature ovaries in female shrimp (Uawisetwathana et al., 2011).

Effects of eyestalk ablation

Eyestalk ablation has been utilized since 1970 to increase the aquaculture output of *Penaeus* spp. larvae (Bray and Lawrence, 1992; Subramoniam, 2011). In commercial hatcheries, it is the most widely utilized method of inducing crustacean reproduction because it decreases aggression, which increases survival and fosters better growth (Asusena et el., 2012).

In many hard-to-spawn, closed-thelycum female prawn brood stock species such *F. aztecus, F. duorarum, Penaeus monodon, and P. orientalis*, unilateral eyestalk ablation is a standard procedure to induce ovarian maturation (Primavera, 1985 and Gandy et al., 2007). This method can be used to encourage spawning both during the reproductive season and in the off-season (Aktas et al., 2003). Research has demonstrated that unilateral eye ablation, in which just one eyestalk is removed, is adequate to induce molting, increase the size of the reproductive gland, and lessen any adverse effects (Arnstein and Beard, 1975). According to Heit and Fingerman (1975), the removal of the eyestalk causes an increase in weight and a decrease in the osmolality of the hemolymph. Unilateral eyestalk ablation can decrease the moult interval and speed up gonad development in shrimps (Lin and Crewell, 2001).

Eyestalk ablation encourages many metabolic changes that enhance reproductive performance in the white shrimp *Litopenaeus vannamei*, *Macrobrachium rosenbergii*, *Farfantepenaeus aztecus*, and *Farfantepenaeus duorarum* (AQUACOP, 1977; Caillouet, 1972). For gonad maturation, copulation, fertility, and larval development to occur normally, there must be synchrony between these several metabolic processes.

Effects of eyestalk ablation on molting

Studies on the removal of the eyestalks in prawns have shown that in some species, including *Macrobrachium lanchesteri* and *Cryphiops caementarius*, the length of the molt cycle and the rate of reproduction are not significantly altered (Ponnuchamy et al., 1980; Reyes et al., 2002). In contrast, several species, including *M. rosenbergii* and *M. acanthurus*, have shorter molting cycles that enable females to lay more eggs over time (Okumura and Aida, 2001; Cunha and Miyaco, 2010).

According to Venkitraman et al. (2004), there were significant differences in the dry moult weight and moult rate of Penaeid prawns *M. dobsoni* of two different sizes that had been ablated both unilaterally (UEA) and bilaterally (BEA). While bilateral eyestalk ablation decreased the dry weight of the moult and this reduction in the rate increased after each succeeding moult, unilateral eyestalk ablation increased the average intermolt period and increased dry weight. According to the findings, unilateral eyestalk ablation can be used to boost growth and conversion rates.

The duration of the molting cycle was significantly shortened in eyestalk-ablated *Lipopenaeus vannamei* compared to unablated shrimp (24 days), bilaterally (10 days), and unilaterally (17 days), as a result of decreased or suppressed molt-inhibiting hormone (MIH) production (Sainz-Hernández et al., 2008).

Effects of eyestalk ablation on metabolism

Biological processes in two penaeid prawns for the research of effects of unilateral (UEA) and bilateral (BEA) eyestalk ablation, *Metapenaeus monoceros* and *M. dobsoni* were investigated. The two size groups of BEA prawns in both species absorbed more oxygen than the UEA prawns, which resulted in increased metabolic activity. The oxygen consumption (OC) trend was a progressively declining function of time in the small-size group, but a steady state condition was seen in the large-size group, in both species. Small-size groups indicated more metabolic activity than the large size ones. More ammonia was excreted (AE) by the small-sized prawns than the large-sized ones. BEA prawns of both size groups excreted more ammonia than UEA with higher ammonia quotient (AQ) values, which resulted in higher metabolic activity. This suggests that steps should be taken to keep the amount of ammonia in the culture to a minimum in order to protect the culture (Venkitraman and Jayalakshym, 2008).

Effects of eyestalk ablation on reproduction and survival

The majority of research on the removal of penaeid shrimp eyestalks has been on reproduction (Bray and Lawrence, 1992; Racotta et al., 2003). The metabolic or immunologic effects were only briefly examined in a few research (Rosas et al., 1993; Perazzolo et al., 2002) discovered that the energy balance of the sexes differed following ablation, despite the fact that these changes were attributed to the divergent reproductive strategies of men and females.

After both eyestalks were removed, it was discovered that the ovary of juvenile *M. japonicus* produced more vitellogenin mRNA and had a higher gonadosomatic index (Tsutsui et al., 2005). When both eyestalks were removed from young female *M. japonicas*, the ovarian weight, hemolymph vitellogenin levels, and vitellogenin mRNA all increased significantly (Okumura 2007).

In order to accelerate crab reproductive development, eyestalk ablation has been used (Bray and Lawrence, 1992). While ovarian maturation may be induced by ablation, growth is also put at risk, the molting cycle is shortened, and there is a rise in energy requirements (Browdy and Samocha, 1985). These effects eventually lead to a reduction in egg quality and increased mortality (Benzie, 1998). Injurious behavior has the potential to cause physiological stress and even have an impact on survival (Indriyani et al., 2021). Accordingly, a long-term objective for the shrimp industry is predictable maturation and spawning in captivity without eyestalk ablation (Quackenbush, 1991).

In an investigation on *P. monodon* broodstock, Uawisetwathana et al. (2011) used a cDNA microarray to pinpoint the cellular processes that eyestalk ablation affected. The outcome revealed 682 transcripts that were differently expressed in the ovaries of eyestalk-ablated and intact brooders. During the ablation, the reproductive genes involved in vitellogenesis were greatly boosted. As compared to untreated shrimp (2%), mortality was significantly higher in unilaterally (35%) and bilaterally (68%) ablated *Lipopenaeus vannamei*. This is likely due to the impairment of a number of physiological functions, which are mediated by hormones from the eyestalk, as well as direct damage to the nervous system (Sainz-Hernández et al., 2008).

Metapenaeus dobsoni

The dobnosi shrimp, also known as *Metapenaeus dobnosi*, is a species of crustacean that is a member of the Penaeidae family. The Indo-West Pacific region, from the west coast of India to the Philippines and New Guinea, is where it is geographically distributed (Holthuis, 1980). *Metapenaeus dobnosi* falls within the Kingdom Animalia, Phylum-Arthropoda, Subphylum-Crustacea, Class-Malacostraca, Order-Decapoda, Family-Penaeidae, and Genus-*Metapenaeus*. It belongs to the diverse class of decapod crustaceans, which also includes shrimp, lobsters, and crabs, according to its taxonomic classification. In the sexually dimorphic *Metapenaeus dobnosi*, females are often larger than males. The species has a variety of hues, including brown, green, and gray tones, which help them blend in with their surroundings and act as camouflage. Depending on the surroundings and the stage of life, the coloration may also change.

Shrimps have been extensively exploited in recent decades for human consumption (Aguirre and Ascencio, 2000). *Metapenaeus dobsoni* is one of the exploited prawns with significant aquaculture potential. These shrimps also have thin shells, an appealing appearance, and tasty meat (Ponce-Palafox et al., 2002). Much of the research has been conducted on other shrimp species, however, very little is known regarding eyestalk ablation in *M. dobsoni*. Here, in this study, the effect of eyestalk ablation has been evaluated by observing the molting and growth of this commercially important species.

MATERIALS AND METHODS

In a thesis, the terms "Materials" and "Methods" typically refer to two distinct sections that provide detailed information about the materials used and the methodology employed in the research study. Here's a brief explanation of each section: The "Materials" section provides a comprehensive description of the materials, tools, equipment, and substances utilized in the research. It includes specific details about the origin, specifications, and quantities of the materials.

The "Methods" section outlines the procedures and techniques employed to conduct the research study. It provides a step-by-step account of how the research was carried out, including the experimental design, data collection methods, data analysis techniques, and any statistical procedures used. This section aims to provide sufficient information for other researchers to replicate the study and verify the results.

Collection of samples

Metapenaeus dobsoni (length 4.55 ± 0.66 and weight 0.59 ± 0.14) was collected from the Dorianogor beach of Cox's Bazar by hand net (Figure 2). About 200 individuals of *Metapenaeus dobsoni* were collected for this research purpose. The shrimp were collected in large plastic buckets, sorted, and transferred to the hatchery of the Institute of Coastal Biodiversity, Marine Fisheries and Wildlife Research Centre of Chattogram Veterinary and Animal Sciences University (CVASU) with aeration. The full strength of seawater (salinity 33ppt) was collected from the near shore area of Dorianogor Beach. The water was filtered and transported in large carboys of 50liters capacity to the hatchery of the research station. The salinity of the filtered seawater was adjusted to the desired level (17 ppt) by adding aerated fresh water. The shrimp was conditioned first for a period of 24h and after conditioning when the observation period started, they were fed once in a day with commercial pellet feed approximately equivalent to 2% of the body weight.

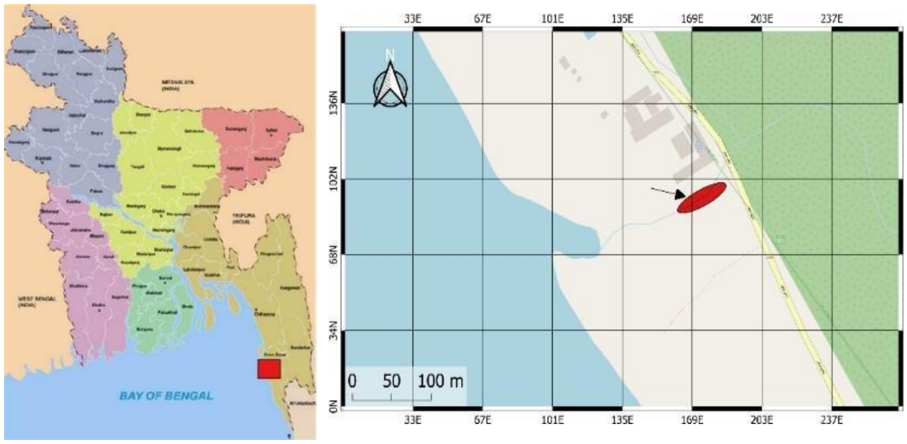

Figure 2: Sample collection site

Experimental design

In this experiment, nine glass aquaria (18 × 12 × 10 inches) were filled with 20L of filtered saline water (17 ppt). Water quality parameters were maintained to keep it at an optimum level that meets up the requirement for a successful experiment. Aerators were used due to keep the water oxygenated. Three treatment groups, including (a) no ablation, (b) unilateral eyestalk ablation, and (c) bilateral eyestalk ablation with three replications were used to examine the effects of eyestalk ablation. Ten *M. dobsoni* were randomly assigned in each aquarium after either unilaterally ablated or bilaterally ablated in each aquarium. The control group was not ablated and also stocked in similar numbers in each aquarium.

Figure 3: *Metapenaeus dobsoni*

Eyestalk ablation

In the present study, eyestalks were removed unilaterally or bilaterally using sterile surgical blades reduce to mortality. Shrimps in the juvenile stage were chosen for eyestalk ablation. At first, *M. dobsoni* was kept in a 100ppm (17ppt) 4L anesthetic solution to be anesthetized. After that, body weight (gm) and lengths (cm) were recorded. The shrimp was carefully held in pre-cooled sea water during ablation in order to lower the pulse rate and hemolymph loss. For bilateral eyestalk ablation, the second eyestalk was taken out at intervals of two to three hours. Zero mortality was attained by using this approach. The shrimp that had been abated were then put into the experimental tanks (Figure 4 −6).

Figure 4: Preparation for eye-stalk ablation

Figure 5: Preparation of experimental tanks with adequate requirements

Figure 6: Conducting eye-stalking ablation

Size and weight measurements

Different lengths (cm) of the shrimp, like standard length (SL), rostrum length (RL), carapace length (CL), pre-carapace length (PCL), and telson length (TL) were measured by vernier calipers. After blotting on filter paper, the weight (g) of the shrimp was measured using an electronic balance (PS 1200. R2) (Figure 7).

Figure 7: Measuring length and weight

Observation of molting and survival

Molting number, molting frequency, and survival of shrimp in the aquarium were observed 4−5 times a day for a period of thirty days. Most of the molting was observed during the early morning. Molting was confirmed by observing the old exoskeleton and soft body of the molted shrimp. Old exoskeletons sometimes remained floated in the aquarium; the number of floated exoskeletons confirmed the number of molting shrimps in the aquarium. The deaths of the shrimp were confirmed by observing unmoved appendages. The dead shrimp were also counted and the final weight (g) was recorded immediately after death (Figure 8).

Figure 8: Observing molting and survival

Monitoring of water quality parameters

Indicators of water quality include physical, chemical, and biological characteristics that were regularly observed in accordance with the intended water parameters of concern. Several parameters were routinely collected or monitored to determine the quality of the water, including temperature, conductivity, salinity, ORP, dissolved oxygen, pH, and turbidity. P^H level, temperature and DO level were kept at 8.5-8.7, 26-28 ^{0}C, 4-6 mg/L respectively in the observation period. In addition, seawater was regularly exchanged to keep the aquarium clean and to provide a suitable environment for the shrimp (Figure 9).

Figure 9: Measuring water quality parameters

Estimation of the growth parameters

After thirty days of experimental trial, weight gain, % weight gain, specific growth rate (SGR %/day), feeding efficiency (FE), feed conversion ratio (FCR), feed conversion efficiency (FCE) and survival (%) were calculated on the basis of the following formulas:

- ✓ Weight gain = Final weight of shrimp- Initial weight of shrimp
- ✓ Percent weight gain = (Final weight of shrimp- Initial weight of shrimp) *100
- ✓ (SGR%/day) = 100* (In W_n-In W_{n-1})/t
- ✓ FCR = Amount of feed fed/ Total weight gain
- ✓ FCE = Total weight gain/ Amount of feed fed
- ✓ No of Molting = Total number of molting / no of treatments
- ✓ Survival (%) = 100*(final no. of shrimp / initial no. of shrimp)

Data analysis

Values were presented as mean ± standard deviation of the mean (SD). All statistical analyses were performed in Microsoft excel and SPSS 26.0 for windows (SPSS Inc.). One-way analysis of variance (ANOVA) was used to examine statistically significant differences, and then Tukey's HSD post-hoc analysis was performed. Statistically significant variations were set as $p < 0.05$ unless described anywhere in the text.

RESULTS

Effect of eyestalk ablation on the molting events in *M. dobsoni*

In the *M. dobsoni*, eyestalk ablation also significantly affected the events of molting during the 1-month study period. According to the findings, the bilateral eyestalk ablated shrimps had the highest molting events (11.00 ± 0.87) in 30 days (Figure 11). The lowest molting events (2.50 ± 0.87) was observed in the no-eyestalk ablated shrimps. In the case of unilaterally eyestalk ablated shrimps, the average molting events was 8.50 ± 0.87 in each aquarium during the study period. In the three-separate eyestalk-ablated shrimps, we found a statistically significant difference in the molting events ($p < 0.05$) (Figure 11).

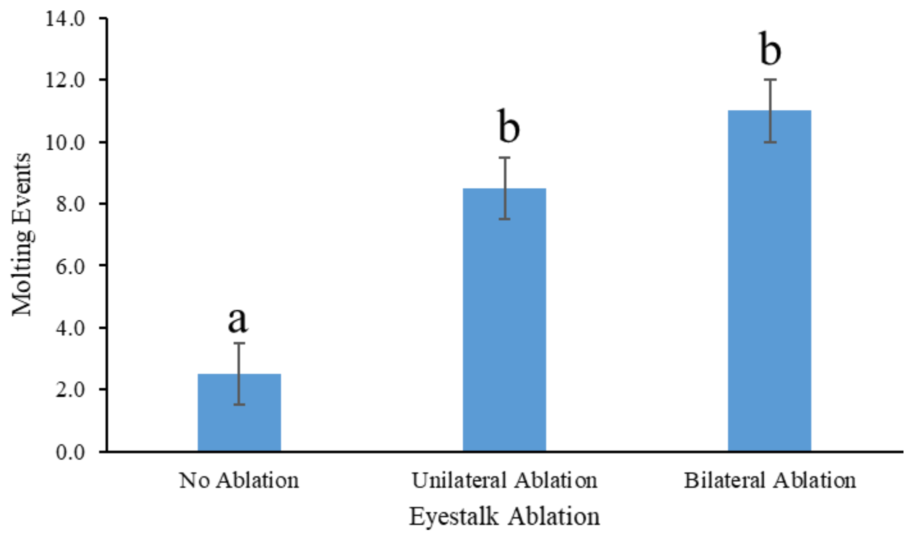

Figure 11: Effect of eyestalk ablation on the molting events in the *M*. *dobsoni* for a period of 30 days. Values are presented as mean ± standard deviation of the mean (SD) (n = 15 for each treatment groups). Different subscripts of alphabets are statistically significant at $p < 0.05$ according to the Tukey's HSD post-hoc test.

Effect of eyestalk ablation on the percentage of molting number in *M. dobsoni*

Eyestalk ablation has a significant impact on the percentage of molting number in *M. dobsoni*. In this study, *M*. *dobsoni* were subjected to three different eyestalk ablations (no ablation, unilateral eyestalk ablation, and bilateral eyestalk ablation), and the number of percentages of molted shrimps were recorded for a period of 30 days in each treatment group. Results found that the highest percentage of molting number (80 ± 10) % was observed in the unilateral eyestalk ablated shrimps (Figure 10). The lowest molting percentage number (42.2 ± 38) % was observed in the no-eyestalk ablated shrimps. In the case of bilaterally eyestalk ablated shrimps, the average molting percentage number was (70 ± 10) % in each aquarium. We found a statistically significant difference ($p < 0.05$) in the three different eyestalk-ablated shrimps (Figure 10).

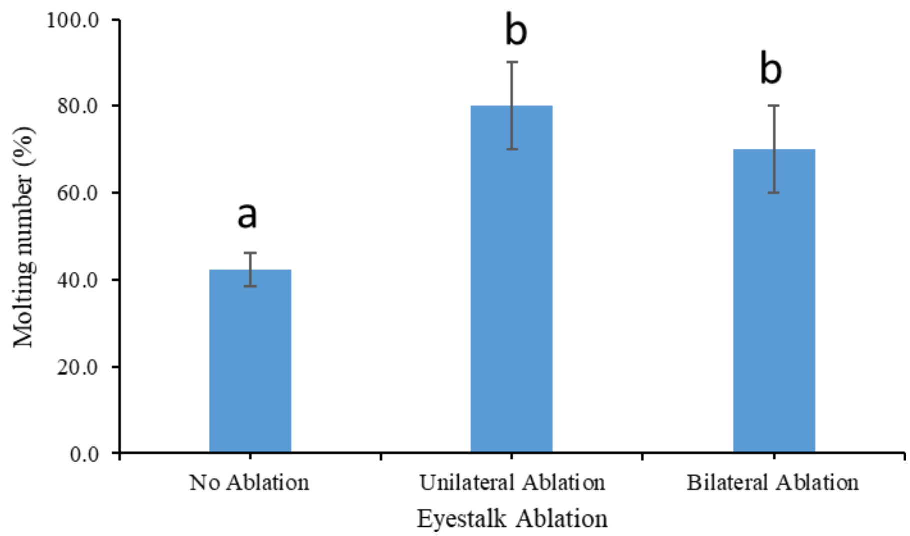

Figure 10: Effect of eyestalk ablation on the percentage of molting number in the *M. dobsoni* for a period of 30 days. Values are presented as (mean ± standard deviation) % of the mean (SD) (n = 15 for each treatment groups). Different subscripts of alphabets are statistically significant at $p < 0.05$ according to the Tukey's HSD post-hoc test.

Effect of eyestalk ablation on the growth performance in *M. dobsoni*

The growth performance of *M. dobsoni* has been greatly impacted by the eyestalk ablation as well. In this study, the weight gain, percentage weight gain, and specific growth rate (SGR) as growth parameters were measured. Results showed that weight gain was significantly higher ($p < 0.05$) in unilaterally eyestalk-ablated shrimps than in no ablated and bilaterally eyestalk-ablated shrimp (Figure 12A).

Besides, higher FCR (1.62 ± 0.05) was found in the non ablated shrimp than in unilateral eyestalk-ablated shrimp (0.82 ± 0.19) and bilateral eyestalk-ablated shrimp (0.44 ± 0.09) (Figure 12B). In contrast, the lowest FCE (0.62 ± 0.02) was observed in the no eyestalk ablated shrimps compared to the unilateral (1.26 ± 0.30) and bilaterally eye-stalked shrimp (2.33 ± 0.49). A statistically significant difference ($p < 0.05$) was observed in the FCR and FCE values among different treatment groups (Figure 12C).

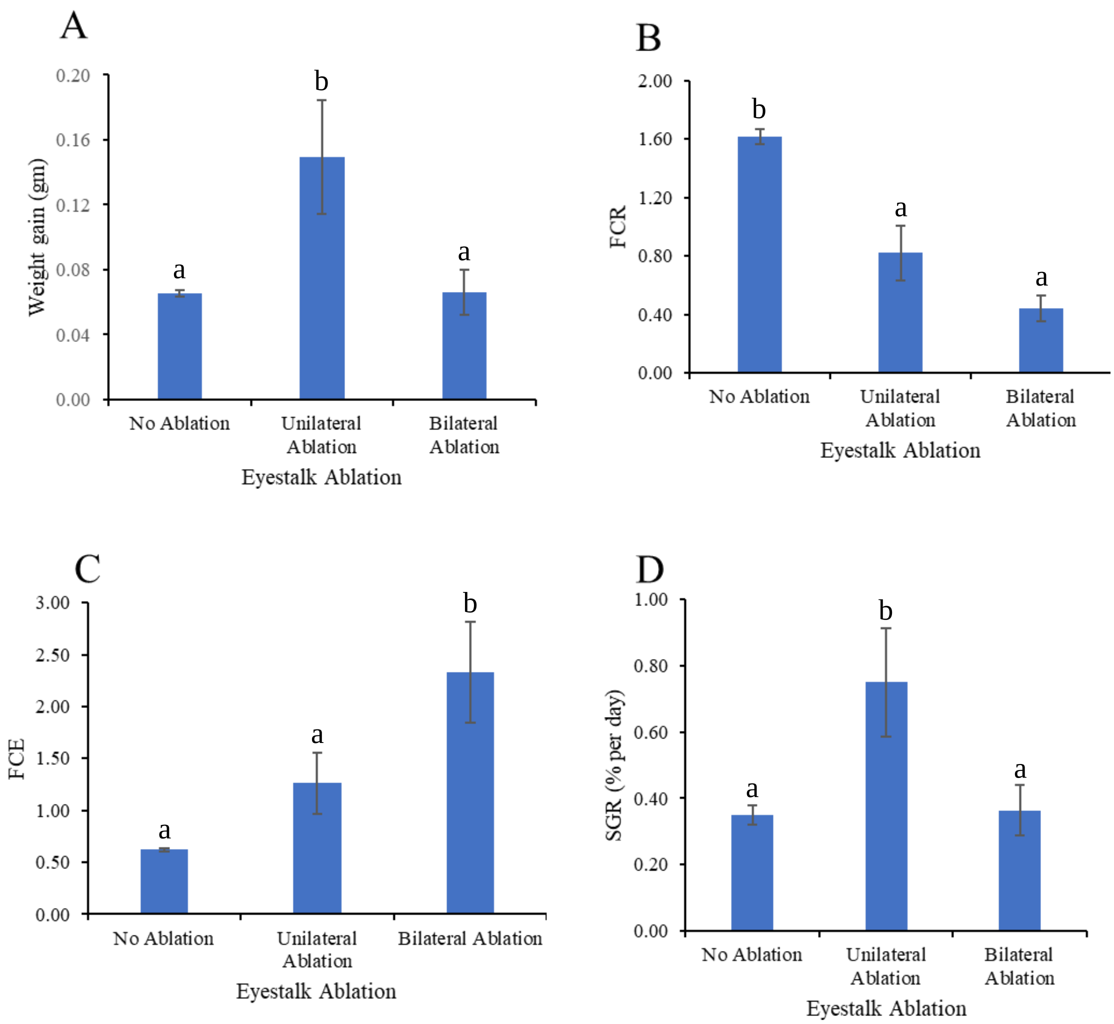

Figure 12: (A to D): Effect of eyestalk ablation on the growth performance in the *M. dobsoni* for a period of 30 days. Values are presented as mean ± standard deviation of the mean (SD) (n = 15 for each treatment groups). Different subscripts of alphabets are statistically significant at $p < 0.05$ according to the Tukey's HSD post-hoc test.

Furtherly, a higher SGR (% per day) was also recorded in the unilaterally eyestalk ablated shrimp than in no eyestalk ablated and bilateral eyestalk ablated shrimp (Figure 4D). The average SGR was 0.35 ± 0.03, 0.75 ± 0.16, and 0.36 ± 0.08 percent per day in no ablated, unilaterally ablated, and bilaterally eyestalk ablated shrimp respectively. We found a statistically significant difference ($p < 0.05$) among different treatment groups (Figure 12D).

Effect of eyestalk ablation on the percentage of mortality in *M. dobsoni*

In this experiment, eyestalk ablation had a significant impact on the percentage of mortality of *M. dobsoni*. The bilateral eyestalk ablated shrimps had the highest percentage of mortalities during the study period (Figure 13). Most of the bilaterally ablated shrimps died periodically after a particular time of molting. Before dying, shrimp recirculate themselves both clockwise and anti-clockwise for a particular period of time especially, the bilaterally ablated shrimps. The lowest percentage of mortality (16.67 ± 5.77) % was observed in the no-eyestalk ablated shrimps during the study period. In the case of unilaterally eyestalk ablated shrimps, the average mortality percentage was (20 ± 10) % in each aquarium. We found a statistically significant difference ($p < 0.05$) in the mortality percentage among the three different eyestalk-ablated shrimps (Figure 13).

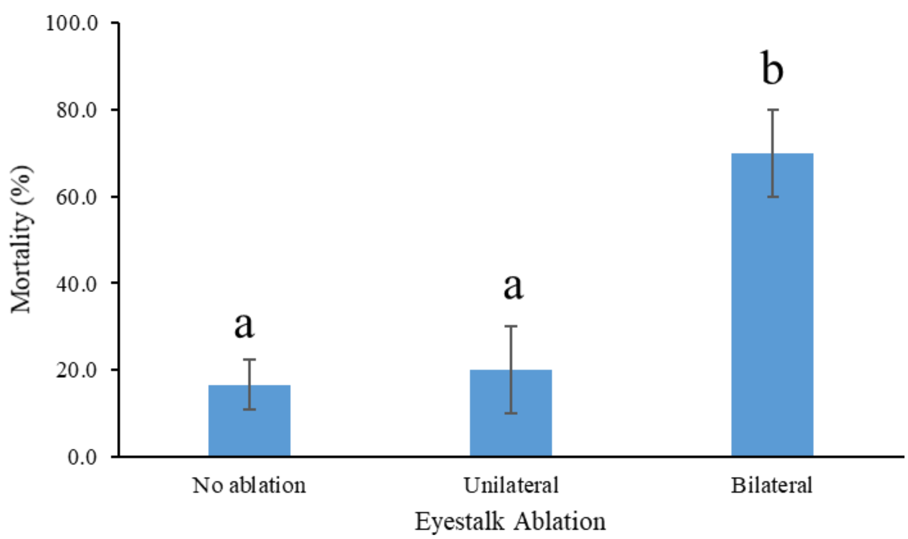

Figure 13: Effect of eyestalk ablation on the percentage of mortality in the *M. dobsoni* for a period of 30 days. Values are presented as (mean ± standard deviation) % of the mean (SD) (n = 15 for each treatment groups). Different subscripts of alphabets are statistically significant at $p < 0.05$ according to the Tukey's HSD post-hoc test.

DISCUSSION

In the present study, higher growth performance was observed in unilaterally eyestalk ablated shrimp than bilateral ablation and no ablation. It was also observed that the survival rate is low in bilateral eyestalk ablation. In addition, it was also noticed that frequent molting events occurred in bilateral eyestalk ablation. So, better results were found in the unilateral eyestalk ablation as less mortality (%) than other treatments and better molting performance and growth than other treatments. The results of this study's findings can be helpful for the sustainable shrimp culture in the coastal region of Bangladesh.

The length of the molt cycle is shortened through the eyestalk ablation in both sexes of white-leg shrimp. Similar findings were also in the study by Chan et al., (1990) who found that shrimp that were intact, unilaterally ablated, or bilaterally ablated underwent a molt cycle that lasted 23.4, 15.9, and 9.1 days, respectively. The effect of eyestalk ablation however was less significant in two similar species of shrimp (blue shrimp *Litopenaeus stylirostris* and white shrimp *L. setiferus*) kept at warmer temperatures (27−29 °C) (Sainz-Hernández et el., 2008). According to Robertson et al. (1987), the molt cycle took 13.6 days for intact shrimp and 11.5 days for unilaterally ablated shrimp. In the present study, we found a significant difference in the molting number (%) as well as molting events among the no ablation, unilateral ablation, and bilateral ablation in the *Metapenaeus dobsoni* which confers the findings of the previous studies in other shrimp species.

The reduced concentration of the putative molt-inhibiting hormone (MIH) brought on by eyestalk ablation is primarily responsible for the reduction in molt cycle duration after eyestalk ablation. Studies found that the biosynthesis of ecdysteroids is inhibited by sinus gland extracts or a recombinant MIH (Lachaise et al., 1993; Lee and Watson, 2002; Zheng et al., 2008). Therefore, 20-hydroecdysone (20E) is produced and secreted at a higher rate in eyestalk-ablated shrimp. However, the duration of the molt cycle did not shorten following eyestalk ablation in *L. stylirostris* (Gendrop-Funes and Valenzuela-Espinoza, 1995). According to Chan et al., (1990), this may vary due to the reproductive or molting stage when eyestalk ablation occurred. The frequency of molts and the growth increment acquired at each molt determine how quickly crustaceans grow. Two hormones, ecdysterone (which speeds up molting) and the molt-inhibiting hormone have an impact on molting (MIH which retards molting). It has been demonstrated that the latter, a peptide, is found in the sinus glands of crustaceans (Bruce & Cheng, 1984), and it is known to interfere with ecdysone's ability to induce proecdysis, delaying the molt cycle (Quakenbush & Herrnkind, 1983). Similar findings were made by Soumoff and O'Conner (1982) and Bruce & Cheng (1984), which demonstrated that the sinus glands in the beach crab and American lobster govern the molt cycle by preventing the Y-organs from secreting ecdysone. The molting cycle was significantly shortened in eyestalk-ablated *Lipopenaeus vannamei* compared to unabated shrimp (24 days), bilaterally (10 days), and unilaterally (17 days) as a result of decreased or suppressed molt-inhibiting hormone (MIH) production (Sainz-Hernández et al., 2008).

Since hormones regulating molt, metabolism, and a number of other activities also come from the sinus gland, the removal of one eyestalk causes a wide range of additional negative effects on the physiology of animals (Browdy, 1992). Because eyestalk ablation lowers levels of GIH and MIH, the shrimp is compelled to reproduce and molt more frequently, both of which require a large amount of energy. Ablation increases food intake and results in more effective physiological use of energy (Peter and Murugadass 1991; Rosas et al. 1993). In the present study, we found a significant increase in the weight gain, and percent weight gain in the unilaterally ablated shrimp compared to the non-ablated and bilaterally ablated shrimp. However, FCR values were higher in no ablated shrimp among three treatments where FCE was found high in the bilateral shrimp group compared to no ablated and unilateral ablated shrimp. Also, the unilateral ablated *M. dobsoni* had the highest specific growth rate compared to the other two groups. However, the weight of dead bilaterally ablated shrimp in the present study had a reduced weight. Reduced weight gain is one of the effects of the elevated energy demand caused by ablation, at least in shrimp that are in the process of reproducing. Accordingly, the shrimps in this study that had undergone ablation were smaller than the unabated controls. There were substantial changes in the dry molt weight and molt rate of *M. dobsoni* of two different sizes that had been ablated both unilaterally (UEA) and bilaterally (BEA), according to Venkitraman et al. (2004). So, it is evident from the study that unilateral ablation can be a better solution for sustainable commercial *M. dobsoni* farming where there is still a question mark on the feasibility of bilateral ablation of this species.

In the present study, we found significantly higher mortality percentage in the bilaterally ablated *Metapenaeus dobsoni*. When compared to untreated shrimp (2%), *Lipopenaeus vannamei* that had undergone unilateral (35%) or bilateral (68%) ablation experienced significantly greater mortality. This is most likely due to direct damage to the nervous system as well as the disruption of several physiological processes regulated by hormones from the eyestalk (Sainz-Hernández et al., 2008). Controlling factors like stocking density, tank size, and rearing techniques may be modified to improve the survival of shrimp. Eyestalk ablation in shrimp and other crustaceans is known to cause significant mortality (Radhakrishnan and Vijayakumaran, 1984, Koshio et al., 1992). Juinio-Meñez and Ruinata (1996) found that just 6% of *Panulirus ornatus* lobsters with bilateral ablations survived after two months, compared to 73% and 75% of lobsters with unilateral ablations and unablated lobsters, respectively. Mortality rates were inversely correlated with the extent of eyestalk ablation. Given the significant physiological stress caused by partial or complete ablation of the primary endocrine gland, especially if eyestalk ablation was performed after two days of molting, this was largely expected. Eyestalk ablation causes severe trauma, destroys a significant section of the nervous system, and leaves the animal blind in addition to removing this organ complex (Chao et al., 1988; Brody and Chang, 1989). The physiology of crustaceans is known to be negatively impacted by eyestalk ablation, leading to significant mortalities. Further studies are required for confirmation of the findings of the present study with more samples, different salinities, and an extended duration of the study period.

CONCLUSION

In brackish water, shrimp farming can increase productivity and boost export possibilities. In commercial shrimp farming, eyestalk ablation in *Metapenaeus dobsoni* and other shrimp species can significantly affect molting, growth, and survival. It is a routinely used technique for induced gonad development and spawning. While it may increase spawning and stimulate reproductive activity, it sometimes can interfere with the natural molting process, stunt growth, and perhaps lower survival rates. Eyestalk ablation should only be done after thorough consideration of the potential short- and long-term effects on shrimp health and overall production efficiency. According to the findings, Increased growth and conversion rates may be achieved with unilateral eyestalk ablation. We can therefore conclude that the use of unilateral eyestalk ablation in *M. dobsoni* commercial cultivation may be favorable and encouraged.

RECOMMENDATIONS

Eye stalk ablation is a popular experiment to understand different biological aspects in crustaceans. We tried to explore some of these aspects in *Metapenaeus dobsoni* to check its potentiality on a commercial scale. Though there are few fields where the research can be improved. These areas are listed below:

- The result could have been better if the experiment set in a larger scale.
- Larger number of samples could be used with larger tanks.
- Different seasons of the year should be used for the research.
- Longer period of time may bring some better results in growth.
- Various age groups of shrimps should be used.
- Different environmental conditions should be taken into consideration.

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