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Effect of environmental variables on the growth of Asian green mussel *Perna viridis* (Linnaeus, 1758), in two different aquaculture systems in Goa, west coast of India

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ABSTRACT

Asian Green mussel *Perna viridis* (Linnaeus, 1758), is a bivalve mollusc with high market demand along India's western coast, specifically in Goa. An experiment was conducted to compare the environmental variables, specific growth rate (SGR) and length-weight progression of the species raised in two different coastal aquaculture systems; a semi-enclosed water body (SEW) and an open-water system (OWS). The variables chlorophyll-*a* and plankton density were high in SEW and these variables were positively correlated with the growth rate of the species. The '*b*' value of the length-weight relationship and SGR were found high and allometric in nature in SEW. The prediction of SGR using the generalised linear model has indicated that plankton density and nitrate level are the variables that influence the growth of *P. viridis*. Thus, being a predominant filter-feeder on plankton, the high plankton density channeled through the rich nutrients and chlorophyll content might have triggered the growth of mussels in SEW. India is blessed with many semi-enclosed coastal water bodies lying unutilised, and the results indicate that there is scope for mussel culture in these systems, which will provide a source of secondary livelihood for the coastal fishers.

Keywords: Bivalves, Coastal aquaculture, GLM, Molluscan culture, Zuari

Introduction

The Asian green mussel *Perna viridis* (Linnaeus, 1758), under the family Mytilidae is a coastal bivalve mollusc and a keystone species indigenous to the Indo-Pacific region (Baker *et al*., 2012). It is widely distributed in the intertidal zones and subtidal ecosystems along the west coast of India. High growth rate, natural abundance, filter-feeding efficiency, adaptability to new environments and simple culture techniques make *P. viridis* an ideal mollusc species for coastal aquaculture in India.

Vakily (1989) as well as Kripa and Mohamed (2008) reported on culture trials of *P. viridis* in various ecosystems like estuaries, semi-enclosed bays and open-sea with encouraging growth rates. Being a filter feeder, *P. viridis* strains water through a set of gills to retain food items such as phytoplankton, zooplankton and other organic materials (Cosling, 2003). The abundance of phytoplankton results in the fast growth of *P. viridis* (Ren and Ross, 2005). Besides, the growth of these organisms also depends on the changes in the environmental variables such as turbidity, temperature, salinity and dissolved organic matter. Literature substantiates the effect of environmental conditions of different culture systems on the growth of *P. viridis*. Therefore, it has been recommended that the environmental variables need to be analysed before starting culture of mussels (Lovatelli, 1988; Frechette and Grant, 1991; McQuaid and Lindsay, 2000).

The morphometric relationship between length and weight is a suitable index for comparing the growth and production traits of *P. viridis* in different culture systems (Moutopoulos and Stergiou, 2002). The length-weight relationships (LWR) in bivalves are generally allometric (Vakily, 1989; Lok *et al.*, 2006; Hemachandra and Thippeswamy, 2008; Sundaram *et al.*, 2011; Thejasvi *et al.*, 2013). The specific growth rate (SGR) is another critical index that compares growth in length and weight for species in aquaculture experiments (Hopkins and Leach, 1992). Thus, studies on the LWR and specific growth rate (SGR) would help to understand the suitability and growth efficiency of *P. viridis* in various aquaculture systems. However, the growth and production of *P. viridis* under different coastal aquaculture systems mediated by environmental variables are still unclear. Considering all the above facts, a comprehensive study was conducted to

evaluate the growth of *P. viridis* and correlate the effects of environmental variables on growth rate in two different aquaculture systems *viz.,* a semi-enclosed water body and an open-water body in coastal ecosystems.

Materials and methods

Site selection and mussel culture

The culture experiment of *P. viridis* was conducted at two sites in the Zuari Estuary, Goa: Batim creek as an open-water system (OWS), which is directly connected to the main estuary and a semi-enclosed water body (SEW); channeled from the main estuary through a sluice gate (Fig. 1 and 2) in Goa Velha.

The aquaculture experiment was carried out in rack structures of 5 x 5 m size constructed with bamboo poles approximately covering an area of 600 m^2 with an average depth of 1.5 m. Mussel spats (seeds) with an average size of 28 mm were procured from the intertidal regions of northern Kerala (Kasaragod) and were used for seeding the ropes. Seeding of mussels was done manually in pre-stitched bags made with a muslin cloth and the core material used was nylon rope of 1 m length. Each bag with 1 kg of mussel spat and seeded strings were suspended from bamboo poles of the rack structure. The culture period was from November to May, where two trials were conducted in each system from 2014 to 2016.

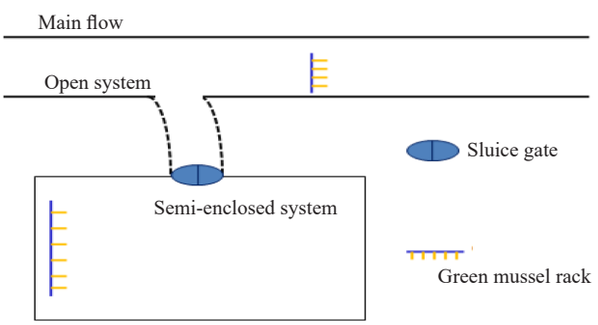

Fig. 1. Schematic diagram of the culture sites

Sampling and data collection

A total of 30 numbers of *P. viridis* were sampled every month from each culture system. The total length (cm) and total weight (g) of individual mussels were measured using a vernier caliper (0.01 mm accuracy) and digital weighing balance (0.01 g accuracy), respectively. The length was measured from the tip of the umbo to the posterior margin of the shell. Water and sediment samples were collected monthly from each culture system in triplicate. Parameters like salinity (SALINITY in ‰), temperature (TEMP in °C), pH, depth (DEPTH in m), electrical conductivity (EC in mS cm^{-1}), ammonia-nitrogen (AmN in mg l^{-1}), nitrite-nitrogen (NITRITE in mg l^{-1}), nitrate-nitrogen (NITRATE in mg l^{-1}), available phosphorus (AP in mg l^{-1}), total suspended solids (TSS in mg l^{-1}), dissolved organic matter (DOM in mg l^{-1}), dissolved oxygen (DO in mg l^{-1}), biochemical oxygen demand (BOD in mg l^{-1}), chlorophyll-a (CHLA in µg l^{-1}) and plankton density (PD in Nos. ml^{-1}) in the water samples were estimated as per APHA (2005). The PD was subjected to log transformation before statistical analyses. Available nitrogen (SEDAN in kg ha^{-1}), organic carbon (SEDOC in %), electrical conductivity (SEDEC in mS cm^{-1}) and pH (SEDPH) were estimated for the sediment samples as per APHA (2005).

Growth of P. viridis

For studying the growth of *P. viridis*, two indices were estimated: (1) Length-weight relationship (LWR) and (2) Specific growth rate (SGR).

LWR is generally determined using the linear regression analysis. The basic form of the LWR (Le Cren, 1951) is given in the following equation:

$$W = a\,xL^{b} \quad \cdots\cdots (1)$$

where W = Total weight (g); L = Total length (cm); a = Intercept and b = Slope.

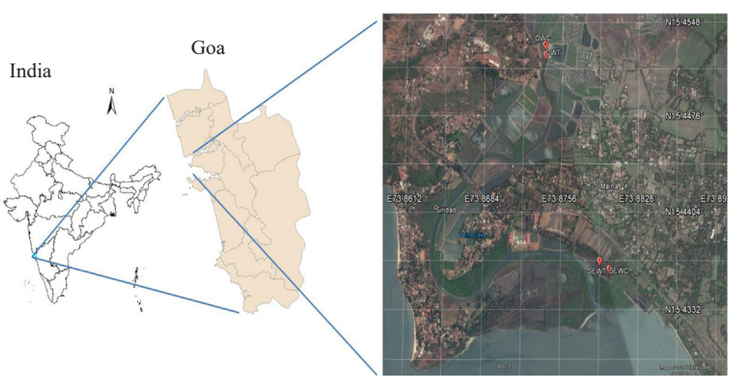

Fig. 2. Map showing the mussel culture sites

The slope 'b' is an exponent indicating isometry in the relationship. Since this is a non-linear model, the length and weight data were log-transformed to run the linear regression. This is represented in the logarithmic equation as:

$$\ln(W) = \ln(a) + b \times \ln(L) \quad \text{..... (2)}$$

The 'b' value in the LWR was estimated every month for each culture system and a graph was plotted using MS EXCEL. Then, the pattern of growth (isometric or allometric) was determined using the plot.

SGR in length was calculated using the following formula (Hopkins and Leach, 1992):

$$SGR = \frac{\ln(L_t) - \ln(L_0)}{t} \quad \text{..... (3)}$$

where, L_t = Length at the time 't' after stocking and L_o = Length at stocking

The SGR was estimated every month for both the culture systems and a comparison plot was drawn. A two-way ANOVA was performed using the culture system and month as two factors to compare the significant difference in SGR between the two culture systems and across months. Further, Tukey's HSD (Linton and Harder, 2007) was used to determine the extent of the difference between the levels in these two factors.

Environmental variables

The environmental variables were measured monthly from the culture systems. Arithmetic mean values were estimated for these variables on a monthly basis and a plot was generated to compare the trends in the two culture systems. To compare these variables during different months and culture systems, a two-way ANOVA was executed using the generalised linear model (GLM) of SAS (2012).

Influence of environmental variables on SGR

To determine the correlation between environmental variables and SGR, Pearson's correlation analysis was carried out using the CORR procedure of SAS (2012). After considering the correlation between SGR and environmental variables and between the environmental variables, a selection was made for the independent (non-collinear) environmental variables, which may influence SGR. Therefore, a GLM was fitted for SGR with class effects (S_i) and independent environmental variables (x_i). Backward elimination method was used for the selection of the model using Schwarz's Bayesian information criterion (SBC) as the fit statistic. The GLM was fitted using the GLMSELECT procedure of SAS (2012).

$$SGR = a + S_i + \sum_{i=1}^{n} b_i x_i + \varepsilon_i \quad \text{..... (4)}$$

where a = Intercept, n = Number of independent environmental variables; b = Coefficient of environmental variables and Ɛ = Error.

Results

Mussel growth parameters

The month-wise regression of body weight on the total length of *P. viridis* was compared in SEW and OWS. The 'b' value ranged from 1.14 to 2.98 and 1.02 to 2.73 in SEW and OWS, respectively (Fig. 3). The 'b' value has shown an allometric growth pattern throughout the growth period in both the culture systems. The 'b' value increased during the initial half of the culture period and decreased in the latter half in both the culture systems. The SEW has shown higher 'b' values during the middle phase of the culture period and generally, it depicted high values compared to OWS throughout the culture period. In the middle phase of the culture period, the 'b' values were approaching the isometric growth pattern (b=3), while during the initial and final phases, the values were far from isometry (b=3). Thus, in both the culture systems, *P. viridis* followed negative allometric growth pattern (b<3) and 'b' value was comparatively higher in SEW than in OWS.

The SGR in terms of length was compared for significant differences between the culture systems and months (Fig. 4). Two way ANOVA indicated significant difference in SGR between the culture systems and months ($p<0.05$). The SGR was significantly higher in SEW compared to OWS. SGR declined from 1.09 to 0.15 in SEW and 1.08 to 0.10 in OWS during the culture period.

Environmental variables

Temperature showed an increasing trend from November to December and declined from January to February. During the last phase of the culture, from March to May, a gradual increase in temperate was noted (Fig. 5). The fluctuating trend of temperature was evident in both

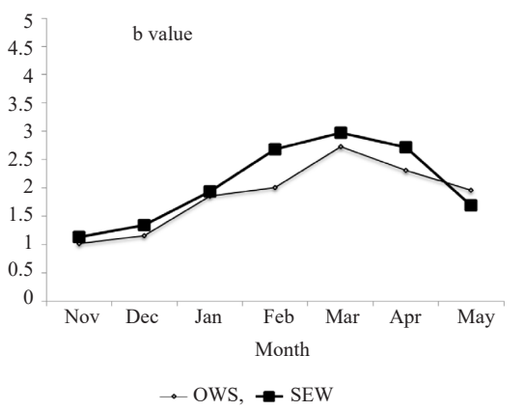

Fig. 3. Month-wise variation in 'b' value for the two culture systems

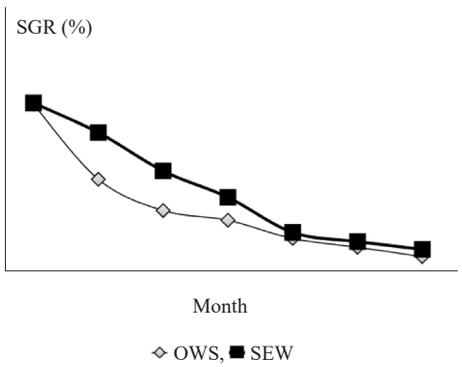

Fig. 4. Month-wise variation in SGR for the two culture systems

the culture systems. The salinity and EC for both the systems gradually increased from November to May. A negatively sloped curve was observed for DOM and nitrate concentration for both the systems; however, the latter variable increased during the last phase. In the case of TSS, an initial decline was followed by an increasing trend from January to February in OWS.

In the case of sediment organic carbon (SEDOC) an initial sharp increase was followed by a steep decline for SEW whereas the curve was flat for OWS in the last phase while sediment OC peaked for both the systems. For both the systems, BOD showed a flat curve till the last phase. The values of BOD increased towards the end of the culture period and maximum value was recorded in SEW. DOM and PD always stood high in SEW than OWS except in March. Depth showed a similar pattern throughout the culture period in both systems (Fig. 5). AmN followed a declining trend in SEW and a fluctuating trend in OWS. After the initial declining trend during November-December, sediment pH (SEDPH) increased gradually towards the end of the culture period in both the systems.

Comparison of environmental variables

The variables PD, BOD, DO, DEPTH, EC and SALINITY were significantly different between the culture systems. Further, the variables AmN, PD, BOD, DOM, DEPTH, EC, NITRATE, SEDAN, SEDEC, SEDOC, SEDPH, SALINITY, TSS and TEMP were significantly different across the months. CHLA and PD were high in SEW compared to OWS ($p<0.05$). However, the DEPTH was more in OWS in comparison with SEW.

Comparison of SGR and environmental variables in culture systems

Two-way ANOVA was carried out for data on environmental variables and SGR exclusively in the culture sites using the month and culture system as the

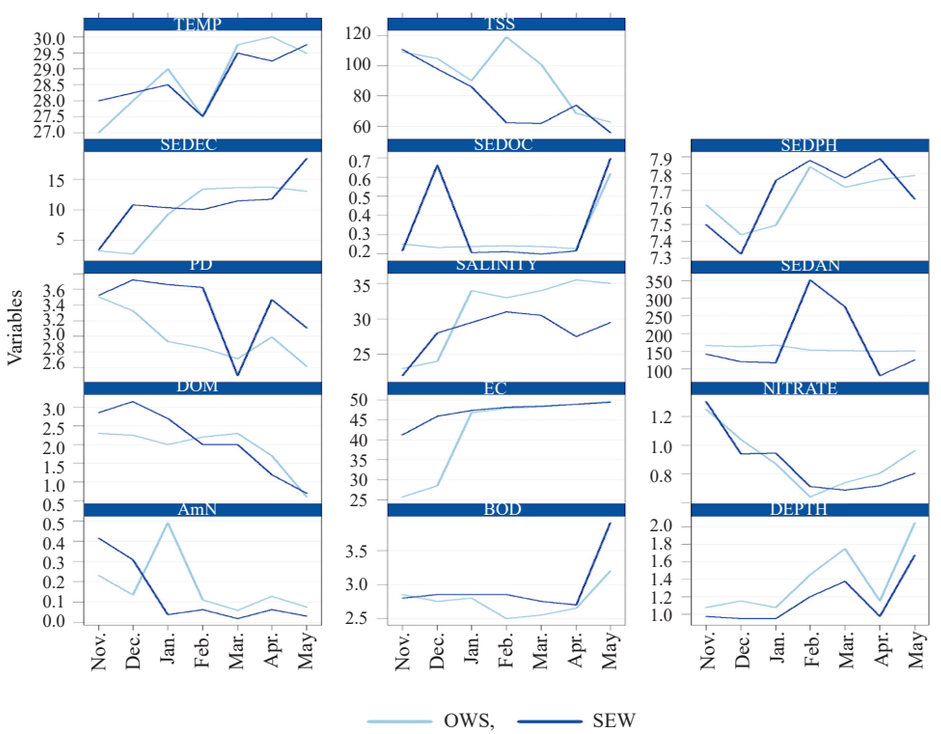

Fig. 5. Monthly patterns in selected environmental variables of water and sediment in the two different culture systems (The graphs were plotted only for variables that showed significant differences between months)

two factors. The variables like PD, BOD, DEPTH, NITRATE, NITRITE, SEDEC, SALINITY, TEMP and CHLA were significantly different across the months ($p<0.05$). Moreover, the variables like PD, DEPTH and CHLA were significantly different between the culture systems. SGR also exhibited variations across months and culture systems. Interestingly, PD, CHLA and SGR were higher in SEW than OWS. It is clearly understood that the parameters like SGR, CHLA, PD and DEPTH were high in SEW than in OWS. Tukey's HSD test showed that there was significant variation in SGR as well as the mean values of CHLA, PD, DEPTH and TEMP between the two culture systems (Table 1).

Influence of environmental variables on SGR

SGR was positively correlated with plankton density, chlorophyll a, dissolved organic matter, nitrate and sediment organic carbon (Table 2). At the same time, SGR showed a negative correlation with depth, salinity and temperature. PD indicated a positive correlation with DOM, SEDOC and CHLA, while it was negatively correlated with DEPTH, SEDEC, SALINITY and TEMP. DEPTH showed a strong negative correlation with CHLA. NITRATE in water showed a positive correlation with CHLA and SEDOC showed a strong positive correlation with CHLA. After considering the correlation between SGR and environmental variables and also between the environmental variables, PD and NITRATE were selected as the unrelated environmental variables which influenced SGR. From the ANOVA and subsequent Tukey's HSD, it is understood that SGR was significantly different between OWS and SEW. HenGLM was fitted for SGR with the system type as the class effect and PD and NITRATE were chosen as the constant variables under this background. The final model fitted is as follows:

$$SGR = -2.1894 + Si + 0.6729 *PD + 0.4604 *Nitrate \cdots\cdots\cdots (5)$$

where $S_i = 0.273$ in OWS, $S_i = 0$ in SEW with an SBC of 121.3

Table 1. Comparison of mean and standard error for SGR and environmental variables in different culture systems using Tukey's HSD test

Variable	OWS	SEW
SGR	0.411±0.13[b]	0.53±0.14[a]
Water parameters		
AP (mg l^{-1})	0.59±0.24[a]	0.25±0.04[a]
AmN (mg l^{-1})	0.16±0.04[a]	0.13±0.06[a]
BOD (mg l^{-1})	2.90±0.14[a]	2.96±0.18[a]
TSS (mg l^{-1})	95.14±8.28[a]	74.20±12.36[a]
DOM (mg l^{-1})	1.76±0.21[a]	1.97±0.36[a]
TEMP (^{0}C)	28.64±0.50[a]	28.86±0.28[a]
DO (mg l^{-1})	4.81±0.17[a]	4.77±0.06[a]
CHLA (ug l^{-1})	2.43±0.10[b]	2.95±0.22[a]
SALINITY (‰)	28.86±1.32[a]	31.57±2.01[a]
DEPTH (m)	1.43±0.15[a]	1.14±0.11[b]
EC (mS cm^{-1})	40.31±4.48[a]	46.97±0.99[a]
NITRATE (mg l^{-1})	0.83±0.09[a]	0.82±0.09[a]
NITRITE (mg l^{-1})	0.32±0.03[a]	0.29±0.02[a]
PD (Nos. ml^{-1})	2.89±0.05[b]	3.48±0.06[a]
Sediment parameters		
SEDAN (kg ha^{-1})	155.98±2.41[a]	165.07±34.58[a]
SEDEC (mS cm^{-1})	9.17±1.67[a]	8.69±1.08[a]
SEDOC (%)	0.31±0.09[a]	0.43±0.14[a]
SEDPH	7.68±0.07[a]	7.67±0.08[a]

Discussion

The value of morphometric relationship has been used to compare the dimensional growth of the same species in varied environments (Hemachandra and Thippeswamy, 2008). In this study, 'b' value varied from 1.14 to 2.98 and 1.02 to 2.73 in SEW and OWS, respectively. The 'b' value exhibited an allometric growth pattern throughout both systems' culture period. Reports state that values of 'b' commonly range between 2.5 and 2.8 in the species (Vakily, 1989). However, a highly negatively allometric value of 1.3 for 'b' was reported by Chatterji *et al.* (1984). During the present study, in both the culture systems, *P. viridis* followed negative allometric growth pattern ($b<3$) and 'b' value was significantly higher in SEW than in OWS. Moreover, the common range of 2.5 to 2.8 for 'b' value was observed during the middle phase of culture in both the systems. There was a significant difference in SGR between the culture systems and across the months. The SGR was significantly high in SEW in comparison with the OWS. SGR was high in the initial phases of culture and decreased towards the end of the culture period. Similar results have been reported (Rivonkar *et al.*, 1993) and it was explained that the variation in SGR could be due to the metabolic activities of the individual mussel since the high values of SGR coincided with the high rates of metabolic activities in the earlier stages. Food availability and quality were the main factors affecting growth rate in mussels (Lok *et al.,* 2007; Celik *et al*., 2009). The SGR of *P. viridis* was reported as 1.03% in February which declined to -0.23% in March 2019 during 7-month culture period in Integrated Multitrophic Aquaculture system at Philippines (Melendres, 2021). Mohamed *et al.* (2003) compared the mean SGR for length and weight of mussels with respect to different seeding materials, using Duncan's multiple range test and found that there was no significant difference in the SGR using different treatments.

Environmental factors including quantity and quality of food principally affects growth in marine

Table 2. Pearson's correlation matrix for SGR and environmental variables

	AP	PD	AmN	BOD	DOM	DO	DEPTH	EC	NITRATE	NITRITE	SEDAN	SEDEC	SEDOC	SEDPH	SALINITY	TSS	TEMP
AP	0.37																
PD	0.82**	0.01															
AmN	0.61*	0.05	0.32														
BOD	-0.03	0.23	0.06	-0.05													
DOM	0.70**	-0.13	0.60*	0.58*	-0.51												
DO	0.65*	0.25	0.46	0.61*	0.03	0.42											
DEPTH	-0.64*	0.06	-0.70**	-0.47	0.36	-0.64*	-0.67**										
EC	-0.63*	-0.69**	-0.29	-0.23	-0.08	-0.24	-0.62*	0.25									
NITRATE	0.72**	0.49	0.44	0.45	0.27	0.23	0.48	-0.27	-0.56*								
NITRITE	0.04	0.58*	-0.22	0.006	0.22	-0.27	-0.04	0.15	-0.15	0.60*							
SEDAN	-0.004	-0.04	0.03	-0.15	-0.15	-0.07	0.05	0.009	0.03	-0.13	-0.2						
SEDEC	-0.85**	-0.31	-0.68**	-0.42	-0.09	-0.46	-0.75**	0.55*	0.78**	-0.73**	-0.007	-0.07					
SEDOC	0.84	0.33	0.60*	0.73**	0.03	0.61*	0.37	-0.42	-0.38	0.66*	0.18	-0.17	-0.56*				
SEDPH	-0.58*	0.02	-0.45	-0.69**	-0.32	-0.49	-0.66*	0.35	0.41	-0.42	0.16	0.27	0.61*	-0.57*			
SALINITY	-0.77**	-0.29	-0.76**	-0.31	-0.14	-0.4	-0.52	0.54*	0.62*	-0.58*	0.047	0.3	0.80**	-0.63*	0.53		
TSS	0.55*	0.19	0.3	0.60*	-0.2	0.53	0.47	-0.35	-0.45	0.34	-0.12	-0.41	-0.41	0.4	-0.4	-0.4	
TEMP	-0.67**	-0.43	-0.54*	-0.35	0.09	-0.39	-0.44	0.41	0.63*	-0.26	0.23	-0.11	0.52	-0.44	0.17	0.55	-0.62*
CHLA	0.86**	-0.06	0.90**	0.60*	-0.05	0.74**	0.5	-0.70**	-0.22	0.57*	-0.12	-0.11	-0.68**	0.77**	-0.58*	-0.71**	0.42

**Indicates correlation is significant at 1% level, *Indicates correlation is significant at 5% level

bivalves where the phytoplankton availability is the most important factor (Jones and Iwama, 1991; Manoj Nair and Appukuttan, 2003; Ren and Ross, 2005, Celik *et al*., 2009). The variables PD, BOD, DO, depth, EC and salinity were significantly different between the culture systems. Further AmN, PD, BOD, DOM, depth, EC, NITRATE, SEDAN, SEDEC, SEDOC, SEDPH, salinity, TSS and temperature were significantly different across the months. Chlorophyll-a and PD were substantially higher in SEW in comparison with OWS. The high plankton density with rich chlorophyll content might have triggered the enhanced growth rate in SEW. Nitrogen is usually considered a nutrient with potential to limit phytoplankton productivity in estuaries and coastal marine systems (Ryther and Dunstan, 1971). The temporal variability of phytoplankton and nitrate concentration are considered important factors that regulate the productivity of mussels in farms (Ogilvie *et al.*, 2000). In the present study, a general decrease in plankton density and nitrate concentration was observed as the growth of mussels progressed from November to May. The active plankton filtration of mussels in open water systems and farms has been reported in earlier studies (Vanderploeg *et al*., 1995; Noren *et al*., 1999; Ogilvie *et al*., 2000). Rejeki *et al.* (2020) reported that the amount of water that the mussels were actually exposed to ultimately restricted weight yield. Lekshmi *et al.* (2018) reported a seasonal variability in phytoplankton populations and its positive correlations with phosphate and nitrate concentrations in similar aquatic systems, like open and semi- enclosed coastal waters. The grazing activity of mussels could reduce the phytoplankton concentrations in shallow coastal areas and bay ecosystems (Wright *et al.*, 1982; Carlson *et al.*, 1984; Nichols, 1985). An earlier report on the culture of *P. viridis* in Goa illustrated that the minimum growth rate of the species coincides with low phytoplankton concentration (Qasim *et al.*, 1977).

Chlorophyll-a concentration is regularly used to assess phytoplankton biomass in the field and therefore, it can be used as an indicator of environmental conditions that control mussel growth (Ren and Ross, 2002). The GLM fitted for SGR in the study, selected plankton density and nitrate concentration as the explanatory variables. Hence, the availability of nitrate in higher concentrations will augment the growth of plankton and thereby increase the SGR in *P. viridis*.

The growth of cultured mussels depends on the type of culture system mediated through environmental conditions, including the physicochemical parameters of water and sediment (Celik *et al*., 2009). Rejeki *et al.* (2020) compared the effectiveness of green mussel (*P. viridis*) between longline culture and the traditional bamboo

stake method using different densities and concluded that cultivation method significantly affected SGR. In this study, *P. viridis* cultured in two different environments were varying in their growth rate. Results from this study also proved significant difference in environmental variables between the two culture systems. The growth rate of mussel was more in SEW than in OWS. There was a positive correlation of PD and chlorophyll content with the growth of *P. viridis*. Besides, PD and chlorophyll-a concentrations were more in SEW than in OWS.

The limiting nutrient nitrogen in the form of nitrate was found to positively influence the mussel's growth. Thus, it is inferred that the growth rate of mussels will depend on the environmental variables, specifically nitrate, chlorophyll-a and PD in the culture system and hence, site selection is an essential criterion for mussel culture. India is bestowed with a rich resource of productive semi-enclosed coastal water bodies which are lying unutilised. These areas can be utilised for mussel culture using wild seeds from the coastal ecosystems which would benefit coastal fishers as a secondary source of livelihood.

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