Vol. 15(2), pp. 10-20, July-December 2023
DOI: 10.5897/IJFA2023.0847
Article Number: C458FBB70969
ISSN 2006-9839

http://www.academicjournals.org/IJFA

International Journal of Fisheries and Aquaculture

Full Length Research Paper

Study on the Green Mussel, *Perna viridis* (L.) distribution, artificial spat collection, and raft culture along the Karwar Coast, Eastern Arabian Sea

Shivakumar Haragi[1]*, Prashant K. C.[2], Saavi N.[1], Rajeshree P.[1], Shreedevi H.[1] and Sreekanth GB[3]

[1]Department of Studies in Marine Biology, Karnatak University Dharwad, Post Graduate Centre, Karwar – 581303, India.
[2]Deputy Conservator of Forest, Department of Forest, Karwar, Karnataka. India.
[3]ICAR- Central Coastal Agricultural Research Institute, Old Goa, Goa – 403402, India.

Received 18 March, 2023; Accepted 16 June, 2023

Green mussel, *Perna viridis* is widely distributed along the Indian peninsula and considered as a nutritious delicacy. The growing demand for edible mussels prospered the farming practices along the Indian coast for which seeds termed "Spats" are derived from nature. In this study, naturally distributed green mussels' beds were surveyed along the intertidal and estuarine regions across the Karwar coast. Subsequently, artificially spat collections were carried out using low-cost floating longline spat collectors. This study reports an approximate 350 m^2 area of potential mussel beds with an average seed abundance of 406.66 no./m^2 from the intertidal and estuarine regions. Artificial spat production varied from 193 to 110 no./m rope and there is no statistical significance between the spat collecting ropes (*P*>0.05) wherein, a statistical significance was observed between the spat collecting sites (*P*<0.05). Mussel raft culture was successfully carried out along the backwater of Ankola with a total production of 420 kg/10 rafts during the 137 days culture period. Overall, this article details the suitability of Karwar Coast for large-scale farming of green mussels.

Key words: Estuary, Green mussel, Mariculture, raft culture, spat collection.

INTRODUCTION

Asiatic green mussel, *Perna viridis* (L.) is an edible marine bivalve with an exquisite source of protein, essential minerals, and vitamins (Chakraborty et al., 2016). Mussels belong to the family Mytilidae, characterized by narrow elliptical fan-shaped shells of equal size, without prominent hinge teeth and anterior adductor muscle besides the presence of byssal threads for anchoring (Siddall, 1980). Mussels generally inhabit the mid-littoral to sublittora zones along the coastal rocky stretch, Islands, intertidal and subtidal regions, and across the high saline estuarine layouts of tropical and subtropical; areas of Indo-Pacific regions (Soon and Ransangan, 2017). Their diet is mainly composed of phytoplankton, ingested through the filter-feeding process around them (Tan and Ransangan, 2016).

The growth rate of mussels can reach up to 10mm per month and is rapid during the juvenile stages (Vural et al., 2015). Green mussels occupy a prime position in

*Corresponding author. E-mail: shivakumarhb@kud.ac.in. Tel. 919663037103.

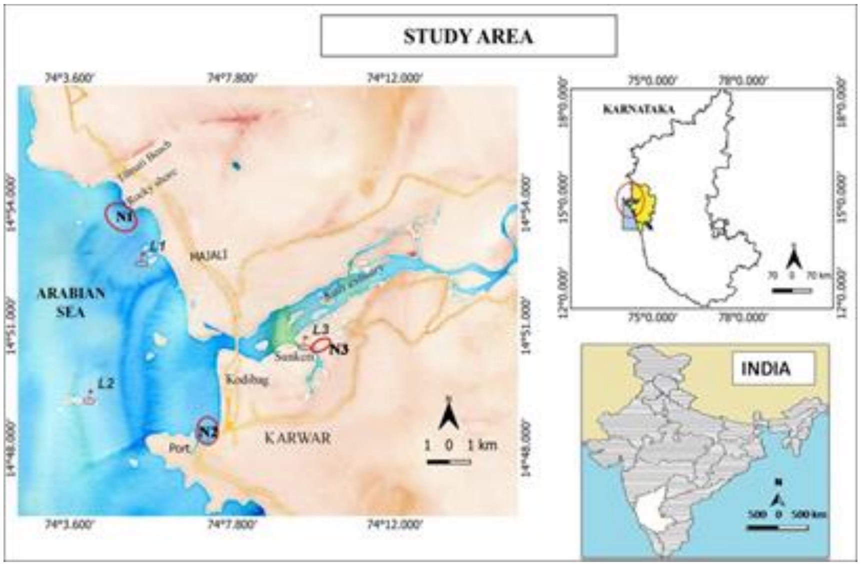

Figure 1. Study site, N representing the native mussel bed and L representing the long line spat collection zones, along the Karwar coast, Karnataka.
Source: Quantum Geographic Information System (QGIS Software version - 3.22.2)

mariculture due to their successful settlements, low production cost, and less environmental impact (Isnain et al., 2020). Moreover, their rapid growth, less predation, high fecundity, early maturity, and the ability to withstand extreme environmental variations are farming features (Eunice et al., 2018). Mussel forms a significant fishery on the Indian coastline and contributes nearly 68% to the total bivalve production with a harvest of 11435 tons during the year 2015 (Sasikumar et al., 2016). However, over the subsequent years, mussel productivity drastically declined along the Indian coast and production stoop to about 2000 tons in 2018 (Shinoj et al., 2021). The crop decline is attributed to combinations of factors and the prevalence of the protozoan parasite *Perkinsus olseni* (Shamal et al., 2018). Green mussel farming in India is mainly centered along the Malabar regions of Kerala coast (Mohammed et al., 2019) and on a limited scale along the southern coast of Karnataka. Hence, multi-location farming trials are required across Karnataka's northern coast to ensure the suitability of the site. Moreover, the seed required for culture is presently derived from naturally distributed mussel beds, hence, exploration of the naturally distributed *P. viridis* and their regional reproductive biology are pivotal in endorsing mussel farming. Though seeds are available along the coast, small-sized seed collection from the subtidal zone through skin diving is laborious and perilous (Sasikumar et al., 2016). Therefore, artificial mussel spat collections are complementary to the farming sector. Various methods of spat collection were successfully demonstrated from the coastal shelf region among polypropylene rope spat collection by Sasikumar et al. (2016) and longline and bamboo stakes by (Sagita et al., 2017; Rejeki et al., 2020) are found to be efficient. In this context, in the present study, potential native mussel beds were surveyed along the intertidal and estuarine regions of the Karwar coast. Subsequently, artificial spat collections of green mussels were carried out using the low-cost floating longline spat collector. Further, a piolet scale green mussel raft culture was executed to determine the feasibility of the site along the Karwar coast, Eastern Arabian Sea.

MATERIALS AND METHODS

The present study carried out along the Karwar coast, Arabian Sea, West coast of India, and its adjoining River Kali estuary and backwaters of Bela Bandar Ankola, Karnataka, India (Figure 1).

Potential mussel bed survey

A naturally distributed *Perna viridis* (L.) habitat (N) survey was conducted along the intertidal areas of Karwar and adjoining Kali estuary, West coast of India. Periodical visits were made to survey the intertidal and estuarine regions to observe the spat settlements during the study period. The approximate area of the mussel bed and their spatio-temporal distributions were recorded covering the 13km coastline (Figure 1).

Spat collection

Wild spat collection was carried out along the coastal shelf and the adjoining Kali estuary, Karwar for the period of nine months from September 2021 to May 2022. Spat collection sites were determined based on the standard conditions mentioned by Sasikumar et al. (2016) and the following sites were selected, 1) subtidal zone - Dandebag (L1) representing an average depth of

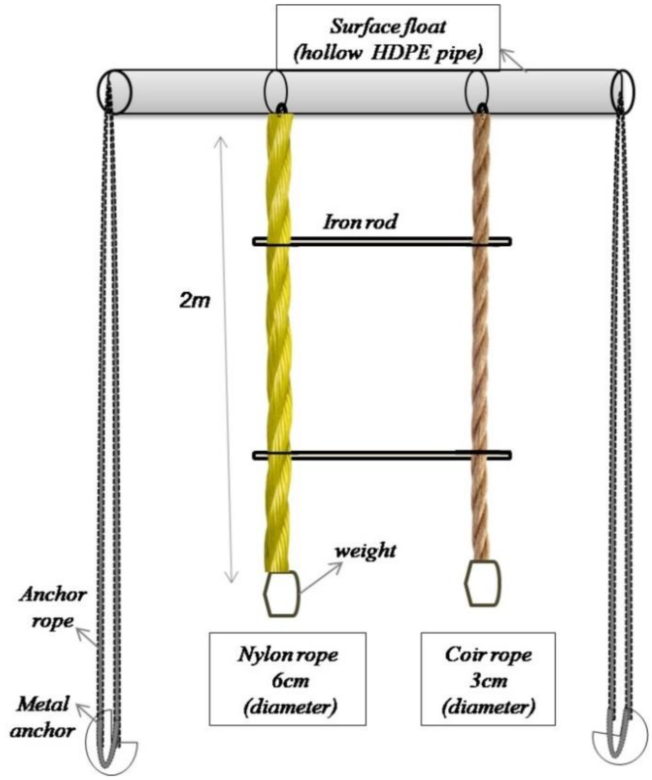

Figure 2. Design of Low-cost spat collector**.**
Source: Authors

5.0m, subsequently, 2) inshore zone - Devgad Island (L2), 12 nautical miles away from the coastline with a depth of 16.0 m and 3) Kali estuary (L3) representing a depth of 2.0m during low tide (Figure 1). A low-cost artificial spat collector was designed to suit the open sea and well estuarine spat collection during the study which consisted of a vertically suspended floating longline with different spat collectors (spacing a distance between the rope was 0.2m), such as a nylon rope diameter of 6.0cm and coir rope 3.0 cm. All spat collector ropes measured a length of 2.0m and ropes were tied with an iron piece in between for stability (Figure 2). Longline spat collectors were deployed on a monthly basis from August 2021 to April 2022 and positioned with a 50kg metal anchor. Later spat collectors were retrieved once in two months to check the spatio-temporal settlements of wild mussel spats and the total spat counted individual no./m according to the method of Sagita et al. (2017). Parallelly, during each spat collection event environmental variables (Figure 5) temperature (°C), pH, dissolved oxygen (mg/l), and salinity (‰) were measured in situ using a hand-held multiprobe instrument (SD400 Oxi - Lovibond). Seawater samples were procured from the spat collection sites to the laboratory in the ice-cooled box to determine Chlorophyll-a (mg/m^3) and Calcium (mg/l) contents (Figure 6) within 24 h by following the Parson et al. (1984) and standard protocol of APHA (2005). Karwar inshore sea surface water current (Figure 7) data were procured from the Indian National Centre for Ocean Information Services (INCOIS), Hyderabad, India.

Statistical analysis

The obtained data for water parameters were grouped seasonally according to the sample sites. Similarly, the data collected for spat collections were categorized seasonally with the variable of the site and spat collector rope. The data were subjected to Analysis of Variance (ANOVA) followed by Tukey's post hoc comparison test for specific significance ($P \leq 0.05$) between spat collection sites. The statistical analysis was carried out through SPSS (Statistical Package for the Social Science ver. 22).

Mussel raft culture

On a pilot scale mussel raft culture was carried out at the backwaters of Bela Bandar (14°39'39" N 74°17'79" E), Ankola (Figure 3) with the logistics support of the Coastal and Marine Ecosystem Cell, Department of Forest, Karwar Division. The backwater area is well protected and sheltered by mangrove vegetation, with minimum influence of wind and wave action, and additionally, there is no intense fishing activity across the farming site. The experiment site had an average depth of 2m during the low tide hence; the floating raft culture method was employed so as to provide a 1m floor distance to the hanging rope. Environmental parameters such as dissolved oxygen, pH, temperature, salinity, and turbidity, were found to be in the ambient conditions as semidiurnal tide significantly exchanges the seawater. Ten rafts (3 x 3m) were fabricated out with a *Casuarina* pole as described by Mohamed (2015). The buoyancy of the raft was maintained with the sealed emptied synthetic barrels of 200 l capacity. Rafts were positioned at the suitable site with retrievable concrete anchors. One unit of raft loaded with 35 nylon ropes of 1m length. *P. viridis* seeds collected from natural beds and through spat collections measuring a size of 15-25mm size were thoroughly cleaned. Approximately 0.7 to 0.8kg (103 ± 15.3 no.) seeds were loaded per 1m rope and secured around the rope by stitching knitted cotton

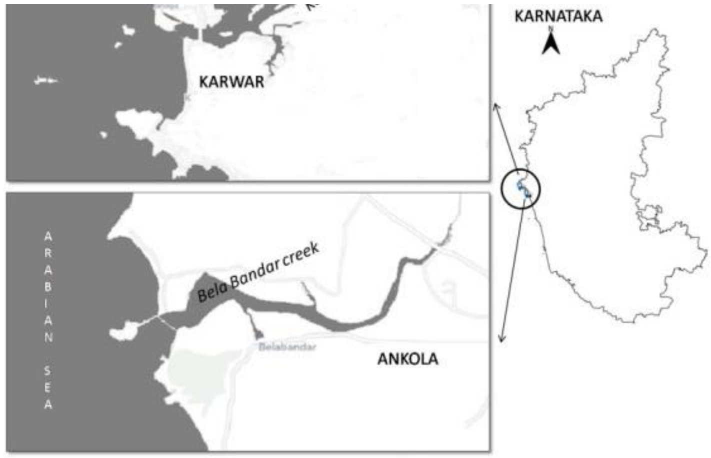

Figure 3. Locations of mussel's raft culture along the Karwar coast, Karnataka.
Source: QGIS 3.22.2

Table 1. Potential natural *Perna viridis* distributions along the Karwar coast, Karnataka.

Zone/sites	Estimated extended mussel bed (m^2)	Nature of seed bed			Longitude and Latitude
		POM	PRM	Habitat	
Majali (N1)	70	✓	✓	IT	14°53'54.80"N ;74°05'07.04"E
Aligadda (N2)	30	✓	-	IT	14°48'14.77"N ;74°07'19.07"E
Kali Estuary (N3)	250	✓	-	Estuary	14°50'16.37"N ;74°10'02.04"E

N- natural green mussel bed, POM- Post-monsoon, PRM- Pre-monsoon, IT-inter tidal.
Source: Authors

gauze. Mussel culture was carried out for a period of four months. The mussel production (P) per meter was derived according to Loo and Rosenberg (1983). Subsequently, the mussels' total shell and meat weights were recorded monthly according to the method Rivonker et al. (1993). The standard Linear Regression Equation was applied to correlate the growth parameter. The Physicochemical parameters of the culture site were recorded simultaneously over the period of culture.

RESULTS

The naturally distributed mussel beds across the Karwar coast were explored and mapped in this study. Two prominent sites along the intertidal zone and one site along the estuary were demarcated for the presence of a natural mussel distribution (Table 1). The potential intertidal mussel beds located along the Karwar coast are at Majali (N1) and Aligadda (N2) rocky shores (Figure 1) and the spat beds extending to an area of 70m^2 (400 ± 28 no/m^2.) and 30m^2 (260 ± 19 no./m^2) respectively, wherein, 260m^2 area mapped (530 ± 12 no./m^2) along the Kali estuary (Table 1). The natural green mussel settlements along the intertidal zone were observed from the post-monsoon (October) season which extended up to the pre-monsoon (February) period. The spawning season was observed from August to September months followed by the second spawning during the January to February month along the inshore region of the Karwar coast. Along the Kali estuary, mussel settlement was observed from the late post-monsoon (December) period.

Spat collection

The average green mussel spat settlement on the coir rope spat collector varied from 134.0 no./m (± 77) at L1 and 149.0 no./m (± 83) along the L2 inshore spat collection sites and 85.0 no./m (±43) at L3 estuary respectively. Similarly, nylon rope spat settlement ranged from 108.0 no./m (±67) at L1, 124 no./m (±77) at L2, and 68 no./m (± 36) at L3 sites. The spat fall onsets from Post monsoon season and extended up to March (Figure 4) along the inshore region. Wherein, spat settlement was observed from the late post-monsoon period (December) along the Kali estuary (L3). The spat collectors such as coir and nylon ropes were found to be suitable spat collectors without statistical significance ($P \geq 0.05$). The spat fall was higher at L2 compared to L1 and L3 sites

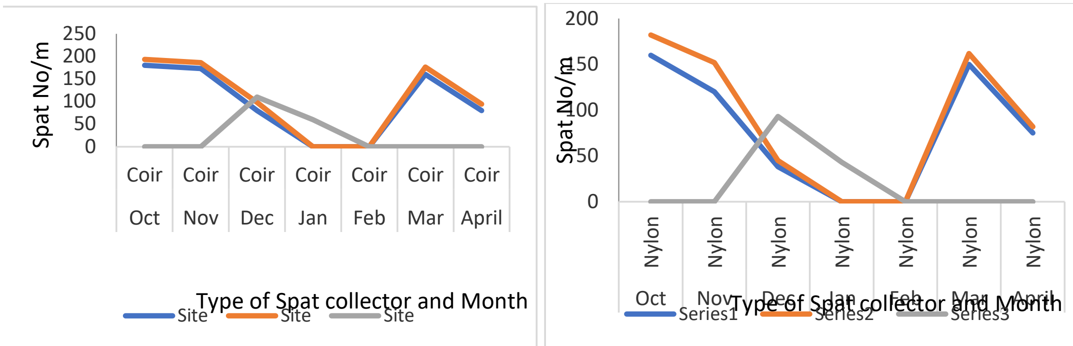

Figure 4. Month-wise average spat settlement on the spat collectors.
Source: Authors

Table 2. Fixed effect two-way ANOVA to evaluate the significant difference in artificial spat collection material and sites. Specific site significant difference through Tukey's Post-hoc test.

Parameter	Sum of squares	df	Mean square	F	p (same)
Rope	1974.86	1	1974.86	0.4437	0.5096
Site	47236	2	23618	5.307	0.009555
Interaction	413.143	2	206.571	0.04641	0.9547
Within	160219	36	4450.53		
Total	209843	41			
Tukey's Post-hoc					
	L1	L2	L3		
L1		0.9007	0.03688		
L2	0.9007		0.01274		
L3	0.03688	0.01274			

Source: Authors

and represented a statistical significance difference ($P \leq 0.05$). The significance observed between the site was analyzed through Tukey's post-hoc test and found to be L1 and L2 inshore sites are statistically significant ($P<0.05$) from the L3 estuary (Table 2). The spat size varied from 4 to 8 mm with an average weight of 30 to 60 mg. The spat settlement was higher from the surface to a depth of 1.5 m with peak density observed between the depths of 0.2 to 1.5 m.

Hydrological parameters

The physicochemical parameters during the spat collection were illustrated in Figures 5 and 6. The hydrological conditions along L1 and L2 represented typically open sea water features, wherein, L3 being an estuary marked salinity variations were observed. During the study period, the average dissolved oxygen represented 5.7mg/L (±0.28), pH 8.0 (±0.2), water temperature 27.9°C (±1.6), and Salinity 29.7ppt (±7.8) respectively. Station-wise (L1, L2 and L3), dissolved oxygen and water temperature were found to be non-significant (p>0.05) wherein pH and salinity exhibited statistically significant (p<0.05) differences. Comparatively season wise environmental parameters such as water temperature, pH, and dissolved oxygen did not exhibit variations, wherein, salinity represented remarkable variation with the lowest value of 12.0 ppt during the monsoon period and a maximum of 30.0 ppt during the pre-monsoon season with an average value of 20.0 ppt (±6.2) along the L3 spat collection site. Chlorophyll-a and Calcium values were higher along the L1 and L2 study sites, with an average value of 1.09 mg/m^3 (±0.2), and 393 mg/L (±30.6) respectively (Figure 6). Sea surface water current data along the inshore waters of Karwar coast varied from 0.9 to 6.5cm/s with an average value of 3.3 cm/s (±1.9) respectively. The sea surface water current along the Karwar coast was found to be higher during December and lowest during September month of

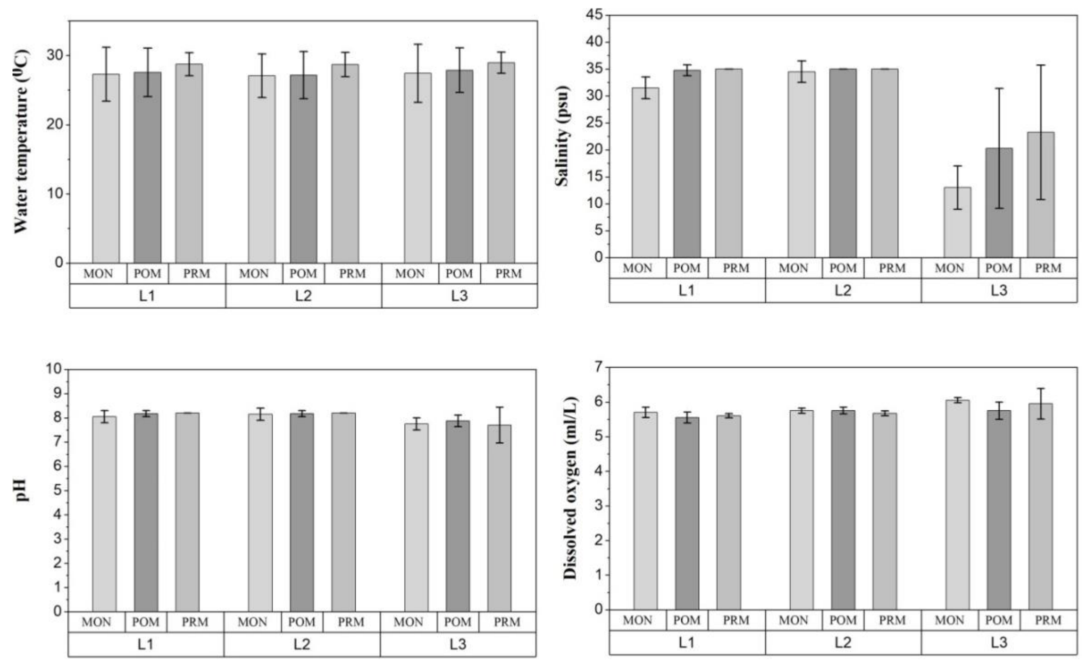

Figure 5. Environmental parameters studied during the spat collection period across the Karwar coast.
Source: Authors

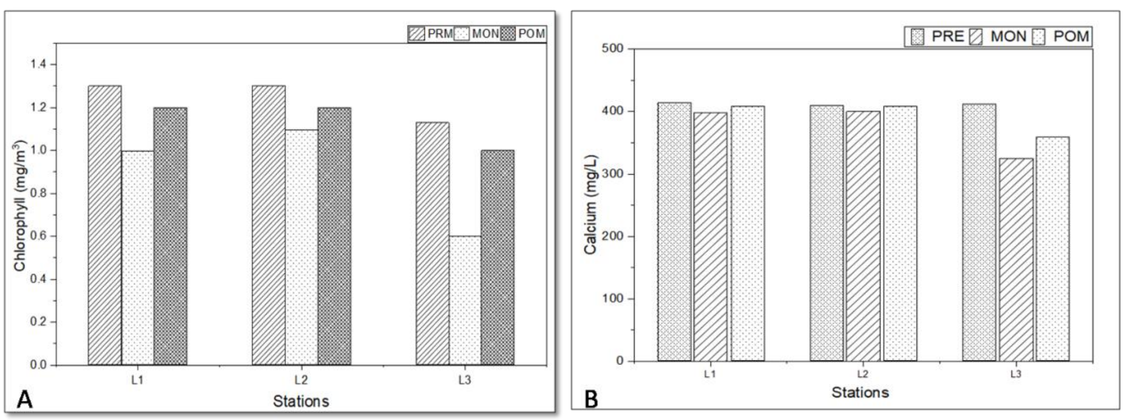

Figure 6. Chlorophyll-a and calcium concentration studied during the spat collection period.
Source: Authors

the study period (Figure 7).

Green mussel raft culture

The pilot scale mussels raft culture was carried out at the backwaters of Bela Bandar, Ankola from 6th January 2022, with a culture period of 137 days, a total of 420kg of mussels was harvested on 23rd May 2022 (Table 3). The maximum production from the single raft was found to be 158kg and the minimum was 25kg. Rope-wise production ranged from a minimum of 4.5kg to a maximum of 8.0kg and harvest size varied from 45 to 70 mm, with an average monthly growth of 10.1mm. The relationship between growth and culture period was found to be linear (b= 0.042) and represented a positive correlation (R^2=0.98) respectively (Figure 8A). The initial meat and shell growth of the mussels are coupled up to 100 days of the culture period, thereafter there was a tendency of an inverse relationship between shell and meat weight (Figure 8B).

The environmental parameters at the culture site were found to be ambient with an average dissolved oxygen of 5.2mg/L (±1.1), pH 7.8 (±1.3), and water temperature of

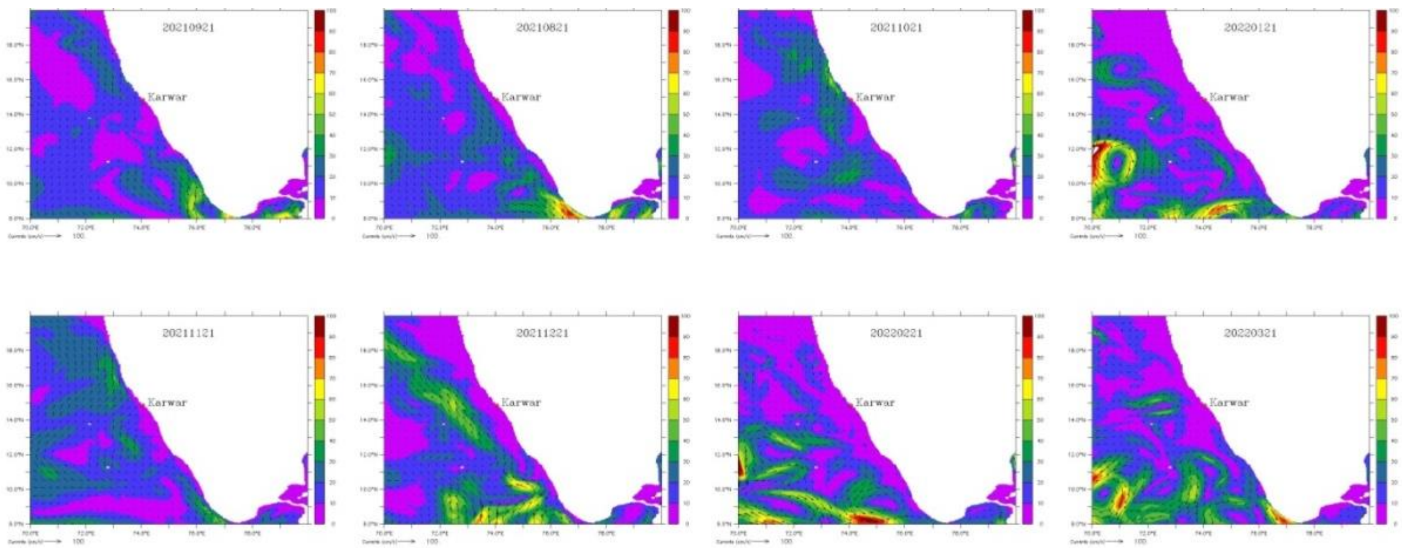

Figure 7. Spatial Sea surface water current (cm/s) from ROMS (Regional Ocean Modeling System) during the study period along the Karwar coast, West coast of India.
Source: INCOIS, Hyderabad, India.

Table 3. Details of *P. viridis* raft culture.

S. No	Descriptions	Site – Backwater Bela Bandar Ankola
1.	Number of raft	10
2.	Size of the raft	3 m x 3 m
3.	Date of seedling	6 January 2022
4.	No of rope/raft	35
5.	Length of seeded rope	1 M
6.	Weight of seed/m rope	700 to 800 gm
7.	Size of seed	15.0 to 25.0mm
8.	Date of harvest	23 May 2022
9.	Total production/raft	420 kg
10.	Maximum production/raft	158 kg
11.	Production/M rope (min/max)	4.5 to 8.0 kg
12.	Size at harvest (min/max)	50 to 70 mm
13.	Shell on weight (min/max)	11.26 to 17.2 gm
14.	Count of Mussels/M rope (min/max)	45 to 70 individuals
15.	Average salinity	25 ppt

Source: Authors

27.3°C (±4.2). Salinity did not show significant variations during the culture period representing a mean value of 25.3 ppt (± 3.2).

DISCUSSION

Green mussel *P. viridis* L. has emerged as one of the prime shellfish species farmed in India, offering income and employment security to coastal hamlets (Shinoj et al., 2021). Currently, mussel farming solely relies on the seeds available from nature defined as spats. Hence to popularize the mussel culture along the coast, knowledge of the spat's availability and their spatio-temporal distribution are of prime requisite.

P. viridis natural distribution

Tropical coastal waters are the most productive zones

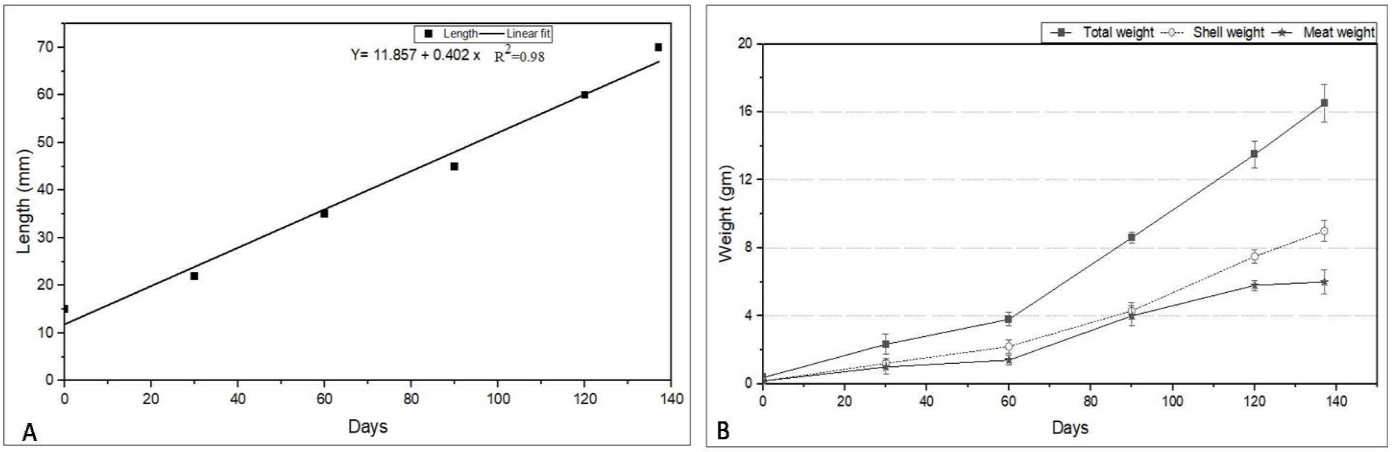

Figure 8. A) Regression analysis of mean growth rate for mussels, B) Mean total, shell, and meat weight during the raft culture at Bela Bandar, Ankola. Karwar coast.
Source: Authors

and offer an ideal environment for the natural growth, recruitment as well farming of *P. viridis* (Asaduzzaman et al., 2019). Extensive studies were carried out to understand the distribution and biological aspects of *P. viridis* along the Indian coast (Rivonkar et al., 1993; Rajagopal et al., 1998; Appukuttan et al., 2001; Kripa and Mohamed, 2009; Laxmilatha, 2013; Sreedevi et al., 2014; Sasikumar et al., 2016). An earlier study on *P. viridis* distribution particularly along the Karwar coast documented an area of 1140m^2 mussel bed from the inshore regions of the Arabian Sea (Appukuttan et al., 2001). In the present study, intertidal and estuarine areas were surveyed for the natural distributions of mussels and an approximate potential area of 350m^2 was demarcated along the Karwar coast. The demarcated regions were found to be suitable for the mussel's natural harvest as semidiurnal low-tide conditions provide smooth access for effortless spat collections

Artificial mussel spat collection

The successful spat settlements depend on the geographic location and environmental conditions along the coastal habitat. The green mussel was reported to spawn year-round (Kripa and Mohamed, 2009) twice a year (Rajagopal et al., 1998), or only once a year (Mohan and Kalyani, 1998). The natural spawning reported along the Karnataka coast was during the post-monsoon period (August to September) and secondary spawning was stated from March to April (Sasikumar et al., 2016). In the present study, similar spawning patterns were noticed considering the results of artificial spat production trends. However, the collection of wild spats from the open sea habitat is perilous during the spawning season as the process is laborious and nonprofitable (Sasikumar et al., 2016). Therefore, artificial mussel spat collection is obligatory, and ample experiments were carried out along the marine ecosystems to validate the regional suitability and to explore the appropriability of the spat collection method (Laxmilatha, 2013; Sasikumar et al., 2016; Sagita et al., 2017). Deploying artificial spat collectors is considered to be the most practical, economical, and sustainable means of seed collection (Sreedevi et al., 2014). In this study, spat collection was achieved along the subtidal and estuarine zone of the Karwar coast through the vertical floating longline method with optimal spat settlements observed on coir and nylon ropes during the post-monsoon period. Wherein, the spat collection was extended to the late post-monsoon period along the Kali estuary due to the spatio-temporal salinity variations.

Environmental conditions

The physicochemical and biological productivity is an imperative parameter in mussel distributions and farming. Ideal locations for mussel culture are open sea, backwaters, and estuaries, as these zones provide suitable environmental, geo-climate, and hydrological conditions. Among the physicochemical parameters dissolved oxygen, pH, temperature, and salinity are vital for metabolic activity, similarly, chlorophyll, nutrients, and water transparency for growth and reproduction (Ong and Ransangan, 2019). Though it is reported that bivalves can tolerate low dissolved oxygen (Wang et al., 2011), but the prolonged exposure induces stress (Sui et al., 2017). During the study period dissolved oxygen concentrations were found to be ambient along the inshore and estuarine area. The water pH along the coastal water fluctuates mainly along the estuarine and nearshore regions because of the biological activity

(Cornwall et al., 2013) and due to organic effluents brought by the terrestrial runoff (Boyd, 2015). During this study period, the pH was found to be significant between the seasons but the pH values were within the ambient conditions. Temperature, salinity, and food availability are fundamental influencers of the growth and reproduction of mussels (Rajagopal et al., 1998; Urian, 2009). Studies indicate that *P. viridis* prefers the prevalent salinity range of 27 to 34 ppt for reproduction and growth (Soon and Ransangan, 2017; Rajagopal et al., 2006) though their tolerance level is wider. In the present study, salinity was constant along the inshore region, wherein, marked seasonal salinity variations were observed at Kali estuary. Salinity variations correlated southwest monsoon which onsets during the June and extends up to September month (Nishita et al., 2023) across the Karnataka coast moreover, the rainfall extends up to the early post-monsoon period (October and November) due to the influence of the northeast monsoon. During this period the amplified riverine discharge dilutes the salinity regime across the estuary. As the rain subsides, higher salinity prevails from the late post-monsoon season due to the pronounced tidal ingress, which is crucial in mussel spat distributions and settlements along the Kali estuary (Rajeshree and Shivakumar, 2023). Similarly, a post-monsoon period is considered to be productive due to the enhanced level of nutrients that boost the phytoplankton abundance. As *P.viridis* is a suspension-feeding bivalve, the higher abundance of phytoplankton favors nourishment (Asaduzzaman et al., 2019). During the study period, higher concentrations of Chlorophyll–a, were observed during the post and pre-monsoon periods and found to be suitable for mussels' growth and farming. Overall, the studied water parameters were found to be ambient in conditions for the green mussel's natural distributions as well as for the farming prospects.

Green mussel culture

Green mussel is generally farmed during the post-monsoon months extending from November-December to April-May when higher salinity conditions prevail in the backwater/estuarine system of India (Shinoj et al., 2021). Among the various mussel culture method, raft culture methods are well suited for commercial-scale production along the open sea (Pai and Kuriakose, 1981) and estuarine habitat (Rivonker et al., 1993; Sasikumar et al., 2000). However, the study indicates that the coastal vulnerability, site selection, and social conflicts are the limitations and challenges for open sea mussels' mariculture. Mussel farming is very well demonstrated along the estuaries when high saline conditions prevail from October to April. Similarly, the suspended rope culture on racks is the most suitable farming method for low-depth and bottom culture (Laxmilatha, 2013). In the present study, green mussel culture was carried out on floating rafts along the backwaters for a period of 137 days with an estimated production of 5.0 to 8.0kg m^{-1}. The growth pattern during the study period was found to be linear and represented a positive correlation (R=0.98) between the growth and culture period indicating the site suitability and ecological conditions. Studies on the raft culture demonstrate the variations in the production from region to region, Rivonker et al. (1993) reported 33.15kg annually from the Zuari estuary. Similarly, a study on *P. indica* at the Vizhijam coast represented a yield of 10-12 kg/m in the bay and 15 kg/m in the open sea (Appukuttan and Alagarswami, 1980). A study on the green mussel production along the Edaiyur backwater of the east coast of India reported an annual production of 47.0kg m^{-1} and biomass of 22.0kg m^{-1} which is relatively higher than the other parts of India (Rajagopal et al., 1998). Similarly, the green mussel raft culture study carried out at the Mulky estuary of South Karnataka represented a monthly growth rate of 9.5mm and a meat content of 26% in 171 days, besides study signifies the requirement of a higher salinity range during the culture period (Sasikumar et al., 2000). Thus, the success of mussel culture is attributed to site selection, environmental parameter, food supply and adoption of suitable type of culture practice. Sea water with high plankton productivity, moderate water current with good exchange of water, and distribution of food are essential for the growth and survivability of the mussel, moreover, a salinity range of 25-35 ppt is essential for green mussel farming (Mohamed et al., 2019). The present study site is located on the coast of the Arabian Sea, the west coast of India, and backwater in nature, where mixed semi-diurnal tidal ingress significantly exchanges of seawater and food dispersal. The backwater area of Bela Bandar is considered to be non-polluted with less traditional fishing activity. Along this backwater average, 25 ppt salinity prevails from the November month of the post-monsoon period, and gradually increases up to the pre-monsoon period. Hence green mussel farming practice is ideal from the post-monsoon to pre-monsoon period along this backwater.

Conclusion

The present study infers that the continental shelf region of the Karwar coast represents profuse natural distributions of green mussels which can be harvested naturally or artificially to expand the mariculture activity. The raft culture success is mainly attributed to higher salinity (>20 ppt) and an area of 40,000m^2 found to be suitable for mussel farming along the Bela Bandar backwater of Ankola.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to Karnatak University Dharwad for providing financial assistance under Research Seed Grant 2022 and Pramod Nayak, RFO, Coastal and Marine Ecosystem Cell, Department of Forest, Karwar for providing logistic support towards mussel raft culture.

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