Influence of Depth and Site-specific Ecological Factor on Shell Morphometrics and Coloration in Green Mussels (*Perna viridis)*

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Abbreviation

DFA	Discriminant Function Analysis
PCA	Principal Component Analysis
GM	Geometric Morphometrics
GMA	Geometric Morphometric Analysis
P	Level of Significance
NS	Not Significant
CVA	Canonical Variates Analysis
CIELAB	Commission Internationale de l'Éclairage L*a*b* Color Space
RGB	Red Green Blue (Color Model)
HSV	Hue Saturation Value (Color Model)
Wilks' λ	Wilks' Lambda – A multivariate statistic indicating group mean differences
MANOVA	Multivariate Analysis of Variance
i.e.	That is
e.g.	For Example
et al.	And Associates / And Others (used in citations)

Abstract

The green mussel (*Perna viridis*) is a commercially valuable bivalve with strong aquaculture potential in Bangladesh. This study examined how water depth and site-specific ecological factors influence shell morphometrics and coloration in *P. viridis*. A total of 450 mussels were cultured at three depths (0.5 m, 1.0 m, and 1.5 m) across three coastal sites Khurushkul, Moheshkhali, and Chowfaldandi. Monthly monitoring of water quality was conducted throughout the study from October 2024 to March 2025. Shell shape and size variations were assessed using geometric morphometric techniques, while shell coloration was analyzed through RGB, HSV, and CIELAB color models. The results revealed significant differences in shell morphology and pigmentation across both site and depth. Mussels from Chowfaldandi displayed broader shells, while those from Khurushkul had a more streamlined form, reflecting local water dynamics. Deeper-dwelling mussels tended to develop rounder, more inflated shells, possibly as an adaptation to calmer, low-light environments. Coloration also varied notably: darker green pigmentation was observed in mussels from shallow depths and from Moheshkhali, likely associated with higher chlorophyll a concentrations and clearer water. In contrast, mussels from Chowfaldandi showed paler shells, potentially due to reduced light penetration and limited food supply. These findings demonstrate how environmental conditions shape key shell traits in *P. viridis*, offering insights for site and depth selection to enhance growth and shell quality in aquaculture. Shell morphology and pigmentation serve as valuable indicators for assessing habitat suitability and environmental quality.

Keywords: *Perna viridis*, shell morphology, coloration, culture depth, ecological stress, aquaculture

Chapter I

Introduction

1.1Background

Bangladesh has water resources that make it a good place for aquaculture to thrive. Bangladesh has a wide range of fisheries, including inland openwater catch fisheries, inland and coastal aquaculture, and marine fisheries. Coastal waters are rich in fish and shellfish, making them one of the most productive ecosystems on Earth. There are still many unknown marine fishery resources, and there are 301 species of mollusks that are important for their size and worth as food (Ahmed, 1990). The coastal waters of Bangladesh are home to three types of mollusks that are significant for business potential: Green mussel (*Perna viridis*), Oyster (*Crassostrea madrasensis*), and Clam (*Meretrix meretrix*) (Ahmed, 1990). Small-scale fishermen and coastal tribal people in Bangladesh who live along the coast harvest these bivalves for food and money. Tribal groups and small-scale fishermen in the Satkhira, Barishal, and Chittagong-Cox's Bazar regions catch about 850 tons of mollusks every year (Shahabuddin *et al*., 2010).

The green mussel (*Perna viridis*), a pivotal species within marine ecosystems, serves as a cornerstone for both ecological balance and economic development across the Indo-Pacific's tropical and subtropical waters, underpinning its role as a critical intertidal filter feeder and a burgeoning source of sustainable mariculture (Vakily, 1989; Hickman, 1992). This species has garnered attention for its capacity to serve as a sustainable protein source, which is crucial in meeting the protein requirements of the expanding global population (Wijsman *et al*., 2019). Recognized for its rapid growth and economic value, the green mussel has seen a notable increase in cultivation, particularly in Southeast Asia, evidencing its significant mariculture potential across various nations (Appukuttan, 2003; Nair and Appukuttan, 2003; Tan and Ransangan, 2014). The ideal conditions for mussel aquaculture are found along the coasts of many Asian countries, characterized by their high environmental productivity, significant tidal movements, strong tidal currents, pristine water conditions, and plentiful phytoplankton. These factors collectively create a conducive environment for advancing bivalve mussel culture technology (Shahabuddin *et al*., 2010; Tan and Ransangan, 2017). In Bangladesh, green mussel (*P. viridis*) populations thrive naturally along the coastline, particularly in areas like St. Martin's Island, the

Moheshkhali channel, Shahporir Dwip, and the Naf River estuary, highlighting the region's conducive conditions for both natural proliferation and mariculture (Shahabuddin *et al*., 2010). The country's coastal zones, known for their high productivity, are optimal for the growth and farming of *P. viridis*, with successful culture practices and methodologies being established in locations such as Moheshkhali, Khurskul, and Chowfaldandi, indicating the viability of green mussel cultivation at various water depths (Khan *et al*., 2019; Hoque *et al*., 2021). Key environmental factors contributing to the successful farming of green mussels include avoiding areas with strong winds and large waves, maintaining a minimum water depth of 1.5 m, ensuring current speeds of 0.1-0.3 m/s, achieving stable high salinity levels between 21-33 ppt, keeping alkalinity within 50-200 ppm, and operating in temperatures of 25-35°C. Additionally, areas with primary production characterized by chlorophyll-α concentrations of 0.7-17 µg L-1 are preferable, underscoring the specific conditions required for the commercial success of green mussel farming (Aypa, 1990; Rajagopal *et al*., 1998; Saxby, 2002; Apukuttan *et al*., 2003;Nayak *et al*., 2014;FAO, 2021). Initially, the classification and identification of marine mussel species relied heavily on the physical characteristics of their shells, such as color, form, texture, and size (Jones *et al*. 2015). While certain aspects of shell shape are genetically determined and may offer evolutionary advantages (Marinho *et al.* 2021), the influence of the surrounding habitat and environmental conditions on shell morphology is substantial (Haag, 2012). Factors such as the physicochemical properties of the water, predation levels, environmental stresses, and the availability of nutrients and food sources play significant roles in the variation of mussel shell shapes across different locations and depths.

To quantify changes in body shape and their relationship with other variables, geometric morphometrics are the best tools because they are able to extract and provide information concerning the physical localization of morphological variation and its magnitude (Zelditch *et al*., 2004). In fishes, the investigation focuses on the correlation between body shape and hydrodynamic efficiency. In recent studies, Rodrigues-Oliveira *et al*., (2023) found notable differences in body shapes among seven populations of the characid *Psalidodon rivularis* in the São Francisco River basin. Holmes *et al*. (2023) observed that body shape variations in damselfish species *Chromis* and *Azurina* align closely with phylogenetic data, illustrating the utility of

geometric morphometrics in phylogenetic studies. This approach has also shed light on morphological evolution and speciation, as Berendzen *et al*. (2022) demonstrated with stream fish *Notropis nubilus*, and has revealed the shape diversity of endemic species, such as in the *Danakilia* genus by Chiozzi *et al.* (2018) and between Sicilian populations of the rainbow wrasse *Coris julis* by Fruciano *et al*. (2011). For bivalves, the emphasis is on the geometric characteristics of shell morphology, aiming to uncover the connections between shell design, defensive capabilities, and environmental suitability. Krapivka *et al*. (2007) utilized geometric morphometrics, specifically Fourier elliptical analysis, to explore how shell shape varies with latitude in *Mytilus chilensis* across its wild populations. They identified notable variations in the convexity of the shell's ventral edge, umbo shape, and shell length, attributing these differences to a latitudinal gradient. A similar trend was noted in *Brachidontes* sp., another key South American mussel species, by Aguirre *et al*. (2006). This methodological approach has also been applied in studies by Palmer *et al*. (2004) on *Chamelea gallina*. Rufino *et al*. (2006) examined both *Chamelea gallina* and *Chamelea striatula*, as well as investigations into *Corbicula fluminea* by Sousa *et al*. (2007), and *Tapes decussatus* and T. *philippinarum* by Costa *et al*. (2010), further showcasing its effectiveness in discerning morphological patterns across various bivalve species.

Such tools allow for direct visualization of the patterns of change in shape (Bookstein, 1991) based mainly on two principles: visualization of shape change by showing the relative displacements of corresponding landmarks in different shapes or by showing the deformation of a regular grid, an outline or a surface that is interpolated from the shape change. These two approaches can also be used in combination (Klingerberg *et al*., 2013; Zelditch *et al*., 2004). In order to investigate morphological changes, landmark geometric morphometrics (GM) were used (Rohlf & Marcus, 1993; Klingenberg *et al*., 1996). Landmark-based morphometrics, despite their prevalence, are limited by the lack of sufficient landmarks on some structures and potential loss of important shape features (Rohlf, 1990; Adams *et al*., 2004). Bookstein's (1997) 'sliding semi-landmarks' method addresses this by digitizing points along a structure's outline, adjusting them to minimize discrepancies, and enhancing the accuracy of shape analysis by treating these adjusted points as traditional landmarks (Zelditch *et al*., 2004). This approach merges the precision of landmark-based methods with the

flexibility of outline analysis, improving morphometric evaluations. Moreover, advances in computer graphics have made it easier to produce appealing illustrations of shape changes in two or three dimensions with both these approaches, and computer animation holds further potential (Zelditch *et al*., 2004;Valentin *et al*., 2008; Klingerberg *et al.*, 2013; Fruciano *et al*., 2014, 2020).

In addition to shape, mussel shell coloration is a key visual and ecological trait. Color variation may result from environmental factors such as light, nutrients, fouling, water chemistry, and also genetic variability (Vermeij, 2002; Wilke & Davis, 2010;Williams, 2017). It can indicate physiological condition, pollutant exposure, or age-related changes (Al-Subiai 2011; Moore *et al*., 2013). Recent studies employ image-based color quantification using RGB, HSV, and CIELAB models for accurate and consistent analysis across samples (Troscianko & Stevens, 2015).

Despite the ecological and commercial significance of *Perna viridis*, there remains a lack of comprehensive research on how environmental gradients, particularly site-specific conditions and water depth affect its shell shape and coloration in Bangladesh. Understanding these relationships is essential for optimizing mussel farming and improving environmental monitoring efforts. This study aims to address these knowledge gaps and support the development of sustainable aquaculture practices in the region.

1.2 Research Objectives:

The study aimed to achieve the following objectives:

- To evaluate the influence of different cultivation depths on shell morphometric traits and coloration patterns in green mussels (*Perna viridis*).
- To assess how site-specific ecological factors (e.g., water temperature, salinity, turbidity, and nutrient availability) affect shell shape and pigmentation in cultured populations of *P. viridis*.

Chapter II

Review of Literature

2.1 Biology of Green Mussel (*Perna viridis*)

Perna viridis, commonly known as the green mussel (Fig. 1) or green-lipped mussel, belongs to the family **Mytilidae**, the only family under the order **Mytiloida** (GSFMC, 2005). Within the genus *Perna*, which includes four known species (*P. canaliculus, P. picta, P. perna,* and *P. viridis*), *P. viridis* is widely distributed throughout the tropical and subtropical waters of the Indo-Pacific region. Other *Perna* species are native to places like South America, Africa, and New Zealand (Sallih, 2005). The scientific classification of *Perna viridis* is,

Phylum Mollusca

Class Bivalvia

Subclass Autobranchia

Order Mytilida

Family Mytilidae

Genus *Perna*

Species *Perna viridis* (Linnaeus, 1758)

Figure 1. External view of Green mussel (*Perna viridis*)

Perna viridis is a relatively large mussel species, typically measuring between 80 and 100 mm in length, though it can occasionally reach sizes of 150 to 165 mm (NIMPIS, 2002; FIGIS, 2005). It possesses two nearly identical valves with smooth, pear-shaped exteriors, marked by concentric growth lines and a slightly concave ventral surface. The shell is covered by a durable, soft periostracum that appears bright green in juveniles and transitions to brown with green edges as the mussel matures (Sallih, 2005). Juvenile individuals exhibit rapid growth, attaining shell lengths of 3–4 mm within 4 to 8 weeks (Aypa, 1990).

2.1.1 Feeding Habit

Perna viridis is a filter feeder, drawing in water and filtering it through its gill filaments to capture microscopic food particles such as phytoplankton, zooplankton, and organic debris. Phytoplankton form the primary part of their diet, although additional carbon sources like macrophyte fragments and resuspended detritus can also contribute (Sallih, 2005).

The species' rapid growth is mainly attributed to areas with high phytoplankton biomass (Ren & Ross, 2005). Year-round availability of phytoplankton, along with favorable temperatures and salinity levels, further enhances their growth (Kamal & Khan, 1998).

2.1.2 Growth

The growth rate of green mussels is largely dependent on environmental conditions in the cultivation area. Subtidal zones, in particular, provide ideal habitats due to their stable conditions and abundant food supply, supporting fast and efficient growth (Rivonker *et al*., 1993; Rajagopal *et al*., 2006). Other key environmental influences include water turbidity, current speed, food availability, and competition for space (Alvarado & Castilla, 1996). Growth rates vary geographically, with first-year growth reported at 49.7 mm/year in Hong Kong and up to 120 mm/year in India (NIMPIS, 2002).

Green mussels are well-suited for aquaculture due to their high fecundity, free-swimming larval stage, and adaptability to a wide range of tropical conditions (Spencer, 2002). Among tropical mussels, *P. viridis* is one of the most productive species, particularly in Asia (Sreenivasan *et al*, 1989; Gallardo *et al*., 1992). In Bangladesh,

commercial farming of *P. viridis* began in 1995 in Anthakaranazhi (Alleppey district), where local fishermen successfully used longline methods (Appukuttan *et al*., 2001).

2.1.3 Habitat

Perna viridis is naturally found in warm tropical waters and is widely distributed across the Indo-Pacific region (Bayne, 1976). Its native range includes coastal waters around India, Southeast Asia, Japan, Malaysia, Papua New Guinea, the South Pacific Islands, and the Persian Gulf (Sivalingam, 1977; Rajagopal *et al*., 2006).

In Bangladesh, green mussels are particularly abundant along the Cox's Bazar coast. Notable populations have been reported in St. Martin's Island, Shahporir Dwip, the Naf River, and the Moheshkhali Channel (Ali, 1975; Ahmed *et al*., 1990; Shahabuddin *et al*., 2010).

2.2 Morphometric Measurements of Mussels

Shell shape analysis is a key tool for understanding how mussels vary in appearance and how they adapt to different environments (Adams *et al*., 2004; Zelditch *et al*., 2012). Traditionally, researchers have used basic linear measurements such as shell length, width, and height to study shell size and growth patterns (MacLeod, 2017; Noor *et al*., 2021). These methods work well in many situations, but they often fail to capture subtle differences in shape that can reflect environmental influences (Adams *et al*., 2004; Fruciano *et al*., 2011).

To address this limitation, geometric morphometrics (GM) has become a powerful alternative. Unlike traditional methods, GM focuses on the shape of the shell rather than just its size (Fruciano *et al.*, 2011; Zelditch *et al*., 2012). It uses landmark points on the shell to analyze how shapes differ among individuals. This method gives a more detailed understanding of shape variation and its potential environmental or evolutionary causes (Fruciano *et al*., 2011). Common tools in GM include Procrustes superimposition, Principal Component Analysis (PCA), Canonical Variate Analysis (CVA), and Discriminant Analysis (DA), which allow researchers to clearly detect shape differences that are not always visible through basic measurements (Fruciano *et al*., 2011; Beyett *et al*., 2020).

GM has been used successfully in many bivalve studies. For example, Trovant *et al*. (2018) used this technique to tell apart closely related mussel species in both living and fossil samples. Rufino *et al.* (2006) applied GM to distinguish between two clam species ***Chamelea gallina*** and ***C. striatula*** where traditional measurements had failed. Beyett *et al*. (2020) also showed that GM, when combined with DNA barcoding, could identify freshwater mussel species with 99% accuracy, compared to only 90% when using simple shell ratios like length-to-height.

GM is also helpful in studies beyond bivalves. Fruciano *et al*. (2011) used it to examine jaw shapes in the fish species ***Coris julis***, revealing small but significant differences between populations that ate different types of food. This shows that even minor environmental differences, such as food availability or sediment type, can lead to visible changes in shape.

In the case of green mussels (*Perna viridis*), most research so far has focused on traditional shell measurements to study growth and allometry (Noor *et al*., 2021). These studies suggest that environmental factors like wave strength, bottom type, and predation might influence shell shape (Sukumar *et al*., 2016). However, detailed geometric analyses in this species are still limited.

A rare example comes from Gamier *et al*. (2019), who applied geometric morphometrics to *P. viridis* from two aquaculture sites in Cavite Province. Using four landmark points and procrustes analysis, they found no significant shape differences between the two sites even though salinity, temperature, turbidity, and pH varied. Their study suggests that *P. viridis* may be highly tolerant of environmental variation. However, they recommended including more variables, such as water depth and nutrient availability, to better detect finer-scale shape variation.

These findings support the growing view that GM is an accurate and sensitive method for detecting shell variation. For instance, Beyett *et al*. (2020) emphasized the importance of using many landmark points and robust statistical tools like CVA and jackknife classification. Their approach increased the reliability of species identification and helped explain how environmental factors shape morphology.

Still, there are gaps in the use of GM in *P. viridis*. Important factors like water depth and hydrodynamics, which are known to affect shell shape in other mussel species, have not been fully explored in green mussels using this method (Sukumar *et al*., 2016; Gamier *et al*., 2019). Moreover, GM has not yet been widely used to study shell deformities caused by environmental stress or pollution in this species (Gamier *et al*., 2019).

Combining geometric morphometrics with ecological data is a promising way forward. This approach could help us better understand how site-specific factors like salinity, temperature, water movement, or habitat disturbance influence shell development, color variation in *P. viridis* (Fruciano *et al*., 2011; Beyett *et al*., 2020). It may also help in early detection of environmental stress in aquaculture, which is important for both conservation and sustainable mussel farming (Gamier *et al*., 2019).

2.3 Shell Coloration in Bivalves

Shell coloration in bivalves is a fascinating and complex trait. It is shaped by a mix of genetic, physiological, and environmental factors. Understanding how these factors work together is important, especially when exploring how environmental conditions like depth or location affect the appearance of the shell.

2.3.1 Biochemical and Structural Basis of Bivalve Shell Coloration

Shell coloration in bivalves is influenced by both pigment-based and structural mechanisms. In species such as *Pinctada margaritifera* var. *cumingii*, biological pigments are the primary contributors to shell color, producing a broad spectrum of hues ranging from dark and pastel tones to silver, gold, green, blue, and even iridescent rainbow-like shades (Iwahashi & Akamatsu, 1994; Ky *et al*., 2014; Stenger *et al*., 2021). Different pigment types are responsible for specific colorations for example, tetrapyrroles like porphyrins and bilins generate red, brown, green, or blue shades, while melanins are linked to darker colors such as black, brown, and yellow (Comfort, 1951;Williams, 2017; Gefaell *et al*., 2023). Alongside pigmentation, shell structure also plays a crucial role in color expression through a phenomenon known as structural coloration, which often results in iridescent colors that shift depending on the angle of light. In *Pinctada margaritifera* var. *cumingii*, both pigment content and the

arrangement of calcium carbonate crystals in the nacre layer contribute to this visual effect (Liu *et al*., 1999; Stenger *et al*., 2021). The optical properties of the shell are influenced by the thickness and organization of nacre tablets. For instance, in *Sinanodonta woodiana*, a thick nacre layer composed of thin tablets produced a vibrant golden hue, while a thinner nacre with thicker tablets yielded a silvery appearance (Sahidin *et al*., 2022). These findings demonstrate that both biochemical pigmentation and microstructural features collectively determine the intensity, hue, and optical qualities of bivalve shell coloration.

2.3.2 Influence of Depth on Bivalve Shell Coloration

Shell coloration in bivalves exhibits notable environmental plasticity, particularly in response to water depth. In *Pinctada margaritifera* var. *cumingii*, individuals transplanted from shallow waters (4 meters) to deeper environments (30 meters) developed significantly darker inner shells, highlighting the influence of depth on pigmentation (Stenger *et al*., 2019; Stenger *et al*., 2021). Remarkably, this darker coloration persisted even after the oysters were returned to shallow water, suggesting that the change was not merely transient but potentially regulated through stable biological mechanisms, possibly epigenetic in nature (Stenger *et al*., 2021). Supporting this hypothesis, depth-induced coloration changes have been linked to modifications in gene regulation via DNA methylation. Genes associated with pigment biosynthesis (e.g., *GART*, *ABCC1*, *MAPKAP1*, *GRL101*) and shell matrix formation (e.g., *perlucin*, *MGAT1*) exhibited differential methylation patterns depending on the depth-related environmental conditions (Stenger *et al.*, 2021). These findings imply that factors such as reduced light availability and increased hydrostatic pressure at greater depths may trigger heritable changes in gene expression, resulting in altered shell coloration through molecular, rather than solely physiological, responses.

2.3.3 Site-Specific Ecological Factors and Shell Coloration

Shell coloration in bivalves is strongly influenced by a range of environmental factors, including temperature, food availability, suspended solids, and habitat characteristics. In *Sinanodonta woodiana*, individuals grown in warmer waters (26–28 °C) developed thicker nacre layers and exhibited a more vibrant golden hue, indicating a temperature-dependent enhancement of pigmentation (Sahidin *et al*., 2022). Food availability—

often estimated through chlorophyll-a concentration—also plays a critical role, with higher chlorophyll-a levels leading to thicker nacre and more intense coloration, demonstrating a direct link between nutrient access and shell color expression (Sahidin *et al*., 2022). Conversely, elevated levels of total suspended solids (TSS) were associated with thinner nacre layers and duller yellow tones, suggesting that sediment-rich waters can hinder shell development and color intensity (Sahidin *et al*., 2022). Beyond water quality, habitat features can also shape shell coloration patterns. In some marine snails, shell colors match their surroundings to avoid predation, a strategy known as crypsis (Gefaell *et al.*, 2023). Although this phenomenon is primarily documented in gastropods, similar camouflage-driven selection may also occur in bivalves, influenced by background substrates such as seafloor coloration or macroalgal cover (Gefaell *et al*., 2023). Furthermore, ecological interactions like the introduction of invasive species can indirectly alter shell traits. For example, in South Africa, the decline of the native Magellan mussel (*Aulacomya atra*) due to competition with the invasive *Mytilus galloprovincialis* (Grobler *et al*., 2023) highlights how changes in habitat structure and resource availability, though not directly linked to shell coloration, could contribute to stress-induced modifications in shell development and pigmentation over time.

2.4 Effect of Depth and Site-specific Ecological Factors on Green Mussels (*Perna viridis*)

The growth, shell morphology and coloration of green mussels (*Perna viridis*) are significantly influenced by water depth and site-specific ecological conditions. These environmental variables can exert their effects either independently or in combination, impacting multiple aspects of mussel biology such as biometric traits (BTs), condition indices (CIs), survival rates, and physiological responses.

2.4.1 Influence of Depth

Water depth is a critical factor influencing the physiological and morphological characteristics of *Perna viridis*. It affects several key environmental parameters, including light availability, temperature, food concentration, and water movement, all of which play a role in the mussels' growth and development (Soon & Ransangan, 2014; Toralde *et al*., 2021). Mussels cultivated in shallow waters typically less than 5 meters

tend to exhibit faster growth rates and higher condition indices due to warmer temperatures, greater phytoplankton abundance, and more favorable metabolic conditions, making such depths ideal for aquaculture (Toralde *et al*., 2021; Marma e*t al*., 2021). For example, in Leyte Bay, mussels grown at a depth of 3.39 meters depth reached a condition index (CI) of 15.34%, which was higher than the 14.83% observed at a depth of 5.12 meters in Villareal Bay (Toralde *et al*., 2021). Likewise, mussels suspended at mid-depths (3–7 m) achieved an average shell length of 5.86 cm within three months, reflecting the benefits of balanced environmental conditions at these depths (Marma *et al*., 2021). In contrast, mussels in deeper waters may experience reduced growth due to lower temperatures, decreased food availability, and increased hydrostatic pressure, which can also affect shell morphology (Rajagopal *et al*., 2006). Depth also influences shell thickness and coloration. Mussels in deeper, calmer waters often develop thicker shells, likely due to reduced wave energy, whereas those in shallow, high-energy environments may form thinner shells as a result of ongoing mechanical stress (Siddall, 1980; Rajagopal *et al*., 1998). Shell pigmentation varies with depth as well, with darker coloration commonly found in mussels from well-lit, shallow habitats potentially as a protective adaptation against increased UV exposure (Tan & Ransangan, 2014).

2.4.2 Influence of Site-specific Ecological Factors

Local environmental conditions significantly influence the growth, shell structure, and overall health of *Perna viridis*. Factors such as temperature, salinity, dissolved oxygen, current speed, turbidity, food availability, and seasonal variation differ across sites and can markedly affect mussel performance. Optimal growth has been observed within a temperature range of 21–32°C and salinity levels between 27 and 35 ppt (Rajagopal *et al*., 2006; Marma *et al*., 2021). However, when temperatures exceed 30°C, condition indices (CIs) tend to decline, indicating heightened thermal stress (Noor *et al*., 2021). Abrupt changes in salinity, often caused by freshwater runoff during heavy rainfall events, can similarly reduce biometric traits (BTs) and CIs (Ma *et al*., 2022). In the context of Bangladesh, gonadal development in *P. viridis* typically begins when water temperatures drop below 30°C, highlighting a strong link between thermal conditions and reproductive timing (Asaduzzaman *et al*., 2019; Noor *et al*., 2021).

Dissolved oxygen levels also play a vital role, as concentrations above 7.8 mg/L have been positively associated with improved metabolic performance and higher dry meat weight (Toralde *et al*., 2021). In contrast, acidified water conditions (pH < 7.9) reduce the availability of carbonate ions essential for shell formation, which may hinder calcification and result in shell deformities (Tan & Ransangan, 2017; Kapsenberg *et al*., 2018). Hydrodynamic conditions further influence mussel physiology, with moderate current speeds (0.1–0.3 m/s) enhancing feeding by increasing water circulation and plankton delivery. However, stronger currents above 0.5 m/s can disrupt normal feeding behavior and reduce condition indices due to impaired filtration efficiency (Lovatelli, 1988; Noor *et al*., 2021). Similarly, high turbidity levels (exceeding 100–300 NTU) decrease light penetration, limit phytoplankton production, and interfere with feeding, ultimately impacting growth and shell quality (Noor *et al*., 2021).

Phytoplankton, the primary food source for *P. viridis*, is closely tied to shell growth and reproductive success, with chlorophyll-a concentration commonly used as a proxy for food availability. Sites with higher chlorophyll-a levels, such as Villareal Bay, often support better condition indices even under suboptimal environmental conditions (Utting, 1988; Smaal & van Stralen, 1990; Ren & Ross, 2005; Toralde *et al*., 2021). Seasonal changes also have a marked effect on the physiological state of mussels. Peak condition indices are generally observed during the gonadal maturation phase from October to February. Following the spawning period, which typically occurs from March to September, mussels often exhibit reduced condition due to energy depletion associated with reproductive activity (Hemachandra & Thippeswamy, 2008; Asaduzzaman *et al*., 2019; Noor *et al*., 2021).

2.4.3 Spat Settlement, Cultivation Methods, and Stocking Density

The success of spat settlement is largely dependent on environmental conditions and the cultivation technique employed. Nutrient-enriched zones with moderate depth are generally more favorable for higher spat densities. For instance, North Khurushkul recorded a high density of 192 spats per foot of rope, indicating optimal conditions for settlement (Marma *et al*., 2021).

Among the available cultivation techniques, rope and cage culture systems have been reported to outperform traditional pole methods. These modern systems facilitate better water circulation, enhance food availability, and result in improved growth rates and survival percentages (Qasim *et al*., 1977; Marma *et al*., 2021).

Stocking density is another critical parameter. Lower densities such as 20 individuals per basket, promote healthier growth and higher survival rates. In contrast, high-density setups lead to intensified competition for space and food resources, which not only slows growth but can also induce stress-related shell deformities (Rusydi *et al*., 2024).

2.4.4 Shell Coloration

Shell coloration in *P. viridis*, ranging from vivid green to brown, is also influenced by local environmental conditions. Elements such as ultraviolet (UV) radiation, dietary composition, and physiological stress can contribute to variations in shell color (Rajesh *et al*., 2001; Tan & Ransangan, 2014). Darker pigmentation is commonly found in mussels from shallow, well-lit environments, which is likely associated with increased melanin production functioning as a photoprotective response.

2.4.5 Synthesis and Research Gaps

Although numerous studies have examined the influence of individual ecological variables on *P. viridis*, there remains a lack of comprehensive insight into how these factors interact with one another. For example, the impact of depth-related variations in light availability on shell color expression is still not well understood. Additionally, the potential role of local genetic adaptation presents a valuable direction for future research. Mussel populations may develop distinct characteristics over time in response to sustained exposure to specific environmental gradients, yet this concept requires further detailed investigation.

Chapter III

Materials and Methods

3.1 Study area

Only the South-Eastern coast of Bangladesh, notably in the Moheshkhali Channel, which is directly connected to the Bay of Bengal, is home to naturally occurring *Perna viridis* (Shahabuddin *et al*., 2010). Therefore, this research recently established some research-based culture systems at the three locations of Moheshkhali Channel (Khurushkul, Moheshkhali & Chaufaldandi). The culture experiments of *P. viridis* were carried out from October 2024 to March 2025 at three locations (Fig. 2). These areas are favorable for the cultivation of green mussels due to their inductive water quality parameters and salinity consistency from the prior research (Asaduzzaman *et al*., 2020). The geographic locations of the study area are given in Table 1.

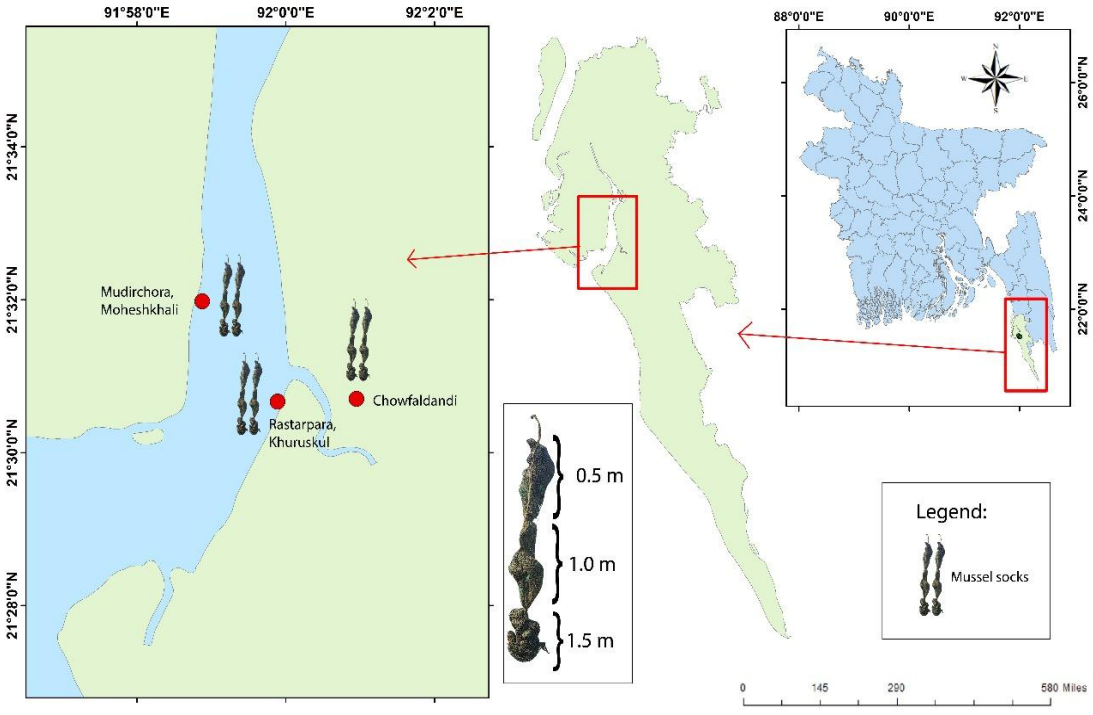

Figure 2: Sampling location of Green mussels (*Perna viridis)* used in this study.

Table 1. Geographic locations of the study area

Station	Study Location	Geographic Position
1	Khurushkul	21º 30' 40.13"N and 91º 59' 53.3"E
2	Moheshkhali	21º 31' 58.8"N and 91º 58' 52.5"E
3	Chaufaldandi	21º 30' 42.3"N and 92º 0' 56.9"E

3.2 Experimental Design

For this study, bamboo raft systems have been set up in three locations along the Moheshkhali Channel (Khurushkul, Moheshkhali, and Chaufaldandi). These culture systems were developed in places with shallow water, with a maximum water depth of 6-7 feet at low tide. For this study, we considered depths of 0.5m, 1 m and 1.5 m . Bamboo rafts were used to connect net socks vertically (Fig. 3). Each mussel sock had 4.5 feet and was suspended from the bamboo raft. There were ten (10) mussel socks at each location. Thus, this study had a 3×3 factorial design with three depths (0.5m, 1 m, and 1.5 m) as the first factor and three locations (Khurushkul, Moheshkhali, and Caufaldandi) as the second factor. Treatments were repeated ten times in every location.

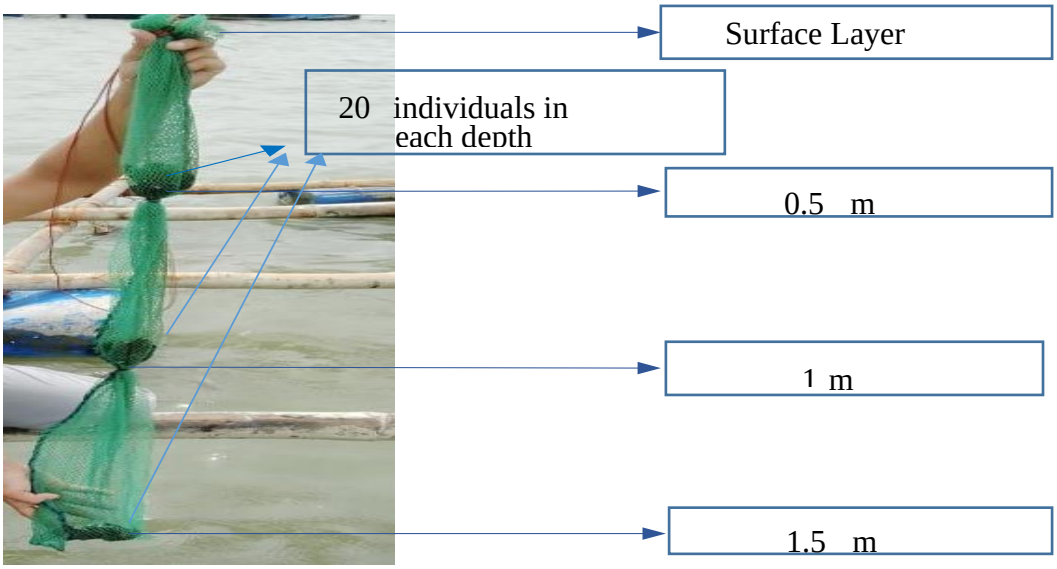

Figure 3. Experimental design of the study. Here, a total of ten mussel socks were installed in the raft in every location and ten replications of each depth associated with twenty spat samples were installed in every location.

3.3 Spat Collection and Inoculation

For this study, the local fishermen used diving equipment to collect green mussel spat from the Moheshkhali Channel's natural bed. After collecting spat, biometric traits of spat were measured, and 20 spat of uniform size were inoculated into each of the three distinct depths (0.5 m, 1 m, and 1.5 m) of each mussel sock. Spats were stocked on 1st October 2024 and attached to the raft.

3.4 Sample Collection

A total of 150 samples were collected from each location, with 50 samples randomly collected from each depth, resulting in a total of 450 samples for the study. Subsequently, these samples were meticulously processed in the laboratory. For all analyses, including geometric morphometrics assessment, only the external shell valves were used without any gut removal or soft tissue dissection. Specifically, the left valve of each mussel was selected for geometric morphometric analysis.

3.5 Digital imaging/ Photographic documentation

The left valves of the mussel samples were meticulously photographed in a controlled photo laboratory, ensuring consistent distance and orientation for all images, which is crucial for accurate geometric morphometric analysis. Each shell valve was labeled with a unique identification number, facilitating precise documentation and tracking throughout the study. To capture the digital images, a Canon EOS 60D 18.0MP DSLR camera equipped with an 18-55MM lens (USA) was employed. This camera setup was chosen for its high resolution and reliability, allowing for detailed and replicable images of the shell valves. The digital imaging process not only enabled precise geometric morphometric measurements but also ensured that any subtle morphological variations could be accurately detected and analyzed (Cardin and Friedland, 1999). Additionally, the controlled environment minimized external variables, such as lighting and shadow, which could potentially affect the quality of the images, thereby enhancing the reliability of the morphometric data collected.

3.6 Water Quality Parameters

Throughout the study period, Physical parameters, including water temperature (using Celsius Thermometer), pH (using Digital pH meter, EcoSence pH10A by YSI), Dissolved oxygen (using Digital Dissolved Oxygen meter, PDO-519 by Lutron), Salinity (using Bellingham and Stanley E-Line Refractometer by Xylen), and Turbidity (using Digital Turbidity Meter, Turb430 IR by WTW) were recorded in situ separately from each culture depth from three locations and they were properly calibrated properly before fileld use. Three replications of water samples were taken from each depth using a vertical water sampler (1200-E Kemmerer, Science First/WildCo, Yulee, FL, USA).

Figure 4: Measurement of water quality parameters from three depths & three locations monthly.

Additionally, total suspended solids (TSS) were assessed following the APHA (2005) methodology by filtering 200 mL of seawater through Whatman filter paper using a vacuum pump. Chemical parameters, including Nitrite, Nitrate, Phosphate, and Ammonia, were analyzed by using the portable photometer (pHotoFlex STD by WTW) and relative reagents when zero adjustments were done by using distilled water (Fig. 4). Nitrite (NO2-N), Nitrate (NO3-N), Ammonia (NH3-N), and Phosphate (PO4-P) concentrations were evaluated (PhotoFlex STD, WTW, Weilheim, Germany) monthly (APHA, 1992).

Chlorophyll-a Measurement

After the collection of water samples from different depths, fresh samples were immediately brought to the laboratory. Using a vacuum pump and Whatman GF/C microfibre glass filter paper, 500 ml of water samples were filtered. In 10 ml of 90% acetone, the filtered membranes were placed and left overnight. Using a glass rod, acetone was extensively incorporated with the filtered papers. After that, centrifugation was carried out for 2.30 minutes at 3500 RPM (Fig. 6). Following Boyd's (1979) procedure, the supernatant contents (extract) were placed in corvettes to have their absorbance measured at wavelengths of 664, 647, and 630 nm (Optizen Pop 2102, Daejeon, Republic of Korea).

The following equation was used to get the chlorophyll-a concentration:

Chlorophyll-a = (11.85 A664 – 1.54 A647 – 0.08 A630) * (V/S) * 1000

Where A664 = Absorbance at 664 nm

A647 = Absorbance at 647 nm

A630 = Absorbance at 630 nm

V = Volume of acetone used (ml) &

S = Volume of sampled filter (ml)

3.7 Landmark-based Geometric Morphometric Analysis:

The shell morphology of mussel samples was analyzed using TPSdig2 software, which digitized five fixed landmarks and 36 semi-landmarks in accordance with Rohlf's protocols (2005b). The designated configuration included: (1) The Umbo, marking the starting point of the hinge plate; (2) The Ligament, denoting the endpoint of the hinge plate; (3) The midpoint of the ventral margin; (4) The midpoint of the dorsal margin; and (5-40) a series of semi-landmarks capturing the shell's curvature. TPSUtil software facilitated the separation of fixed landmarks from semi-fixed landmarks.

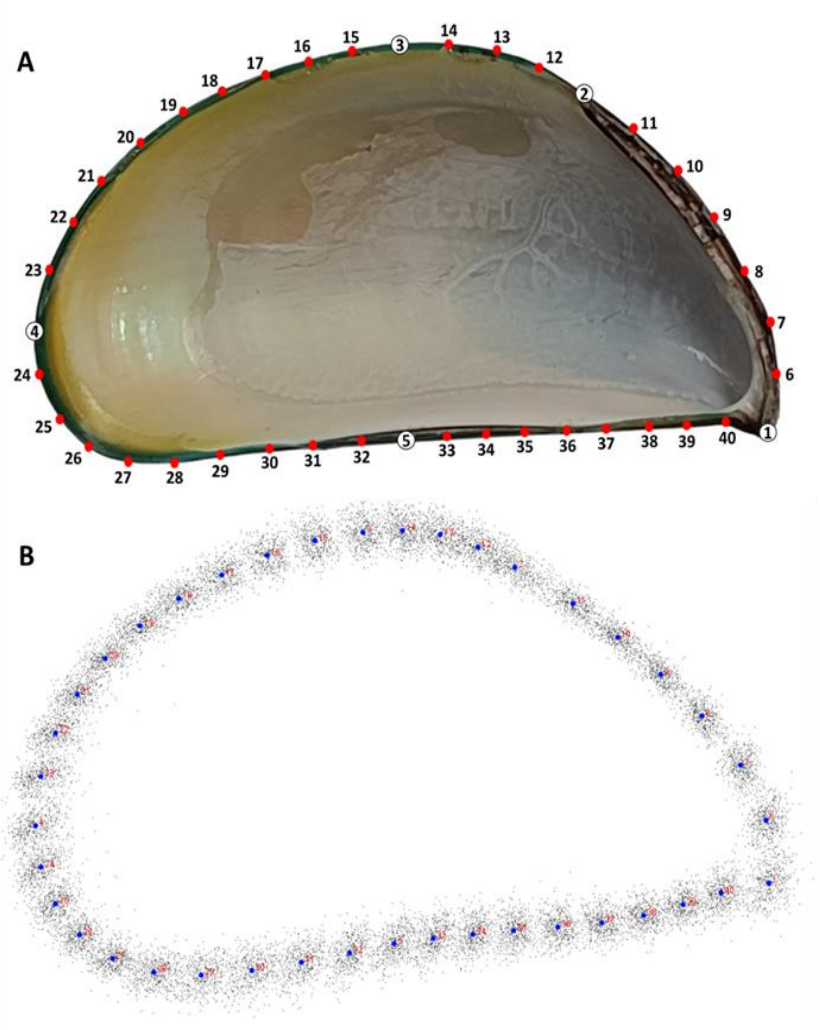

Figure 5: Key geometric morphometric landmarks points for analyzing the shell morphology of green mussels (*Perna viridis*) collected from three coastal regions in Bangladesh (Choufaldandi, Khurushkul, and Moheshkhali). (A) A set of 40 two-dimensional (2D) landmarks includes: 1) The Umbo, marking the initiation point of the hinge plate; 2) The Ligament, indicating the terminus of the hinge plate; 4) The midpoint of the ventral margin; 5) The midpoint of the dorsal margin; and (6-40) A series of semi-fixed or auxiliary landmarks, serving to elucidate the curvature within the mussel shell structure. (B) Procrustes Superimposition visualization of landmark configurations for *Perna viridis*, facilitating a comprehensive understanding of the shell morphology in this species.

Table 2. Landmark points and their description

Sl. No.	Landmark Point	Description
1	Point 1	Anterior ventral tip (shell hinge or opening start)
2	Point 2	Upper anterior curve (transition to dorsal arc)
3	Point 3	Maximum shell height (dorsal mid-point)
4	Point 4	Posterior dorsal curve transition point
5	Point 5	Posterior ventral base (curve turning downward toward ventral margin)
6	Point 6	Anterior margin, near the ventral start
7	Point 7	Slight dorsal-ward along the anterior slope
8	Point 8	Anterior sloping margin
9	Point 9	Moving up the anterior margin
10	Point 10	Mid-anterior slope
11	Point 11	Nearing the anterior-dorsal junction
12	Point 12	Transition from anterior to dorsal curvature
13	Point 13	Beginning dorsal arc
14	Point 14	Lower dorsal arc
15	Point 15	Mid dorsal arc
16	Point 16	Upper dorsal arc
17	Point 17	Dorsal curve nearing posterior side
18	Point 18	Posterior dorsal arc
19	Point 19	Posterior upper arc
20	Point 20	Posterior upper slope
21	Point 21	Mid-posterior curve
22	Point 22	Steep posterior arc
23	Point 23	Lower posterior curve
24	Point 24	Base of posterior margin
25	Point 25	Posterior tip, near hinge
26	Point 26	Posterior-ventral turn
27	Point 27	Beginning ventral margin
28	Point 28	Lower ventral arc
29	Point 29	Mid-ventral curvature

30	Point 30	Continuation of the ventral margin
31	Point 31	Ventral arc center
32	Point 32	Ventral outline trending anteriorly
33	Point 33	Anterior-lower ventral margin
34	Point 34	Curve up toward the anterior
35	Point 35	Slope toward the anterior end
36	Point 36	Base margin before the upward anterior curve
37	Point 37	Near the anterior bottom end
38	Point 38	Approaching the anterior curve apex
39	Point 39	Bottom slope of the anterior
40	Point 40	Curve connecting back to Point 1

To standardize the landmark configurations and remove the effects of rotation, translation, and scaling, a generalized Procrustes analysis was applied using TPSRelw software, as per the methodologies of Rohlf & Slice (1990) and Slice *et al*. (1996). This software also includes an algorithm that adjusts semi-landmarks along the contour tangents, minimizing bending energy to match a reference configuration (Bookstein, 1991). The analysis generated Relative Warp (RW) scores, which quantify shape variation as deviations from a consensus shape, functioning similarly to principal components in principal component analysis (PCA) (Adams *et al*., 2004).

MorphoJ software was then utilized to visually represent the variations in shell shape among different groups, based on these RW scores (Klingenberg, 2011). For enhanced clarity, the statistical programming language R was employed to produce detailed graphical representations. Data for these visualizations were extracted from MorphoJ analyses. Subsequently, PCA was performed in R using the 'FactoMineR' package (Lê *et al*., 2008), version 4.0.5. Additionally, canonical variate analysis (CVA) and discriminant function analysis (DFA) were conducted using the 'MASS' package (Venables and Ripley, 2002).

3.8 Shell Coloration Analysis

To evaluate shell color variation across depths and locations, digital photographs were captured from 50 mussel shells per depth across the three study sites, yielding a total of 450 images. Each image focused on the left lateral valve and was taken under standardized lighting and camera settings (Fig. 6A), as described previously. Maintaining consistent lighting conditions and camera angles is essential to avoid artificial color shifts and ensure reproducibility in digital color analysis. This principle is strongly emphasized by Akkaynak *et al*. (2014), who highlight the importance of linearizing raw images and enforcing uniform capture protocols for accurate color quantification.

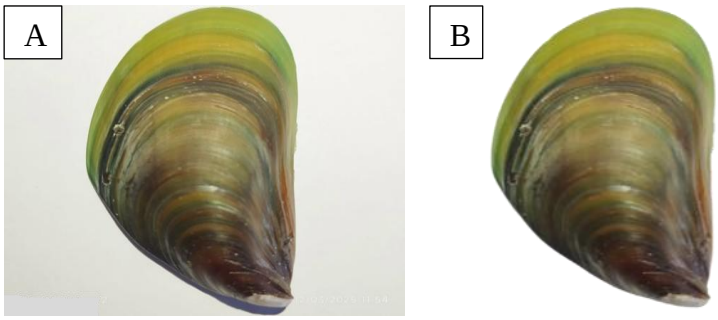

Figure 6. Example of shell image processing for digital coloration analysis of *Perna viridis*. (A) Original shell image captured under standardized white background conditions. (B) Corresponding shell image after background removal using Adobe Photoshop to isolate shell coloration for accurate color quantification.

Following image acquisition, backgrounds were carefully removed using Adobe Photoshop to isolate the shell region and eliminate visual noise, adhering to recommended preprocessing procedures for digital color studies (Stevens *et al*., 2007) (Fig. 6B). Objective color measurements were then guided by the protocols established in the Image Calibration and Analysis Toolbox, which provides a validated framework for quantifying reflectance and color from digital images (Troscianko & Stevens, 2015).

The processed images were analyzed using the Image Color Summarizer tool (https://mkweb.bcgsc.ca/color-summarizer), a web-based application designed to

extract quantitative color information from digital images. This tool provided detailed data on dominant shell colors, including values from RGB (Red, Green, Blue), HSV (Hue, Saturation, Value), and CIELAB (L*, a*, b*) color spaces, along with pixel percentages associated with each color cluster. These color models are widely used in ecological and biomonitoring studies due to their ability to capture both device-dependent and perceptual color characteristics (Westland *et al*., 2012). Notably, the CIELAB model is recognized for its perceptual uniformity, making it particularly effective for detecting subtle biological color differences (Fairchild, 2013).

All output data were compiled and organized in Microsoft Excel, then imported into R (v. 4.2.2) for statistical analysis. Canonical Variate Analysis (CVA) was employed to investigate patterns in shell coloration across depths and sites using the extracted CIELAB values and corresponding pixel proportions. CVA enables the identification of group separation based on multivariate color traits and is especially well-suited for analyzing biological color variation derived from digital images (Bergman & Beehner, 2008; Delhey & Peters, 2008). While CVA is not universally applied in all coloration studies, the influence of environmental and genetic factors on animal coloration is well-established (Endler, 1990; Roulin, 2014), supporting its relevance here.

In addition, hierarchical clustering was applied to the color profiles using Euclidean distance and Ward's linkage method. This approach is effective for visualizing similarity structures among samples and detecting site-specific or depth-related color groupings. Hierarchical clustering has been widely used in ecological and phenotypic datasets for uncovering underlying patterns in multivariate data (Murtagh & Legendre, 2014; Borcard et al., 2018). The resulting relationships were visualized as dendrograms, facilitating interpretation of grouping tendencies among mussel populations.

Overall, this methodology provides a robust, objective, and replicable framework for assessing shell coloration in bivalves. As shell color has increasingly been recognized as an ecological indicator of environmental stress, developmental condition, and age-related change (Williams et al., 2017), the analytical workflow adopted here is particularly well-suited for addressing the research questions of this study.

Chapter IV

Results

4.1 Water Quality Parameters Across Depths and Sites

The variations in different water quality parameter measures over the experimental periods are displayed in Table 3. No ecological factor showed a significant difference ($p>0.05$) among the culture depths except for chlorophyll a (Table 3). On the contrary, ecological parameters, except for DO, turbidity, and chlorophyll a, did not differ significantly ($p>0.05$) between the locations (Table 3). Chlorophyll a showed a highly significant variation between culture depth and culture site ($p<0.001$). Chlorophyll a was higher at 0.5 m depth (5.67 ± 0.17µg/L) rather than at 1m depth (4.83 ± 0.17 µg/L) and 1.5m depth (4.27 ± 0.17µg/L). Likewise, chlorophyll a was higher in Moheshkhali (5.7 ± 0.17µg/L) than Khurushkul (5.02 ± 0.17µg/L) and Chaufaldandi (4.06 ± 0.17µg/L) culture site. The lowest chlorophyll a was found in the Caufaldandi culture site, which was 4.06±0.17µg/L. The temperature varied from 27.7-29.1ºC and the DO level ranged from 5.28-5.98 among the three culture sites. The salinity ranged from 20.5-31.7 ppt among three locations during the study period. The water turbidity fluctuated from 9.5-17.6 NTU in three locations. Besides, nitrate (NO_3-N) levels were found to differ significantly ($p<0.05$) among the culture sites. Nitrate (NO_3-N) content in Khurushkhul (1.35±0.15 ppm) was significantly higher than in Moheshkhali (0.99±0.15 ppm) and Chaufaldandi (0.65±0.15 ppm) culture site (Table 3).

Table 3. Variations in water quality parameters among different depths and locations using two-way ANOVA. Asterisks are used to indicate the significance levels (p) (*<0.05, ***<0.001, NS= Not significant). Superscripts denote significant differences (p<0.05)

Variables	Means (Tukey test)						Significance (p-value)		
	Depth (D)			Location (L)					
	0.5m	1m	1.5m	Khurushkul	Moheshkali	Caufaldandi	D	L	D×L
Temp (˚C)	28.34±0.32^{a}	28.34±0.32^{a}	28.34±0.32^{a}	28.2±0.32^{ab}	27.74±0.32^{b}	29.10±0.32^{a}	NS	*	NS
PH	7.78±0.03^{a}	7.76±0.03^{a}	7.77±0.03^{a}	7.78±0.03^{a}	7.79±0.03^{a}	7.74±0.03^{a}	NS	NS	NS
DO (ppm)	5.73±0.12^{a}	5.73±0.12^{a}	5.73±0.12^{a}	5.98±0.12^{a}	5.92±0.12^{a}	5.28±0.12^{b}	NS	***	NS
Salinity (ppt)	30.00±3.42^{a}	24.44±3.42^{a}	24.45±3.42^{a}	31.7±3.42^{a}	26.67±3.42^{a}	20.57±3.42^{a}	NS	NS	NS
Turb (NTU)	12.35±1.00^{a}	12.35±1.00^{a}	12.35±1.00^{a}	9.86±1.00^{b}	9.57±1.00^{b}	17.64±1.00^{a}	NS	***	NS
PO_4-P (ppm)	1.45±0.32^{a}	1.11±0.32^{a}	1.21±0.32^{a}	1.49±0.32^{a}	1.23±0.32^{a}	1.06±0.32^{a}	NS	NS	NS
NO_2-N (ppm)	0.19±0.01^{a}	0.15±0.01^{a}	0.16±0.01^{a}	0.14±0.01^{a}	0.18±0.01^{a}	0.18±0.01^{a}	NS	NS	NS
NO_3-N (ppm)	0.95±0.15^{a}	0.93±0.15^{a}	1.11±0.15^{a}	1.35±0.15^{a}	0.99±0.15^{ab}	0.65±0.15^{b}	NS	*	*
NH_3-N (ppm)	0.17±0.03^{a}	0.23±0.03^{a}	0.18±0.03^{a}	0.22±0.03^{a}	0.17±0.03^{a}	0.19±0.03^{a}	NS	NS	NS
Chl a (μg/L)	5.67±0.17^{a}	4.83±0.17^{b}	4.27±0.17^{b}	5.02±0.17^{b}	5.70±0.17^{a}	4.06±0.17^{c}	***	***	NS

4.2 Shell Morphological and Color Variation Across Sites and Depths

Geometric morphometric and digital coloring analyses demonstrated significant spatial and vertical variation in the shell phenotype of *Perna viridis* obtained from three coastal habitats Chowfaldandi, Khurushkhul, and Moheshkhali along the Moheshkhali Channel. Morphological characteristics were evaluated using landmark-based geometric morphometrics, while pigmentation was measured by digital image analysis utilizing RGB and HSV color spaces.

4.2.1 Shell Shape Variation by Site

Geometric morphometric analysis, based on 40 landmark points (5 fixed and 35 semi-landmarks) on the left valve, revealed statistically significant shape variation among populations from the three study sites. Principal Component Analysis (PCA) of Procrustes-aligned coordinates revealed that the first three components captured the major axes of shape variation, with populations forming distinguishable clusters (Figure 7A–C). The associated wireframe plots demonstrated shape deformations along the anterior-posterior axis and variation in ventral curvature, which appeared site-dependent.

Canonical Variate Analysis (CVA) provided further evidence of inter-site shape differentiation. CV1 and CV2 jointly described the most prominent spatial separation, especially in the umbo and ventral shell regions (Figure 7D). All pairwise comparisons among sites showed statistically significant differences in both Procrustes and Mahalanobis distances ($p < 0.0001$), as shown in Table 4. Notably, the greatest Procrustes distance (0.0490) and Mahalanobis distance (4.3104) were observed between Chowfaldandi and Khuruskul, while Chowfaldandi–Moheshkhali and Khuruskul–Moheshkhali comparisons also exhibited significant but relatively lower distances.

Discriminant Function Analysis (DFA) results mirrored these findings, with Procrustes and Mahalanobis distances corroborating CVA outputs (Table 4). The T-square statistics for all comparisons were highly significant ($p < 0.0001$), indicating robust multivariate separation among populations.

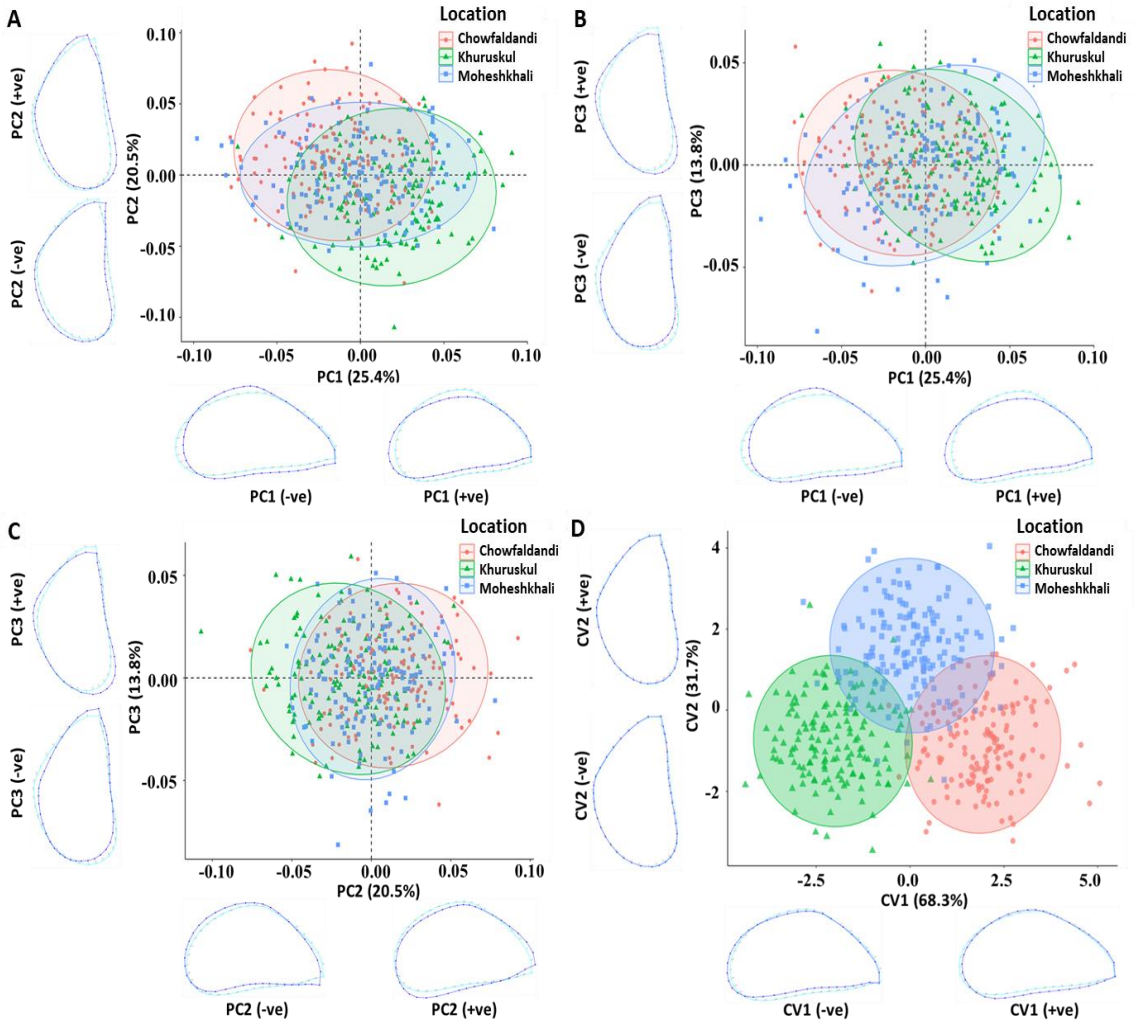

Figure 7: Principal component analysis (PCA), Canonical variate analysis (CVA) and wireframe plots showing the morphological change described by each component due to habitat variation through geometric morphometrics of green mussel (*Perna viridis)*. **(A-C)** Principal component analysis (PCA) plot for the first three principal components showed percent contribution with shape variation in positive and negative wireframe plots and **(D)** Canonical variate analysis (CVA) plots with percent comparison between CV1 and CV2 revealed the population shape variation with positive and negative wireframe plots. In wireframe plots, light blue outlines represent the average shape, and dark blue outlines represent extreme shape changes.

Table 4. Results of Canonical variate analysis (CVA) and Discriminant function analysis (DFA) statistic tests of Procrustes distance, Mahalanobis distance and T-square values for compared groups of *P.viridis* from different habitats in coastal areas of Bangladesh.

Compared Groups	Canonical variate analysis (CVA)		Discriminant Function Analysis (DFA)			
	Procrustes Distance (Permutation test p-value)	Mahalanobis Distance (Permutation test p-value)	Procrustes Distance (Permutation test p-value)	Mahalanobis Distance (Permutation test p-value)	T-square	T-square p-value (permutations)
Chowfaldandi-Khuruskul	0.0490 (<.0001)	3.9993 (<.0001)	0.04895167 (<.0001)	4.3104 (<.0001)	1393.4530	<.0001
Chowfaldandi-Moheshkhali	0.0222 (<.0001)	3.0634 (<.0001)	0.02220428 (<.0001)	3.2383 (<.0001)	786.5164	<.0001
Khuruskul-Moheshkhali	0.0302 (<.0001)	3.1268 (<.0001)	0.03024034 (<.0001)	3.6199 (<.0001)	982.7806	<.0001

Thin-plate spline deformation grids and DFA histograms (Figure 8A–C) further visualized shape differences between each population pair. In these plots, prominent shell deformation patterns were evident particularly in anterior expansion and umbo elevation highlighting the localized morphological adaptations of each habitat.

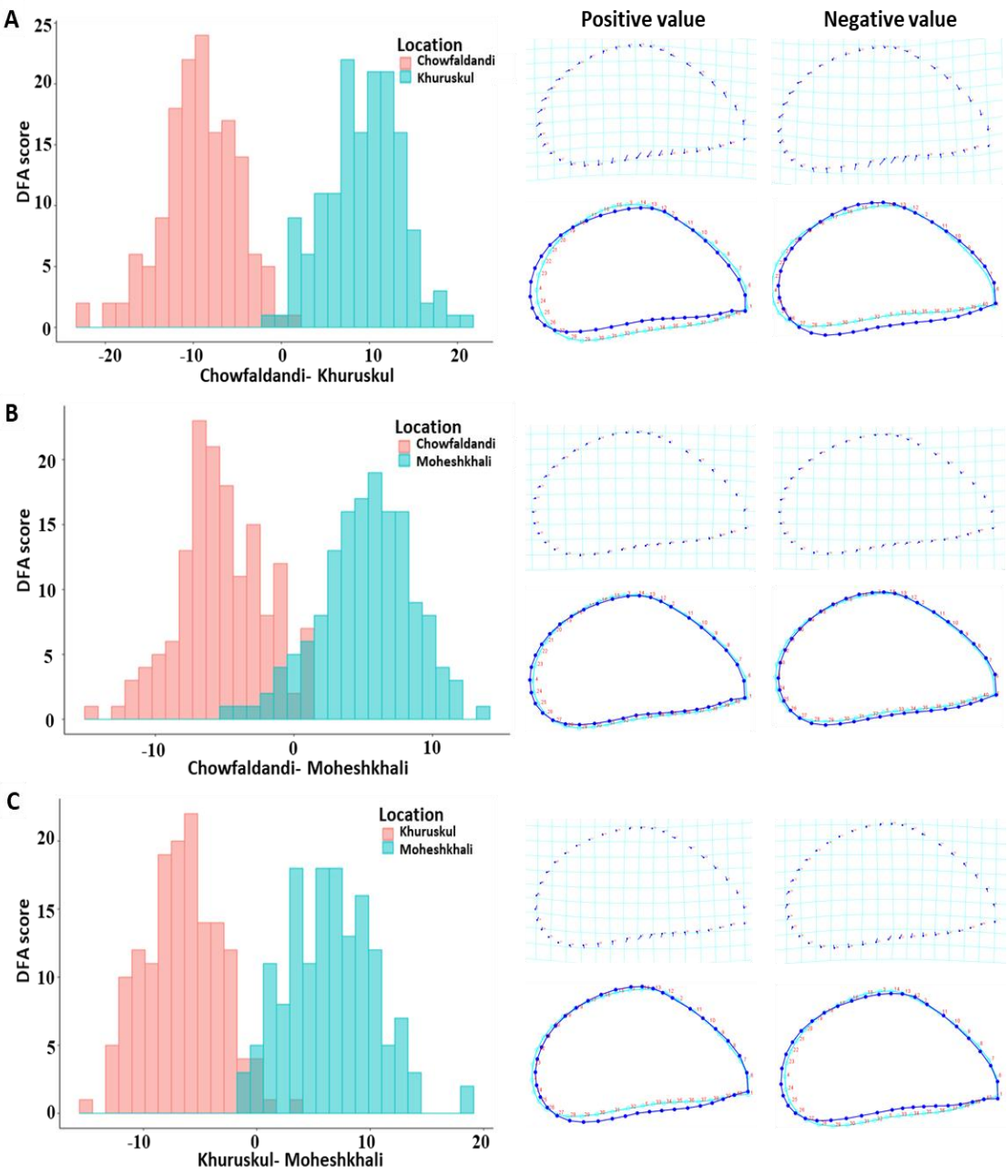

Figure 8: Discriminant Function Analysis (DFA) for pairwise comparison and corresponding thin-plate spline of Procrustes deformations and wireframe plots illustrating shape variation of green mussel (*Perna viridis*). DFA histogram with positive and negative wireframe plots showed mean shape variation between Chowfaldandi-Khurushkul **(A)**, Chowfaldandi-Moheshkhali **(B)**, Khurushkul-Moheshkhali **(C)** populations. Light blue

outlines represent the average shape and dark blue outlines represent extreme shape changes.

Centroid-size analysis (Figure 9A) revealed clear, statistically significant differences in shell size among the three coastal habitats: mussels from Chowfaldandi were consistently larger, whereas those from Khuruskul and Moheshkhali were smaller. Procrustes deformation plots based on centroid size (Figure 9B) showed that these size differences were accompanied by systematic shape changes along the anterior–posterior and ventral–dorsal axes, indicating an allometric component to the variation. Wireframe diagrams (Figure 9C) further highlighted localized shell expansion and compression, underscoring habitat-specific morphological adjustments.

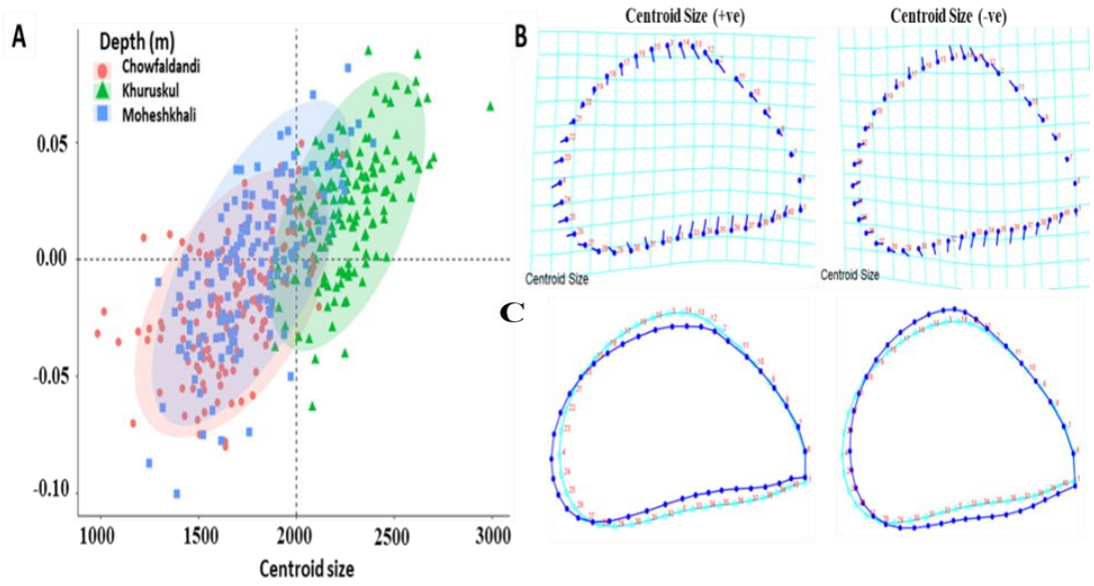

Figure 9: (A) Variation in centroid size in *P. viridis* population from three coastal habitats (Chowfaldandi, Khuruskul, Moheshkhali). **(B)** Procrustes deformations based on centroid size for both positive and negative value, where each dot indicates the mean and line indicates the shape variation from the mean. **(C)** Shape variation in positive and negative wireframe plot for centroid size due to habitat variation.

Taken together, the size and shape patterns point to local environmental drivers especially water flow, sediment regime, and food availability—that promote phenotypic plasticity in *Perna viridis*. The robustness of these habitat-specific signatures was confirmed by discriminant-function analysis: cross-validated classification accuracy remained high across sites (Chowfaldandi = 92.0 %, Khurushkul = 91.33 %, Moheshkhali = 86.67 %; Table 5). Thus, centroid size, its associated shape deformations, and DFA statistics collectively demonstrate that each site imposes a distinctive ecological imprint on mussel shell morphology.

Table 5. Results of the cross-validation test in Discriminant function analysis **(**DFA) for the compared groups of *P. viridis* from different habitats in coastal areas of Bangladesh. Numbers in brackets represent the correctly assigned individuals in relation to the total number of compared individuals.

Sampling Locations	Predicted Group Membership	Chowfaldandi	Khuruskul	Moheshkhali	Total
Original (%)	Chowfaldandi	–	98.67% (148/150)	94.67% (142/150)	150
	Khurushkhul	99.33% (149/150)	–	98.67% (148/150)	150
	Moheshkhali	92% (138/150)	96.67% (145/150)	–	150
Cross-validated (%)	Chowfaldandi	–	92% (138/150)	82% (123/150)	150
	Khuruskul	91.33% (137/150)	–	90.67% (136/150)	150
	Moheshkhali	86.67% (130/150)	85.33% (128/150)	–	150

4.2.2 Shell Shape Variation by Depth

The shell shape of *Perna viridis* exhibited significant variation across depth strata (0.5 m, 1.0 m, and 1.5 m) within each study site Chowfaldandi, Khurushkul, and Moheshkhali.

Centroid size analysis revealed a general trend of increasing shell size with increasing depth, though the magnitude and consistency of this trend varied among sites (Figure 10A–C). Procrustes deformation plots associated with centroid size showed localized expansions and compressions of shell shape, suggesting depth-related morphological shifts potentially influenced by ecological gradients such as water movement, sediment deposition, and light availability.

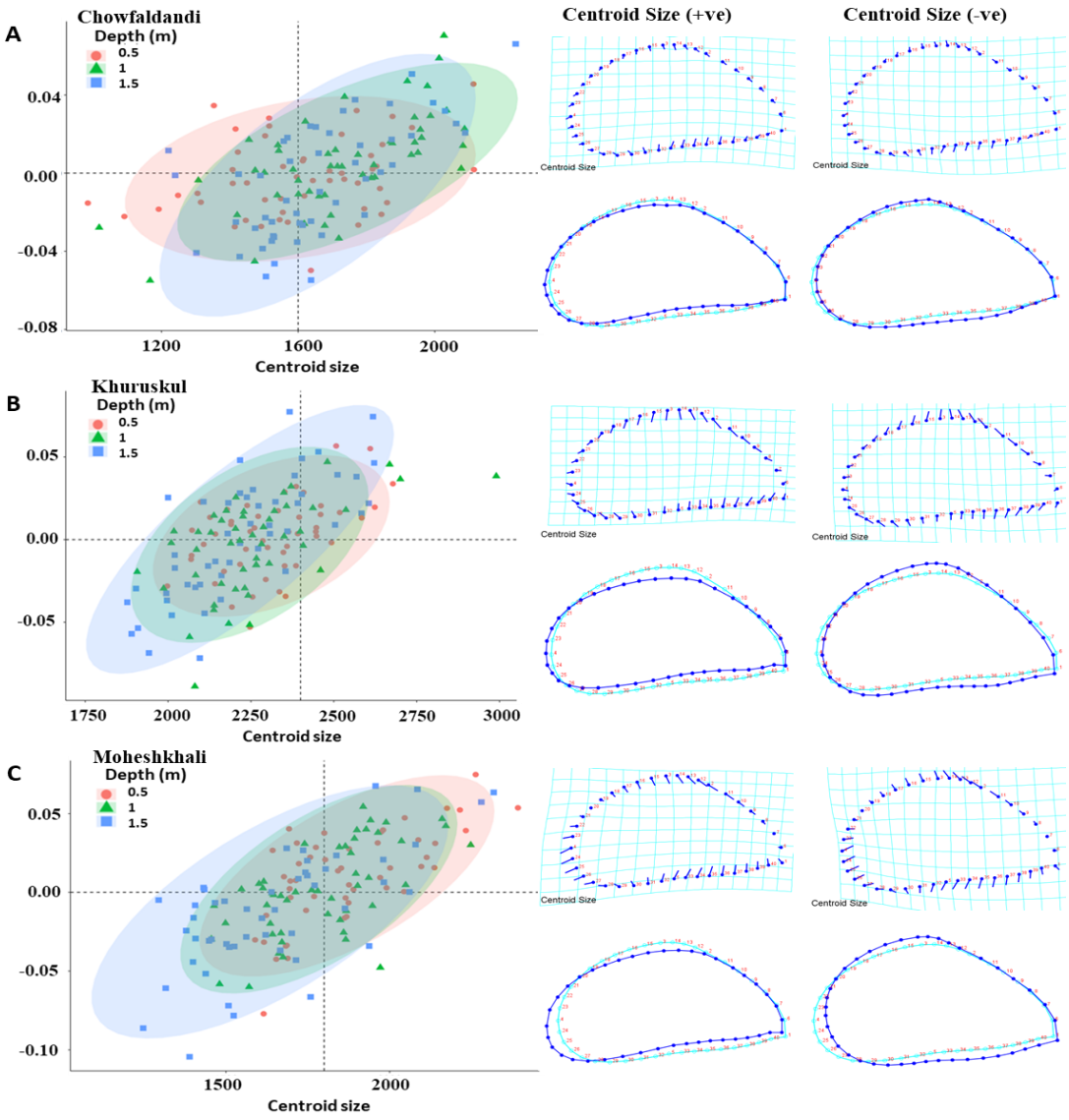

Figure 10: Centroid size variation of *Perna viridis* across depth strata within three coastal sites: **(A)** Chowfaldandi, **(B)** Khurushkul, and **(C)** Moheshkhali. Mean centroid size values (dots) and corresponding Procrustes deformation plots (lines) illustrate depth-related shell

size changes and associated shape variation. Wireframe diagrams indicate localized expansions and compressions in shell morphology potentially driven by habitat conditions at varying depths.

Principal Component Analysis (PCA) conducted separately for each site illustrated distinct patterns of depth-related shape variation (Figure 11A–C). The first two principal components (PC1 and PC2) captured the dominant deformation modes, primarily along the dorsal and ventral margins of the shell. Wireframe visualizations associated with PCA axes supported these findings, indicating gradual yet measurable morphological adjustments along depth gradients.

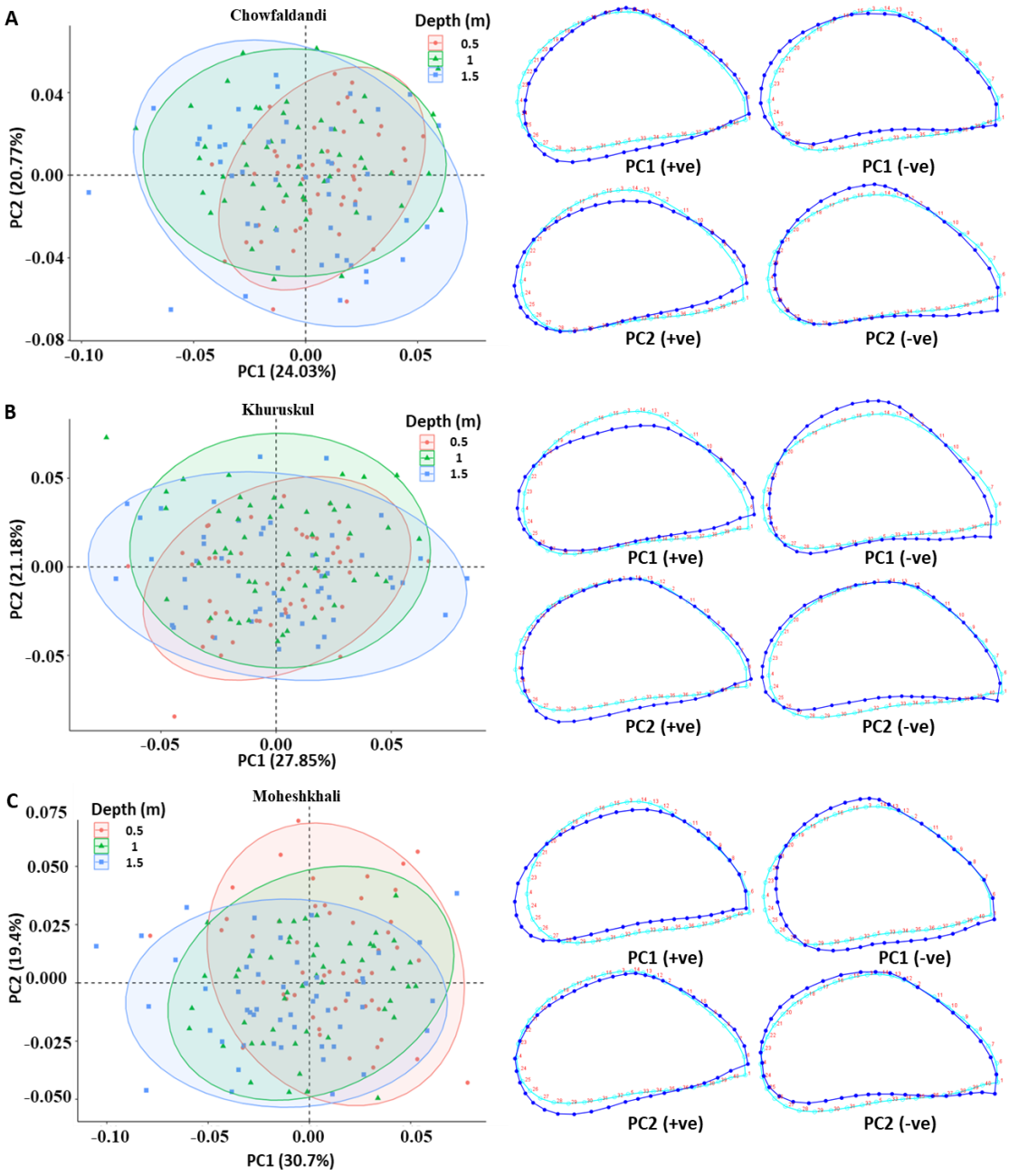

Figure 11: Principal Component Analysis (PCA) of shell shape variation in *Perna viridis* across depth levels (0.5 m, 1.0 m, 1.5 m) at: **(A)** Chowfaldandi**, (B)** Khurushkul, and **(C)** Moheshkhali. Each PCA plot shows PC1 vs. PC2 with associated variance percentages. Wireframe plots display representative shape deformations along each principal axis, indicating site-specific depth-induced changes in dorsal and ventral shell margins.

Canonical Variate Analysis (CVA) further revealed depth-based clustering of shell shape within all three locations (Figure 12A, D, G). While the shallow (0.5 m) and deep (1.5 m) groups were more clearly separated, the intermediate depth (1.0 m) often overlapped with both extremes, suggesting moderate phenotypic plasticity, particularly at transitional depths. This pattern was reinforced by the CVA density plots (Figure 12B–C, E–F, H–I), which indicated continuous shape variation rather than strict group segregation.

Discriminant Function Analysis (DFA) pairwise comparisons among depth groups showed statistically significant Procrustes and Mahalanobis distances in most cases (Table 6; Figure 13A–I). The most pronounced shape differences were observed between the shallowest (0.5 m) and deepest (1.5 m) strata across all three regions. DFA classification accuracy using cross-validation ranged from 52.67% to 66% (Table 7), highlighting that while depth-driven shape variation was present and statistically supported, it was relatively subtle and overlapping, especially compared to inter-site variation.

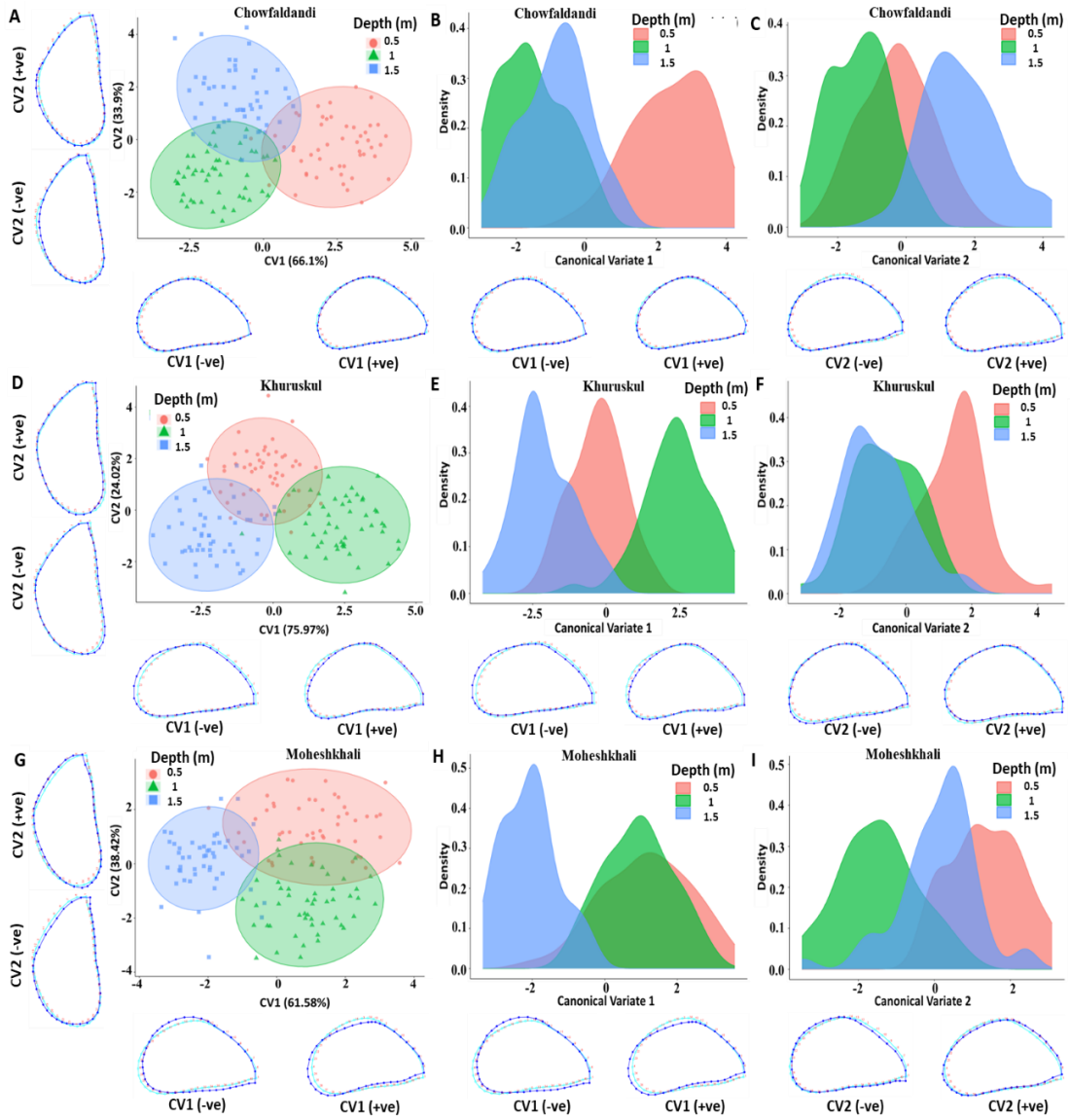

Figure 12: Canonical Variate Analysis (CVA) of *Perna viridis* shell shape variation across depth gradients in **(A–C)** Chowfaldandi, **(D–F)** Khuruskul, and **(G–I)** Moheshkhali. CVA scatterplots **(A, D, G)** contrast CV1 and CV2 scores, revealing group clustering by depth. Corresponding density plots **(B–C, E–F, H–I)** highlight phenotypic overlap among depth groups, with the highest convergence at intermediate depths (1.0 m), suggesting moderate morphological plasticity.

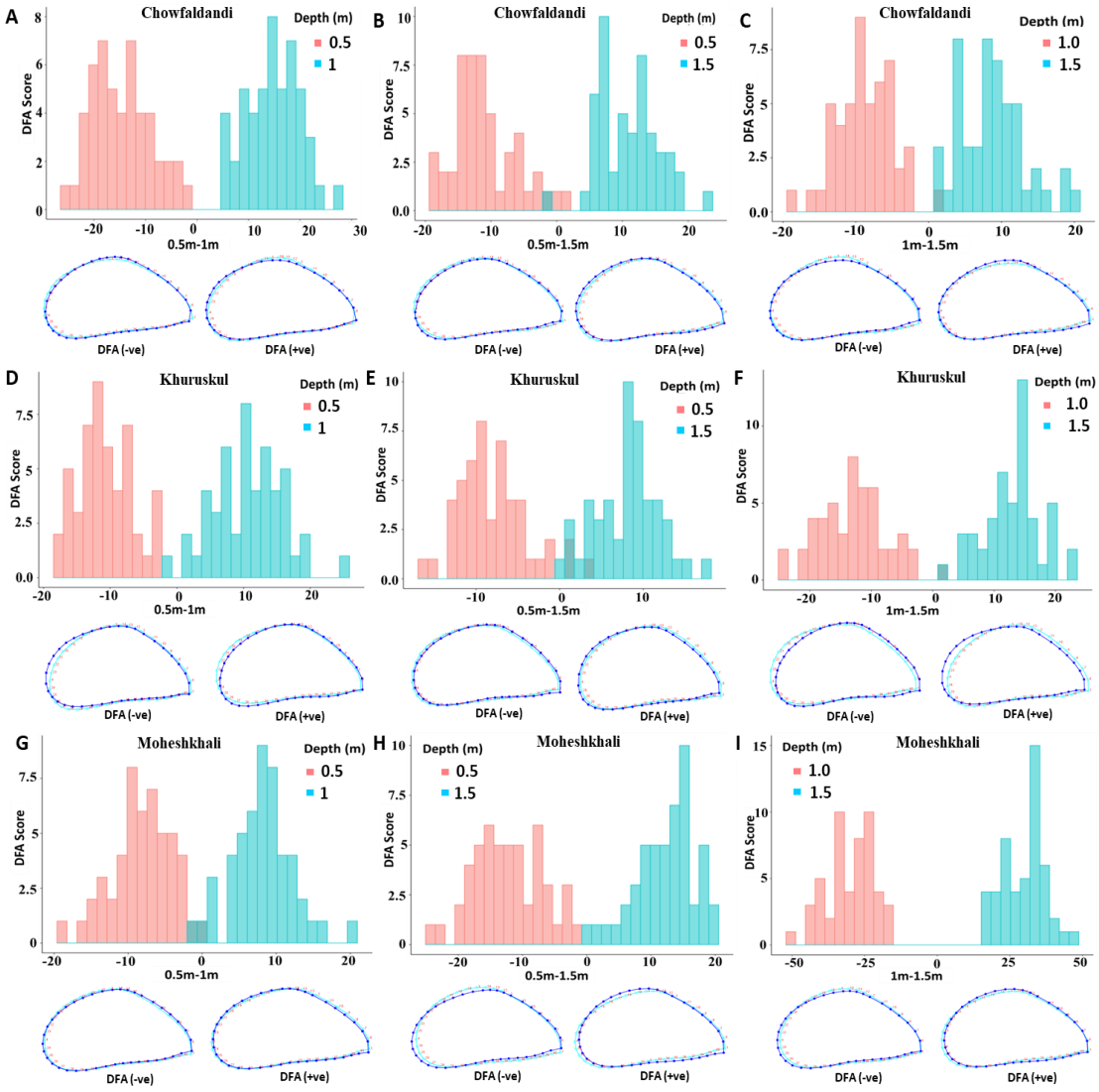

Figure 13: Discriminant Function Analysis (DFA) for pairwise comparison and corresponding wireframe plots for both positive and negative values illustrating shape variation for three distinct regions: Chowfaldandi **(A-C)**, Khuruskul **(D-F)**, and Moheshkhali **(G-I).** Each region has three plots comparing two depth levels within specific depth ranges. Chowfaldandi's plots **(A-C)** reveal the DFA score distribution for depths 0.5m and 1m **(A)**, 0.5m and 1.5m **(B)**, and a comparison between 1m and 1.5m **(C)**. Khuruskul's distributions **(D-F)** follow a similar pattern with scores between 0.5m and 1m **(D)**, 0.5m and 1.5m **(E)**, and 1m versus 1.5m **(F)**. Moheshkhali (**G-I)** mirrors this depth analysis with plots for 0.5m and 1m **(G)**, 0.5m and 1.5m **(H)**, and a 1m to 1.5m comparison

(I). Light blue outlines represent the average shape and dark blue outlines represent extreme shape changes.

Table 6. Results of Canonical variate analysis (CVA) and Discriminant function analysis (DFA) statistic tests of Procrustes distance, Mahalanobis distance and T-square values for compared groups of *P.viridis* from different depths in coastal areas of Bangladesh.

Compared Groups	Canonical variate analysis (CVA)		Discriminant Function Analysis (DFA)			
	Procrustes Distance (Permutation test p-value)	Mahalanobis Distance (Permutation test p-value)	Procrustes Distance (Permutation test p-value)	Mahalanobis Distance (Permutation test p-value)	T-square	T-square p-value (permutations)
0.5m-1m	0.0089 (0.1219)	1.4262 (<.0001)	0.00889155 (0.0003)	1.5784 (0.0003)	186.8465	0.0003
0.5m-1.5m	0.0124 (0.0139)	1.4351 (<.0001)	0.01240234 (0.0040)	1.4767 (0.0040)	163.5479	0.0040
1m-1.5m	0.0125 (0.0102)	1.6580 (<.0001)	0.01250234 (<.0001)	1.7993 (<.0001)	242.8090	<.0001

Table 7. Results of the cross-validation test in Discriminant function analysis **(**DFA) for the compared groups of *P. viridis* from different depths in coastal areas of Bangladesh. Numbers in brackets represent the correctly assigned individuals in relation to the total number of compared individuals.

		Sampling Locations	Predicted Group Membership			Total
			0.5m	1m	1.5m	
Original	%	0.5m	-	76% (114/150)	76% (114/150)	150
		1m	78% (117/150)	-	79.33% (119/150)	150
		1.5m	77.33% (116/150)	82% (123/150)	-	150
Cross-validated	%	0.5m	-	60% (90/150)	60.67% (91/150)	150
		1m	61.33% (92/150)	-	66% (99/150)	150
		1.5m	52.67% (79/150)	65.33% (98/150)	-	150

4.3 Shell Coloration Variation by Site and Depth

Digital colour analysis of 450 left-valve images produced quantitative values in RGB, HSV, and CIELAB colour spaces. Multivariate MANOVA confirmed a strong overall effect of site and depth on shell pigmentation (Wilks' $\lambda = 0.22$, $p < 0.001$).

4.3.1 Site-specific variation

Canonical variate analysis (CVA) separated the three populations Chowfaldandi (orange), Khuruskul (green), and Moheshkhali (blue) along CV1 and CV2, which together explained 78 % of colour variance (Figure 14A). Ninety-five percent confidence ellipses showed minimal overlap. Hierarchical clustering of group centroids (Figure 14B) paired Khuruskul with Moheshkhali, while Chowfaldandi formed its own branch, suggesting local turbidity or nutrient differences drive distinct pigmentation.

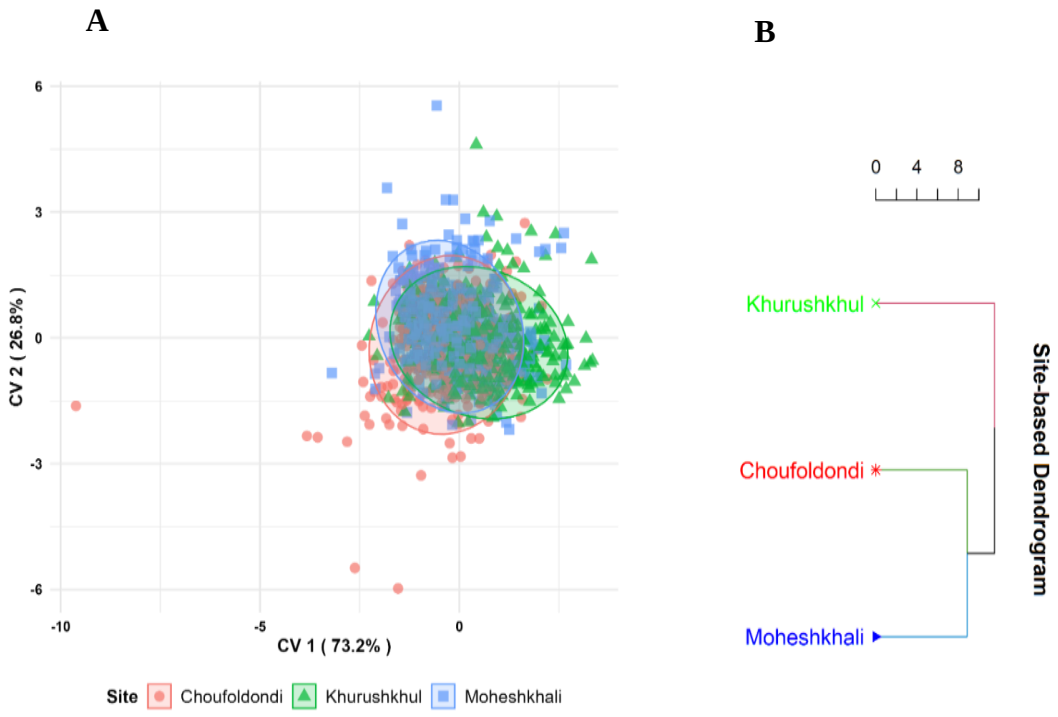

Figure 14. Canonical variate analysis (CVA) and hierarchical clustering of shell coloration in *Perna viridis* across habitats**.(A)** CVA plot showing site-wise separation along CV1 and CV2 with percent variance explained, reflecting group-level shell color differentiation. **(B)** A dendrogram based on Euclidean distances of color traits illustrates clustering patterns among populations. Shell color data include RGB and HSV variables; ellipses denote 95% confidence limits. Color groups: Choufoldondi (orange), Khurushkhul (green), and Moheshkhali (blue).

4.3.2 Depth-specific variation

CVA also distinguished shells from 0.5 m (red), 1.0 m (green), and 1.5 m (blue) depths (Figure 15A). The shallow group (0.5 m) was clearly isolated from mid- and deep-water groups, indicating possible photoprotective pigmentation under high light. Hierarchical clustering (Figure 15B) confirmed greater similarity between 1.0 m and 1.5 m samples versus 0.5 m.

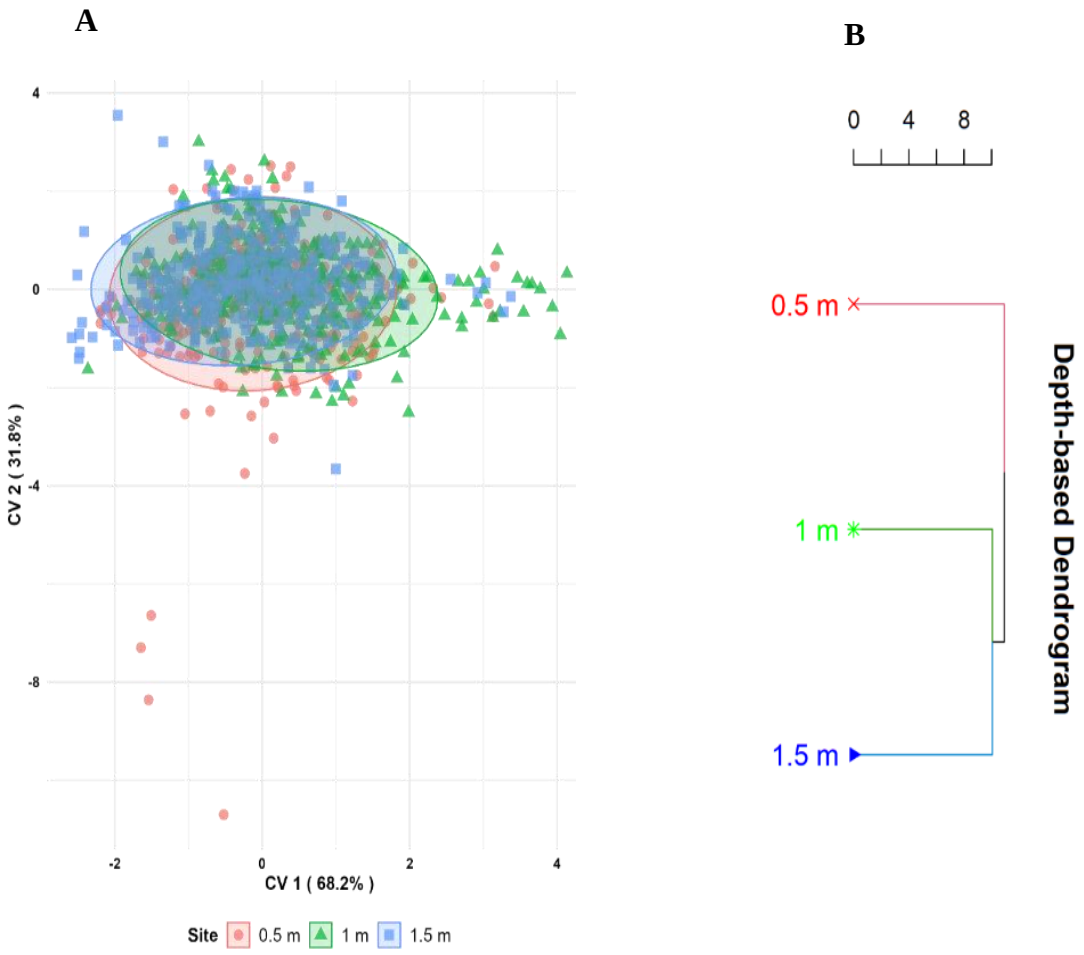

Figure 15. Canonical variate analysis (CVA) and hierarchical clustering of shell coloration in *Perna viridis* across depths**. (A)** CVA plot illustrating depth-wise variation in shell coloration across 0.5 m (red), 1 m (green), and 1.5 m (blue) depth strata. CV1 and CV2 represent axes of maximum group separation based on color variables; ellipses indicate 95% confidence intervals for each depth group. **(B)** Depth-based hierarchical clustering dendrogram using Euclidean distances of group means reveals close similarity between 1 m and 1.5 m populations, with 0.5 m forming a distinct cluster. These patterns suggest depth-driven shifts in shell color expression.

4.3.3 Joint site × depth effects

When site and depth were analysed together, CVA resolved nine discrete clusters corresponding to every site–depth combination (Figure 16). Shallow groups from all sites

clustered tightly, indicating convergent colour traits under shallow conditions. The accompanying dendrogram (Figure 16) corroborated this pattern, first grouping by depth and then by site.

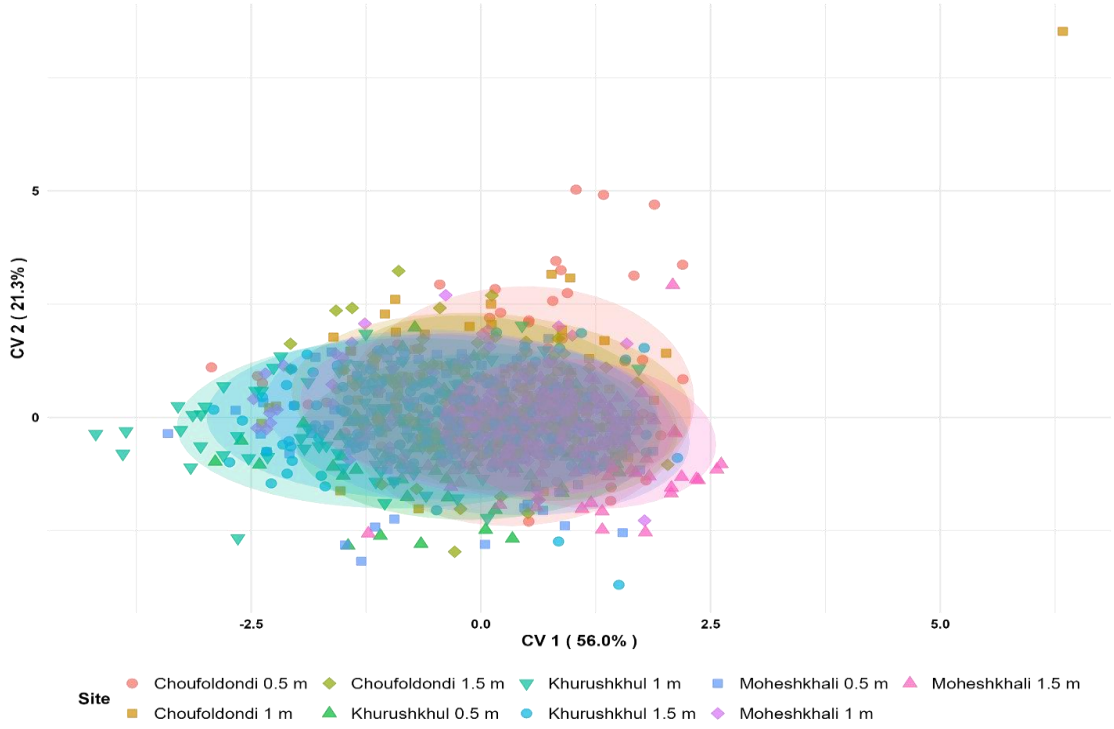

Figure 16. Depth-driven differentiation in shell coloration of *Perna viridis* revealed by canonical variate analysis and hierarchical clustering**.** Canonical variate analysis (CVA) of shell color metrics demonstrates distinct clustering of individuals collected from three depth strata 0.5 m (red), 1 m (green), and 1.5 m (blue) along the first two canonical axes (CV1 and CV2), which capture the major axes of intergroup variance. Elliptical confidence regions (95%) highlight the within-group dispersion, with minimal overlap suggesting clear phenotypic segregation by depth. Hierarchical clustering of group-wise color centroids, based on Euclidean distance, further supports depth-associated divergence, showing closer affinity between the 1 m and 1.5 m populations, and distinct separation of the shallowest (0.5 m) group. These patterns underscore a potential role of light availability, depth-related stressors, or environmental filtering in driving adaptive shifts in shell pigmentation.

Chapter V

Discussion

5.1 Shell Morphometrics: Influence of Site and Depth

5.1.1 Site-Specific Variation in Shell Shape and Size

In marine bivalves, shell morphology is influenced by both genetic and environmental factors, including water movement, sediment texture, food availability, and salinity (Seed, 1976; Sarà *et al*., 2004). These environmental variables often cause site-specific variations in shell shape, which can be used as indicators of habitat condition (Norkko *et al*., 2020).

In the present study, clear shape differences were observed among *Green mussel (Perna viridis)* populations from three coastal sites Chowfaldandi, Khurushkul, and Moheshkhali (Figure 7–8). Mussels from Chowfaldandi exhibited broader shells with pronounced anterior expansion and umbo elevation. In contrast, shells from Khurushkul were more elongated and streamlined, while Moheshkhali showed intermediate features.

These shape differences were accompanied by variations in shell size, as centroid size analysis (Figure 9A) showed that individuals from Chowfaldandi were larger than those from Khurushkul and Moheshkhali. However, the larger size at Chowfaldandi was not associated with increased shell robustness, as seen in the shape deformation plots (Figure 9B–C), suggesting that shell growth was primarily linear and influenced by environmental factors rather than mass gain.

Such habitat-specific morphometric divergence is well-documented in marine bivalves and often attributed to adaptive plasticity in response to environmental gradients (Koehn *et al*., 1983; Trussell, 2001). For example, in environments with higher water flow, mussels often develop more streamlined and curved shells to reduce drag and resist dislodgement (Bell & Gosline, 1997). Conversely, in calmer waters with higher sedimentation, flatter or

broader shells may be favored to increase stability and reduce burial risk (Frandsen *et al*., 2002).

The morphological differences observed here are consistent with these patterns, as Chowfaldandi known for lower water current exhibited shells with more anterior expansion and umbo elevation. In low water flow environments like Chowfaldandi, mussels tend to develop broader and shorter shells. This shape enhances their ability to anchor more effectively by increasing burrowing efficiency and stability in softer substrates (Levine *et al*., 2014). Thinner and more uniformly thickened shells are typical here, facilitating easier burrowing and anchoring in calm waters where strong hydrodynamic forces are less of a threat (Keogh *et al*., 2024).

In high water flow environments such as Khurushkul, mussels evolve slimmer, elongated shells that are often thicker relative to body size, especially in the part of the shell buried deepest in the sediment. This morphology helps them withstand the pressure of stronger currents and resist being dislodged from their burrowed positions (Keogh *et al*., 2024). The streamlined shape reduces drag and mechanical stress from flowing water, while increased shell thickness provides structural strength against turbulent forces (Bell & Gosline, 1997; Hornbach *et al*., 2010).

This morphological convergence, driven by flow rates, reflects an evolutionary response where hydrodynamic forces are a major selective pressure (Keogh *et al*., 2024). Mussels adapt their shell shape and thickness to balance the need for anchorage and resistance to current-induced dislodgement, with broader shells favoring stability in low flow and slender, robust shells favoring survival in high flow (Hornbach *et al*., 2010; Keogh *et al*., 2024).

The influence of food availability is also noteworthy. Shell growth rates are fundamentally linked to food availability, with chlorophyll a serving as the primary proxy for food supply in marine environments (Bonitz *et al*., 2018; Silina, 2023). The availability of food resources influences not only growth rates but also shell morphology, including thickness and elongation patterns (Alunno-Bruscia *et al.,* 2001). Well-fed bivalves typically exhibit

faster growth rates and develop shells with different proportional characteristics compared to food-limited individuals (Alunno-Bruscia *et al*., 2001). When food is abundant, as indicated by high chlorophyll-a concentrations, bivalves can allocate more energy to shell thickening while maintaining rapid linear growth (Fitzer *et al.*, 2019). Conversely, under food-limited conditions, energy allocation prioritizes essential functions, potentially resulting in thinner shells (Fitzer *et al*., 2019).

Therefore, although the centroid size was higher in Chowfaldandi, the associated thinness and shape deformation (Figure 9C) likely reflect a trade-off between food scarcity and energy allocation, prioritizing expansion over structural robustness. This supports the concept of environmental allometry, where size and shape changes are driven by both physiological and ecological constraints (Hickman, 1979; Bookstein, 1991).

5.1.2 Depth-Specific Variation in Shell Shape and Size

Water depth influences several physical and biological parameters that affect shell morphology in bivalves, such as light penetration, wave turbulence, food distribution, and sedimentation (Seed, 1976; Bayne & Worrall, 1980). The present study found that shell shape and size varied significantly among the three depth levels 0.5 m, 1.0 m, and 1.5 m. Centroid size increased with depth, although the shape deformation plots (Figure 10) showed that deeper mussels developed rounder and more inflated shells, while shallow mussels had streamlined, compact forms (Figures 11–13). These findings are consistent with earlier observations that mussels in shallow, wave-exposed environments often develop smaller, narrow shells to reduce hydrodynamic drag and resist dislodgement (Bell & Gosline, 1997; Kandratavicius & Brazeiro, 2014). According to the physical limitation hypothesis, organisms living in turbulent environments face mechanical constraints that shape their body form (Kandratavicius & Brazeiro, 2014). Streamlined shapes help minimize the impact of water movement, allowing mussels to maintain their position on rocky or loose substrates (Hunt *et al*., 2001; Kandratavicius & Brazeiro, 2014).

In contrast, mussels cultured at 1.5 m depth were less exposed to wave action and developed rounder and more expanded shells. This can be attributed to reduced mechanical

stress and stable environmental conditions at greater depths. The rounder, more inflated shell shape may also serve to increase the mussel's anchoring weight, helping them remain settled in soft sediments typical of deeper habitats (Fitzer *et al*., 2019). Additionally, rounder shells may provide enhanced protection against certain predators, compensating for the reduced threat from physical forces (Fitzer *et al*., 2019).

Environmental stability at depth supports more symmetrical and uniform growth. Bonitz et al. (2018) demonstrated that under steady food availability, shell calcification proceeds continuously, leading to more consistent morphologies. This matches the smooth shape transitions seen in the centroid and DFA plots (Figures 10–13).

Overall, depth-specific morphological variation in *P. viridis* likely results from differences in wave energy and food stability. Mussels in shallow water adapt for anchorage and resistance, while deeper mussels optimize shape for volume and tissue investment under calmer conditions.

5.2 Shell Coloration: Variation Across Sites, Depths, and Their Interaction

5.2.1 Site-Specific Divergence in Shell Coloration

Shell coloration in the Green mussel *(Perna viridis)* is not just a visual feature but a dynamic response to local environmental conditions. Numerous studies suggest that pigmentation in marine mollusks is influenced by factors such as light penetration, food quality, salinity, turbidity, and sediment load, all of which can vary substantially across coastal habitats (Williams *et al*., 2022; Dallas & Day *et al.*, 2004).

In this study, Canonical Variate Analysis (CVA) revealed a strong separation in shell pigmentation among the three sites Chowfaldandi, Khurushkul, and Moheshkhali with 95% confidence ellipses showing minimal overlap (Figure 14A). The hierarchical clustering dendrogram (Figure 14B) further supported this differentiation, where Chowfaldandi formed a distinct branch, while Khurushkul and Moheshkhali appeared more closely related. These patterns were reinforced by the site-wise heatmap analysis

(Figure 16), which showed consistent grouping of pigment profiles, confirming that each site produced a unique shell coloration pattern likely driven by ecological differences.

At Chowfaldandi, mussels generally displayed paler or chalkier shells. This is likely due to high turbidity and suspended solids, as observed in water quality data (Section 4.1), which limit light penetration and reduce the growth of pigmented surface biofilms. Such conditions may also suppress pigment biosynthesis or disrupt dietary pigment uptake, both of which are essential for shell coloration. These visual differences were clearly captured in the CVA plot and dendrogram (Figure 14A–B), where Chowfaldandi samples clustered away from the other populations.

Khurushkul mussels showed intermediate pigmentation, often with olive-green or mottled appearances. These variations may result from fluctuating environmental stressors, such as moderate turbidity surges and variable salinity levels. Despite these stressors, moderate chlorophyll-a concentrations suggest a relatively steady food supply, which may help maintain pigment levels but with some variation across depth layers (Raby *et al*., 1994; Saxby, 2002). The slight overlap in clustering with Moheshkhali observed in the CVA (Figure 14A) reflects this ecological and phenotypic in-between position.

Moheshkhali mussels, by contrast, exhibited the most vivid and saturated shell colours, ranging from deep green to brown tones. This site benefits from stronger water currents, reduced sedimentation, and higher chlorophyll-a levels, all of which promote continuous feeding and optimal pigment assimilation. The darker pigmentation was reflected in their distinct position in both CVA and heatmap analyses (Figures 14 A and 16), reinforcing that Moheshkhali provides the most favourable conditions for pigment expression.

Shell coloration may also be indirectly affected by water chemistry, such as salinity gradients and trace element concentrations which can impact physiological pathways responsible for pigmentation (Clark *et al*.,2020; Piwoni-Piórewicz *et al*.,2021; Guillot *et al*.,2024). External biofilms, which differ based on site-specific light and nutrient conditions, could also play a role in shell appearance (Kauser *et al*.,2016; De Carvalho *et*

al., 2018; Shree *et al*., 2023), especially in image-based analyses (Troscianko & Stevens, 2015; Relucenti *et al*., 2021; Mhade *et al*., 2023).

5.2.2 Depth-Specific Variation in Shell Coloration

In addition to site-specific differences, shell coloration in the Green mussel *(Perna viridis)* also varied clearly with culture depth. Canonical Variate Analysis (CVA) showed a strong separation among mussels grown at 0.5 m, 1.0 m, and 1.5 m depths, with 95% confidence ellipses revealing distinct clustering of color profiles (Figure 15A-B). The corresponding dendrogram (Figure 15B) showed that mussels from 1.0 m and 1.5 m were more similar to each other, while those from 0.5 m formed a separate cluster. These findings were supported by the heatmap analysis of depth-wise pigment variables (Figure 16), which confirmed consistent color patterns tied to cultivation depth.

In general, mussels grown at 0.5 m exhibited the most vivid and saturated shell colors, particularly rich green hues, compared to those from deeper layers. This trend is likely linked to greater light availability near the surface, which promotes the development of pigmented surface biofilms and may influence internal pigment metabolism as well (Wagner *et al*., 2015; Bengtsson *et al*., 2018; Villa *et al*., 2022). Shallow depths also allow for higher chlorophyll a exposure, suggesting greater access to dietary pigments, especially carotenoids and porphyrins, which are known to affect shell color in mollusks (Williams *et al*., 2016; Saenko *et al*.,2021; Wang *et al*., 2024).

At 1.0 m depth, pigmentation appeared more variable and less intense. Mussels at this level may experience moderate light limitation and slightly reduced food quality, resulting in uneven pigmentation or mottled shell patterns, as captured in the deformation plots and color clustering in Figure 15A. Additionally, fluctuating turbidity or suspended particles may interfere with feeding and pigment uptake (Li *et al*.,2018; Luesiri *et al*.,2022; Brouwer *et al*., 2023).

The palest and least pigmented shells were generally observed at 1.5 m depth. Mussels grown at this level showed duller hues and reduced saturation, which could result from multiple overlapping stressors. Reduced light penetration limits both the growth of

pigmented epibionts and the expression of pigment-related genes (Elfwing *et al*., 2003; Mainwaring *et al*., 2014; Bertolo *et al*., 2015). Simultaneously, deeper water layers often contain more suspended sediment and lower dissolved oxygen, which can impair shell biomineralization processes and hinder the deposition of pigmented materials (Fitzer *et al*., 2014,2015). These patterns were consistently reflected in the clustering seen in Figure 15A–B, where the 1.5 m group formed a visibly distinct cluster with lower color intensity.

Such depth-related variation is consistent with findings in other bivalve studies, where depth influences light, food composition, and water movement, all factors that affect pigmentation either directly or indirectly (Medcof *et al*., 1965; Lauerma *et al*., 2021; Williams *et al.*, 2022). In *P. viridis*, this depth gradient appears to act as a filter on shell color expression, with shallow-water mussels showing higher visual pigmentation due to favorable light and food conditions.

The interaction of site and depth also played a defining role. Mussels from 0.5 m in Moheshkhali showed the deepest green coloration likely due to synergistic effects of shallow depth and a favorable site (light, food, low turbidity). In contrast, 1.5 m Chowfaldandi mussels were exposed to both depth-related (e.g., low oxygen, less light) and site-specific (e.g., high turbidity, low phytoplankton) stressors, resulting in the dullest shells. Even 0.5 m Chowfaldandi mussels showed limited pigmentation, indicating that shallow placement alone cannot offset poor environmental quality. Khurushkul mussels, particularly at 1.0 m and 1.5 m, had patchy or mottled pigmentation likely caused by fluctuating salinity and food supply (Troscianko & Stevens, 2015; Rakib *et al*., 2022).

Altogether, shell pigmentation in *P. viridis* proved highly plastic and ecologically sensitive, shaped by both independent and interactive effects of site and depth. For aquaculture, optimizing both environmental location and cultivation depth could improve not only growth but also shell aesthetics, an important quality trait for market value.

Chapter VI

Conclusion

This study demonstrates that shell morphology and coloration of *Perna viridis* are strongly shaped by ecological gradients across sites and depths. Mussels from Khurushkhul developed the largest, broadest shells under high-flow conditions, while Chowfaldandi mussels showed smaller, compressed forms in stagnant, sediment-rich waters, and Moheshkhali mussels exhibited intermediate morphologies under mixed regimes. Depth further influenced form, with streamlined, compact shells in shallow mussels adapted to wave exposure, and rounder, inflated shells at greater depth reflecting stability and reduced hydrodynamic stress. These findings highlight the adaptive plasticity of *P. viridis* in response to environmental pressures.

Shell coloration also showed marked ecological sensitivity. Chowfaldandi mussels displayed pale, chalky shells under turbid, light-limited conditions, Khurushkhul mussels exhibited olive-green to mottled pigmentation under moderate stress, while Moheshkhali mussels developed deep green to brown shells in stable, food-rich habitats. A vertical gradient was also evident, with vivid coloration in shallow mussels diminishing with depth. Together, these traits reflect not only ecological adaptation but also the potential of shell features as bioindicators of habitat quality.

By integrating geometric morphometrics and color profiling, this study emphasizes the dual role of shell traits as functional adaptations and aquaculture-relevant indicators. The results underscore that site and depth selection are central to optimizing both growth performance and commercial value in mussel farming. Future research should build on these findings by exploring genomic mechanisms of trait expression, microbial biofilm interactions, and climate resilience under acidification, turbidity, and warming scenarios. Ultimately, this work reinforces that mussel aquaculture must be grounded in ecological understanding, where phenotype and environment are inseparably linked.

Chapter VII

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