Effect of eco-physiological factors on biometric traits of green mussel *Perna viridis* cultured in the south-east coast of the Bay of Bengal, Bangladesh

Aysha Rahi Noor, Abrar Shakil, Nayeema Ferdausy Hoque, Md Moshiur Rahman, Sumi Akter, Avijit Talukder, Sk Ahmad-Al-Nahid, Md Abdul Wahab, Md Nahiduzzaman, Md Jalilur Rahman, Md Asaduzzaman

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- Multivariate approach

**ABSTRACT**

Information on allometry, condition indices (CIs), and biometric traits (BTs) of marine bivalves are necessary for determining the growth pattern and the size at which harvest can be intensified to maximize production in aquaculture. These parameters of marine bivalves are largely regulated by the complex interactive effects of various ecological and physiological factors. Therefore, the present study aimed to apply an integrated multivariate approach to explore a comprehensive knowledge about how allometry, condition indices (CIs) and BTs are interlinked with various eco-physiological factors of cultured green mussel (*Perna viridis*), with a particular focus on the effect of seasonality. Six selected length-weight dimensions (shell length, shell width and height against total weight and soft tissue weight) were proved to be positively correlated with negative allometric relation (slope < 3) by regression analysis in male, female and sexually undifferentiated individuals. Linear discriminant function analysis (LDFA) displayed that CIs and BTs of green mussel were not differentiated by the sex, rather evidenced a pronounced discrimination by the seasonality and reproductive cycle. The correlation analysis demonstrated that different CIs and BTs were significantly correlated with different ecological factors, ingested plankton and gonadosomatic index (GSI) of the green mussel. Taken together all datasets, the principal component analysis (PCA) showed that seasonality and reproductive cycle explained >60 % of the variability. In both cases, PCA analysis further revealed that CIs and BTs increased with the increase of salinity, dissolved oxygen, food availability, plankton ingestion and GSI value, while they decreased with the increase of temperature, current speed and turbidity. Thus, our results clearly demonstrated that CIs and BTs of the green mussel were influenced by seasonality, ecological factors, plankton ingestion and reproductive cycle of the species. The findings of the present study represent a step toward a better comprehension of the CIs and BTs of *P. viridis* in relation to different eco-physiological factors and would be helpful to determine the ideal period for commercial exploitation, biodiversity conservation and future potential aquaculture of this commercially important species to a greater extent.

1. Introduction

The green mussel (*Perna viridis*), a keystone bivalve species, is extensively distributed along the intertidal and subtidal areas of the tropical and subtropical estuaries and seas in the Indo-Pacific region (Vakily, 1989; Hickman, 1992). Being a sustainable source of protein production, the global production of mussels is expected to grow further to fulfil the protein demand of the growing world population (Wijsman et al., 2019). It is a large and fast-growing commercially important bivalve species, which has been already demonstrated their impressive culture potentialities in many countries (Tan and Ransangan, 2014).

*Perna viridis*, like other bivalves, progressively changes its body weight in relation to their different shell size dimensions such as length, width and height (Hemachandra and Thippeswamy, 2008; Singh, 2017).
Such relationship between two measurable variables of body weight and shell size of *P. viridis* is often termed as allometry (Hemachandra and Thippeswamy, 2008; Singh, 2017). The allometry equation has been using extensively in physiological investigation and flesh weight estimation relative to the measurement of shell length in mussel production (Gosling, 1992). When allometry equation is established, measurement of shell length is an adequate substitute for estimating total flesh and biomass production. Therefore, studies on allometry growth of marine bivalves are necessary for successful determining the size at which harvest can be intensified to maximize production in aquaculture (Palmer, 1990).

The condition indices (CIs), like allometry, are an important basic measurements of the physiological status of marine bivalves, and the relative allocation of resources for tissue or shell growth. When the CI is used to indicate growth, it is defined as the ratio of the dry weight of soft tissues over shell weight which appears to be a good indicator of growth rate (Yap et al., 2002; Thebault et al., 2005). For marine bivalves, a lot of physiological CIs have been used in the eco-physiological studies to assess the muscle quality at a particular time and to determine the harvesting time. Moreover, CIs have been used for economic and ecophysiological purposes in exploitation of bivalves from wild population and aquaculture facility (Lucas and Beninger, 1985). In economic purpose, CIs are often used to designate the quality of the marketed product, whereas they are used for the characterization of the health condition in ecophysiological purpose. They are also often used to identify the well-being, i.e. meat portion (fattiness) of the species on the basis of assumption that organism with heavy weight on a specific length are good in condition (Froese, 2006). Therefore, a comprehensive understanding about these parameters and their linkage to different eco-physiological factors is very essential for the commercial exploitation of this species from the aquaculture farms.

As an estuarine and intertidal bivalve species, *P. viridis* has to function within the constraints of changing environmental conditions. To better understand the dynamics of this relationship, an increased knowledge about the CIs and biometric traits (BTs) that link the environmental conditions of the farming sites to the ecological responses is imperative (Niemi and McDonald, 2004). As like other marine bivalves, *P. viridis* displays marked seasonal variations in different BTs and biochemical content of the soft tissue (Ansell et al., 1986; Williams and McMahon, 1989). Such seasonal variation in shell growth and shape of *P. viridis* are influenced by biotic (physiological) and abiotic (environmental) factors of their farming sites. In many marine bivalves species, a variety of environmental factors are known to influence shell morphology and relative proportions such as latitude (Beukema and Meehan, 1985), shore level (Franz, 1993), type of bottom (Claxton et al., 1998), type of sediment (Newell and Hidalgo, 1982), currents (Fuiman et al., 1999), tidal level (Dame, 1972), depth (Claxton et al., 1998), water turbulence (Hinch and Bailey, 1988) and wave exposure (Akester and Martel, 2000). Like the allometry, CIs and BTs of marine bivalves are also reported to exhibit obvious seasonal variation and influenced by many ecological factors such as salinity, temperature and environmental contaminants (Lucas and Beninger, 1985; Loesch and Evans, 1994; Iglesias et al., 1996). Being a planktivorous species, CIs and BTs of *P. viridis* largely depend on the plankton abundance of their culture sites (Smaal and van Stralen, 1990), and have been found to be strongly correlated with chlorophyll-a concentration (Brown, 1986). Similarly, poor growth performance and condition indices of marine bivalves have also been reported in high stocking density area due to the intensive competition for food resources among themselves (Filgueira et al., 2013).

Seasonal patterns in allometry and CIs of bivalves typically include increases in weight and condition before spawning, and are usually tightly coupled to the cycle of gonadal development (Newell and Bayne, 1980; Acarlı et al., 2018). Allometry and CIs are often linked to spawning through the loss of mass in the form of gametes, serving as an indicator of spawning events (Lambert and Dutil, 1997; Hemachandra and Thippeswamy, 2008). In most bivalves, gonad development and growth prior to onset of spawning result in fattening and increase meat yield. However, marine bivalves require additional energy to meet the special and unique metabolic activities of crucial stages during the gametogenesis (Asaduzzaman et al., 2019).

Green mussel can adopt an opportunistic scheme to build up their gonads using energy from the available food (Tan and Ransangan, 2017; Asaduzzaman et al., 2019). Plankton is the main component of diet in *P. viridis*, and an indispensable source of nutrients and energy for the gametogenesis (Lopes-Lima et al., 2014; Tan and Ransangan, 2017; Asaduzzaman et al., 2019). Again, the abundance of plankton in water and its ingestion from the water column by green mussel are widely influenced by the seasonality and environmental factors (Lopes-Lima et al., 2014; Tan and Ransangan, 2017; Kim et al., 2019), indicating a further complex interrelation of allometry and biometric traits with different eco-physiological factors. However, such multiplex interrelationships among allometry, CIs and BTs, and various eco-physiological factors are poorly investigated in cultured *P. viridis*, even for other marine bivalves. Therefore, in this study, we applied a multivariate approach to explore a deeper understanding about how allometry, CIs and BTs are interlinked with different eco-physiological factors like environmental parameters, food availability, plankton ingestion and reproductive cycle, highlighting a pertinent effects of seasonality.

Although it is very complex and tortuous to separately claim an individual or couple of factors that can trigger the growth and different life history patterns of this species, the present study would be helpful to point out which eco-physiological factors interactively influence their growth and reproduction in a trial culture system. Furthermore, the present study would provide information on: (1) the factors that are favorable to grow out the target size of this species in an aquaculture system; (2) the parameters that need to regulate to enhance their production (i.e. growth and reproduction) and quality (i.e. desired market size and appearance); and (3) the season that is appropriate for their commercial exploitation and aquaculture in the coastal region of Bangladesh and other south Asian countries, etc.

2. Materials and methods

2.1. Sampling sites

In Bangladesh, the natural distribution of *P. viridis* is confined to the south-eastern coasts, particularly in the Moheshkhali Channel, which is directly connected to the Bay of Bengal (Shahabuddin et al., 2010). Therefore, we recently established a number of pilot-scale research-based culture systems to evaluate the potentiality of green mussels’ culture at the five locations of Moheshkhali Channel, namely Choufaldandi 1 (21°30′20″ N and 91°59′19″ E), Choufaldandi 2 (21°29′09″ N and 91°57′21″E), North Khurushkul (21°27′54″ N and 91°58′09″ E), Khurushkul 1 (21°26′46″ N and 91°58′29″E) and Khurushkul 2 (21°33′04″ N and 92°03′14″E). In each of these locations, we established suspended culture system in the water column using rafts or floats to support ropes and cages on which the mussel spat settlement occurred and grew naturally. For this study, about 60 green mussels and water samples were collected on monthly basis for the period of July 2019 to June 2020 from the same five locations of these pilot-scale research-based culture sites. After collection, they were immediately brought to the laboratory and kept them alive for biometric measurements, gut plankton analysis and gonadal histological study.

2.2. Biometric measurements

The collected mussels were initially cleaned from all the encrusting organisms and their byssuses were removed carefully. From the collected mussels of each month, shell length (maximum distance of anterior and posterior end of the shell), shell width (maximum spacing...
between the outer edge of the valves) and shell height (maximum dorso-ventral distance) of randomly taken 20 mussels were measured using a Vernier calliper with an accuracy of 0.01 mm. The weight of individual mussel was also recorded after draining the fluid from intervalvral region (mantle) to the nearest 0.1 g using an electronic balance (PS 1200.R2, Radwag, Poland). Subsequently, the meat portion was dissected and removed from the shell, and soft tissue weight and shell weight were also separately recorded. After measuring the soft-tissue weight, the gonads were gently separated and weighed for calculating the gonado-somatic index (GSI).

2.3. Calculation of allometry, condition indices and biometric traits

Allometry was examined through length-weight relationships using the equation of \( W = a \times L^b \) (Le Cren, 1951) where ‘a’ and ‘b’ are constants (Pauly, 1983). The logarithm of the equation was taken so that the exponential relationship could be expressed by a linear equation-log \( \log(W) = \log(a) + b \times \log(L) \). Here, \( W \) is the weight (total weight and soft-tissue weight), \( L \) is the length (shell length, shell width and shell height), whilst ‘a’ is the intercept (initial growth coefficient) and ‘b’ is the slope (relative growth rate of variables). The condition factor (K) was calculated using the formula: \( K = W/L^3 \), where \( W \) indicated total weight and \( L \) denoted shell length and ‘b’ is the slope of length-weight relationship (Devaraj, 1973; Acharya and Dwivedi, 1985). The dried soft tissue weight (DSTW) was measured after drying the soft tissue samples at 105 °C until the weight became constant in a hot air oven. Ash free dry weight (AFDWSTW) was calculated after subtracting the ash content, which was measured by combusting the dried soft tissue samples at 550 °C for 6 h in a muffle furnace. The CI in dry weight basis (CI-dry) was calculated using the formula: \( CI_{dry} = (g/cm^2) = \frac{[total \ soft \ tissue \ dry \ weight \ (g)/shell \ volume \ (cm^3)]}{1000} \) (Yap et al., 2002). The CI-body was measured following the equation: \( CI_{body} = (g/soft \ tissue \ weight/total \ weight) \times 100 \) (Rao, 1956; Baird, 1958). The GSI is expressed as the percentage of the gonad weight in relation to the total body weight and was calculated as: \( GSI(\%) = \frac{[weight \ of \ gonad \ (g)/total \ weight \ of \ mussel \ (g)]}{100} \) (Devlaming et al., 1982).

2.4. Determination of sex and gonad developmental stages

About 20 green mussels were used in each month for the histological analysis of gonads throughout the annual spawning season (n = 242). A section of gonadal masses was collected from the mantle lobes of individuals and fixed in Bouin’s solution for 24 h, and subsequently dehydrated in series (80–100 %) of ethanol and xylene. Afterwards, the dehydrated gonads were embedded in paraplast, cut in transverse serial sections (7 μm), and mounted on glass slides. An increasing alcohol concentration protocol was adopted for dehydration of the mounted slide, and then stained with Harris’s hematoxylin and eosin (Pearse, 1985). The stained slides were observed and photographed using digital microscope (Optika B-190TB, Ponteranica, Italy) with magnification 10–40× for the identification of sex and gonad developmental stages. The condition of gonads was classified into five main categories including resting, development, mature, spawning, and spent (Asaduzzaman et al., 2019).

2.5. Analysis of gut plankton

The gut plankton contents from 20 individuals of collected mussels per month were examined throughout the year (n = 240). Immediately after collection, the mussels were preserved in 20 % buffered formalin for 7 days and then transferred to the 70 % ethanol for 7 days followed by the distilled water for another 4 days. Afterwards, the stomach content was collected by using a glass Panteur pipette through a small slit beneath crystalline style and diluted in 50 mL of filtered sea water. A 1-mL subsample was transferred to a Sedgewick Rafter Counting Cell (S-R cell), and all plankton in 10 randomly selected squares were counted using a binocular microscope (Optica B-190TB with digital facilities; magnification 10×). The abundance of the ingested gut plankton was calculated based on the formula: \( N = (P \times C \times 100) \), where \( N \) = numbers of plankton cells or units in the whole guts; \( P \) = total number of plankton counted in 10 fields; and \( C \) = volume of final concentrate of the sample in mL. For each gut sample, three subsamples were examined following the same procedure.

2.6. Measurement of water quality parameters and plankton abundance

For water quality parameter analyses, water samples were collected monthly from the same location of green mussel collection sites. A multifunction environmental sensor (YSI, Loveland, CO, USA) was used to measure in situ the temperature (°C), salinity (ppt), pH and dissolved oxygen (DO, mg L⁻¹). The water current (m sec⁻¹) was measured with a digital water velocity meter (Flow Probe FP311, Global Water, TX, USA) positioned 0.5 m below the water surface. The water turbidity of collected samples was measured using a digital turbidity meter (Turb 430 IR, WTW, Weilheim, Germany). Total alkalinity was measured following the titrimetric method (APHA, 1992). For chlorophyll-a determination, water samples were collected using a vertical water sampler (1200-E Kemmerer, WildCo, FL, USA) and filtered through microfiber glass filter paper (Whatman GF/C), using a vacuum pressure air pump. Chlorophyll a was determined at 750-nm and 664-nm wavelength (Optipen Pop 2102, Daejeon, Republic of Korea), following the method of Boyd (1979). For water plankton measurements (cells L⁻¹), 20 L of pooled water samples was passed through a 45-μm mesh plankton net, and the concentrated samples were preserved in small plastic bottles with 5% buffered formalin. A quantitative estimation of plankton was done using a Sedgewick–Rafter (S–R) cell containing 1000 1-mm² cells (Asaduzzaman et al., 2019). A 1-mL sample was put in the S–R cell, and the plankton in 10 randomly selected cells were identified up to genus level and counted under a binocular microscope (Optika B-190TB, Ponteranica, Italy). Plankton abundance was calculated using the following formula: \( N = \frac{(P \times C \times 100)}{L} \), where \( N \) = the number of plankton cells or units per liter of original water, \( P \) = the number of plankton counted in 10 fields, \( C \) = the volume of final concentrate of the sample (mL), and \( L \) = the volume (L) of the water sample.

2.7. Statistical analyses

All analyses were done using R, version 3.5.2 (R Development Core Team, 2017). The percentage data were expressed in percentages at first and then underwent an arcsine square root transformation before statistical analysis. The assumptions of normal distributions of all datasets were checked with the Shapiro–Wilks test, and homogeneity of variances were checked with Levene’s test using the ‘onewaytests’ package (Dag et al., 2017). The univariate analysis of variance (ANOVA) model was applied using the “car” package (Fox and Weisberg, 2011) of R followed by the Tukey multiple comparison test using the “multcomp” package (Hothorn et al., 2008) to compare the monthly variation of all the datasets. Significant differences were evaluated at the 95 % confidence level. One-way ANOVA analysis was performed for the water quality dataset, which demonstrated that seasonality influenced most of the water quality parameters significantly (p < 0.001) except for pH and alkalinity (Supplementary Table 1). Therefore, these two parameters were discarded for further multivariate analyses. The regression analysis for various length-weight relationships was also done by using the “car” package. The correlations among variables were tested and plotted using the “PerformanceAnalytics” packages (Peterson and Carl, 2012). The principal component analysis (PCA) for all datasets was performed using the ‘FactoMineR’ package (Sebastien et al., 2008). The Linear Discriminant Function Analysis (LDAF) was performed by using ‘MASS’ package (Venables and Ripley, 2002). All the plots were made using the ‘ggplot2’ package (Wickham, 2016).
3. Results

3.1. Allometry relationship

Among the collected 242 individuals of green mussels, the histological analysis precisely identified 67 (27.69 %) as males and 62 (25.61 %) as females, while the remaining 113 (46.69 %) individuals were almost impossible to distinguish their sexes due to the gonadal resting phase in the month of May to September (Table 1). The monthly variation of different biometric traits parameters were summarized in Supplementary Table 2. The univariate ANOVA analysis revealed that different shell dimensions (length, width and height) and weights (total weight and soft tissue weight) were not significantly (p > 0.05) different between male and female individuals (Table 1). However, a significant (p < 0.05) lower values of these parameters were observed in sexually undifferentiated individuals as they mostly represented resting phase in their gonadal developmental stage. In the present study, six length-weight dimensions (shell length, shell width and shell height against total weight and soft tissue weight) demonstrated significant (p < 0.001) linear relationships with the higher values of coefficient of determination, R² (please see Fig. 1A-F) for male, female and sexually undifferentiated individuals. The "b" values (slope of regression curve) obtained from the equation of regression analysis of different length-weight dimensions ranged from 1.67 to 2.67 (~3) in various sex groups, indicating a negative allometry growth pattern of the species (Fig. 1). However, "b" values of ≥ 2.0 only obtained from the regression equations of shell length-total weight and shell length-soft tissue weight for all sex groups (Fig. 1).

3.2. CIs and BTs depending on seasonality, sex and reproductive cycle

The monthly variability of the two CIs (CI-dry and CI-body) of P. viridis is presented by the box and whiskers plots in Fig. 2. An increasing shift of CI-dry value was observed as early as October, which reached towards it’s significantly (p < 0.05) highest value in December to January, and then a decreasing inclination towards its lower value from March to September (Fig. 2A). As like CI-dry, a similar trend of seasonal variation of CI-body with their significantly (p < 0.05) higher value during the month of October to February was also observed (Fig. 2B). Based on the data on condition, it is suggested that the ideal period for commercial exploitation of P. viridis from the culture sites of south-east coast of Bay of Bengal from October to January, when the meat yield is the highest.

The multivariate approaches (LDFA and PCA) were applied using all the CIs and BTs to better understand how they were discriminated by the seasonality, sex and reproductive cycle. It was observed that the multivariate spaces of CIs and BTs were not discriminated between male and female individuals of green mussels, while the sexually undifferentiated groups were clearly differentiated (Fig. 3). The LDFA analysis revealed that multivariate spaces exhibited extensively overlapping patterns among different months as well as different gametogenesis stages (Fig. 4). In seasonality dataset, it was observed that multivariate spaces from May to September tended to form one cluster, and formed another cluster from October to February, while the multivariate spaces of March and April overlapped between these two clusters (Fig. 4A, B). In gametogenesis stage dataset, it was observed that the multivariate space of gonadal resting stage is completely differentiated from the other gametogenesis stages (Fig. 4C, D). For each of the seasonality and gametogenesis stage dataset, PCA extracted four significant and cross-validated PCs having eigenvalues ≥ 1, all of which together accounted 87.78 % of the total variability in the original data (Table 2). The first PC (51.9 % variability) was dominated by the different lengths and weight-related BTs parameters, whereas the second component (16.2 % variability) was dominated by the CI-dry and CI-body (Table 2). The third and fourth PCs explained only 12.21 % and 7.45 % of the total variability, and therefore, we did not consider them for further analysis. As like LDFA biplots, PCA biplots also displayed that the multivariate spaces of different months and gametogenesis stages exhibited very overlapping patterns (Fig. 5). However, multivariate spaces of gonadal development and matures during the months of October to February were somewhat differentiated and were mostly discriminated by the higher values of the CIs and BTs.

A correlation analysis with GSI was conducted to better understand about how various CIs and BTs were influenced by the reproductive cycle of the green mussel. The correlation test revealed that shell length (r = 0.24, p < 0.001), shell width (r = 0.24, p < 0.001), total weight (r = 0.23, p < 0.001), soft tissue weight (r = 0.13, p < 0.05), shell weight (r = 0.26, p < 0.001), dry soft tissue weight (r = 0.35, p < 0.001), ash free dry soft tissue weight (r = 0.38, p < 0.001), CI-dry (r = 0.34, p < 0.001) and CI body (r = 0.40, p < 0.001) had significant positive correlation with GSI, while shell height (r = 0.10, p > 0.05) and condition factor (r = 0.028, p > 0.05) had no significant correlation with GSI value (Fig. 6).

3.3. CIs and BTs depending on the ecological factors and plankton ingestion

The influence of various ecological factors and plankton ingestion on different CIs and BTs of green mussel was conducted by a comprehensive correlation analysis (Fig. 7). The correlation test revealed that temperature had a significant (p < 0.001) negative correlation with DSTW (r = -0.31), AFDSTW (r = -0.38), CI-dry (r = -0.35) and CI-body (r = -0.38). Turbidity also had a significant (p < 0.001) negative correlation with DSTW (r = -0.23), AFDSTW (r = -0.32), CI-dry (r = -0.11) and CI-body (r = -0.35). Similarly, the current speed of waterbody also negatively influenced DSTW (r = -0.30, p < 0.001), AFDSTW (r = -0.37, p < 0.001), CI-dry (r = -0.19, p < 0.01) and CI-body (r = -0.26, p < 0.001). In contrast, salinity was found to be positively correlated with DSTW (r = 0.27, p < 0.001), AFDSTW (r = 0.30, p < 0.001), CI-dry (r = 0.21, p < 0.01) and CI-body (r = 0.31, p < 0.001). Similarly, dissolved oxygen and chlorophyll-a were significantly (p < 0.001) positively correlated with DSTW (r = 0.35, 0.27), AFDSTW (r = 0.39, 0.29), CI-dry (r = 0.23, 0.31) and CI-body (r = 0.31, 0.32). Water plankton abundance was found to be positively influenced only by CI-dry (r = 0.19, p < 0.01) and CI-body (r = 0.16, p < 0.05) of green mussels. Interestingly, it was observed that DSTW (r = 0.27, p < 0.001), AFDSTW (r = 0.30, p < 0.001), CI-dry (r = 0.21, p < 0.01) and CI-body (r = 0.31, p < 0.001) were positively influenced by the quantity of the ingested plankton. However, none of these factors (except turbidity) were found to be significantly correlated with the condition factor of the green mussel.

3.4. Interrelationship among CIs, BTs and eco-physiological factors

A multivariate PCA analysis was performed to attain a deeper understanding about the interrelationship of the multiplex scenario of CIs and BTs with seasonality, ecological factors and reproductive cycles that could summarize the information obtained from the previously
Applying the PCA to these datasets, five PCs with eigenvalue > 1 were extracted that combinedly explained 87.22% of the total variability in the original data (Table 3). The PC1 accounted for 38.26%, PC2 for 22.32%, PC3 for 11.92%, PC4 for 8.70% and PC5 for 6.02% of the variability. As the PC3, PC4 and PC5 accounted for smaller amount of variability, they were not considered for further steps. From the loading variables of PCs (Table 3), it was observed that both PC1 and PC2 were associated to different allometry and biometric traits of green mussels and were positively correlated with salinity, dissolved oxygen, chlorophyll-a, plankton ingestion and GSI, and negatively correlated with temperature, current speed and turbidity. During gonadal development, maturation and spawning stages (October to March), higher salinity, dissolved oxygen, chlorophyll-a, food availability and plankton ingestion were positively interacted among themselves, which positively triggered allometry and biometric traits of green mussels (Fig. 8). In contrast, the higher temperature, current speed and turbidity negatively influenced allometry and biometric traits of green mussels during their gonadal resting phase in the months of May to September (Fig. 8).
Fig. 2. Box and whiskers plots of the monthly variation of (A) condition index-dry (CI-dry) and (B) condition index-body (CI-body) of green mussel *Perna viridis*. Small letters indicate significant differences in CIs values among months at $P < 0.05$.

Fig. 3. Linear Discriminant Function Analysis (LDFA) showing the gender variation of condition indices and biometric traits parameter of green mussel. (A) Biplot of the LDFA analysis of gender variation of different condition indices and biometric traits; (B) Density plot of linear discriminant function 1 (LD1) scores of gender variation of different condition indices and biometric traits.
4. Discussion

4.1. Allometry growth patterns

Allometry relationship is often used to measure the growth and production of an organism (Meher et al., 2006), and is considered as the most reliable method for marine bivalves in determining the growth pattern and size at which harvest can be intensified to maximize production (Hemachandra and Thippeswamy, 2008; Ashwin et al., 2013). In general, living organisms gain weight with increase in length, and marine bivalves are not exception. However, weight gain pattern against increase in length varies from species to species. In our study, it was observed that allometry growth in cultured P. viridis from southeast coast of Bangladesh showed a significant linear relationship in all dimensions of shell size (shell length, width and height) against body weight (total weight and soft tissue weight) for different sex groups (Fig. 1). Moreover, it was observed that some individuals of P. viridis of the same length showed different weights, and these differences could be due to physiological condition, reproductive strategies and environmental variables (Bauer, 1983; Ravera et al., 2007), which are discussed more elaborately below.

From the regression line and growth equation, a constant value of the slope (b), also known as coefficient of allometry relationship, was derived which are often used to compare between dimensional growth of the same species dwelling diverse habitats and are influenced by numerous biotic and abiotic factors (Winberg, 1971; Ashwin et al., 2013). In this study, results showed that values of ‘b’ in all dimensions of shell size against body weight ranged from 1.67 to 2.72 for different sex groups, indicating that P. viridis followed negative allometry growth pattern where the rate of increase in shell length is faster than the rate of increase in weight (Hemachandra and Thippeswamy, 2008; Sundaram et al., 2011; Aban et al., 2017). Moreover, the highest b value (2.11–2.37) was observed in shell length-total weight and shell length-soft tissue weight relationships for both the male and female individuals, indicating that these two allometry relationships more precisely displayed (b value more close to 3) the growth pattern of P. viridis than the others. Different b values in various allometry relationship (Fig. 1) further represented that the growth rate of the various parts of the body of P. viridis, as in other animals, may not be uniform, with the result that the relative proportions of various parts of the body change differently with increase in size and age (Hemachandra and Thippeswamy, 2008; Singh, 2017). However, the b value of different allometry relationship of this study also corroborated the findings of other studies on allometry relationship in P. viridis (Qasim et al., 1977;
Table 2
Principal Component Analysis (PCA) of condition indices and biometric traits parameter in relation to the seasonality and reproductive cycle of the green mussel. Eigenvalues, explained and cumulative variance, loadings of the variables for the first four PCs.

| Principal Components | PC1     | PC2     | PC3     | PC4     |
|----------------------|---------|---------|---------|---------|
| Variance explained   |         |         |         |         |
| Eigenvalues          | 7.79    | 2.43    | 1.83    | 1.12    |
| % of variance        | 51.94   | 16.18   | 12.21   | 7.45    |
| Cumulative %         | 51.94   | 68.13   | 80.34   | 87.78   |
| Factor loadings      |         |         |         |         |
| Shell length         | 0.944   | –0.214  | –0.061  | 0.083   |
| Shell width          | 0.894   | –0.244  | –0.125  | 0.075   |
| Shell height         | 0.833   | –0.369  | –0.106  | 0.149   |
| Shell volume         | 0.936   | –0.290  | –0.098  | 0.043   |
| Total weight         | 0.960   | –0.076  | 0.201   | –0.117  |
| Soft tissue weight   | 0.881   | 0.106   | 0.393   | 0.076   |
| Shell weight         | 0.951   | –0.142  | 0.120   | –0.186  |
| Dry soft tissue weight| 0.898  | 0.380   | –0.123  | 0.084   |
| Ash free dry soft tissue weight | 0.859 | 0.348 | –0.262 | 0.102 |
| Condition index-dry  | 0.073   | 0.961   | –0.038  | –0.111  |
| Condition index-body | 0.241   | 0.695   | –0.543  | 0.319   |
| Meat yield           | –0.042  | 0.368   | 0.566   | 0.587   |
| Condition factor     | 0.218   | 0.372   | 0.622   | –0.522  |
| Ash                  | 0.414   | 0.223   | 0.492   | –0.041  |
| Gonadosomatic index  | 0.303   | 0.247   | –0.497  | –0.542  |

Narasimham, 1980; Sundaram et al., 2011; Aban et al., 2017). In contrast, a relatively higher b values (2.7173–2.995) for shell length-total weight and shell length-wet weight relationship in P. viridis was recorded from India (Hemachandra and Thippeswamy, 2008). This is mainly because of the size difference between two studies, where they considered wide size range (shell length 0.61–13.32 cm) of P. viridis than this study (5.56–14.70 cm).

4.2. CIs and BTs truly reflect reproductive cycle with seasonality

CIs and BTs of marine bivalves displayed marked seasonal variation and closely followed the cycles of gonadal development (Ansell et al., 1998; Williams and McMahon, 1989). This study also proclaimed a marked seasonal variation in dry condition index and body condition index in P. viridis (Fig. 2). Significant higher condition indices values were recorded during the months of October to February (Fig. 2), which might be due to the facts that P. viridis started developing gonad from October and reached at maximum maturity for spawning until February along the southeast coast of Bangladesh (Asaduzzaman et al., 2019). When gonadal growth and maturation occurred in the collected P. viridis, they resulted in bulkiness of soft body and consequent high body weights. As condition indices mostly depended on the weight of individuals, an increasing shift in the CI values indicated the onset of maturation and gonadal growth in P. viridis.

After January-February, condition indices started to decline until they reached at the lowest level as found in April because almost all the individuals completely spawned by then, and the thickness of the mantle tissue reduced due to gametes release (Asaduzzaman et al., 2019). The similar uncoupled growth rates and condition indices between shell and soft tissues due to soft tissue weight loss resulting from spawning, was also reported in P. viridis from the east coast of India (Rajagopal et al., 1998). From April to September, monthly mean CIs were quite similar, indicating the resting phase of the reproductive cycle of P. viridis as the histological analysis revealed that the gonad consisted mostly of storage cells during those months (Asaduzzaman et al., 2019). Consistently, both LDFA (Fig. 4A, B) and PCA (Fig. 5A) analysis further confirmed that CIs and BTs showed a marked seasonal variation. During the months from May to September, the multivariate spaces aggregated to one cluster, which mostly represented gonadal resting stages, while the months of October to February, they aggregated into another cluster, which mostly represented gonadal development and maturation stages. However, the multivariate spaces of March and April overlapped between these two clusters, which mostly represented the spawning and gonadal spent stages. Such linkages of seasonal variation of CIs and BTs with the reproductive cycle were further confirmed by the LDFA (Fig. 4C, D), and PCA analysis (Fig. 5B). These multivariate analyses further ensured that gonadal resting stage was completely differentiated from the other gametogenesis stages (Fig. 4C, D) and higher values of various CIs and BTs mostly discriminated the gonadal development and maturation stages (Fig. 5B).

![Fig. 5. Principal Component Analysis (PCA) showing the discrimination of condition indices and biometric traits parameter based on the seasonality and reproductive cycle of green mussel. (A) Biplot of the PCA analysis of monthly variation of different condition indices and biometric traits parameter; (B) Biplot of the PCA analysis of gonadal stage-specific variation of different condition indices and biometric traits.](image-url)
During the growth of marine bivalves, the shell volume enlargement allows the individuals to generate more visceral mass, which increases considerably during the maturation and reproduction phases of the *P. viridis* life cycle (Bayed, 1990). As like most bivalves, gonadal growth during the pre-spawning period resulted in increasing the bulk of the gonad, which forms the bulk of the visceral mass in *P. viridis*. Therefore, the corresponding increase in different CIs and BTs of *P. viridis* were due to the accumulation of gametes in follicles and the resultant bulkiness of gonads during the gonad development and maturation stages (Hemachandra and Thippeswamy, 2008). However, the release of gametes from the follicles and emptying and shrinking of the gonad during the spent and resting stages governs the corresponding decrease values of CIs and BTs (Hemachandra and Thippeswamy, 2008). However, our study using the histological characteristics of the gonad during annual breeding cycle confirmed that variation in CIs and BTs truly reflected the breeding periodicity and gonadal status of the marine bivalves. Although a pronounced discrimination by the seasonality and reproductive cycle was observed, however, the findings of our study confirmed that CIs and BTs were not differentiated by the sex of *P. viridis* (Fig. 3).

### 4.3. Ecological factors and plankton ingestion influenced CIs and BTs

In marine bivalves, CIs and BTs response to the ecological factors are often species specific and varies between and within geographical locations (Rainer and Mann, 1992; Marsden and Pilkington, 1995). These ecological factors regulate the CIs and BTs by influencing food availability, feeding activity, the growth, and reproductive cycles of *P. viridis* (Lopes-Lima et al., 2014; Tan and Ransangan, 2017; Asaduzzaman et al., 2019). Moreover, all of these factors interact among themselves often synergistically, which ultimately make it difficult to quantify the precise

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**Fig. 6.** Interrelationship among different condition indices and biometric traits parameter with the gonado-somatic index (GSI) of the green mussel (*Perna viridis*). Here, the variables’ full names are: SL, shell length (cm); SW, shell width (cm); SH, shell height (cm); TW, total weight (g); STW, soft-tissue weight (g); SW.1, shell weight (g); DSTW, dry soft tissue weight (g); AFDSTW, ash free dry soft tissue weight (g); CI. Dry, condition index dry (g/cm$^3$); CI.body, condition index body; CF, condition factor; GSI, gonado-somatic index (%). The values given around all the axes are the range of each individual parameter’s measured unit values. Correlation coefficients ($r$) are indicated with numeric values, while significance levels ($p$) are denoted by asterisks (* $< 0.05$, ** $< 0.01$, *** $< 0.001$).
Influence of a single environmental factor on CIs and BTs in *P. viridis* as well as other marine bivalves (Gosling, 1992).

In our study, it was observed that temperature, turbidity and current speed were negatively correlated with CIs and BTs of the *P. viridis* (Fig. 7). In the southeast coast of Bangladesh, during pre-monsoon and monsoon seasons (April to September), heavy rainfall and suspended particles bearing freshwater runoff created high current speed (≥0.5 ms⁻¹) and turbidity (>300 NTU) together with high temperature (>30 °C), which are all above the recommended optimum level of temperature 27 °C-30 °C (Aypa, 1990), turbidity <150 NTU (Aypa, 1990) and current speed 0.1–0.3 ms⁻¹ (Lovatelli, 1988) for green mussel. In fact, water temperature act as a crucial factor in eco-physical performance of mussel (Sokołowski et al., 2010). In mussels, growth and CIs increased in a linear fashion between 5 and 20 °C, but declined sharply above 20 °C (Almada-Villela et al., 1982). Temperatures above 20 °C (Incze et al., 1980) have been found to decrease the growth and CI in the blue mussel *Mytilus edulis*. Moreover, CIs are largely affected by the reproductive cycle and feeding activity, which are also temperature-dependent. In *P. viridis*, temperatures below 30 °C have shown to enhance filtration rate and significantly increase their CI (Rajagopal et al., 1998; Power et al., 2004). In the southeast coast of Bangladesh, gonad development of *P. viridis* initiated and induced by the falling of temperature from its higher value of above 30 °C, which ultimately led temperature to be negatively correlated with CIs and BTs (Asaduzzaman et al., 2019).

Excessively high current speeds, however, negatively influenced CIs and BTs of *P. viridis* mainly by inhibiting feeding activity. The underlying mechanism of negative correlation with current speed is believed to be a build-up of a pressure differential between inhalant and exhalant apertures that interferes with feeding (Wildish and Kristmanson, 1985). Similarly high turbidity negatively influenced the CIs and BTs mainly by exerting their influence on food availability and feeding activity of *P. viridis*. High turbidity often results in low primary productivity due to
limited light penetration, which causes insufficient supply of food and failure of the filtering activities of *P. viridis* (Lovatelli, 1988). In contrast, salinity and dissolved oxygen were positively correlated with CIs and BTs (Fig. 7), indicating higher values of these factors positively influenced physiological mechanism of *P. viridis*. As a marine water mussel species, *P. viridis* requires high salinity of 27–35 ppt for optimum growth (Rajagopal et al., 2006; Tan and Ransangan, 2014). In a previous study, it was observed that CI of Asian wedge clam, *Donax scortum* was also influenced by the salinity and dissolved oxygen as demonstrated by the positive correlation between them (Singh, 2017). It was also observed that high salinities (25–32 ppt) increased the filtration rate (Rajesh et al., 2001) as well as stimulate gametogenesis and spawning in *P. viridis* (Rajagopal et al., 2006; Tan and Ransangan, 2014; Asaduzzaman et al., 2019).

Food availability has consistently been shown to be the most important factor in determining bivalve growth and reproduction in both hatchery and wild populations (Utting, 1988; Smaal and van Stralen, 1990). As other suspension feeding bivalves, a variety of planktons with different shape and size contribute as main component in the diet of *P. viridis* (Lopes-Lima et al., 2014; Tan and Ransangan, 2017).

Consistently, we observed that chlorophyll-a and plankton abundance were positively correlated with the CIs and BTs of *P. viridis* (Fig. 7). In the wild marine bivalves, CIs and BTs have been shown to be positively correlated with phytoplankton abundance. For example, growth and CI of *M. edulis* on an offshore platform off the Californian coast positively correlated with chlorophyll-a concentrations and variations in phytoplankton abundance (Page and Hubbard, 1987), while growth of *Crassostrea gigas* on nets at ten different locations correlated with chlorophyll b concentrations (Brown, 1988). Similarly, poor growth and CI of mussel have been documented in lower level of chlorophyll-a (Ren and Ross, 2005) and plankton abundance. Ingestion of high phytoplankton biomass usually results in faster growth and increase in condition of *P. viridis* (Ren and Ross, 2005). In our study, it was also observed that CIs and BTs were positively correlated with the quantity of ingested plankton in the gut (Fig. 7). As allometry and CIs were closely associated with the reproductive cycle, the positive correlation with the quantity of ingested plankton further suggest that *P. viridis* ingested higher quantity of planktons to meet the increasing energy demand during their crucial gametogenesis stages (Tan and Ransangan, 2017; Asaduzzaman et al., 2019). Previous studies confirmed that *P. viridis* adopted opportunistic strategies to build up their gonads using energy from the available food (Lopes-Lima et al., 2014; Tan and Ransangan, 2017; Asaduzzaman et al., 2019).

### 4.4. Interrelations among CIs, BTs, and eco-physiological factors depending on seasonality

CIs and BTs of marine bivalves are largely influenced by seasonality, environmental variables, food availability, feeding activity, reproductive strategies and other physiological conditions (Bauer, 1983; Ravera et al., 2007). Although individual or combined influence of these eco-physiological factors are well documented, a comprehensive study by integrating all of these factors to provide a deeper knowledge about CIs and BTs has been rarely documented in *P. viridis*, even for other
marine bivalve species.

During May to September, water temperature raised more than 30 °C and coupled with heavy rainfall and freshwater run-off in the southeast coast of Bangladesh. Heavy rainfall and freshwater run-off carried large amount of suspended sediment particles that result in increased turbidity (>300 NTU) and current speed (>0.5 ms⁻¹) above the recommended optimum level (27–32 ppt) due to the dilution effects by huge freshwater runoff (Asaduzzaman et al., 2019). During these periods, high turbidity and lower nutrients concentrations result in decreased abundance of food availability in the southeast coast of Bangladesh. Due to the unfavorable environmental condition, P. viridis adapted to maintain gonadal resting stage during these periods, which ultimately resulted in poor CIs and BTs as revealed in this study (Figs. 2 and 8).

In contrast, environmental conditions started to shift during post monsoon (October to January) season as revealed temperature started to fall down with occasional and almost no rainfall and surface run-off. Water turbidity and current speed decreased, while salinity increased towards the optimum levels required for growth and reproduction of P. viridis. Due to the concentration effects, nutrients abundance and increased food availability, P. viridis had an opportunity to ingest more amounts of plankton. During these periods, gonad development and maturation were triggered by a set of environmental conditions, food availability and plankton ingestion (Fig. 8). The accumulation of gametes in follicles resulted in bulkiness of the gonad, which increased CIs and BTs in green mussel during the months of October to January (Fig. 8). From January to April, environmental factors triggered to release the gametes from the follicles, and emptying of the gonad, which resulted in falling the CIs and BTs parameters of P. viridis.

5. Conclusions

In the present study, we used a multivariate approach to provide an extensive understanding about the allometry, CIs and BTs of P. viridis cultured in the southeast coast of Bangladesh. The integrated approach revealed that CIs and BTs of P. viridis are strongly interlinked and influenced by the seasonal variation of a set of environmental factors, food availability, feeding behaviors, and cycle of gonadal development. This innovative research provides a model toward a better understanding about how CIs, BTs and eco-physiological factors are interrelated among each other in P. viridis and their culture environment. The findings of the present study from these broad datasets represent a step toward a better comprehension of the CIs and BTs of the species and would be helpful for sustainable commercial exploitation of P. viridis from the aquaculture farms as well as the wild environment.

Author contributions

Conception and design of study: M.A. Acquisition of data: A.R.N., A.S., S.A. and N.F.H. Analysis and/or interpretation of data: M.A. and M.M.R. Drafting of the manuscript: A.R.N., M.A., A.T., S.A.N. Revising the manuscript critically for important intellectual content: M.M.R., M.A.W., M.N. and M.J.R. Funding acquisitions and project management: M.A., A.S., M.A.W, M.N. and M.J.R. All authors approved the final version of the manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi:https://doi.org/10.1016/j.aqrep.2020.100562.

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