MORPHOMETRIC ANALYSIS OF GREEN MUSSELS (*Perna viridis*) CULTURED AT DIFFERENT WATER DEPTHS IN THE SOUTH-EAST COAST OF BANGLADESH

Md. Hassibul Hossain Shanto

Roll No. 0122/09

Registration No. 1109

Session: 2022-2023

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Marine Bioresource Science

Department of Marine Bioresource Science
Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University
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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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DEDICATED TO MY BELOVED PARENTS AND FAMILY MEMBERS

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The Author

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Abbreviation

DFA	Discriminant Function Analysis
PCA	Principal Component Analysis
GM	Geometric Morphometrics
GMA	Geometric Morphometric Analysis
ANOVA	Analysis of Variance
SPSS	Statistical Package for Social Science
F Value	Variation between sample means/variation within the samples
P	Level of Significance
SD	Standard Deviation
NS	Not Significant
CVA	Canonical variates analysis
PCC	The percentage of Correctly Classified
DF	Degrees of freedom
LDA	Linear Discriminant Analysis
i.e	That is
e.g.	For Example
et al.	Associates

Abstract

Perna viridis is a bivalve native to the Asia-Pacific region including the coastal waters of Bangladesh. The analysis of the morphometric variations of *P. viridis* (Green Mussels) through the truss network method and body shape morphometrics is urgently needed. This research holds crucial importance for economic benefit and food security, given its substantial commercial value and potential for cultivation in tropical countries. Additionally, there's a lack of information concerning its morphometric variations among populations from distinct locations. A total of 450 samples were taken at three different depths (1.5 feet, 3 feet, and 4.5 feet) from the three culture sites of Khuruskul, Chowfaldandi, and Moheshkhali. A total of 150 samples were taken from each location, with 50 samples each at 1.5 feet, 3 feet, and 4.5 feet in depth. A truss network was generated with 24 distance variables using digital images of the components and 15 morphometric factors using the SigmaScan Pro software platform. The truss measurements were transformed before being subjected to factor analysis and a cross-validation discriminant analysis. Factor analysis revealed a statistically significant difference among ten of fourteen morphometric lengths and twenty of twenty-four truss network measurements for *P. viridis* at the 0.05, 0.01, and 0.001 levels of significance. The factor analysis showed that the *P. viridis* population in these three areas varies significantly in terms of its morphology. The principal component analysis (PCA) suggested that the morphometric differences were most significant in Khuruskul and Moheshkhali than in Chowfaldondi. The morphometric differences were higher in 1.5ft water depth than 3ft and 4.5ft water depths in each location. To ensure long-term viability of *P. viridis*, it is essential to consider these morphological characteristics when making decisions about management strategies, conservation efforts, and extensive seed production. Different physical and biological conditions might occur in the estuaries and the Bay of Bengal, which could help to explain the reason behind such discrimination among various stocks.

Keywords: Green Mussel, Morphometric, Truss network, Discriminant analysis, Principal component analysis

Chapter-1: Introduction

1.1 Background of the study

Perna viridis, sometimes referred to as the Asian green mussel, is a bivalve that is significant commercially and is a member of the Mytilidae family. *P. viridis* typically grows to a length of 80 to 100 millimeters but can occasionally grow as long as 165 millimeters (Carpenter and Niem, 1998). Its shell ends in a downward-pointing beak. The smooth periostracum is dark green in color and becomes paler and more brownish the closer it gets to its attachment point (umbo). Younger mussels have a brilliant green color, which darkens with age. The interior of the shell has a light blue gloss. The mussel has a large mobile foot which it used to climb vertically and it should be covered by sediments. In order to help it adhere to its substrate, it also creates byssus (Benson et al., 2001). It is known that the green mussel *P. viridis* is an indigenous brackish water bivalve of the Indo-Pacific region, which stretches from the Arabian Gulf to the southern Chinese provinces of Guangdong and Fujian and southern Japan (Ye et al., 2015). Because of its quick growth and abundance, it has commercial significance. They serve as markers for heavy metal, organic chloride, and petroleum hydrocarbon pollution. It can normally withstand salinities up to 80 PSU, but it can also endure lower salinities (Villaluz et al., 2016).

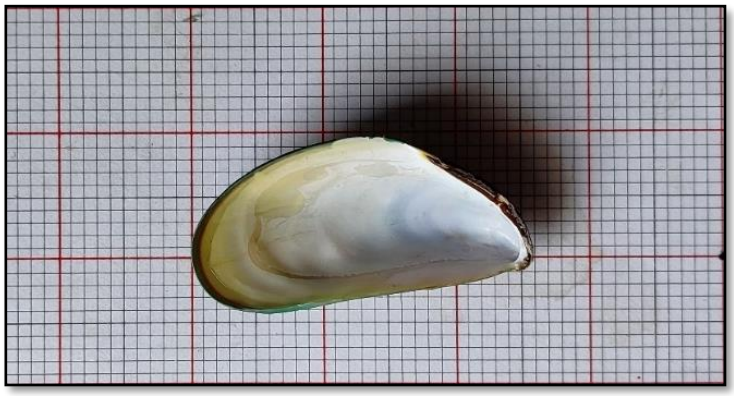


Figure-01: Inner side view of *Perna viridis* shell

The Asian green mussel fertilizes externally and has distinct sexes. There are incredibly few (less than 0.1%) functioning hermaphrodites. The temperature has an impact on the mussel's sexual development (Lee, 1988). However, the mussels found in the Philippines and Thailand are known to spawn all year round. Spawning typically takes place twice a year, between early spring and late fall, Following the spawning, zygote forms and after 7-8 hours of development, the zygote develops into a larva. The larvae

spend 10 to 12 days in the water column before changing into a juvenile and landing on a surface. The juveniles become sexually mature when they are 15–30 millimeters in length, a size reached within 2–3 months (Rajagopal et al., 2006). Although it boosts marketability and can keep predators and barnacles out, cage culture slows the growth of the mussel. Adults can live for up to two or three years. Its rapid development allows it to compete with other marine fouling species and alter the interactions between marine creatures. This mussel is a filter feeder that consumes suspended organic matter, zooplankton, and phytoplankton. They are eaten by fishes, crustaceans, sea stars, octopuses, and humans (Rajagopal et al., 2006).

Mussel is a sizable, quickly expanding species with significant commercial value that has shown astounding cultural potential in several nations. Since 1990, the output of marine mussels (Mytilidae) has increased globally at an average rate of 5% per year, reaching roughly 1.1 million tons in 2015, with 85% of that production coming from Asian nations. Since marine mussels are a sustainable source of protein, it is projected that worldwide production of mussels, particularly in Asia, would increase in order to meet the rising global population's demand for protein. *P. viridis* is only found on the southeastern shores of Bangladesh including Moheshkhali Channel and the Naaf River estuary. The most prolific areas in Bangladesh are the coastal waters, which provide the best conditions for *P. viridis* cultivation as well as for natural growth and recruitment. The development of green mussel farming technology and the sustainable management of the wild population has received top attention from the Bangladeshi government, which will result in the generation of employment as well as improve the livelihood condition of the poor fishermen and mussel farmers (Asaduzzaman et al., 2019).

The use of morphometric traits is considered to be one of the most popular, accessible, and economical techniques for classifying and identifying green mussel populations (Cadrin and Silva, 2005; Chaklader et al., 2015; Siddik et al., 2016). By identifying shape changes, morphometric characteristics may be utilized to measure a property of evolutionary importance (Chaklader et al., 2016). Consequently, studies of morphology may enhance population management and conservation strategies, as well as knowledge of species' ecology, behavior, and stock assessment (Muchlisin et al., 2014; Anvarifar et al., 2011; Chaklader et al., 2016).

There is currently a lack of information on the morphometric relationships among the mussel species found in Bolinao Bay. Not only in the municipality of Bolinao but also throughout the entire province of Pangasinan, this species is currently in high demand (Aban et al., 2017). As a result of their significance as a cheap source of animal protein for human consumption, they are currently extensively cultivated in several Asian nations. Mussel morphology may be related to its growth as consequently on the environmental factors. Its growth is affected by a number of factors, including age, caging, environmental factors and the availability of food. Transparency plays a crucial role in mussel culture as it indicates the presence of suspended organic and inorganic matters and determines the level of primary production in the culture area (Lovatelli, 1988). In mussel culture, transparency has a crucial relation with water depth, so growth can differ according to water depth and turbidity of the water (Lovatelli, 1988). They are utilized in some Asian nations as a biomonitoring tool for heavy metal contamination in addition to being consumed as a protein-rich food (Monirith et al., 2003). As there is diversified significance, morphometric analysis of *P. viridis* will indicate the highest growth of *P. viridis* according to different water depths and locations. So, a level of knowledge is needed to know about the morphological variation of *P. viridis*. This study will highlight the morphometric variation of green mussel (*P. viridis)* in different locations on the southeast coast of Bangladesh.

1.2 Objectives of the research

The specific objectives of the study are:

- To identify the morphometric variation of *Perna viridis* cultured at different water depths
- To ascertain the significant morphometric differences and growth of *P. viridis* in aspects of three different locations (Khuruskul, Chowfaldondi, and Moheshkhali).

1.3 Significance of the Study

For the quantitative investigation of green mussel size and form, morphometric analysis is a key tool. Through a number of multivariate studies, it often delineates the morphometric variances of green mussels in the aspects of different locations and water depths. The morphometrics of different populations, which are the most easily

observable markers of how well a species has evolved to adapt to its environment, will be clarified through this research. Close observation is necessary to determine how it affects the immediate environment. To ascertain its impact on the immediate surroundings, close observation is required. To maintain the long-term sustainability of the species, these morphological variations also aid in efficient management, conservation, and mass seed production.

Chapter-2: Review of Literature

2.1 Traits of Green mussel (*Perna viridis*)

Perna viridis is a prodigious mussel species that manifests a considerable size range, attaining lengths spanning from 8 to 16 cm. Regarding their size or any other exterior characteristics, there is no sexual dimorphism. The elongated, smooth shell has concentric growth lines. The shell tapers in size as it extends to the anterior (Rajagopal et al., 2006). The shell's long, concave ventral border is known as the hinge. The shell is covered with the periostracum, a thin external layer. The periostracum is a vibrant green color in juveniles. The periostracum of the adult mussel fades to a dark brown color with green borders (McGuire and Stevely, 2015). The kidney-shaped scar from the posterior adductor muscle. At the beak, there are teeth that bite together. While the right valve only has one tooth, the left valve has two. The foot has been uniquely designed for vertical mobility and is long and flat. The ligamental ridge (hinge) is finely pitted (Sidall, 1980).

2.2 Habitat and Feeding Behavior of *Perna viridis*

P. viridis are located in the coastal areas at or below 10 meters depth and their lifespan is about 3 years (Power et al., 2004). They frequently inhabit areas that are intertidal, subtidal, and estuary. It thrives in environments with salinities of around 18 to 33 ppt (parts per thousand) and temperatures of 10 to 42 ℃. By creating attachment fibers known as byssus threads, they bind themselves to rocky surfaces, bridges, ships, and other hard substrates (McGuire and Stevely, 2015). They can endure murky waters and dwell in regions with heavy water flow. They actively use their siphons to pull in and pump out water. Since they are most active at night, they are nocturnal (Robson et al., 2010). They feed on sessile bivalves that filter water. Microscopic phytoplankton, zooplankton, and water-borne organic debris make up the majority of its diet (Gosling, 2004). The mussel feeds on suspended food in the water via its gills and ciliary-mucus processes. The mussel rapidly draws water into its inhalant siphon while securely fastened to a hard substrate. The gill filaments are used to pump water. Cilia filter and capture the proper-sized organic food particles in the food-filled water. The exhalant siphon is used to remove the water and sediment particles. The labial palps receive the organic food particles from the cilia. According to Rajagopal et al. (2006), the labial palps control how much food enters the mouth and expel extra food as pseudofeces.

They develop quickly in close-knit colonies. In mid-littoral and sublittoral environments, they create dense communities on a variety of hard substrata including ships, buoys, and pipelines. There have been instances of larvae population densities as high as 39,500 individuals per cubic meter (Rajagopal et al., 2006). The existence of other species, the quality of the habitat, and the accessibility of food all have an impact on population density (Rajagopal et al., 2006). Depending on location, the precise quantity changes. Depending on the location in Tampa Bay, the population density ranged from 3,600 to 4,100 individuals per square meter. They could develop in layers, as observed in the Little Manatee River, where there were between 9,000 and 12,000 mussels per square meter (Rajagopal et al., 2006). According to Gobin et al. (2013), the population density distribution along the coast is often described as being isolated and patchy in several places, including Trinidad and Tobago. It is a common species with a lifetime of around 2-3 years and a wide range of tolerance to pollutants and environmental conditions (Gobin et al., 2013).

2.3 Reproductive Biology of *Perna viridis*

They are a dioecious species with distinct sexes, although it is impossible to distinguish a male from a female by looking at their exterior characteristics. Less than 0.1% of individuals occasionally exhibit hermaphroditism (Lee, 1988). While females have vibrant red gonads, males have white gonads. External fertilization takes place. Male and female gametes are discharged into the water in streams during spawning. Few of the many eggs that are generated are fertilized. The egg is surrounded by many sperm, but only one fertilizes it, creating a zygote with a circular shape. After 7-8 hours of fertilization, a free-swimming larva form. Predation causes the loss of a lot of eggs and larvae. After 16 to 19 hours, the soft tissue-enclosing shell starts to take shape. The larva may survive in the water for up to 10–12 days before going through metamorphosis, at which point it becomes a juvenile and sticks to a hard substrate. At the age of two to three months, they become sexually mature (Rajagopal et al., 2006). When predators like crabs and whelks are present, they thicken their shells to keep them from getting to their sensitive interior tissue. The size of the adductor muscle rises when it contracts hard to prevent against predators. In order to firmly attach themselves to a surface and keep predators from detaching or removing them, they also enhance the production of byssus threads (Wong and Cheung, 2003). They feature sensors that can

identify alterations in the water's composition. They are temperature touch-sensitive. If disturbed, they quickly seal their shell (Wong and Cheung, 2003).

2.4 Environmental Parameters for Culturing *Perna viridis*

The growth of green mussels heavily relies on ecological water factors. Environmental factors stand as crucial parameters as they directly impact the requirements and feeding patterns of green mussels, thereby significantly influencing their growth (Pattikawa and Ferdinandus, 2009). *Perna viridis*, a marine mussel species, thrives in high salinity (27-35 ppt) for optimal growth (Aypa, 1990; Rajagopal et al., 2006; Tan and Ransangan, 2017). Ideal temperature ranges from 26 to 32 ºC for green mussel growth (Hickman, 1992), while dissolved oxygen levels of 7-10 mg/l are considered favorable for mussel culture (Bayne, 1983).

For successful green mussel cultivation, maintaining a water depth below the 1-meter mean tide level is advisable (Lovatelli, 1988). The ideal depth varies depending on the cultivation method employed. Bottom culture, for instance, can be effectively conducted in areas where the mean tide level remains under 1.5 meters (Lovatelli, 1988). Conversely, off-bottom culture methods such as raft and long-line systems generally require a minimum water column height during low water spring tides. Specifically, for these methods, the hanging ropes carrying mussel seeds should be positioned at least 1 meter above the seabed during extreme low water spring tides (Lovatelli, 1988). This elevation serves to protect against ground predators, mitigate high water turbidity near the ocean floor, and prevent friction with the bottom. Transparency is pivotal in mussel culture, reflecting the presence of suspended organic and inorganic substances and influencing the primary production in the culture area (Lovatelli, 1988). Lovatelli (1988) suggests that any site with a disc reading below 25 cm is unsuitable for mussel culture. Moreover, to ensure optimal conditions for seed collection and mussel cultivation, a favorable water depth of 2 meters or more is recommended. This depth facilitates favorable growth conditions and overall success in the cultivation process (Aypa, 1990).

2.5 Global Aspects of *Perna viridis*

Globally, the Indo-Pacific area, which stretches from Japan to New Guinea and from the Persian Gulf to the South Pacific islands, is native to the green mussel (Tan and Ransangan, 2016). *P. viridis* is a widespread species found in tropical nations like

Indonesia, disseminated from Sumatera in the Malacca Strait, Lampung Strait, and Sunda Strait, while Java Island is found in Lada Bay, Jakarta Bay, Java Sea, and the Indian Ocean. It may be found in the coastal seas of Nusa Tenggara in eastern Indonesia, from Makassar Strait to Ambon Bay (Evans et al., 1995; Sudharyanto et al., 2005; Arifin et al., 2012; Yaqin et al., 2011; Huhn et al., 2015). According to Davy and Graham (1982), the earliest *P. viridis* cultivations in Indonesia took place in Jakarta and Banten Bay in the late 1970s. Followed by cultivation in Belawan, North Sumatera and Surabaya, Java Sea (Sudharyanto et al., 2005), and Lampung Strait (Noor, 2015). *P. viridis* is a vital species in aquaculture and its flesh is used for human food in Indonesia. They were also a cheap and quick-growing source of protein (Rajagopal et al., 2006). Green mussels often resided close to the estuary and were found perched on wood, bamboo, coral, and ropes through the byssus as their substrate (Yonvitner and Sukimin, 2009). After 6-7 months of growing, the seeds are organically connected to collectors and ready to be harvested (Noor, 2015). In the coastal waters of Pasaran Island, which is situated to the west of the Teluk Betung district in Bandar Lampung City, *P. viridis* is grown. According to Ali et al. (2015), due to the low currents in the waters and the presence of *P. viridis* seeds that are readily available, this area is suitable for growing *P. viridis*. Due to its simplicity of maintenance, raft culture is used for *P. viridis* cultivation (Noor, 2015). The biological properties of the *P. viridis* in the Lampung Strait, as well as growth performance, morphometric, condition index performance, and gonad index, which have a significant impact on shaping the future culture, have not yet been described (Noor et al., 2019).

Indian mussels include the brown mussel *P. indica* and the Asian green mussel *P. viridis. P. indica* is restricted to the southernmost portion of the Indian coast, which stretches from Kanyakumari to Tiruchendur, whereas *P. viridis* has a larger range that stretches from Gujarat on the West Coast of India to West Bengal on the East Coast as well as along the remote Andaman Islands. The green mussel (*P. viridis*), one of the two species recorded from the Indian coast, is a shellfish species grown in mariculture. During the height of the fishing season, mussel seeds around 10-20 mm in diameter together with adults are indiscriminately harvested in several regions along the Indian coast (Kanyakumari-Vizhinjam and Calicut-Tellicherry zones of South India), depleting the supply in the natural mussel beds (Shanmugam, 2020). In this case,

the mussel population becomes increasingly susceptible to changes in environmental conditions and may become more prone to extinction (Divya et al., 2012).

2.6 Morphometric Measurements of Bivalves

Over the past 50 years, a set of traditional measurements have served as the basis of morphometric investigations (Rohlf, 1990). The truss network system is being used for morphometric measurements with the aim of species and/or stock differentiation (Parsons et al., 2003; Turan and Erguden, 2004; Mustafi et al., 2008; Akbarzadeh et al., 2009; AnvariFar et al., 2011). In its place, the truss Network System has been used, notably for stock differentiation (Strauss and Bookestein, 1982). In comparison to a typical set of data, a regionally unbiased network of morphometric measurements should provide additional details regarding regional body variations. For defining morphological variation across closely related aquatic organisms (e.g., stocks), it has been demonstrated that the Truss network technique is substantially more successful than standard measurements. Former studies suggested that morphometric character variation was solely inherited, but more recent studies (Cabral et al., 2003; Nahar et al., 2015) have shown that environmental factors, such as water physicochemical parameters, habitat types, and substrate types, also influence it.

Bivalves have been the object of several morphometric research. These studies were done specifically to identify species, and determine shell characterize the population dynamics and dimension-volume interactions. The *Polymesoda* species found in the Indo-Pacific mangroves were reviewed by Morton (1984). He attempted to distinguish between *P. expansa* and *P. erosa* by measuring shells from Singaporean specimens. He suggested that the correlations between ligament length and shell length and shell height and length as traits that could potentially permit a species to be separated. According to Gimin et al. (2004), the length, height, and breadth of the northern Australian *P. erosa* shell were reliable indicators of growth when applied to live weight. Aban et al. (2017) studied the morphometric relationships of *P. viridis* in Bolinao Bay to acquire information that may be utilized to manage and conserve the mollusk resources of the Bay. The study's specific objective was to determine the morphometric relationships between *Perna viridis* specimens collected in Bolinao Bay in terms of: (1) shell length and total weight; (2) shell width and total weight; (3) shell thickness and total weight; and (4) shell length and soft tissue weight. In Bolinao Bay, samples of live

P. viridis were collected from mussel farmers and gatherers. Before making the necessary morphometric measurements, the samples were cleaned by removing any algae and debris that had been adhered to their shells. Vernier calipers were used to measure the length, breadth, and thickness of the shell. A computerized weighing scale with a 0.01-gram sensitivity was used to calculate the total weight of each sample. The individual sample's flesh (soft tissue) was cleaned and removed from the shell before being weighed to calculate soft-tissue weight (STW).

The morphometric relations between the mussel species present in Bolinao Bay are still undefined. This species is now in great demand not just in the municipality of Bolinao but also across the entire province of Pangasinan. (Aban et al., 2017). There was sexual variation in accordance with morphometric differences of males and females of *P. viridis.* The significant variation in males located at the ligament region. There was a downward bending of the ligament and a slight bending to the left of the posterior adductor border. In females, the significant variation is located at the ligament, posterior adductor border, and height. The variation was higher in female than male *P. viridis* in the aspect of morphometric differences (Villaluz et al., 2016). Therefore, research on morphometric analysis may help develop more effective management and conservation methods for a population (Muchlisin et al., 2014) and it also can result in a greater comprehension of species evolution, ecology, behavioral characteristics, and stock evaluation (Anvarifar et al., 2011; Chaklader et al., 2016).

Chapter-3: Materials and Methods

3.1 Study area

Spread across 1,19,000 square kilometers in the lap of the Bay of Bengal, Bangladesh's vast maritime area accounts for the country's diverse biodiversity. Covering nearly one-third of the land area, the coastal area of Bangladesh, directly and indirectly supports the livelihood of 29 percent of the people. This vast waterbody, full of marine resources, is divided into three coastal zones (southeast zone, southwest zone, and central zone). According to the environmental parameters and nutrients, there is the presence of bivalves in these coastal zones, but the southeast zone plays an important role. Our present study was conducted at three locations (Moheshkhali, Khuruskul, and Chowfaldandi) for green mussel cultivation in the southeast zone of Bangladesh. These locations have been selected because of the natural abundance of spat and favorable water quality in this zone.

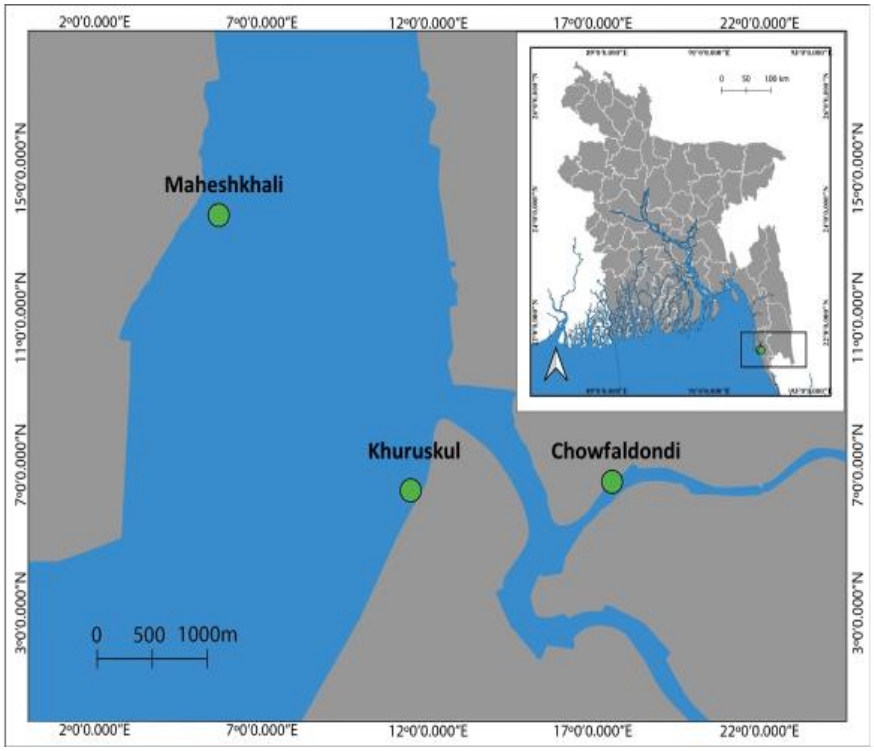


Figure-02: Location of study sites

Coordinates of the locations are shown below:

Location	Latitude	Longitude
Rastarpara, Khuruskul	21°30'40.13"N	91°59'53.3"E
Mudirchora, Moheshkhali	21°31'58.8"N	91°58'52.5"E
Chowfaldandi	21°30'42.3"N	92°0'56.9"E

3.2 Methods of the Study

3.2.1 Sample collection

A total of 450 samples were collected from three locations (Khuruskul, Chowfaldandi, and Moheshkhali) at three depths (1.5 feet, 3 feet, and 4.5 feet). From each location, 150 samples were collected from 1.5 feet, 3 feet, and 4.5 feet depth and each depth contained 50 samples. Later, the samples were well prepared in the laboratory. Their gut content was removed. Thereafter, the posterior adductor muscle and other tissues were dissected and the left valve was taken for morphometric research. Each pair of shell valves was labeled with a single key number. Shell area was estimated in the intact animal followed by Beadman et al. (2003). The measurements of shell length, height, and width was performed using a hand caliper (±0.05 mm).

Figure-03: Samples (*Perna viridis*) collection from three different locations

3.2.2 Image Analysis

The camera was set up in the portable photo lab to capture pictures of the biological samples. The internal aspect (face) of the left valve from each individual was scanned using a desktop scanner (CanoScan D646U, 300 dpi) and stored in *.jpg format. Images were later processed for balance, brightness, and contrast with Adobe Photoshop Image, version 7.0. A data sheet was prepared initially to keep up the records of landmarks.

Figure-04: Photo Lab

3.2.3 Quantitative research (Landmarks adjustment and branding)

Phenotypic variation occurs in the same raced biological organism. Morphometric analysis can determine phenotypic changes and estimate how much distortion occurs in an organism's body shape and size. Landmark counting happens by mapping some homogenous points knitted together (Giducos et al., 2015).

A total of 15 points and 24 landmarks' positions are listed below. These points and distances were measured in the laboratory with the help of SigmaScan Pro software to evaluate the morphometric distortion and truss network.

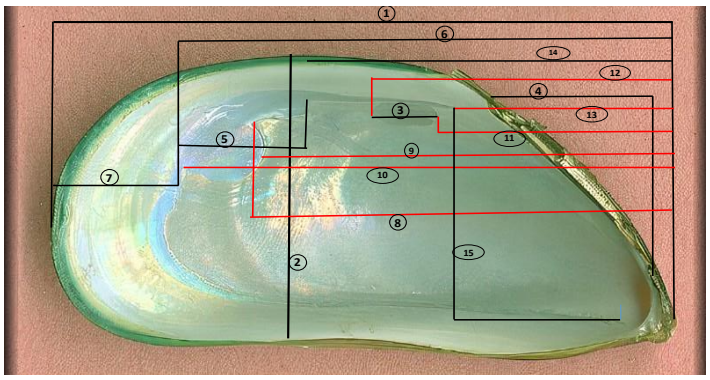


Figure-05: Morphometric lengths (See Table-1)

Table-1: Morphometric lengths

Parameters	Indicator	Description
1	SL	Shell length
2	SW	Shell width
3	PPRMRSL	Posterior pedal retractor muscle scar length
4	HPL	Hinge plate length
5	PAMSL	Posterior adductor muscle scar length
6	UTPS	Umbo to tip of palillal sinus
7	TPSPB	Tip of palillal sinus to posterior border
8	PAMS2U	Posterior adductor muscle scar 2 to umbo
9	PAMS3U	Posterior adductor muscle scar 3 to umbo
10	PAMS4U	Posterior adductor muscle scar 4 to umbo
11	UATPPRM	Umbo to anterior tip of PPRM
12	UPPRM	Umbo to PPRM
13	UEPL	Umbo to end of pallial line
14	UMDM	Umbo to mid dorsal margin
15	DBSEPL	Distance between start and end point of pallial line

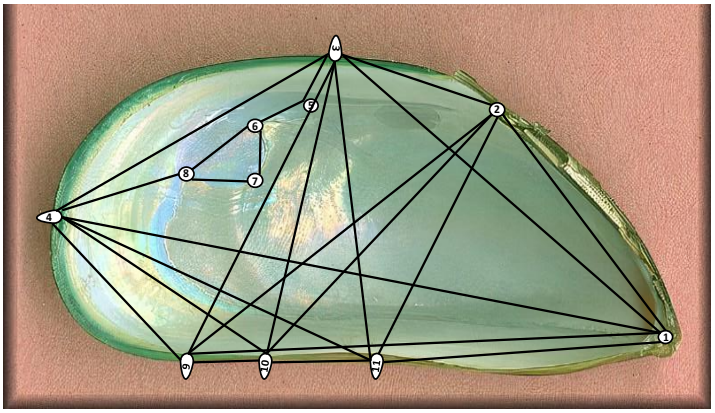


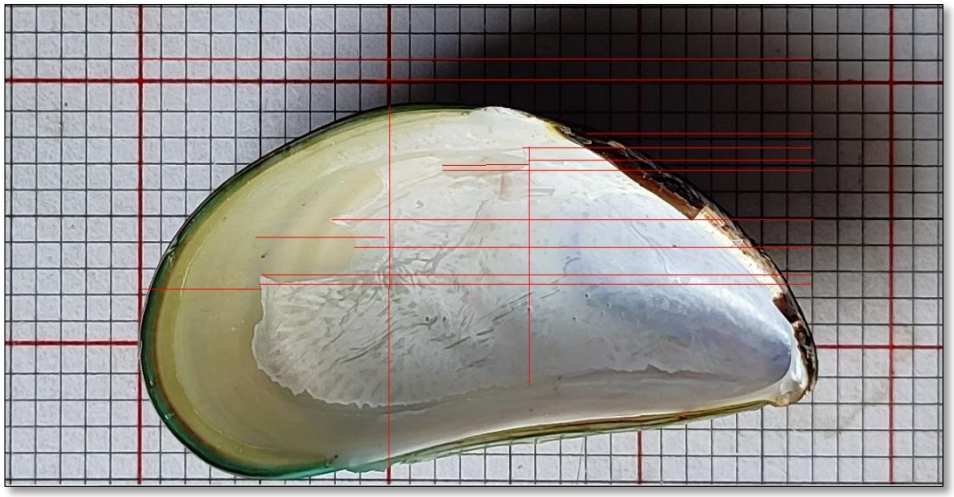
Figure-06: Landmarks points (See Table-2)

Table-2: Landmarks points and their description

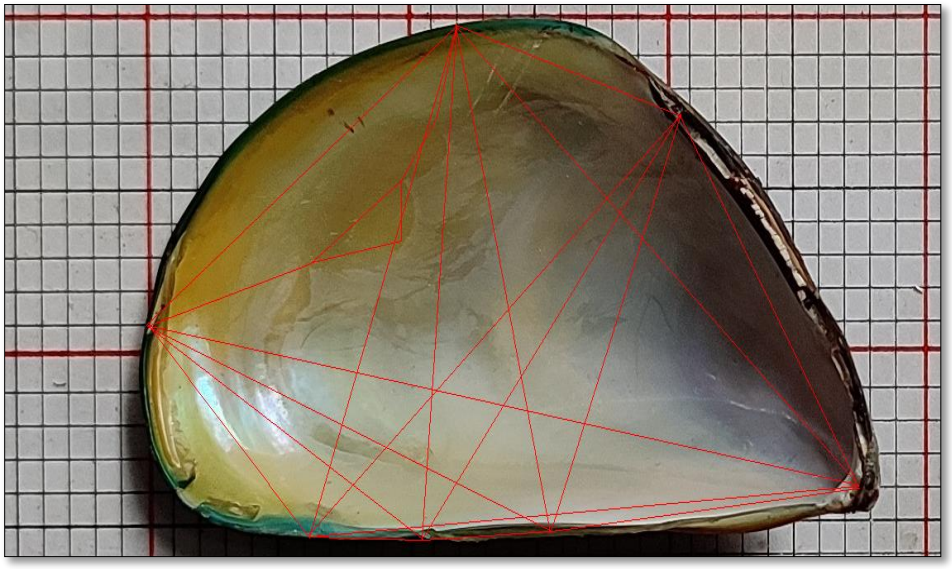
SLNO	Landmarks Points	Description
1	D1-2	From the umbo to the end of the hinge plate.
2	D1-3	From the umbo to maximum anterior curvature
3	D1-4	From the umbo to maximum posterior curvature
4	D1-9	From the umbo to the mid-point of the curve below to posterior
5	D1-11	From the umbo to the bottom point along the width
6	D2-3	From the end of the hinge plate to maximum anterior curvature
7	D2-9	From the end of the hinge plate to mid-point of the curve below to posterior
8	D2-10	From the end of the hinge plate to the middle of 9 and 11
9	D2-11	From the end of the hinge plate to bottom point along the width
10	D3-4	From maximum anterior curvature to maximum posterior curvature
11	D3-9	From maximum anterior curvature to mid-point of the curve below to posterior
12	D3-10	From maximum anterior curvature to the middle of 9 and 11
13	D3-11	From maximum anterior curvature to bottom point along the width
14	D3-5	From maximum anterior curvature to the end of posterior adductor muscle scar
15	D4-8	From maximum posterior curvature to the start of posterior adductor muscle scar
16	D4-9	From maximum posterior curvature to mid-point of the curve below to posterior
17	D4-10	From maximum posterior curvature to middle of 9 and 11
18	D4-11	From maximum posterior curvature to bottom point along the width
19	D5-6	From the end of the posterior adductor muscle scar to the notched point of that
20	D6-7	From the notched point of the posterior adductor muscle scar to the tipping point of that
21	D6-8	From the notched point of the posterior adductor muscle scar to the start point of that
22	D7-8	From the tip of posterior adductor muscle scar to the start point of that
23	D9-10	From the mid-point of the curve below to middle of 9 and 11
24	D10-11	From the middle of 9 and 11 to the posterior to the bottom point along the width

3.2.4 Size Adjustment

Data sets were created by using SigmaScan Pro software to measure morphometric and meristic properties prior to the commencement of the initial investigation. Figure-07 shows how the measurement was observed.



Morphometric



Truss Networking

Figure-07: Landmark data collection through SigmaScan Pro.

Prior to doing the analysis, size measurements were adjusted to exclude size effects from datasets and ensure that the morphological changes were caused by differences in body shape rather than relative sizes (Elliott et al., 1995). All parameters had a strong correlation with the standard length of the gathered *P. viridis.* Therefore, using the technique provided by Elliot et al. (1995), size-dependent variances were eliminated.

$$M_{adj} = M\ (SL_{av}/SL_{ob})^{b}$$

where M is the actual measurement, M_{adj} is the size-adjusted measurement, SL_{ob} is the standard length of the *P. viridis*, SL_{av} is the average mean, the standard length for all samples in each analysis, and b is the slope of the regression of Log M on log SL_{ob} for each character. The outcomes of the allometric method were verified by evaluating the significance of the correlation between transformed variables and the standard length of mussels (Turan, 1999).

3.2.5 Statistical Analysis

Samples of *P. viridis* were compared to show the morphological variations among environments. SPSS version 26.0 and Microsoft Office Excel 2019 software were used for statistical analysis. Analysis of variance (ANOVA) was used to determine the statistical significance of morphological differences ($P < 0.01$) using size-adjusted morphological and landmark distance data. All metric-free morphological and landmark distance data were subjected to a Discriminant Functional Analysis (DFA) and a Principal Components Analysis (PCA). In order to reduce the number of chosen morphological characteristics to a few composite measures of morphological attributes, we investigated the variance between the population according to specified areas and measured features using this PCA. PCAs were used to estimate the specimen distribution patterns over the three designated locations using R's 'FactMineR' package (Sebastien et al., 2008), version 3.5.2 (R development core team, 2018). Every graph was created using the "ggplot2" software (Wickham, 2016). The Linear Discriminant Function Analysis (LDFA) was executed to calculate the percentage of the correctly classified (PCC) population of *P. viridis* in three different locations and in accordingly three different depths in each location. Additionally, the morphometric distances of individuals were assessed using cluster analysis, which makes use of the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering algorithm and the Euclidean distance to measure dissimilarity (Veasey et al., 2001). Canonical Variates

Analysis (CVA) was also conducted to explore the relationship among multiple sets of variables and identify significant patterns of variation within the data of Morphometric characteristics for *P. viridis*. K-means clustering algorithm was also used to identify distinct groups of mussels based on their shell length and shell width. A cluster plot for K-means will display the placement of the K centroids (representing the K clusters) in the data space and the assignment of the data points to each cluster.

Chapter-4: Result

Primary data of green mussel samples of the species *Perna viridis* was acquired from the southeast coastal region of Bangladesh including Khuruskul, Chowfaldondi, and Moheshkhali; the descriptive portion of the differences in morphological data was analyzed and presented in this section. Specifics of systematic analytical observations of the morphology were also evaluated and demonstrated.

4.1 Analysis of variance for *Perna viridis*

The absence of a significant correlation between typical length and the other measurements at the significance level ($p>0.05$) or within the 95% confidence interval indicates that the size effects were successfully eliminated through the application of algometric transformation. Then univariate (ANOVA) analysis was performed through the measurements, and Shell width (SW), Posterior pedal retractor muscle scar length (PPRMSL) along with 24 truss network landmarks were showed significantly different at the level of significance ($p<0.5$*; $p<0.01$**; $p<0.001$***) for the *P. viridis* based on location (Table-3) and for depth (Table-4, 5, 6).

The tables show univariate analysis at $p<0.05$; $p<0.01$; $p<0.001$ levels of significance for *P. viridis* which reveals significant differentiation occurs among variables of the samples. Three stars (***) make highly significant whereas one star (*) makes a slight significant relationship among group means of the variables.

In the case of Land-mark distances, Twenty (1 to 2, 1 to 3, 1 to 4, 1 to 9, 1 to 11, 2 to 3, 2 to 9, 2 to 10, 2 to 11, 3 to 4, 3 to 9, 3 to 10, 3 to 5, 4 to 8, 4 to 9, 4 to 11, 5 to 6, 6 to 7, 6 to 8, 7 to 8) truss measurements were significantly different among samples in varying degrees ($p<0.05$ or $p<0.01$ or $p<0.001$) based on three locations revealed through univariant statistics (See Table-3 and Appendix-A).

Hinge plate length (HPL), Posterior adductor muscle scar length (PAMSL), Umbo to tip of palillal sinus (UTPS), Posterior adductor muscle scar 2 to umbo (PAMS2U), Posterior adductor muscle scar 3 to umbo (PAMS3U), Posterior adductor muscle scar 4 to umbo (PAMS4U), Umbo to anterior tip of PPRM (UATPPRM), Umbo to PPRM (UPPRM), Umbo to mid-dorsal margin (UMDM), Distance between start and end point of pallial line (DBSEPL) along with 20 truss network landmarks were showed significantly different at the level of significance ($p<0.5$*; $p<0.01$**; $p<0.001$***) for

P. viridis based on three locations (Khuruskul, Chowfaldondi, and Moheshkhali). (See Table-3 and Appendix-A).

Table-3: Descriptive data from ANOVA test for each morphometric character of *Perna viridis* for three locations Khuruskul, Chowfaldondi, and Moheshkhali.

Morphometric Parameters	Location			F Value	Level of Sign
	1.Khuruskul (Mean±SD)	2.Chowfaldondi (Mean±SD)	3.Moheshkhali (Mean±SD)		
SW	3.45±.18^{c}	3.42±.19^{c}	3.40±.19^{c}	2.078	0.126NS
PPRMSL	.78±.08^{c}	.80±.09^{c}	.78±.08^{c}	2.55	0.079NS
HPL	1.94±.21^{c}	2.01±.22^{b}	1.98±.25^{bc}	4.371	0.013*
PAMSL	1.26±.09^{c}	1.24±.17^{c}	1.30±.12^{b}	12.267	0***
UTPS	5.00±.11^{c}	5.05±.12^{b}	4.98±.13^{c}	17.399	0***
TPSPB	1.43±.11^{b}	1.35±.10^{c}	1.48±.11^{a}	51.058	0***
PAMS-2U	4.33±.17^{c}	4.38±.17^{b}	4.28±.18^{c}	11.352	0***
PAMS-3U	4.11±.14^{b}	4.19±.14^{a}	4.06±.16^{c}	30.562	0***
PAMS-4U	4.99±.12^{c}	5.04±.14^{b}	4.98±.14^{c}	9.679	0***
UATPPRM	2.48±.19^{c}	2.50±.19^{c}	2.46±.20^{c}	1.879	0.154NS
UPPRM	3.26±.20^{bc}	3.31±.20^{b}	3.25±.21^{c}	3.13	0.045*
UEPL	2.43±.22^{c}	2.44±.20^{c}	2.41±.23^{c}	1.006	0.367NS
UMDM	3.82±.19^{b}	3.89±.19^{a}	3.75±.21^{c}	17.471	0***
DBSAEPPL	2.39±.18^{b}	2.31±18^{c}	2.35±.15^{bc}	8.598	0***
D1-2	2.91±.20^{b}	2.93±.18^{b}	2.79±.16^{c}	26.928	0***
D1-3	4.58±.23^{b}	4.62±.19^{b}	4.48±.17^{c}	21.402	0***
D1-4	6.22±.08^{bc}	6.23±.10^{b}	6.20±.11^{c}	3.736	0.025*
D1-9	4.69±.11^{b}	4.76±.11^{a}	4.63±.12^{c}	45.822	0***
D1-11	2.85±.15^{c}	2.91±.17^{b}	2.82±.15^{c}	12.503	0***
D2-3	1.89±.16^{bc}	1.91±.15^{b}	1.87±.15^{c}	3.009	0.05*
D2-9	4.21±.15^{b}	4.22±.16^{b}	4.12±.16^{c}	20.936	0***
D2-10	3.56±.15^{b}	3.55±.15^{b}	3.45±.15^{c}	21.815	0***
D2-11	3.03±.15^{b}	3.03±.15^{b}	2.94±.14^{c}	19.638	0***
D3-4	3.52±.16^{b}	3.42±.17^{c}	3.66±.19^{a}	70.086	0***
D3-9	3.74±.19^{b}	3.68±.19^{c}	3.69±.18^{bc}	3.921	0.021*
D3-10	3.48±.18^{b}	3.43±.18^{c}	3.43±.18^{c}	4.083	0.017*
D3-11	3.45±.18^{c}	3.41±.18^{c}	3.40±.18^{c}	2.791	0.062NS
D3-5	.72±.07^{b}	.67±.08^{c}	.76±.07^{a}	48.31	0***
D4-8	1.49±.13^{b}	1.45±.12^{c}	1.58±.15^{a}	38.373	0***
D4-9	2.01±.19^{b}	1.95±.14^{c}	1.93±.13^{c}	10.242	0***
D4-10	2.74±.15^{c}	2.70±.16^{c}	2.71±.15^{c}	1.742	0.176NS
D4-11	3.54±.15^{c}	3.49±.25^{c}	3.53±.19^{c}	3.231	3.231NS
D5-6	.63±.07^{bc}	.62±.07^{c}	.64±.07^{b}	4.503	0.012*
D6-7	.59±.09^{b}	.55±.07^{c}	.56±.08^{c}	8.624	0***
D6-8	.88±.07^{bc}	.86±.07^{c}	.89±.06^{b}	6.829	0.001**
D7-8	.73±.10^{b}	.70±.11^{c}	.84±.10^{a}	76.493	0***
D9-10	.93±.13^{c}	.95±.10^{c}	.93±.10^{c}	2.486	0.084NS
D10-11	.92±.14^{c}	.90±.14^{c}	.90±.11^{c}	0.378	0.378NS

In the aspect of three different water depths; 1.5ft, 3ft, and 4.5ft in Khuruskul**,** Fifteen Land-mark distances (1 to 2, 1 to 3, 1 to 4, 1 to 9, 1 to 11, 2 to 3, 2 to 9, 2 to 10, 2 to 11, 3 to 4, 4 to 8, 4 to 10, 5 to 6, 7 to 8, 10 to 11) truss measurements were significantly different among samples in varying degrees ($p<0.05$ or $p<0.01$ or $p<0.001$) based on three locations revealed through univariant statistics (See Table-4 and Appendix-B).

Posterior adductor muscle scar length (PAMSL), Umbo to tip of palillal sinus (UTPS), Posterior adductor muscle scar 2 to umbo (PAMS2U), Posterior adductor muscle scar 3 to umbo (PAMS3U), Posterior adductor muscle scar 4 to umbo (PAMS4U), Umbo to anterior tip of PPRM (UATPPRM), Umbo to PPRM (UPPRM), Distance between start and end point of pallial line (DBSEPL) along with 15 truss network landmarks were showed significantly different at the level of significance ($p<0.5$*; $p<0.01$**; p,0.001***) for *P. viridis*.

Table-4: Descriptive data from ANOVA test for each morphometric character of *P. viridis* for three different water depths; 1.5ft, 3ft, and 4.5ft in Khuruskul.

Morphometric Parameters	Khuruskul			F Value	Level of Sign.
	1. Depth 1.5ft (Mean±SD)	2. Depth 3ft (Mean±SD)	3. Depth 4.5ft (Mean±SD)		
SW	3.44±.17^{c}	3.46±.19^{c}	3.44±.18^{c}	0.171	0.843NS
PPRMSL	.77±.09^{c}	.77±.08^{c}	.79±.08^{c}	0.804	0.449NS
HPL	1.93±.19^{c}	1.94±.25^{c}	1.94±.19^{c}	0.063	0.939NS
PAMSL	1.29±.09^{b}	1.25±.10^{bc}	1.24±.09^{c}	3.642	0.029*
UTPS	4.96±.10^{c}	5.03±.13^{b}	5.00±.08^{bc}	5.443	0.005**
TPSPB	1.44±.10^{c}	1.40±.12^{c}	1.43±.09^{c}	2.164	0.118NS
PAMS-2U	4.26±.14^{c}	4.37±.19^{b}	4.35±.14^{b}	6.656	0.002**
PAMS-3U	4.07±.14^{c}	4.15±.16^{b}	4.12±.11^{bc}	4.41	0.014*
PAMS-4U	4.94±.12^{c}	5.02±.14^{b}	5.00±.09^{bc}	5.413	0.005**
UATPPRM	2.41±.17^{c}	2.49±.22^{bc}	2.54±.15^{b}	6.529	0.002**
UPPRM	3.20±.16^{c}	3.28±.23^{bc}	3.32±.17^{b}	5.274	0.006**
UEPL	2.37±.19^{c}	2.45±.26^{c}	2.46±.20^{c}	2.489	0.086NS
UMDM	3.78±.18^{c}	3.84±.22^{c}	3.83±.15^{c}	1.84	0.162NS
DBSAEPPL	2.44±.16^{b}	2.42±.18^{b}	2.31±.19^{c}	7.795	0.001**
D1-2	2.85±.19^{c}	2.94±.21^{bc}	2.95±.18^{b}	3.378	0.037*
D1-3	4.55±.21^{c}	4.67±.26^{b}	4.52±.18^{c}	6.094	0.003**
D1-4	6.19±.09^{c}	6.26±.08^{a}	6.22±.05^{b}	13.006	0***
D1-9	4.66±.11^{c}	4.75±.10^{b}	4.66±.10^{c}	12.857	0***
D1-11	2.78±.15^{c}	2.91±.16^{b}	2.86±.13^{b}	10.643	0***

D2-3	1.91±.15^{b}	1.96±.16^{b}	1.80±.13^{c}	16.185	0***
D2-9	4.18±.12^{c}	4.26±.15^{b}	4.20±.17^{bc}	4.472	0.013*
D2-10	3.50±.15^{c}	3.62±.14^{b}	3.55±.15^{bc}	7.368	0.001**
D2-11	2.96±.12^{c}	3.06±.14^{b}	3.06±.17^{b}	7.918	0.001**
D3-4	3.47±.14^{c}	3.49±.17^{c}	3.59±.15^{b}	8.743	0***
D3-9	3.71±.16^{c}	3.76±.20^{c}	3.74±.20^{c}	0.885	0.415NS
D3-10	3.46±.16^{c}	3.51±.18^{c}	3.48±.18^{c}	0.938	0.394NS
D3-11	3.44±.17^{c}	3.47±.18^{c}	3.43±.19^{c}	0.789	0.456NS
D3-5	.71±.07^{c}	.73±.08^{c}	.73±.06^{c}	1.773	0.173NS
D4-8	1.47±.10^{c}	1.45±.14^{c}	1.55±.10^{b}	10.146	0***
D4-9	2.02±.10^{c}	1.98±.29^{c}	2.03±.13^{c}	0.976	0.379NS
D4-10	2.76±.17^{bc}	2.69±.16^{c}	2.77±.12^{b}	3.848	0.023*
D4-11	3.57±.12^{c}	3.52±.19^{c}	3.53±.13^{c}	1.754	0.177NS
D5-6	.62±.06^{c}	.65±.07^{b}	.62±.06^{c}	4.405	0.014*
D6-7	.59±.11^{c}	.58±.07^{c}	.60±.08^{c}	0.57	0.567NS
D6-8	.88±.08^{c}	.88±.07^{c}	.88±.06^{c}	0.045	0.956NS
D7-8	.71±.10^{c}	.76±.07^{b}	.72±.10^{bc}	3.97	0.021*
D9-10	.95±.17^{c}	.90±.10^{c}	.94±.09^{c}	2.461	0.089NS
D10-11	.93±.17^{bc}	.95±.11^{b}	.87±.11^{c}	5.152	0.007**

In the aspect of three different water depths; 1.5ft, 3ft, and 4.5ft in Chowfaldondi, Eleven Land-mark distances (1 to 4, 1 to 9, 1 to 11, 3 to 4, 3 to 9, 4 to 8, 4 to 9, 4 to 10, 6 to 7, 9 to 10, 10 to 11) truss measurements were significantly different among samples in varying degrees ($p<0.05$ or $p<0.01$ or $p<0.001$) based on three locations revealed through univariant statistics (See Table-5 and Appendix-C).

Posterior adductor muscle scar length (PAMSL), Umbo to tip of palillal sinus (UTPS), Posterior adductor muscle scar 3 to umbo (PAMS3U), Umbo to mid-dorsal margin (UMDM), Distance between start and end point of pallial line (DBSEPL) along with 11 truss network landmarks were showed significantly different at the level of significance ($p<0.5$*; $p<0.01$**; p,0.001***) for *P. viridis.*

Table-5: Descriptive data from ANOVA test for each morphometric character of *P. viridis* for three different water depths; 1.5ft, 3ft, and 4.5ft in Chowfaldondi.

Morphometric Parameters	**Chowfaldondi**			**F Value**	**Level of Sign**
	1. Depth 1.5ft (Mean±SD)	**2. Depth 3ft (Mean±SD)**	**3. Depth 4.5ft (Mean±SD)**		
SW	3.40±.20^{c}	3.41±.19^{c}	3.43±.18^{c}	0.452	0.637NS
PPRMSL	.81±.08^{c}	.80±.08^{c}	.77±.08^{c}	1.998	0.139NS
HPL	1.20±.19^{c}	2.06±.20^{c}	1.98±.25^{c}	2.128	0.123NS
PAMSL	1.21±.10^{c}	1.23±.10^{bc}	1.26±.15^{b}	3.121	0.047*
UTPS	5.08±.10^{c}	5.04±.13^{c}	5.03±.10^{c}	3.089	0.049*
TPSPB	1.33±.10^{c}	1.36±.12^{c}	1.36±.10^{c}	1.882	0.156NS

PAMS-2U	4.42±.20^{c}	4.35±.20^{c}	4.36±.20^{c}	2.291	0.105NS
PAMS-3U	4.23±.12^{b}	4.18±.15^{bc}	4.15±.12^{c}	3.439	0.035*
PAMS-4U	5.06±.20^{c}	5.03±.15^{c}	5.02±.11^{c}	1.142	0.322NS
UATPPRM	2.52±.15^{c}	2.51±.20^{c}	2.46±.25^{c}	1.188	0.308NS
UPPRM	3.32±.20^{c}	3.32±.20^{c}	3.26±.21^{c}	1.339	0.265NS
UEPL	2.47±.15^{c}	2.44±.20^{c}	2.40±.23^{c}	1.367	0.258NS
UMDM	3.94±.14^{b}	3.86±.20^{bc}	3.84±.21^{c}	4.324	0.015*
DBSAEPPL	2.33±.15^{b}	2.24±.21^{c}	2.33±.20^{b}	4.401	0.014*
D1-2	2.95±.20^{c}	2.91±.20^{c}	2.92±.20^{c}	0.917	0.402NS
D1-3	4.60±.15^{c}	4.60±.22^{c}	4.70±.17^{c}	2.05	0.132NS
D1-4	6.20±.10^{c}	6.24±.10^{b}	6.25±.10^{b}	6.233	0.003**
D1-9	4.70±.10^{c}	4.77±.10^{b}	4.81±.10^{b}	18.047	0***
D1-11	2.82±.12^{c}	2.97±.16^{b}	2.95±.17^{b}	13.332	0***
D2-3	1.89±.14^{c}	1.89±.16^{c}	1.95±.14^{c}	2.641	0.075NS
D2-9	4.20±.16^{c}	4.20±.16^{c}	4.25±.15^{c}	1.268	0.284NS
D2-10	3.54±.16^{c}	3.52±.14^{c}	3.56±.14^{c}	0.822	0.441NS
D2-11	3.00±.15^{c}	3.05±.14^{c}	3.04±.14^{c}	1.185	0.309NS
D3-4	3.37±.16^{c}	3.41±.15^{bc}	3.48±.18^{b}	6.361	0.002**
D3-9	3.63±.19^{c}	3.68±.18^{bc}	3.73±.18^{b}	3.74	0.026*
D3-10	3.39±.19^{c}	3.43±.16^{c}	3.48±.17^{c}	2.737	0.068NS
D3-11	3.37±.19^{c}	3.40±.16^{c}	3.45±.18^{c}	2.613	0.077NS
D3-5	.68±.07^{c}	.65±.08^{c}	.69±.08^{c}	2.863	0.06NS
D4-8	1.38±.09^{c}	1.48±.12^{b}	1.49±.11^{b}	14.277	0***
D4-9	1.95±.13^{b}	1.99±.13^{b}	1.89±.14^{c}	7.352	0.001**
D4-10	2.69±.15^{bc}	2.76±.15^{b}	2.67±.16^{c}	4.34	0.015*
D4-11	3.54±.17^{c}	3.45±.33^{c}	3.47±.22^{c}	1.82	0.166NS
D5-6	.62±.06^{c}	.61±.07^{c}	.63±.07^{c}	1.129	0.326NS
D6-7	.55±.07^{bc}	.52±.06^{c}	.57±.08^{b}	6.176	0.003**
D6-8	.87±.06^{c}	.85±.05^{c}	.87±.08^{c}	1.37	0.257NS
D7-8	.70±.12^{c}	.71±.09^{c}	.69±.12^{c}	0.609	0.545NS
D9-10	.92±.09^{c}	.97±.09^{c}	.97±.09^{c}	3.342	0.038*
D10-11	.95±.12^{b}	.83±.13^{c}	.90±.15^{b}	10.35	0***

In the aspect of three different water depths; 1.5ft, 3ft, and 4.5ft in Moheshkhali, Ten Land-mark distances (1 to 2, 1 to 3, 1 to 4, 3 to 4, 3 to 11, 4 to 8, 4 to 10, 4 to 11, 7 to 8, 9 to 10) truss measurements were significantly different among samples in varying degrees ($p<0.05$ or $p<0.01$ or $p<0.001$) based on three locations revealed through univariant statistics (See Table-6 and Appendix-D).

Hinge plate length (HPL), Posterior adductor muscle scar length (PAMSL), Umbo to tip of palillal sinus (UTPS), Posterior adductor muscle scar 2 to umbo (PAMS2U), Posterior adductor muscle scar 3 to umbo (PAMS3U), Umbo to anterior tip of PPRM (UATPPRM), Umbo to PPRM (UPPRM), Umbo to end of pallial line (UEPL), Umbo to mid dorsal margin (UMDM), Distance between start and end point of pallial line

(DBSEPL) along with 10 truss network landmarks were showed significantly different at the level of significance (p<0.5*; p<0.01**; p<0.001***) for *P. viridis.*

Table-6: Descriptive data from ANOVA test for each morphometric character of *P. viridis* for three different water depths; 1.5ft, 3ft, and 4.5ft in Moheshkhali.

Morphometric Parameters	Moheshkhali			F Value	Level of Sign
	1. Depth 1.5ft (Mean±SD)	2. Depth 3ft (Mean±SD)	3. Depth 4.5ft (Mean±SD)		
SW	3.40±.16^{c}	3.39±.18^{c}	3.42±.22^{c}	0.271	0.763NS
PPRMSL	.79±.08^{c}	.80±.08^{c}	.77±.09^{c}	1.642	0.197NS
HPL	1.98±.26^{bc}	2.07±.22^{b}	1.89±.23^{c}	7.035	0.001**
PAMSL	1.28±.15^{c}	1.27±.09^{c}	1.34±.10^{b}	5.049	0.008**
UTPS	4.99±.11^{c}	5.00±.12^{c}	4.95±.15^{c}	1.816	0.166NS
TPSPB	1.47±.10^{bc}	1.45±.11^{c}	1.51±.12^{b}	3.739	0.026*
PAMS-2U	4.29±.16^{bc}	4.34±.16^{b}	4.22±.19^{c}	5.800	0.004**
PAMS-3U	4.08±.15^{b}	4.11±.13^{b}	4.00±.17^{c}	7.685	0.001**
PAMS-4U	4.99±.12^{c}	4.99±.14^{c}	4.95±.17^{c}	1.569	0.212NS
UATPPRM	2.49±.20^{b}	2.52±.17^{b}	2.37±.20^{c}	8.945	0***
UPPRM	3.27±.20^{b}	3.32±.18^{b}	3.16±.21^{c}	8.074	0***
UEPL	2.44±.22^{b}	2.47±.20^{b}	2.31±.23^{c}	7.293	0.001**
UMDM	3.77±.20^{b}	3.84±.18^{b}	3.64±.20^{c}	14.613	0***
DBSAEPPL	2.31±.15^{c}	2.32±.14^{c}	2.42±.16^{b}	7.969	0.001**
D1-2	2.76±.15^{c}	2.77±.14^{bc}	2.84±.17^{b}	3.917	0.022*
D1-3	4.44±.15^{c}	4.47±.15^{bc}	4.53±.19^{b}	3.738	0.026*
D1-4	6.20±.06^{b}	6.14±.10^{c}	6.26±.12^{a}	19.190	0***
D1-9	4.65±.12^{c}	4.62±.10^{c}	4.63±.13^{c}	0.944	0.391NS
D1-11	2.81±.15^{c}	2.84±.15^{c}	2.81±.16^{c}	0.598	0.551NS
D2-3	1.86±.15^{c}	1.88±.13^{c}	1.87±.17^{c}	0.115	0.892NS
D2-9	4.15±.14^{c}	4.08±.16^{c}	4.12±.18^{c}	2.153	0.120NS
D2-10	3.45±.13^{c}	3.44±.15^{c}	3.47±.16^{c}	0.385	0.681NS
D2-11	2.94±.14^{c}	2.92±.14^{c}	2.95±.15^{c}	0.829	0.439NS
D3-4	3.62±.17^{c}	3.58±.17^{c}	3.76±.18^{b}	15.041	0***
D3-9	3.69±.14^{c}	3.66±.17^{c}	3.73±.22^{c}	1.767	0.174NS
D3-10	3.41±.16^{c}	3.42±.17^{c}	3.48±.21^{c}	2.156	0.119NS
D3-11	3.37±.17^{c}	3.38±.17^{c}	3.45±.19^{c}	3.109	0.048*
D3-5	.75±.07^{c}	.75±.07^{c}	.77±.08^{c}	1.504	0.226NS
D4-8	1.58±.11^{bc}	1.52±.16^{c}	1.63±.16^{b}	7.474	0.001**
D4-9	1.95±.14^{c}	1.91±.13^{c}	1.95±.12^{c}	2.116	0.124NS
D4-10	2.74±.14^{b}	2.66±.15^{c}	2.75±.14^{b}	6.848	0.001**
D4-11	3.54±.16^{bc}	3.46±.18^{c}	3.59±.20^{b}	6.376	0.002**
D5-6	.63±.07^{c}	.65±.07^{c}	.65±.08^{c}	1.501	0.226NS
D6-7	.54±.08^{c}	.57±.08^{c}	.55±.09^{c}	1.817	0.166NS
D6-8	.89±.06^{c}	.89±.06^{c}	.90±.08^{c}	0.832	0.437NS
D7-8	.84±.09^{b}	.79±.10^{c}	.89±.08^{a}	14.023	0***
D9-10	.96±.09^{b}	.90±.09^{c}	.92±.10^{bc}	6.441	0.002**
D10-11	.89±.10^{c}	.90±.11^{c}	.91±.13^{c}	0.420	0.658NS

For morphometric and landmark measures, discriminant function analysis (DFA) generated three sets of predicted group membership. The first group analysis in location 1 (Khuruskul) for original resolved 74.7%, and the other 2 locations (Chowfaldondi and Moheshkhali) determined 16.7% and 8.7% respectively, of the total variability for both morphometric and landmark measurements. In contrast, cross-validated resolved 62% for location 1 and other locations revealed 26% and 12% respectively. They all explained 100% of the total variability for both original and cross-validated results. The following table shows the other predicted group membership with original and cross-validated results for *P. viridis* based on three different locations; Khuruskul, Chowfaldondi, and Moheshkhali (See Table-7).

Table-7: Predicted group membership result for *P. viridis* based on location:

		Location	Predicted Group Membership			Total
			1	2	3	
Original	Count	1	112	25	13	150
		2	30	117	3	150
		3	10	4	136	150
	%	1	74.7	16.7	8.7	100
		2	20	78	2	100
		3	6.7	2.7	90.7	100
Cross-validated[b]	Count	1	93	39	18	150
		2	44	102	4	150
		3	14	4	132	150
	%	1	62	26	12	100
		2	29.3	68	2.7	100
		3	9.3	2.7	88	100

For morphometric and landmark measures, discriminant function analysis (DFA) generated three sets of predicted group membership. The first group analysis about depth 1 (1.5ft) in Khuruskul for original resolved 88%, and the other 2 depths (3ft and 4.5ft) determined 10% and 2% respectively, of the total variability for both morphometric and landmark measurements. In contrast, cross-validated resolved 74% for depth 1 and other depths revealed 20% and 6% respectively. They all explained 100% of the total variability for both original and cross-validated results. The following table shows the other predicted group membership with original and cross-validated results in different water depths; 1.5ft, 3ft, and 4.5ft in Khuruskul. (Table-8).

Table-8: Predicted group membership result for *P. viridis* based on different water depths; 1.5ft, 3ft, and 4.5ft in Khuruskul.

		Depth	Predicted Group Membership			Total
			1	2	3	
Original	Count	1	44	5	1	50
		2	3	44	3	50
		3	0	4	46	50
	%	1	88	10	2	100
		2	6	88	6	100
		3	0	8	92	100
Cross-validated[b]	Count	1	37	10	3	50
		2	4	36	10	50
		3	2	8	40	50
	%	1	74	20	6	100
		2	8	72	20	100
		3	4	16	80	100

The first group analysis about depth 1 (1.5ft) in Chowfaldondi for original resolved 98%, and the other 2 depths (3ft and 4.5ft) were determined 2% and 0% respectively, of the total variability for both morphometric and landmark measurements. In contrast, cross-validated resolved 86% for depth 1 and other depths revealed 4% and 10% respectively. They all explained 100% of the total variability for both original and cross-validated results. The following table shows the other predicted group membership with original and cross-validated results in different water depths; 1.5ft, 3ft, and 4.5ft in Chowfaldondi (Table-9).

Table-9: Predicted group membership result for *P. viridis* based on different water depths; 1.5ft, 3ft, and 4.5ft in Chowfaldondi.

		Depth	Predicted Group Membership			Total
			1	2	3	
Original	Count	1	49	1	0	50
		2	1	42	7	50
		3	1	8	41	50
	%	1	98	2	0	100
		2	2	84	14	100
		3	2	16	82	100
Cross-validated[b]	Count	1	43	2	5	50
		2	5	32	13	50
		3	4	10	36	50
	%	1	86	4	10	100
		2	10	64	26	100
		3	8	20	72	100

The first group analysis about depth 1 (1.5ft) in Moheshkhali for original resolved 80%, and the other 2 depths (3ft and 4.5ft) determined 18% and 2% respectively, of the total variability for both morphometric and landmark measurements. In contrast, cross-validated resolved 64% for depth 1 and other depths revealed 22% and 14% respectively. They all explained 100% of the total variability for both original and cross-validated results. The following table shows the other predicted group membership with original and cross-validated results in different water depths; 1.5ft, 3ft, and 4.5ft in Moheshkhali (See Table-10).

Table-10: Predicted group membership result for *P. viridis* based on different water depths; 1.5ft, 3ft, and 4.5ft in Moheshkhali.

		Depth	**Predicted Group Membership**			**Total**
			1	2	3	
Original	Count	1	40	9	1	50
		2	7	39	4	50
		3	3	3	44	50
	%	1	80	18	2	100
		2	14	78	8	100
		3	6	6	88	100
Cross-validated[b]	Count	1	32	11	7	50
		2	17	29	4	50
		3	7	8	35	50
	%	1	64	22	14	100
		2	34	58	8	100
		3	14	16	70	100

4.2 Linear Discriminant Analysis

A linear discriminant analysis was done to overview the dataset and explain the samples from different places apart. The result showed that 62.2% of the differences were due to LD1, and 37.8% were due to LD2 for *P. viridis* (Figure-8). In the aspect of depth (1.5 ft, 3 ft, and 4.5 ft) 77.4% of the differences were due to LD1, 22.6% were due to LD2 (Figure-09) in Chowfaldondi; 66.0% of the differences were due to LD1, 33.9% were due to LD2 (Figure-09) in Khuruskul; 81.0% of the differences were due to LD1, 19.0% were due to LD2 (Figure-09) in Moheshkhali for *P. viridis.*

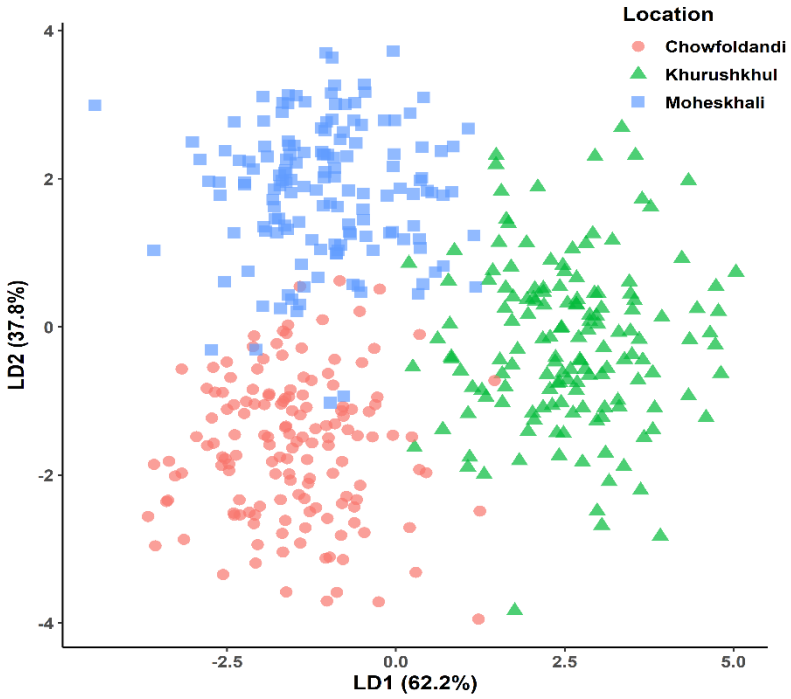
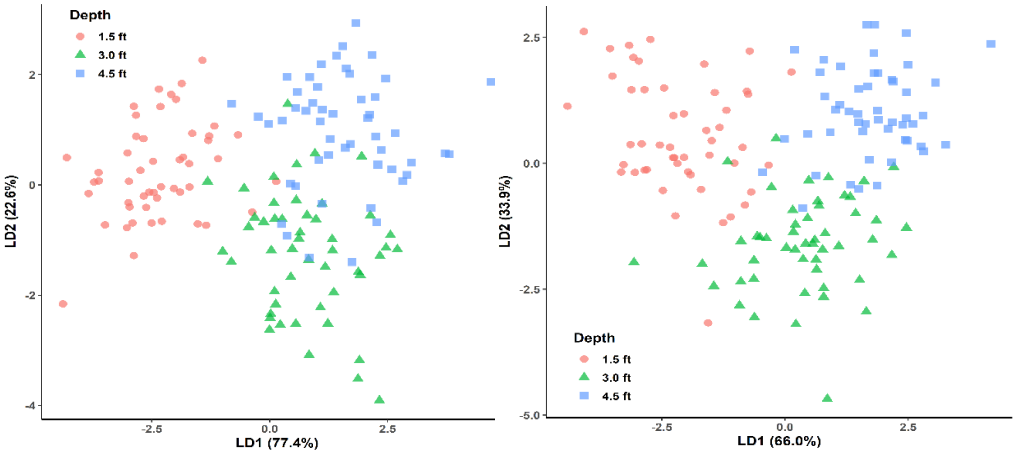
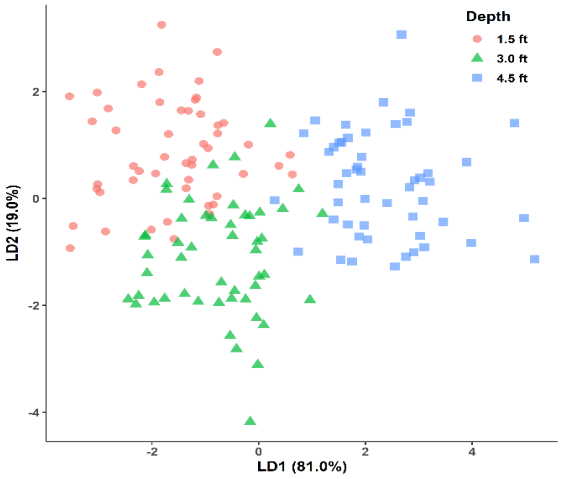


Figure-08: Linear Discriminant Analysis of *P. viridis* in Chowfaldondi, Khuruskul, and Moheshkhali.



Chowfaldondi **Khuruskul**



Moheshkhali

Figure-09: Linear Discriminant Analysis of *P. viridis* in the aspect of three different depths including 1.5ft, 3ft, and 4.5ft in Chowfaldondi, Khuruskul, and Moheshkhali.

4.3 Hierarchical Clustering

A dendrogram was constructed for *P. viridis* populations from three locations based on geographical distances and morphological analysis among cluster centroids. Using the squared Euclidean dissimilarity and the UPGMA (unweighted pair group method with arithmetical average) for the clustering technique (Veasey et al., 2001), we identified our samples from three locations: Chowfaldondi, Khuruskul, and Moheshkhali form one cluster. In contrast, there was another cluster form for depth in each location. The following figure shows the Cluster variation among samples of green mussels in three different depths including 1.5ft, 3ft, and 4.5ft according to each location.

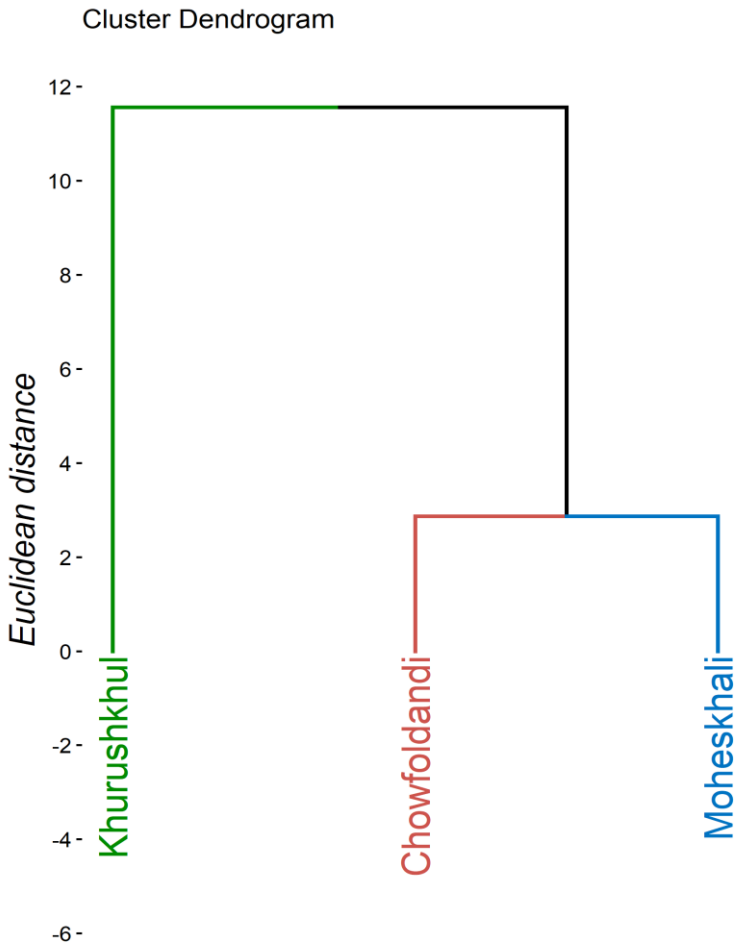
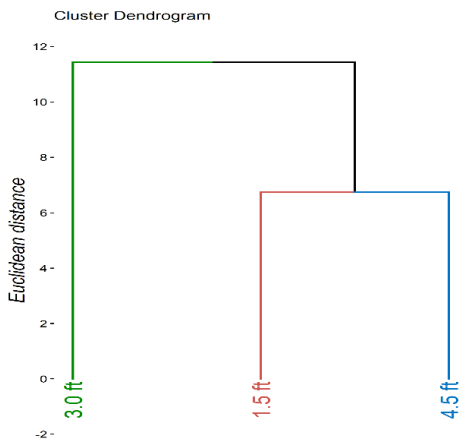
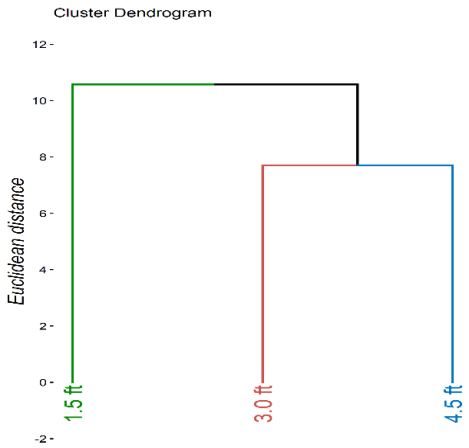


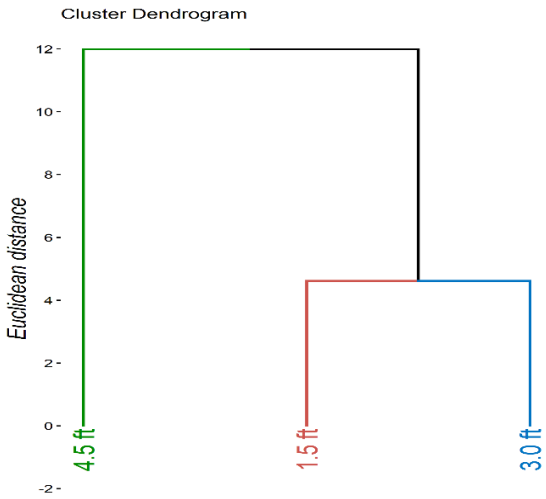
Figure-10: Hierarchical Clustering using UPGMA process based on location.



Chowfaldondi



Khuruskul



Moheshkhali

Figure-11: Hierarchical Clustering using UPGMA process based on water depth.

4.4 Principal Component Analysis

Bartlett's test of sphericity was used to overview at the data's eligibility for principal component analysis, and it was found to be significant (P<0.01). The Principal component analysis (PCA) was used to discover which morphometric measurement best distinguishes among the populations. All samples of three locations could be divided into two groups, with the first principal component (PC1) explaining 84.3% of the variance and the second (PC2) explaining 4% and the variables mostly associated with Khuruskul. On the other hand, in the aspect of three different depths including 1.5ft, 3ft, and 4.5ft in Chowfaldondi, Khuruskul, and Moheshkhali; morphometric variables are mostly associated with 1.5ft water depth.

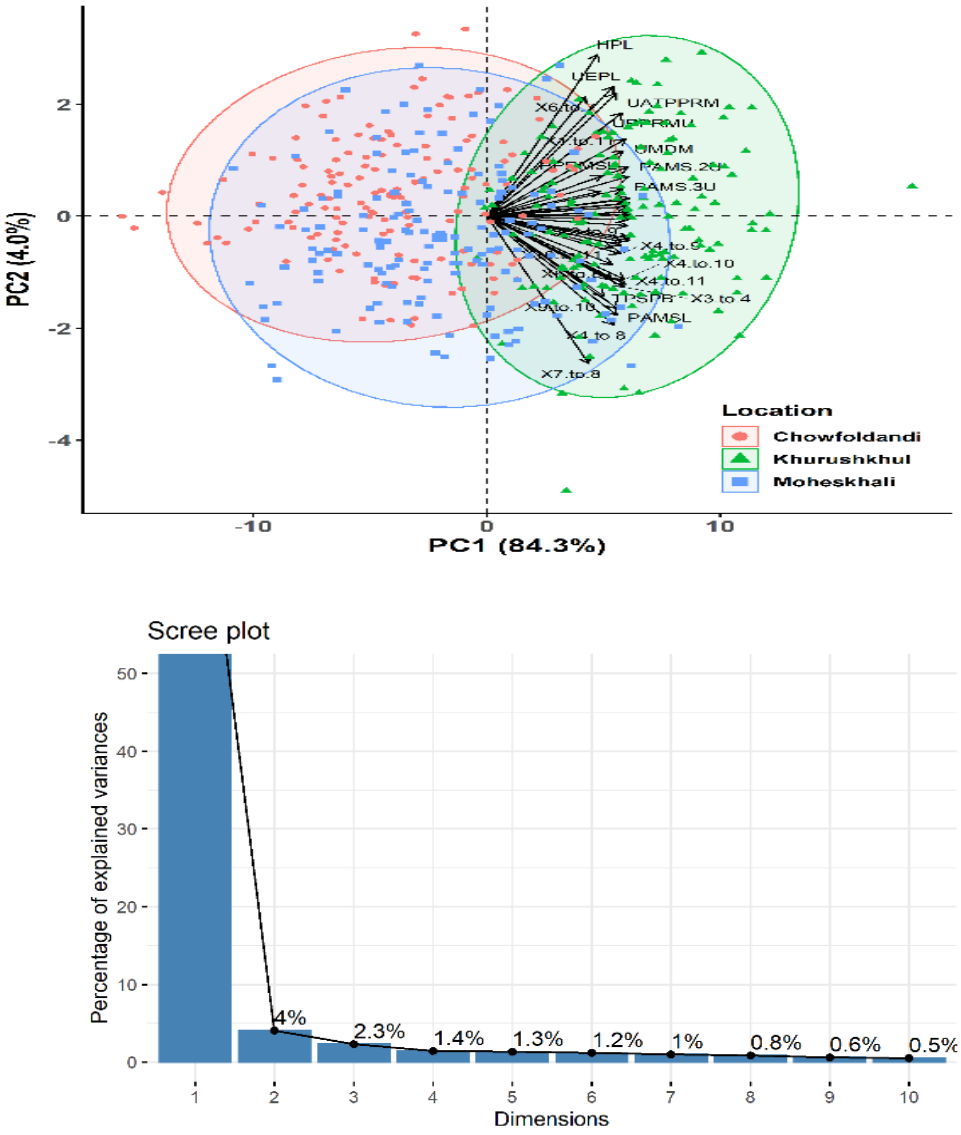


Figure-12: Principal Component Analysis for *P. viridis* in three locations; Chowfaldondi, Khuruskul, and Moheshkhali.

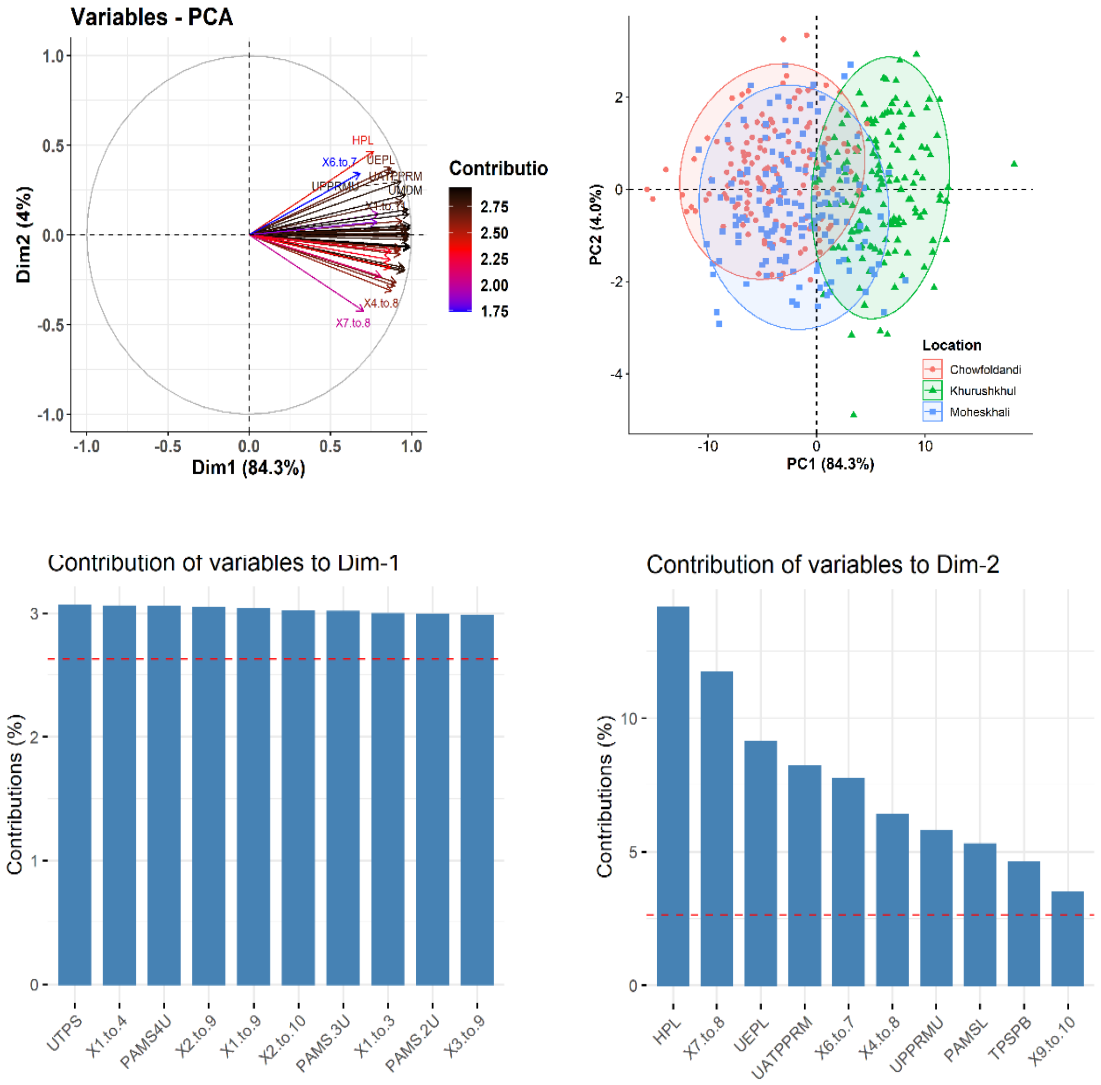


Figure-13: Principal Component Analysis for *P. viridis* in three locations; Chowfaldondi, Khuruskul, and Moheshkhali.

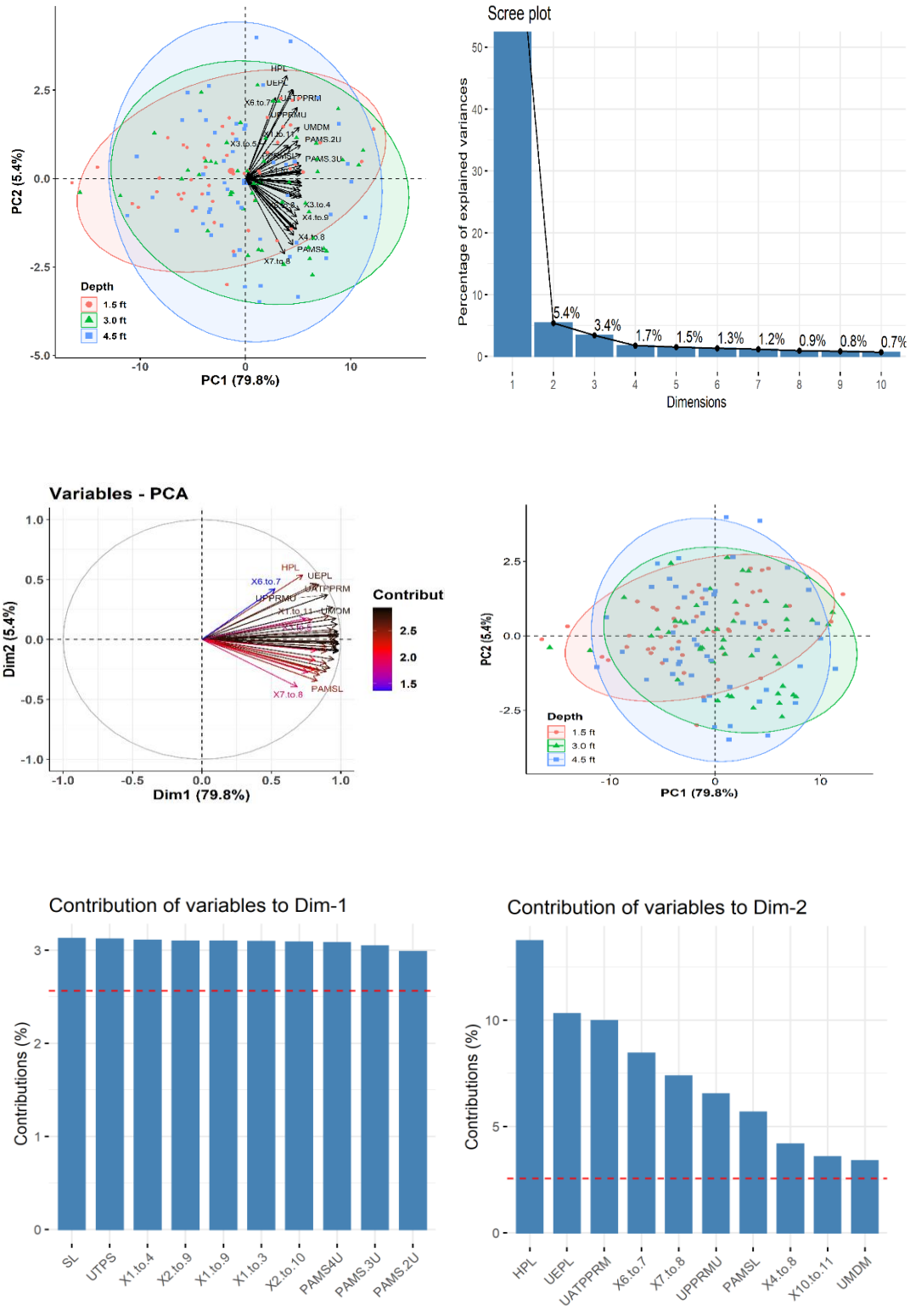


Figure-14: Principal Component Analysis for the *P. viridis* in the aspect of three different depths including 1.5ft, 3ft, and 4.5ft in Chowfaldondi.

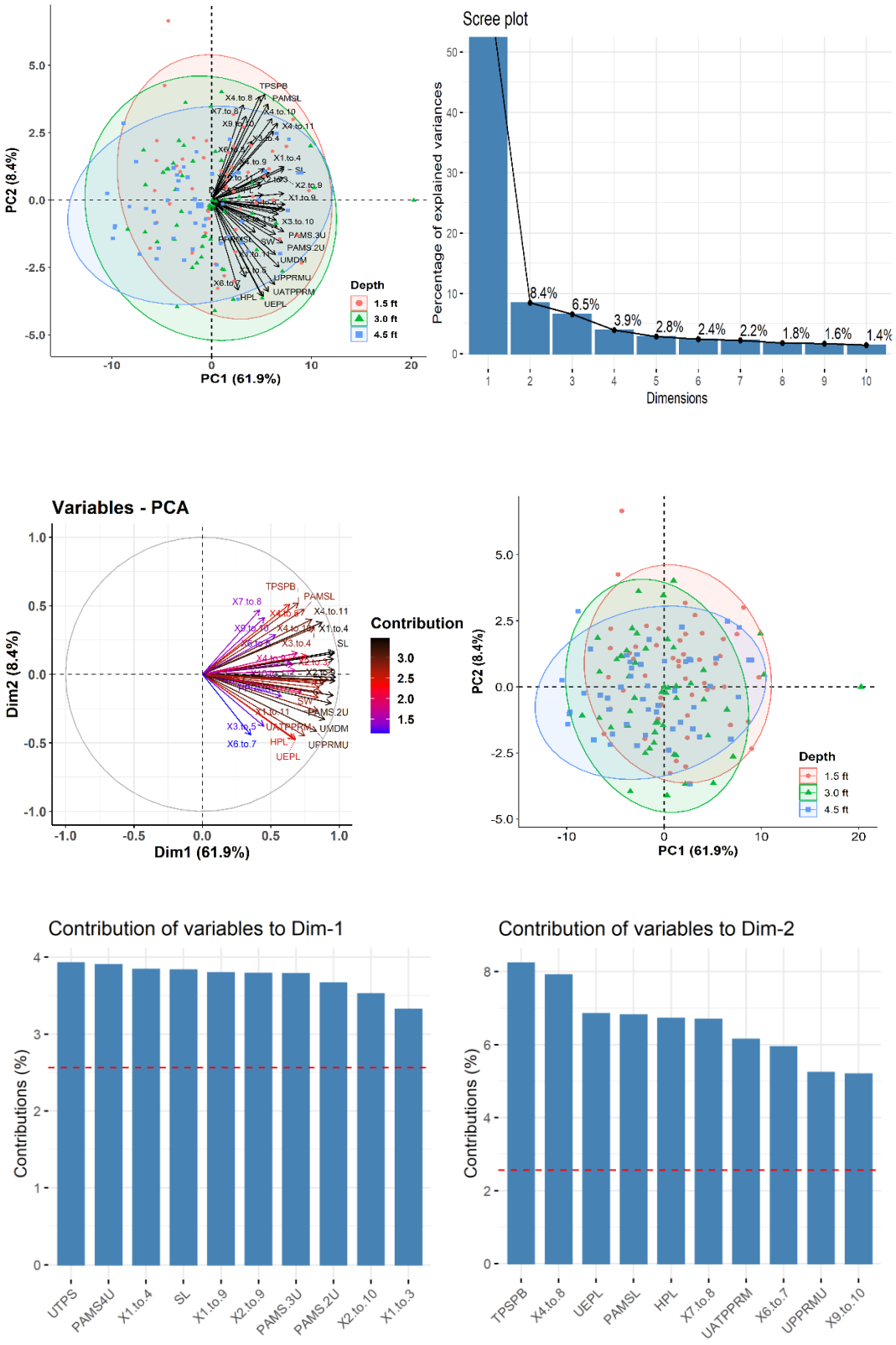


Figure-15: Principal Component Analysis for the *P. viridis* in the aspect of three different depths including 1.5ft, 3ft, and 4.5ft in Khuruskul.

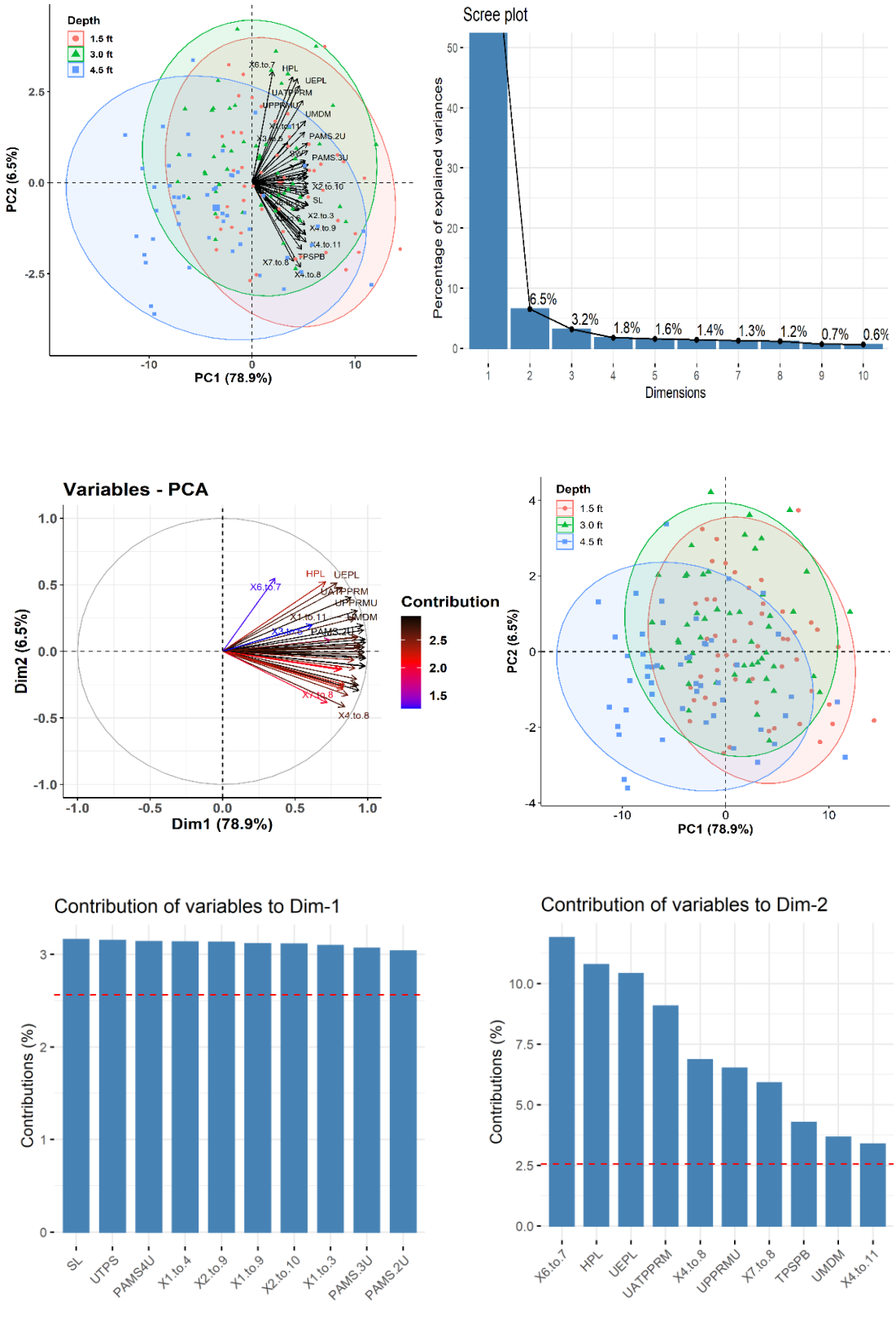


Figure-16: Principal Component Analysis for the *P. viridis* in the aspect of three different depths including 1.5ft, 3ft, and 4.5ft in Moheshkhali.

4.5 Canonical variates analysis

Canonical variate analysis (CVA) is a widely used method for analyzing group structure in multivariate data. It is mathematically equivalent to a one-way multivariate analysis of variance and often goes by the name of canonical discriminant analysis. In this study, conducted Canonical Variates Analysis (CVA) was conducted to explore the relationship among multiple sets of variables and identify significant patterns of variation within the data. The result showed that 62.2% of the differences were due to CV1, and 37.8% were due to CV2 for *P. viridis* (Figure-16). In the aspect of depths (1.5 ft, 3 ft, and 4.5 ft) 77.4% of the differences were due to CV1, and 22.6% were due to CV2 (Figure-17) in Chowfaldondi; 66.0% of the differences were due to CV1, and 33.9% were due to CV2 (Figure-17) in Khuruskul; 81.0% of the differences were due to CV1, and 19.0% were due to CV2 (Figure-17) in Moheshkhali for *P. viridis.*

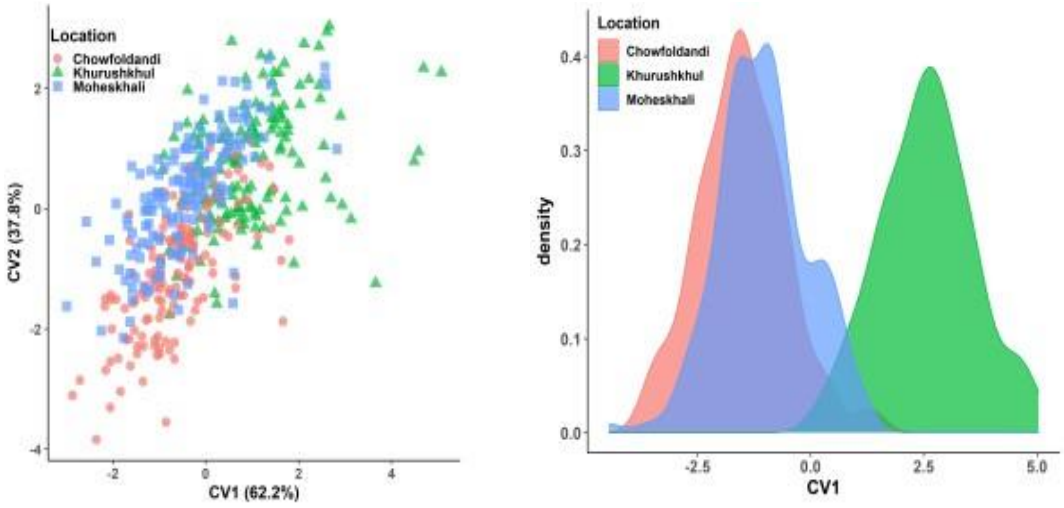
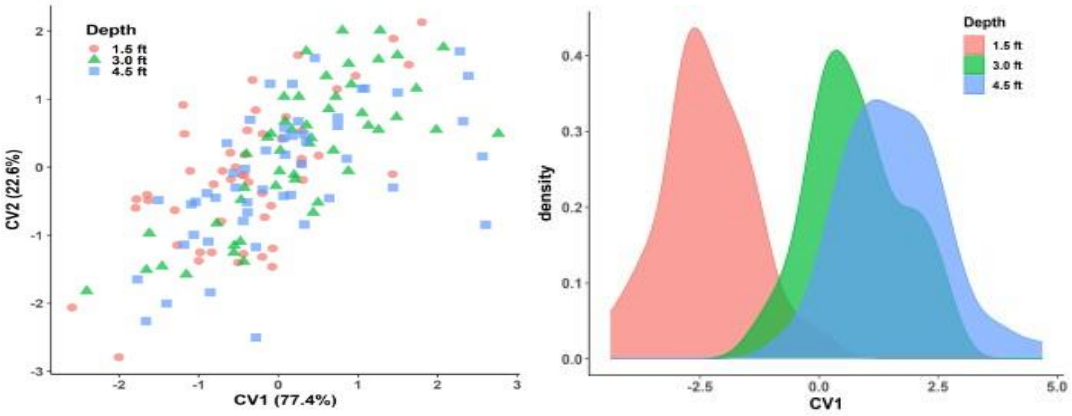
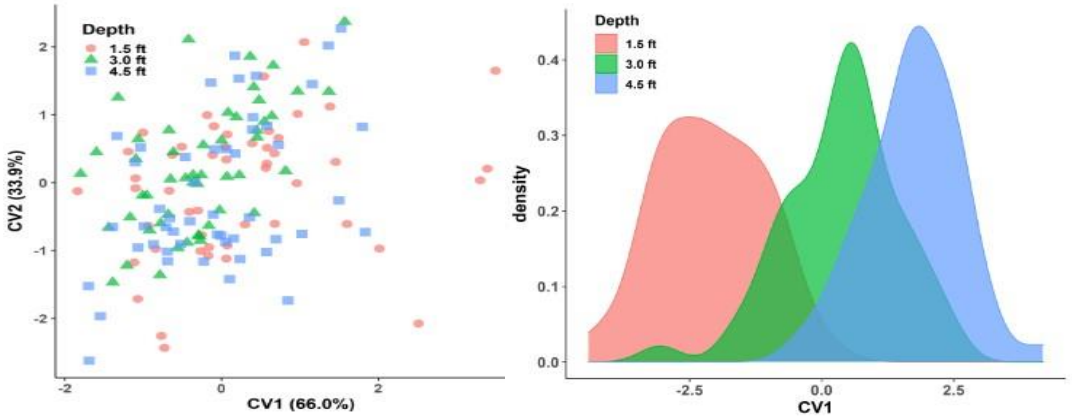


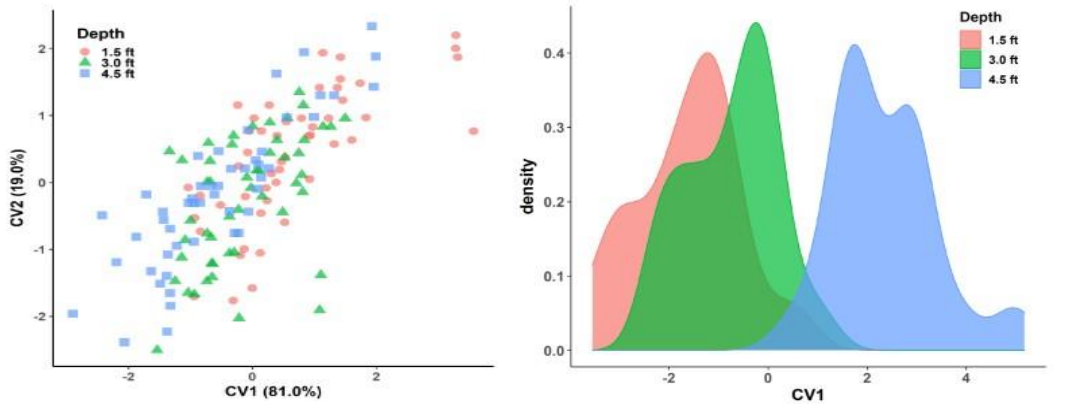
Figure-17: Canonical variates analysis of *P. viridis* in three locations; Chowfaldondi, Khuruskul, and Moheshkhali.



Chowfaldondi



Khuruskul



Moheshkhali

Figure-18: Canonical variates analysis for the *P. viridis* in the aspect of three different depths including 1.5ft, 3ft, and 4.5ft in each location.

4.6 K-means Cluster Plot

The k-means algorithm searches for a pre-determined number of clusters within an unlabeled multidimensional dataset. The "cluster center" is the arithmetic mean of all the points belonging to the cluster. A cluster plot for K-means will display the placement of the K centroids (representing the K clusters) in the data space and the assignment of the data points to each cluster. In this analysis, we applied the K-means clustering algorithm to a dataset containing morphometric measurements of green mussels. The goal was to identify distinct groups of mussels based on their shell length and shell width. After running the K-means algorithm with K=3 (representing three clusters), we obtained an insightful result. The data points have been successfully grouped into three distinct clusters based on their morphometric characteristics. The K-means clustering algorithm has effectively segmented the dataset into meaningful clusters, enabling us to gain valuable insights into the morphometric diversity within the green mussel population. The result showed that 84.3% of differences for Dim 1 and 4.0% due to Dim 2 for *P. viridis* population in three locations including Chowfaldondi, Khuruskul, and Moheshkhali.

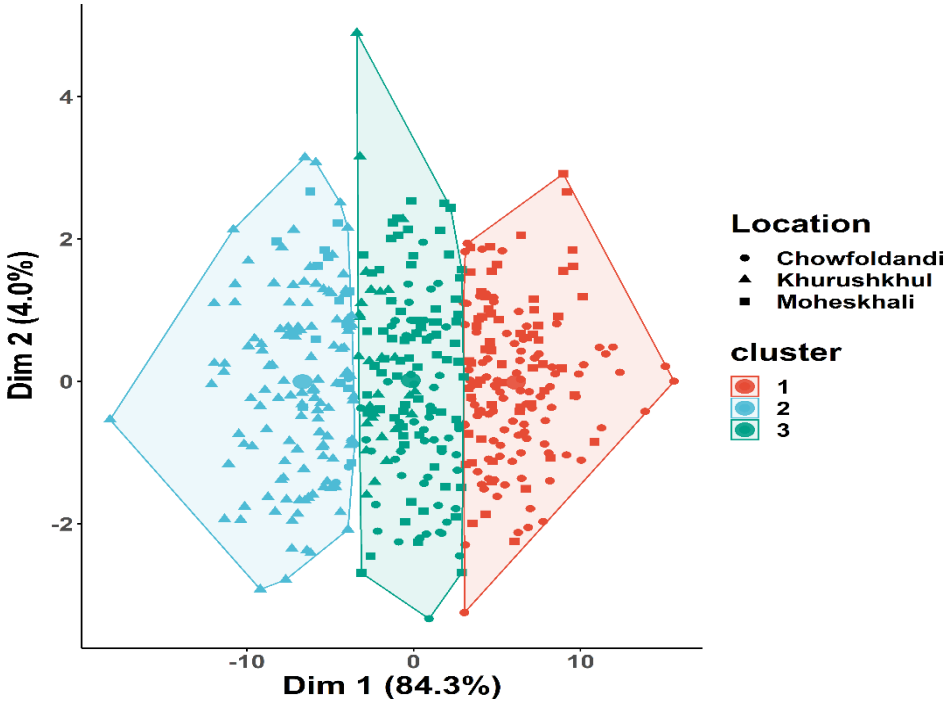


Figure-19: K-means Cluster Plot for the *P. viridis*

Chapter-5: Discussion

The morphological variations observed within *Perna viridis* populations could stem from their varied geographical distribution, diverse environmental factors present in their habitats, or the possibility that these populations have descended from multiple progenitors (Allendorf et al., 1980). Aquatic creatures possess a sensitivity to environmental shifts, adapting swiftly by altering their fundamental morphological traits to align with these changes (Allendorf et al., 1980). It is well known that morphological characteristics can change significantly in response to environmental changes (Swain et al., 1991). Therefore, the distinctive ecological traits of these habitats may help to explain the physical variation across the populations from different places. According to Naz et al. (2022), the presence of different-sized green mussels at the location suggests that coastal currents and daily tide flow allow pelagic larval distribution along the coast. Previous research has been conducted on the growth parameters, nutritive value, and heavy metal analysis of *P. viridis* along the Pakistani coast, including distribution and abundance (Ahmed et al., 1982), condition cycling (Meher et al., 1985), allometric relationships (Barkati and Choudhary, 1988), and growth pattern (Fatima et al., 2006). In addition, the differential shape between *C. gallina* and *C. striatula* from Portuguese coastal waters were investigated using traditional linear and geometric morphometric analysis, including contour (elliptic Fourier analysis) and landmark-based methods (Haines et al., 2000). Differences in habitat have a substantial impact on physical differentiation in a variety of groups (Ferrito et al., 2007).

Twenty truss network landmarks were found significantly different at different level of significance ($p<0.5$*; $p<0.01$**; $p<0.001$***) in Analysis of Variance (ANOVA) test for *Perna viridis* based on three locations; Khuruskul, Chowfaldondi, and Moheshkhali. Several researchers (Ahmed et al., 1982; Meher et al., 1985; Barkati and Choudhary, 1988; Awan et al., 2012; Jayalakshmy et al., 2013; Thejasvi et al., 2014; Villaluz et al., 2016; De Messano et al., 2019; Noor et al., 2019; Arrieche et al., 2020; Noor et al., 2021; Patterson et al., 2021) studied the growth of mussels from wild and mariculture and have reported different types of growth respectively. Morphological variations were found greater in the case of Khuruskul followed by Maheshkhali and Chowfaldondi. It may be due to optimum culture conditions, minimal turbidity, water pressure, and current flow in Khuruskul rather than others. This result is also

enumerated by Allendorf et al. (1980) and Swain et al. (1991). Populations of the same species from various geographic locations had distinct morphologies (Paugy and Leveque, 1999).

In the truss network, twenty truss measurements were significantly different among samples in varying degrees ($p<0.05$ or $p<0.01$ or $p<0.001$) based on three locations. Truss measurements were significantly different mostly in Khuruskul according to water depth. Green mussels from Khuruskul showed the highest morphological traits at 1.5ft depth. According to Nahar et al. (2015), the possible reason behind this result is the transparency of the water as the culture water of Khuruskul is less turbid and clean. Due to higher turbidity, growth was limited to 3ft in the case of Chowfaldondi.

According to Divya et al. (2012), Canonical discriminant function analysis revealed that the first discriminant function accounted 60.6% of the variation of *P. viridis* in Andaman which closely reflects the Canonical discriminant function analysis of this study (62.2%) for three locations, whereas the second discriminant function explained 86.4% of the variation. Divya et al. (2012) also found that the UPGMA dendrogram revealed a lower genetic distance between Kollam, Chennai, and Dona Paula samples of *P. viridis* and a greater separation amongst Andaman groups and also the Andaman seas' (2500km) geographical remoteness from Indian mainland waters may explain the Andaman population's stronger genetic differentiation from the other three coastal groups. On the other hand, Morphometric relationships established for *P. viridis* revealed that the length, width, and thickness of the shell had a substantial positive association with overall weight and soft tissue weight. Correlation coefficients were near to one in all cases, while SW (shell width) - STW (soft-tissue weight) was bigger than all corresponded morphometric measures (Aban et al., 2017).

Discriminant Function Analysis (DFA) may be used to distinguish between many stocks of the same species, which may be of interest to stock management systems (Karakousis et al., 1991). To ensure this difference, another multivariate technique, PCA (Principal Component Analysis), which included visual analysis of projected PC1 and PC2 values for each specimen, was applied. Principal component analysis (PCA) suggested that morphometric differences are more pronounced in Khuruskul and Moheshkhali than in Chowfaldondi. In each location, however, 1.5ft water depth

resembles significantly greater morphometric variances than 3ft and 4.5ft water depths. The unique geographic locations of each population, as well as the physiological and environmental restrictions that each population experiences, including as salinity, temperature, turbidity, water pressure, current flow, and food availability, may explain this inter-population diversity (Allendorf, 1988; Swain et al., 1991). Guo et al. (2017) found in the examined populations that the first two major components accounted for 68% of the overall variation associated with the ten morphometric features for pearl oyster *Pteria penguin* based on Principal component analysis (PCA).

Fisher (1936) created LDA as a strategy for discovering the optimal linear combinations of variables for categorizing or dividing data. Researchers can use these linear combinations to assess which characteristics contribute the most to group separation and the most likely categorization for a case with unobserved group membership. A Linear Discriminant Analysis (LDA) biplot was used to examine intra-colonial diversity in the scleractinian coral *Acropora millepora*, which demonstrated that tiny colonies significantly influenced size class separation (Conlan et al., 2018). Other factors, on the other hand, drove the separation of larger colonies (Conlan et al., 2018). The species diversity and stock structure of the mud crab *Scylla sp*. were studied in a study along the Bangladeshi coast. *S. olivacea* and *S. serrata* could be differentiated using LDF analysis (Asaduzzaman et al., 2021). It accounted for 83.92% of the variance for *P. viridis* in three locations (Chowfaldondi, Khuruskul, and Moheshkhali) and also for three different depths (1.5ft, 3ft, 4.5ft). The LDA plot clearly showed that samples of *P. viridis* were significantly different in each location and according to depth, samples from every depth showed significant variations. A greater size variation of *P. viridis* was found at 1.5ft depth in Khuruskul than 3 ft and 4.5 ft water depth.

The dendrogram was created using morphological analysis and centroids from *P. viridis* populations collected from three distinct locations. Along the samples from three locations; samples of Khuruskul clustered with Chowfaldondi and Moheshkhali. In Chowfaldondi, samples of 3.0ft water depth clustered with samples of 1.5ft and 4.5ft water depth. In Khuruskul, samples of 1.5ft water depth clustered with samples of 3.0ft and 4.5ft water depth. Besides in Moheshkhali, samples of 4.5ft water depth clustered with samples of 1.5ft and 3.0ft water depth. These changes in habitats might be the result of genetic and environmental factors. The UPGMA dendrogram demonstrated a smaller genetic distance between *P. viridis* samples from Kollam, Chennai, and Dona

Paula and a bigger separation among Andaman groups (Divya et al., 2012). A dendrogram based on physical characteristics observed in populations of Japanese charr, *Salvelinus leucomaenis* (Nakamura, 2003); mullet, *Rhinomugil corsula* (Hossain et al., 2015); *Eutropiichthys vacha* (Parvej et al., 2014); and *Labeo calbasu* (Hossain et al., 2010). These markers could be employed to back up our findings in the future.

In terms of management of the coast's green mussel fishery, the current study concluded that the mainland's wild populations of *P. viridis* are genetically different. These findings have far-reaching consequences for the management of green mussel stocks in the countries where they occur. There is significant gene flow among green mussels in Bangladesh's coastal region, which means fisheries managers must collaborate on joint management plans for the green mussel species. There are some major political obstacles to the expansion of this type of structure. Each country should be aware that variations in available biomass within their waters may be caused by fishing pressure from neighboring countries. As a result, in both aquaculture and open water management, it is essential to choose genetically superior populations with improved characteristics. More studies, particularly morphometric studies, and studies of the effects of environmental conditions, are required for conservation and mass seed production of selected populations in order to save this species from extinction.

Chapter-6: Conclusion

The morphometric variance of several characteristics within the *Perna viridis* population revealed considerable variations at the 5%, 1%, and 0.01% levels of significance. The variables exhibit the greatest variability at 0.01% level of significance, and at larger than 5% level of significance, such variables cannot be justified or reflect similarity. In the aspect of both location and depth, the maximum variability was found from umbo to bottom point along width; from maximum anterior curvature to maximum posterior curvature; from maximum anterior curvature to the end of posterior adductor muscle scar; from maximum posterior curvature to the start of posterior adductor muscle scar; from the notched point of posterior adductor muscle scar to the tip point of that; from the tip of posterior adductor muscle scar to the start point of that. Truss network measurements show significant variations and Hierarchical Clustering using UPGMA process successfully reflects the morphometric variations among three locations and water depths.

The morphometric features examined allowed for some distinction among the study groups. An organized set of field-friendly morphometric characteristic was employed to accomplish this. These factors were discovered using principal component and discriminant analyses. Because determining how populations correspond is an important element of managing, breeding, and protecting species, it appears that shell width, hinge plate length, and umbo to tip of palillal sinus might be employed for this purpose in the current subtropical environment.

These findings provide critical morphological data that can be used to more precisely define and distinguish this *P. viridis*. The current findings may serve as a springboard for additional research in this area. This study gives basic information on the diversity of *P. viridis* populations in various aquatic habitats in Bangladesh. It implies that morphometric traits and truss measurements can be employed to offer valid data for stock discrimination of *P. viridis* in order to ensure the species' long-term existence. The findings of the study will lay the groundwork for managing stocks, allowing better regulation of fisheries, and facilitating the creation of more impactful, long-term conservation programs. Therefore, findings of this study will be useful to fishermen, biologists, and taxonomists in their respective sectors.

Chapter-7: Recommendations

Digital tools for geometric morphometric analysis provide a new method for determining morphometric length and truss network distance. In comparison to earlier methods, it is a superior technique to assess meristic counts. The more complex the outcome, the more distinct the differences between the factors are. If you do an excellent job of analyzing, the following steps will help with future research:

- When taking a photo that has to be used for calculating measures, a photo lab with a higher resolution camera should be used to ensure the highest possible image quality.
- The samples are needed to be sorted carefully, and any damaged samples are to be discarded.
- Handling the sample with great care is crucial because rough treatment might cause damage to the external compartment of *P. viridis* shell in the future.
- Proper knowledge about software programs and their operations is necessary; failing to do so may result in incorrect calculations.
- A broad understanding of PCA, DFA, and other statistical approaches is required for this research.
- The higher the total number of samples, the more accurate the results. To conduct proper research, researchers must utilize the most significant number of samples.
- The final and pivotal aspect revolves around securing essential funding for enhancing research through improved software procurement and comprehensive data collection.

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Appendices

Appendix-A: Tests of equality of group means for *P. viridis* due to three different locations.

Variables	Wilks' Lambda	F	df1	df2	Sig.
SW	0.991	2.078	2	447	0.126
PPRMSL	0.989	2.55	2	447	0.079
HPL	0.981	4.371	2	447	0.013*
PAMSL	0.948	12.267	2	447	0***
UTPS	0.928	17.399	2	447	0***
TPSPB	0.814	51.058	2	447	0***
PAMS-2U	0.952	11.352	2	447	0***
PAMS-3U	0.88	30.562	2	447	0***
PAMS4U	0.958	9.679	2	447	0***
UATPPRM	0.992	1.879	2	447	0.154
UPPRMU	0.986	3.13	2	447	0.045*
UEPL	0.996	1.006	2	447	0.367
UMDM	0.927	17.471	2	447	0***
DBSAEPPL	0.963	8.598	2	447	0***
1 to 2	0.892	26.928	2	447	0***
1 to 3	0.913	21.402	2	447	0***
1 to 4	0.984	3.736	2	447	0.025*
1 to 9	0.83	45.822	2	447	0***
1 to 11	0.947	12.503	2	447	0***
2 to 3	0.987	3.009	2	447	0.05*
2 to 9	0.914	20.936	2	447	0***
2 to 10	0.911	21.815	2	447	0***
2 to 11	0.919	19.638	2	447	0***
3 to 4	0.761	70.086	2	447	0***
3 to 9	0.983	3.921	2	447	0.021*
3 to 10	0.982	4.083	2	447	0.017*
3 to 11	0.988	2.791	2	447	0.062
3 to 5	0.822	48.31	2	447	0***
4 to 8	0.853	38.373	2	447	0***
4 to 9	0.956	10.242	2	447	0***
4 to 10	0.992	1.742	2	447	0.176
4 to 11	0.986	3.231	2	447	0.04*
5 to 6	0.98	4.503	2	447	0.012*
6 to 7	0.963	8.624	2	447	0***
6 to 8	0.97	6.829	2	447	0.001**
7 to 8	0.745	76.493	2	447	0***
9 to 10	0.989	2.486	2	447	0.084
10 to 11	0.996	0.975	2	447	0.378

Appendix-B: Tests of Equality of Group Means for *P. viridis* due to three different water depths; 1.5ft, 3ft, and 4.5ft in Khuruskul.

Variables	Wilks' Lambda	F	df1	df2	Sig.
SW	0.998	0.171	2	147	0.843
PPRMSL	0.989	0.804	2	147	0.449
HPL	0.999	0.063	2	147	0.939
PAMSL	0.953	3.642	2	147	0.029
UTPS	0.931	5.443	2	147	0.005
TPSPB	0.971	2.164	2	147	0.118
PAMS-2U	0.917	6.656	2	147	0.002
PAMS-3U	0.943	4.41	2	147	0.014
PAMS4U	0.931	5.413	2	147	0.005
UATPPRM	0.918	6.529	2	147	0.002
UPPRMU	0.933	5.274	2	147	0.006
UEPL	0.967	2.489	2	147	0.086
UMDM	0.976	1.84	2	147	0.162
DBSAEPPL	0.904	7.795	2	147	0.001
1 to 2	0.956	3.378	2	147	0.037
1 to 3	0.923	6.094	2	147	0.003
1 to 4	0.85	13.006	2	147	0
1 to 9	0.851	12.857	2	147	0
1 to 11	0.874	10.643	2	147	0
2 to 3	0.82	16.185	2	147	0
2 to 9	0.943	4.472	2	147	0.013
2 to 10	0.909	7.368	2	147	0.001
2 to 11	0.903	7.918	2	147	0.001
3 to 4	0.894	8.743	2	147	0
3 to 9	0.988	0.885	2	147	0.415
3 to 10	0.987	0.938	2	147	0.394
3 to 11	0.989	0.789	2	147	0.456
3 to 5	0.976	1.773	2	147	0.173
4 to 8	0.879	10.146	2	147	0
4 to 9	0.987	0.976	2	147	0.379
4 to 10	0.95	3.848	2	147	0.023
4 to 11	0.977	1.754	2	147	0.177
5 to 6	0.943	4.405	2	147	0.014
6 to 7	0.992	0.57	2	147	0.567
6 to 8	0.999	0.045	2	147	0.956
7 to 8	0.949	3.97	2	147	0.021
9 to 10	0.968	2.461	2	147	0.089
10 to 11	0.934	5.152	2	147	0.007

Appendix-C: Tests of Equality of Group Means for *P. Viridis* due to three different water depths; 1.5ft, 3ft, and 4.5ft in Chowfaldondi.

Variables	Wilks' Lambda	F	df1	df2	Sig.
SW	0.994	0.452	2	147	0.637
PPRMSL	0.974	1.998	2	147	0.139
HPL	0.972	2.128	2	147	0.123
PAMSL	0.959	3.121	2	147	0.047
UTPS	0.96	3.089	2	147	0.049
TPSPB	0.975	1.882	2	147	0.156
PAMS-2U	0.97	2.291	2	147	0.105
PAMS-3U	0.955	3.439	2	147	0.035
PAMS4U	0.985	1.142	2	147	0.322
UATPPRM	0.984	1.188	2	147	0.308
UPPRMU	0.982	1.339	2	147	0.265
UEPL	0.982	1.367	2	147	0.258
UMDM	0.944	4.324	2	147	0.015
DBSAEPPL	0.944	4.401	2	147	0.014
1 to 2	0.988	0.917	2	147	0.402
1 to 3	0.973	2.05	2	147	0.132
1 to 4	0.922	6.233	2	147	0.003
1 to 9	0.803	18.047	2	147	0
1 to 11	0.846	13.332	2	147	0
2 to 3	0.965	2.641	2	147	0.075
2 to 9	0.983	1.268	2	147	0.284
2 to 10	0.989	0.822	2	147	0.441
2 to 11	0.984	1.185	2	147	0.309
3 to 4	0.92	6.361	2	147	0.002
3 to 9	0.952	3.74	2	147	0.026
3 to 10	0.964	2.737	2	147	0.068
3 to 11	0.966	2.613	2	147	0.077
3 to 5	0.963	2.863	2	147	0.06
4 to 8	0.837	14.277	2	147	0
4 to 9	0.909	7.352	2	147	0.001
4 to 10	0.944	4.34	2	147	0.015
4 to 11	0.976	1.82	2	147	0.166
5 to 6	0.985	1.129	2	147	0.326
6 to 7	0.922	6.176	2	147	0.003
6 to 8	0.982	1.37	2	147	0.257
7 to 8	0.992	0.609	2	147	0.545
9 to 10	0.957	3.342	2	147	0.038
10 to 11	0.877	10.35	2	147	0

Appendix-D: Tests of Equality of Group Means for *P. viridis* due to three different water depths; 1.5ft, 3ft, and 4.5ft in Moheshkhali.

Variables	Wilks' Lambda	F	df1	df2	Sig.
SW	0.996	0.271	2	147	0.763
PPRMSL	0.978	1.642	2	147	0.197
HPL	0.913	7.035	2	147	0.001
PAMSL	0.936	5.049	2	147	0.008
UTPS	0.976	1.816	2	147	0.166
TPSPB	0.952	3.739	2	147	0.026
PAMS-2U	0.927	5.8	2	147	0.004
PAMS-3U	0.905	7.685	2	147	0.001
PAMS4U	0.979	1.569	2	147	0.212
UATPPRM	0.892	8.945	2	147	0
UPPRMU	0.901	8.074	2	147	0
UEPL	0.91	7.293	2	147	0.001
UMDM	0.834	14.613	2	147	0
DBSAEPPL	0.902	7.969	2	147	0.001
1 to 2	0.949	3.917	2	147	0.022
1 to 3	0.952	3.738	2	147	0.026
1 to 4	0.793	19.19	2	147	0
1 to 9	0.987	0.944	2	147	0.391
1 to 11	0.992	0.598	2	147	0.551
2 to 3	0.998	0.115	2	147	0.892
2 to 9	0.972	2.153	2	147	0.12
2 to 10	0.995	0.385	2	147	0.681
2 to 11	0.989	0.829	2	147	0.439
3 to 4	0.83	15.041	2	147	0
3 to 9	0.977	1.767	2	147	0.174
3 to 10	0.972	2.156	2	147	0.119
3 to 11	0.959	3.109	2	147	0.048
3 to 5	0.98	1.504	2	147	0.226
4 to 8	0.908	7.474	2	147	0.001
4 to 9	0.972	2.116	2	147	0.124
4 to 10	0.915	6.848	2	147	0.001
4 to 11	0.92	6.376	2	147	0.002
5 to 6	0.98	1.501	2	147	0.226
6 to 7	0.976	1.817	2	147	0.166
6 to 8	0.989	0.832	2	147	0.437
7 to 8	0.84	14.023	2	147	0
9 to 10	0.919	6.441	2	147	0.002
10 to 11	0.994	0.42	2	147	0.658

Brief Biography of the Author

This is Md. Hassibul Hossain Shanto, son of Md. Rafiqul Islam Akand and Hasina Momtaz from Kapasia Upazila under Gazipur District of Bangladesh. He passed the Secondary School Certificate Examination in 2013 from North Kafrul High School, Dhaka Cantonment, and Higher Secondary Certificate in 2015 from Adamjee Cantonment College, Dhaka Cantonment. He obtained his B. Sc. in Fisheries (Hons.) Degree in 2020 from the Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh. Now, he is a candidate for the degree of MS in Marine Bioresource Science under the Department of Marine Bioresource Science, Faculty of Fisheries, CVASU. He has a great interest in scientific research on marine.