

## Habitat characteristics mediated partitioning of economically important bivalves in a tropical monsoon-influenced estuary

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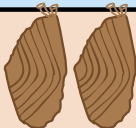
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### Highlights

- Elucidated potential causes for bivalve habitat partitioning in an estuary.
- Short neck bivalve, *Paphiamalabarica* preferred silty-sand substratum.
- Suspension feeding bivalve, *Meretrix casta* preferred sandy substratum.
- Fatty acids are indicators of feeding habit in bivalves.
- Physical forcing, habitat characteristics and quality and quantity of food influence bivalve partitioning.

### Graphical Abstract

Chicalim		Siridao		Water Column
Facultative deposit feeder	Feeding habit	Suspension feeder	Water Column	
 <i>Paphia malabarica</i>		Silty-sandy		Sandy
High	Habitat (Sediment Texture)	Low	Sediment	
Degraded-detrital	Food Quantity (TOM, LOM)	Fresh, nutritional		
High	Food Quality (PRT:CHO, LPD:CHO)	Low		
Oxic	Chlorophyll <i>a</i>	Anoxic		
	Redox status			

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### Abstract

Bivalves are benthic organisms inhabiting coastal marine habitats, especially estuarine tidal and mudflats. Due to their high stocking density and rich protein content, they form a major part of the artisanal fishery resource around the world. A clear partitioning in the population of bivalves, *Paphiamalabarica* (Chemnitz, 1782), and *Meretrix casta* (Gmelin, 1791) in southern (Chicalim) and northern (Siridao) bank of a tropical Zuari estuary influenced by the monsoon along the Indian west coast is evidenced. This study unravels the reasons for their partitioning in this estuary.

*Paphiamalabarica* is an exclusive inhabitant of Chicalim which has silty-sandy sediment, whereas *M. casta* is exclusive to Siridao, a sandy habitat. Observations showed that this segregation is facilitated by the semi-enclosed nature of habitat at Chicalim with the high amount of degraded and aged sediment organic carbon, high chlorophyll-*a*, elemental, and biochemical components. Whereas, Siridao experiences the high impact of tidal currents, low sediment organic carbon, and high water column chlorophyll-*a*. The habitat in Siridao gets exposed to UV radiation during low tide, reducing the photosynthetic oxygen production, turning the habitat to periodic anoxia indicated by differences in the TOC:TS ratio. However, such conditions may not influence *M. casta*, which can derive oxygen from the water column. The fatty acids specific to diatoms, dinoflagellates, higher plants and partially degraded organic matter in the tissues of *P. malabarica* indicate their ability to source the food from the sediment and water column. Whereas in tissues of *M. casta*, higher dinoflagellate specific fatty acids followed by diatom and bacteria indicate water column derived food. Chicalim can be considered as an actively coupled benthic-pelagic habitat, and Siridao as an uncoupled habitat. Thus, the diverse flux of food particles, species-specific feeding ecology, and local hydrodynamics operating at these habitats could be the determining factors in the partitioning of the bivalves.

**Keywords** *Paphiamalabarica*. *Meretrix casta*. Zuari estuary. Resource Partitioning. Sediment

## **Introduction**

Estuaries are rich in biodiversity and inhabited by a wide range of benthic artisanal resources which include bivalves, oysters, crabs, etc. along with other macrobenthic organisms. The sand and mudflats within the estuaries on which a significant fraction of the artisanal fisheries are dependent often anthropogenically stressed. The organisms inhabiting these sensitive ecosystems live close to their environmental tolerance limits, and subtle environmental changes can provoke a significant biological response in them, modifying the food web and affecting the stock of economically important artisanal resources. The important primary producers in shallow estuarine sediment are the microphytobenthos, which contribute considerably to the chlorophyll biomass and benthic ecology (MacIntyre et al. 1996; Cahoon 1999; Hope et al. 2019). The autochthonous biomass of microphytobenthos (dominated by benthic diatoms) acts as vital food resource for large numbers of consumers inhabiting in the sediment (Plante-Cuny and Plante 1986; MacIntyre et al. 1996; Miller et al. 1996; Cahoon 1999; Kanaya et al. 2005). Sediment characteristics, mainly texture, organic carbon, chlorophyll, and microbial biomass play an important role in the feeding ecology and biology of benthic consumers (Rhoads 1974; Lopez and Levinton 1987; Graf and Rosenberg 1997) especially bivalves (Kanaya et al. 2005).

The bivalves in the estuarine systems are important mediators of energy transfer from benthic and pelagic primary producers to the consumers (Heip et al. 1995; Carmichael et al. 2010; Wang et al. 2015) and also helps in organic matter cycling. Organic matter (OM) is a vital component of the estuarine and coastal sediments and plays a significant role in the food web (Zimmerman and Canuel 2001). Estuarine sediment receives OM from different sources, including primary producers, consumers, decomposers and, allochthonous inputs through river discharge including land run-off, industrial and, domestic waste (Meziane and Tsuchiya 2002; Hu et al. 2006; Dunn et al. 2008). Fatty acids (FAs), the main components of lipids act as energy source and nutrients for the organisms such as bivalves, snails, shrimps, crabs, etc. and can be used to identify the diet of dominant fauna of the estuarine and coastal sediments (Meziane and Tsuchiya 2000; Kharlamenko et al. 2001; Meziane and Tsuchiya 2002; Bachok et al. 2003; Alfaro et al. 2006; Bachok et al. 2009; Wang et al. 2015). FAs are source-specific and diverse in structure and are commonly used to identify the OM sources and their fate in the sediments (Zimmerman and Canuel 2001; Meziane and Tsuchiya 2000, 2002; Dunn et al. 2008; Venturini et al. 2012; Guo et al. 2019).

The benthic organisms might experience different responses based on the quantity of food, and competition would be greater between the consumers in food limited environments (Wei et al. 2010;

Campanyà-Llovet et al. 2017). However, few studies have also recognized the importance of nutritional quality of the available food, and its influence on the distribution of benthic consumers (Wiekling and Kroncke 2005; Quitana et al. 2010). Müller-Navarra (2008) defined the term “food quality” as the degree of the quantity and nutritional composition of food which is accessible to satisfy the basic nutritional demand of the consumers and this cannot be unraveled from quantity alone, since, large quantities of nutritionally-poor food may be as valuable as low quantities of nutritionally rich food. Therefore, to understand the influence of food on the benthic community structure, it is important to examine the quality and quantity of food along with other characteristics of the habitat. The bivalves are filter-feeding organisms and generally found in the estuarine sediments and are commercially important food resources being harvested from the estuarine areas (Parulekar et al. 1984; Alexander et al. 1993; Narasimham and Laxmilatha 1996). In the present study area, Zuari estuary, Goa, two commercially important bivalves, namely, *Paphiamalabarica* Chemnitz, 1791 (Sukumaran et al. 2019) and *Meretrix casta* Gmelin, 1971 (Huber 2010) inhabit. These two species are also marketed on a regular basis by the local fishermen during the harvesting season. The fishery of the bivalves has shown a declining trend in the state of Goa and also large inter-annual fluctuations in their stock. In the year 2012 the production of major bivalve resource, *Paphia malabarica* was 442 tonnes and in the year 2017 it was 82 tonnes. From 2012 to 2015 the production of this species showed fluctuations, however, from 2015 onwards it showed a sharp decline (2015 - 614 tonnes; 2017 – 82 tonnes) (Directorate of Fisheries, Goa 2018). However, such data is not available for other species (*Meretrix casta*).

The bivalve, *P. malabarica* is a short-necked bivalve known to survive in diverse habitats in the subtidal zone and is a facultative deposit feeder, wherein it can take food both from the sediment and water column. Whereas, *M. casta* is a suspension feeder and take their food from the water column. These two bivalves have segregated habitats on either side of the banks of Zuari estuary. The bivalve, *M. Casta* is found at Siridao, the northern bank, and *P.malabarica* is found at Chicalim, which is the southern bank of the Zuari estuary. However, the reasons for their partitioning in this estuary are not known. We hypothesize that the possible reasons for the partitioning of these bivalve species could be the variations in the habitat characteristics, quality and the quantity of the organic matter, type of food, the physical processes, and local hydrodynamics acting in the bivalve habitat. In order to delineate this, we evaluated (a) the distribution and nature of sedimentary organic matter (b) sources of fatty acids in the sediment and (c) seasonal variation in the abundance of bivalves and their diet in order to understand the possible reasons for partitioning of the bivalve (*Paphiamalabarica* and *Meretrix casta*) population. The results obtained through this study will be

useful in managing the stocks of these bivalves in these habitats as they are the major spawning grounds of *Paphia malabarica* and *Meretrix casta*. The data obtained on the seasonal variation in the population of both the bivalves will be helpful in interlinking stock assessment and harvesting practices for the development of the conservation strategy. Inferences drawn from the scientific assessment of stocks would provide information on the life cycle strategies of these species and will help in understanding their production, leading the way for implementing harvest potential in a given area and their conservation for population replenishment through management of stocks.

## **Materials and methods**

### **Study area**

The study was conducted in the Zuari estuary, situated in the state of Goa, central west coast of India. The study region experiences three main seasons in a year, which can be described as the pre-monsoon (February-May), south-west monsoon (June-September), and post-monsoon (October-January). Zuari River has a catchment area of about 550 km<sup>2</sup> and receives 491×10<sup>6</sup> m<sup>3</sup> of run-off annually (Qasim and Sengupta 1981; Shetye et al. 2007). The study site Chicalim (15°24'10.92"N and 73°51'8.55"E) is situated on the southern bank and is influenced by different anthropogenic activities such as shipbuilding, barge cleaning, etc., whereas, Siridao (15°25'41.89"N and 73°52'38.84"E) is situated on the northern bank of Zuari estuary (Fig. 1) and is relatively pristine.

### **Sampling and analytical methodology**

Sampling was carried out in April (Pre-monsoon, PreM), July (Monsoon, MON) and, November 2014 (Post-monsoon, POM) during the low tide. Sediment samples were collected by using PVC cores of 20 cm length and 25 mm internal diameter. Each core was sliced into 4 segments (0-5; 5-10; 10-15 and 15-20 cm) and kept frozen at -20°C for further analysis. The bivalves were handpicked from the sediment, brought to the laboratory, and kept frozen at -20°C for not more than 2 weeks for further analysis of fatty acids. In the laboratory, the soft tissues of bivalves were removed carefully, washed thoroughly by filtered, autoclaved sea water, and used for the extraction of lipids. The temperature and salinity of seawater were determined using digital thermometer and refractometer, respectively during the sampling.

### **Analysis of sediment grain size and chlorophyll-*a***

The sediment grain size was determined by the pipette analysis method (Buchanan 1984), and composition is expressed as the percentage of sand, silt and clay. The chlorophyll-*a* was extracted from sediment with 90% acetone in the dark at 4°C (24 h), centrifuged on the next day, and the

supernatant was estimated for the content of chlorophyll-*a* and expressed as  $\mu\text{g g}^{-1}$  of sediment. The chlorophyll-*a* of the seawater was determined by filtering 500 ml of seawater through GF/F filter paper and followed the above procedure for the extraction of chlorophyll-*a* and is expressed as  $\mu\text{g L}^{-1}$ .

### **Estimation of the elemental and biochemical composition of the sediment core**

The samples of sediment which were frozen at  $-20^{\circ}\text{C}$  were thawed, oven-dried at  $50^{\circ}\text{C}$  (Bhosle and Dhople 1988), and ground into fine powder for elemental and biochemical composition. Sediments were weighed in tin boat and the content of Total Carbon (TC), Nitrogen (TN), and Sulfur (TS) was determined using a Vario MICRO Select Elemental Analyzer (Germany) and expressed as percentage of sediment dry weight. Sulfanilamide was used as a standard for calibration. The analysis of Total Organic Carbon (TOC) of the sediment was carried out according to El Wakeel and Riley (1957). The analyses were carried out in duplicate and content is reported as  $\text{mg g}^{-1}$  of sediment. TOC content was converted to the amount of Total Organic Matter (TOM) by multiplying with factor 1.724 (Bhosle and Dhople 1988). Carbohydrates (CHO) in the sediment were estimated following the method by Dubois et al. (1956) using UV-1800 spectrophotometer (Shimadzu). The concentration of proteins (PRT) in the sediment was determined according to Hartree (1972). Lipids (LPD) were extracted from the sediment using chloroform: methanol (2:1 v/v) mixture (Bligh and Dyer 1959) and estimated according to Barnes and Blackstock (1973). Glucose, bovine serum albumin, and cholesterol were used as calibration standards for CHO, PRT, and LPD, respectively. All these analyses were carried out in triplicate, and the concentration is expressed as  $\text{mg g}^{-1}$  of sediment. The pre-combusted sediments ( $450-500^{\circ}\text{C}$  for 4 h) were used to prepare blanks. The sum of PRT, CHO, and LPD content of sediment was reported as labile organic matter (LOM).

### **Enumeration of Total Viable bacterial Count (TVC) in the sediment core**

The sediment samples were analysed by standard spread plate technique for the enumeration of TVC by using specific media. Wet sediment (1 g) was suspended in filtered, autoclaved seawater (10 ml) and diluted serially. Subsequently, 0.1 ml was spread on Zobell Marine Agar (ZMA) for the enumeration of total viable bacterial count, and these agar plates were incubated for 24 h at room temperature ( $28\pm 2^{\circ}\text{C}$ ). The number of colonies were counted after 24 h and are expressed as Colony Forming Unit (CFU)  $\text{g}^{-1}$  of sediment.

### **Enumeration of Total Bacterial Count (TBC) in the sediment core**

Sediment sample (1 g) was suspended in filtered (0.22 µm), autoclaved seawater (10 ml), and fixed with formaldehyde (1-2%). The samples were then sonicated in the water bath sonicator (50% power) for 1 min. This step was repeated 3 times to separate bacterial cells from sediment particles (Luna et al. 2002) and then centrifuged at 3000 rpm for 1 min. Subsequently, the supernatant (1 ml) was mixed with 10 µL of SYBR Green I (Molecular Probes, USA) and incubated in the dark for 15 min at room temperature (Marie et al. 2001). The internal calibration of the instrument was carried out using fluorescent beads (1 µm size). The BD FACSAria™ II Flow cytometer (BD Biosciences, USA), with a nuclear blue laser (488 nm wavelength) was used for the analysis of samples. The events (10000 nos.) were measured and were gated as SSC versus green fluorescence, and bacterial abundance is expressed as cells g<sup>-1</sup> of sediment. The BD FACS Diva software was used for data analysis.

### **Analysis of proteins using MALDI-TOF from the surface sediments**

The extraction of proteins from the sediment was carried out using the method described by Wohlbrand et al. (2017) with some modifications. Briefly, 2 g~~m~~ (wet weight) sediment was mixed with 5 ml of SDS-Phenol buffer. This mixture was sonicated on ice at 90% pulsing and 40% energy for 60 secs (Ultrasonic Probe Sonicator, Life Care, Mumbai) (Keiblinger et al. 2012). This treatment was repeated twice, followed by shaking for 1 h. Subsequently, homogenates were incubated at 90°C (20 min) in the water bath with intermittent mixing followed by cooling down on the ice. The residual sediment was then removed by centrifugation at 12,000 rpm at 20°C (10 min). Supernatants were transferred to another tube, combined, and proteins were precipitated by ammonium acetate (0.1 M) in methanol (fivefold volume), with overnight incubation at -20°C. On the next day pellets were washed with ice-cold acetone, and proteins were recovered by centrifugation. The pellets were then digested overnight (37°C) with trypsin (Promega). Subsequently, the pellets were re-suspended in 100 µl of acetonitrile and stored at -20°C, and the aliquots were used for further analysis.

The aliquots (1 µl) were mixed with matrix (saturated solution of α-cyano-4-hydroxy cinnamic acid in 30% acetonitrile with 0.1% trifluoroacetic acid) and this mixture was then overlaid on the 384 target plate (stainless steel), and air-dried. The samples were analysed using Bruker Ultraflex MALDI TOF mass spectrometer (Bruker Daltonics, Germany). Peptide calibration standard-II and Bovine Serum Albumin (BSA) digest (Bruker Daltonics, Bremen, Germany) were used for calibration. Mass spectra were obtained by the accumulation of laser shots on the sample spot and were processed using Flex Analysis 3.0 software (Bruker Daltonics). Subsequently, selected parent

masses were fragmented under the lift method. The BioTools 3.1 software (Bruker Daltonics) and MASCOT search engine (Matrix Science, London, UK) were used to identify the MS/MS spectra. The Mascot search database: NCBI nr database in SwissProt, taxonomy: all entries, fixed modification: carbamidomethylation (C), variable modification: oxidation (M), mass tolerance: 100 ppm and digestion enzyme: trypsin.

### **Enumeration of *P. malabarica* and *M. casta* in the study area**

An area of one square meter was selected for the sampling of bivalves from both the stations. The collection of bivalves was carried out during low tide by placing the quadrat in the intertidal area. The collection of bivalves was carried out in triplicate at each site during different seasons. The sediment samples were collected in a metal sieve (1 mm mesh size and 50 cm diameter), and the individual bivalves were collected, enumerated, and their abundance is expressed as No m<sup>-2</sup>.

### **Analysis of fatty acids from the sediment core and bivalve tissues**

Sediment samples and bivalve tissues were used for the extraction of lipids according to Bligh and Dyer (1959) method. Samples were sonicated with chloroform:methanol (1:2 v/v) mixture for 20 min. Then, additional chloroform and water (1:1 v/v) were added, mixed well and kept overnight in separation funnel. On the next day, the lower organic phase was separated and evaporated in a rotary vacuum evaporator. Subsequently, it was re-suspended in mixture of methanol-toluene (1:1 v/v) and converted to fatty acids methyl esters (FAMES) with mild alkaline methanolysis method (White et al. 1979). FAMES were then analysed using Gas Chromatography-Mass Spectrometry (GC-MS, QP-2010, SHIMADZU), with a capillary column (Stabilwax, 30m × 0.25mm internal diameter, 0.50µm film thickness). One microliter of sample was injected by auto sampler and helium gas was used as a carrier. The temperature of the oven was started from 50°C (maintained for 2 minutes) and increased to 200°C with rate of 10°C min<sup>-1</sup> and then at 5°C min<sup>-1</sup> from 200 to 240°C. The injector and detector temperature was maintained at 240°C. FAME mix (Supelco 37 Component, 18919-1AMP, Sigma Aldrich, India) was used for the calibration of the instrument. The WILEY7 mass spectral library was used for the qualitative identification of individual FAME. The concentration of FAME has been reported as area percentage.

### **Data analyses**

Spearman's correlation analyses were carried out between sedimentary biogeochemical components (TOC, TC, TN, TS, PRT, CHO and, LPD), grain size composition (sand, silt, and clay) and bacterial abundances (TVC and TBC; log x+1 transformed) at a significance level of ≤ 0.05. Two-way



analysis of variance (ANOVA) was applied for the abundance of bivalves, bacteria (TVC, TBC), and the content of biogeochemical variables (TOC, PRT, CHO, LPD, LOM) of sediments (0-5cm) with stations and seasons as independent factors. SPSS statistical software program (Version 16) was used to perform correlation and ANOVA. To determine the suitable multivariate analysis, data were processed using Detrended Correspondence Analysis (DCA), and its results showed that the length of the gradient of the first axis was low ( $< 2.0$ ), suggesting linearity in the data set (terBraak and Smilauer 2002). The relationship between bivalves, bacteria, and biogeochemical variables was then evaluated using Redundancy Analysis (RDA) under a reduced model on the set of variables using a forward selection of environmental variables with 999 numbers of permutations. These analyses were carried out using CANACO version 4.5 (terBraak and Verdonschot 1995).

## **Results**

The seawater temperature ranged from 25 to 32°C at Chicalim and 27 to 31°C at Siridao. At both the stations, the temperature was low during MON (25°C and 27°C at Chicalim and Siridao respectively), moderate during POM (29°C at Chicalim and 30°C at Siridao) and high during PreM (32°C at Chicalim and 31°C at Siridao). Salinity varied between 12 to 32 at Chicalim and 12 to 29 at Siridao and, it was low during MON at both the stations (12 at both Chicalim and Siridao). During PreM, the salinity was 32 and 29 at Chicalim and Siridao, respectively. The POM season was characterized with moderate salinity, i.e. 28 at Chicalim and 26 at Siridao.

### **Seasonal variation in the sediment texture**

The sediment texture showed a significant (ANOVA;  $p < 0.0001$ ) variation in their composition among Chicalim and Siridao (Fig. 2a and b; Table 1). At Chicalim, the sediment was dominated by silt followed by sand and clay. The content of sand significantly varied with the seasons (Fig. 2a; Table 1). The average percentage of sand, silt, and clay at Chicalim was  $34.4 \pm 9\%$ ,  $64.9 \pm 9\%$  and  $0.7 \pm 0.2\%$  during PreM;  $30.3 \pm 7$ ,  $68.9 \pm 7$ ,  $0.78 \pm 0.8\%$  during MON and  $48.1 \pm 2$ ,  $50.8 \pm 1$ ,  $1.1 \pm 2\%$  during POM respectively indicating an increase in the percentage of sand during POM. At Siridao, the sediment was dominated by sand (Fig. 2b) and the average percentage composition of sand, silt and clay were  $93.8 \pm 2$ ,  $5.82 \pm 2$  and  $0.41 \pm 0.4\%$  during PreM,  $94.3 \pm 0.6$ ,  $5.32 \pm 0.9$ ,  $0.38 \pm 0.4\%$  during MON and  $94.9 \pm 2$ ,  $4.52 \pm 2$ ,  $0.6 \pm 0.9\%$  during POM respectively. The seasonal variation in the sediment texture was more pronounced in Chicalim than Siridao (Fig. 2a and b).

### **Seasonal variation in chlorophyll-*a***

The sediment chlorophyll-*a* was high in Chicalim than Siridao, except during POM season. It was almost similar to both the stations during POM. At Chicalim, the chlorophyll-*a* was high during MON ( $2.58 \pm 0.43 \mu\text{g g}^{-1}$ ) followed by POM ( $2.41 \pm 0.75 \mu\text{g g}^{-1}$ ) and PreM ( $2.11 \pm 0.53 \mu\text{g g}^{-1}$ ) (Fig. 3), whereas, it was high during POM ( $2.66 \pm 0.19 \mu\text{g g}^{-1}$ ) when compared to PreM ( $1.73 \pm 0.85 \mu\text{g g}^{-1}$ ) and MON ( $1.27 \pm 0.11 \mu\text{g g}^{-1}$ ) seasons in Siridao (Fig. 3; Table 1, ANOVA;  $p < 0.043$ ). The water column chlorophyll-*a* was high in Siridao when compared to Chicalim. At Siridao, it was almost similar during PreM ( $1.54 \mu\text{g l}^{-1}$ ) and POM ( $1.50 \mu\text{g l}^{-1}$ ) seasons and low during MON ( $0.76 \mu\text{g l}^{-1}$ ) season. However, at Chicalim, the water column chlorophyll-*a* was similar during POM ( $1.10 \mu\text{g l}^{-1}$ ) and MON ( $1.00 \mu\text{g l}^{-1}$ ) and was low during PreM ( $0.70 \mu\text{g l}^{-1}$ ) season.

### **Seasonal variation in the elemental, biochemical and bacterial abundance in the sediment core of Chicalim (Southern bank)**

The vertical distribution of TC, TN, TS, TOM, TOC, PRT, CHO, and LPD at different depths in Chicalim sediment core (total depth of 20cm) is shown in Fig. 4a-i. The elemental and biochemical components of sediments showed significant irregular down core variations between the seasons (Table 2). The TC content was high during POM compared to other seasons (Fig. 4a). The TN was high during MON and, it varied between 0.02 and 0.07% sediment dry weight during the study (Fig. 4b). The TS content (0.11 – 0.30%) was high at the surface during MON, whereas it was high at 15-20cm depth during PreM (Fig. 4c). A sharp decline in the sulfur content from the surface to 5-10cm depth of sediment was observed during MON. The TOC was high during the POM followed by MON and low during PreM, indicating significant seasonal variation in the surface sediments (Table 2). An increase in the TOC and TOM content was evident with depth (0-20cm) during MON season (Fig. 4d and e).

The content of PRT in surface sediment (0-5 cm) was almost similar during all three seasons but, it varied with the depth, especially during PreM and POM (ANOVA;  $p < 0.0001$ , Table 2). During MON, it was almost similar from 0-20cm depth of the core, whereas, during PreM and POM, it decreased sharply at 5-10cm depth. The PRT content was almost similar between 15-20cm depth during PreM and MON (Fig. 4f). The CHO concentration was maximum during MON followed by POM and PreM at the surface, and it was maximum in 15-20 cm depth during MON (Fig. 4g). The LPD content ranged from 0.29 – 1.37  $\text{mg g}^{-1}$  of dry sediment (Fig. 4h). At the surface, the concentration of LPD was maximum during POM followed by MON and PreM. During POM and MON, the LPD content increased with the depth of the core, whereas during PreM, it increased (5-

10cm) and further decreased with the depth the core (Fig. 4h). The PRT, CHO, and LPD showed significant variations between the seasons and depth at Chicalim (Table 2).

The TVC was high at 5-10 cm depth during PreM( $1.14 \pm 0.4 \times 10^6 \text{CFU g}^{-1}$ ) and MON ( $9.94 \pm 0.14 \times 10^5 \text{CFU g}^{-1}$ ) season (Fig.4i),and it varied with the depth between these seasons (Table 2). However, during POM, the TVC was low and almost similar from 0-20cm depth (Fig. 4i). The TBC was higher during PreM season (Fig.4j), and it was almost similar during MON and POM (Table 3a) and did not vary with the depth during different seasons. A decreasing trend in the abundance of TVC and TBC was observed with seasons (PreM>MON>POM) in the Chicalim sediment.

### **Seasonal variation in the elemental, biochemical and bacterial abundance in the sediment core at Siridao (Northern bank)**

The vertical distribution of TC, TN, TS, TOM, TOC, PRT, CHO, LPD, TVC, and TBC in Siridao sediment core is shown in Fig.5a-i. The TC was maximum at the surface during PreM and decreased with the depth; however, it was low at the surface during MON and POM and increased with the depth (Fig. 5a). The TS in the sediment varied from 0.06 – 0.26%(Fig.5b), and it was maximum during POM at 10-15cm depth. The TN content was below the detection level in the sediments at all the depths. The TOM and TOC content in the sediment was higher during POM (Fig. 5c and d; Table 3a) and ranged from 4.16 – 10.9 mg g<sup>-1</sup> and 2.41 – 6.32 mg g<sup>-1</sup>, respectively. A decrease in the TOM and TOC was observed during PreM. TOC showed a significant seasonal variation (ANOVA;  $p < 0.002$ , Table 2).

The concentration of PRT ranged from 0.33 - 0.80 mg g<sup>-1</sup> of dry sediment weight (Fig. 5e), and it was maximum during MON (10-15cm depth). At the surface, the PRT concentration was higher during PreM and POM, and it decreased with depth (up to 20 cm) (Fig. 5e). Significant depth-wise variability in the PRT was observed (ANOVA;  $p < 0.024$ ); however, CHO varied significantly with the seasons (ANOVA;  $p < 0.002$ , Table 2). The concentration of CHO showed wide variation at the surface (0-5cm depth) during all the seasons, and it was maximum during MON followed by PreM and POM (Fig. 5f). The average CHO content was high during MON (Table 3a). The LPD content showed wide seasonal variation at the surface (0-5cm) and was maximum during PreM followed by POM and MON season (Fig. 5g; Table 3a). It was observed that the LPD content gradually increased with depth during POM and MON (Fig. 5g). LPD showed significant seasonal and depth-wise variation at Siridao (Table 2).

The TVC was high in the bottom (15-20 cm) of the core during PreM and MON, whereas it was high at the surface during POM (Fig. 5h). The TBC was maximum at 0-5 cm depth during PreM and was almost similar throughout the core (Fig. 5i). A significant temporal variability (ANOVA;  $p < 0.0001$ , Table 2) was observed in the distribution of TVC and TBC, with a decreasing trend (PreM>MON>POM) from PreM to POM (Table 3a).

### **Distribution of proteins in the surface sediments**

The mass spectrum analysis of proteins from the surface sediments of Chicalim and Siridao showed seasonal variations. The MS/MS spectra revealed that the proteins in Chicalim sediments were predominantly of anthropogenic origin, whereas those in Siridao of bacterial origin. The identified proteins were found to involve in different metabolic processes such as glycolysis, protein synthesis, flavonoid, amino acid and nucleotide metabolism (Table 3b).

### **Seasonal variation in the abundance of *P. malabarica* and *M. casta***

The abundance of bivalve *P. malabarica* at Chicalim was higher than *M. casta* at Siridao during all the seasons (Fig. 3). The higher abundance of *P. malabarica* ( $183 \pm 80$  no.  $m^{-2}$ ) at Chicalim and *M. casta* ( $104 \pm 78$  no.  $m^{-2}$ ) at Siridao was observed during POM season (Fig. 3), and their abundance was low during PreM season. ANOVA revealed a significant seasonal variation in the abundance of *P. malabarica* (ANOVA;  $p < 0.004$ ) than *M. casta* (ANOVA;  $p < 0.017$ ). A significant spatial (between stations) variation (ANOVA;  $p < 0.002$ ) was also observed in the abundance of bivalves (Table 1).

### **Spatio-temporal and vertical distribution of fatty acids (FAs) in the sediment**

A seasonal variation in the fatty acid composition was observed in the sediments of both the regions and their composition also varied with the depth of the core. The short-chain even carbon numbered saturated FAs (SCFA: C12:0, C14:0, C16:0, C18:0, C20:0) were dominant in the sediments at both the sites (Table 4). Long-chain fatty acid (LCFA), C22:0 were also observed in the surficial (0-5 cm depth) sediment of the core during PreM and POM seasons at Chicalim (Table 4). However, at Siridao, C22:0 was dominant between 5-10 cm depth during PreM and recorded between 15-20 cm and 5-10 cm depth during MON and POM respectively. The LCFA, C24:0 was also found at Chicalim at different depth of the core during PreM and MON (Table 4). At Siridao, C24:0 was dominant between 15-20 cm (40.9%) during PreM and was absent during MON and POM seasons (Table 4). Polyunsaturated fatty acid (PUFA), C18:2 $\omega$ 6 was dominant between 0-10 cm at Chicalim, whereas in Siridao, it was dominant at 5-10 cm during PreM. The PUFA, C18:3 $\omega$ 3 was observed at Siridao during PreM and at Chicalim during POM season (Table 4). Monounsaturated

fatty acid (MUFA), C22:1 $\omega$ 9 was recorded in the surficial sediment at Chicalim and at Siridao from 5-10 cm during the PreM season. Another MUFA, C24:1 $\omega$ 9 was observed exclusively to Chicalim (Table 4).

Bacteria specific FA such as C15:0 (6.4%) was observed between 10-15 cm at Chicalim during PreM and throughout the core except at surface during MON. It was also recorded at 5-15 cm depth during POM. While at Siridao, C15:0 was observed in the surface sediments during all the seasons and from 10-15 cm during MON. The FA, C17:0 was observed during PreM, MON, and POM seasons, however, at different depths of the core (Table 4).

### **Seasonal variation of FAs in the tissues of *P. malabarica* and *M. casta***

The FAs reported in *P. Malabarica*, and *M. Casta* differed between seasons, and some of the FAs were species-specific. During PreM season, FAs C14:0, C16:0, and C18:1 $\omega$ 9 were common to both the species, whereas the FAs, C20:4, C18:0, C16:1 $\omega$ 7 and C24:0 were specific to *P. Malabarica* and C12:0 and C20:0 were specific to *M. casta*. During MON season, C12:0 was observed in the tissues of *P. malabarica* and *M. casta*. However, FAs such as C15:0, C16:1 $\omega$ 7 and C18:2 $\omega$ 6 were specific to *P. malabarica* and C17:0, C18:1 $\omega$ 9 and C18:3 $\omega$ 3 were specific to *M. casta*. The POM season was characterized by the presence of short-chain FAs such as C12:0, C16:0, and C16:1 $\omega$ 7 in the tissues of both *P. Malabarica* and *M. casta*. The FAs, C18:0, and C20:0 were specific to *P. malabarica* and C17:0 and C18:1 $\omega$ 9 were specific to the tissues of *M. casta* (Table 4). However, the percentage of FAs varied with the species and seasons (Table 4).

## **Discussion**

### **The distribution and nature of sedimentary OM**

This study investigated the impact of different sedimentary parameters on the partitioning of two different bivalves on the banks of Zuari, a tropical monsoon-influenced estuary. A marked difference in the sediment grain size was evident between the two sites. At Chicalim, the sediment was rich in silt followed by sand with poorly sorted grains, whereas, in Siridao, sediment was dominated by sand, and these differences could be attributed to tidal currents and water flow in the estuarine system. The tidal (ebb and flood) currents are reported to be stronger in the Zuari estuary (Manoj and Unnikrishnan 2009). Sundar et al. (2015) reported longitudinal flow in the channel and cross-shore flow at the estuarine mouth of the Zuari and the magnitude and duration of ebb and flood flow was nearly similar during pre- and post-monsoon seasons, however, during monsoon an increase in the magnitude and duration of ebb flow is observed as compared to flood flow owing to discharge of

large amount of fresh water. It is also reported that the flow of coastal water is directed towards the northern bank of the estuary (Siridao) during high tide and towards the south side (Chicalim) when the water flushes out during low tide (Shirodkar et al. 2012). Such a flow pattern might possibly wash out fine particles, leaving behind the medium to fine sand particles in the sediment of Siridao. The habitat in Chicalim being sheltered, the impact of water flow during low tide will be comparatively less than at Siridao, and this will not disturb the fine particles (silt and clay) in the sediment of Chicalim. The high TOC content at Chicalim compared to Siridao could be attributed to the accumulation of fine particles and detrital matter from the water column in the sediment. The fine-grain sediments with large surface areas (Meyers 1997) have high adsorptive capacity due to close hydraulic equivalence for the organic molecules (Cotano and Villate 2006). Rao et al. (2011), while studying the suspended sediment dynamics indicated that suspended particulate matter (SPM) was high during high rainfall and low salinity and also during moderate/low rainfall peaks with high salinity during non-monsoon seasons. Overall, the content of SPM in the channel stations was lower during post and pre-monsoon when compared to monsoon season. However, SPM was maximum in the bay stations (stations located near to the Siridao and Chicalim), and this might influence the OM distribution and availability. Thus, higher TOC at Chicalim as compared to Siridao could be attributed to sediment texture and the estuarine hydrodynamics. The water column depth at both the stations is shallow and range between 1-3 m. Since, Zuari is a shallow estuary, combined effect of wind-generated and ebb currents during the neap tide may cause suspension of sediments owing to shallow bottom (Rao et al. 2011) and such a phenomenon will influence the OM dynamics in the bivalve habitats which will benefit the deposit feeders (*P. malabarica*) rather than suspension feeders (*M. casta*). A significant negative correlation of TOC with sand ( $r = -0.594$ ;  $p < 0.002$ ) and positive correlation with silt ( $r = 0.601$ ;  $p < 0.002$ ) reveals higher accumulation of OM in Chicalim sediment which is rich in silt. The TC content was also high in Chicalim compared to Siridao, indicating that sediment grain size composition plays a significant role in the distribution of organic molecules in sediments, and such condition is conducive for deposit feeders as they obtain food from the sediment organic matter for their survival and growth. The specificity and higher abundance of *P. Malabarica* at Chicalim can be attributed to such habitat characteristics.

The redox status of benthic habitat is also crucial as it provides information on the availability of oxygen for the benthic life, and can be determined using TOC to TS ratio (Raiswell et al. 1987). When TOC to TS ratio  $>5$ , the sediments have been categorized as oxic, TOC:TS ratio between 1.5-5 indicates sediment under periodic anoxic condition and TOC:TS  $<1.5$  indicate sediment is anoxic (Raiswell et al. 1987; Akhil et al. 2013; Salas et al. 2015). The sediment from Siridao showed

periodic anoxic conditions with low TOC:TS ratio (PreM: 2.6, MON: 3.1 and POM: 3.5) when compared to Chicalim (PreM:2.6, MON: 5.9 and POM: 5.6). Rasheed et al. (2003) reported higher solute exchange and permeability to enhance the organic carbon decomposition and oxygen consumption rate (3-folds) in the coarse sand when compared to the silt-clay sediments. Shallow water also enables UV radiation to reach the sediment surface, which causes damage to the aquatic organisms and reduces the photosynthetic uptake of atmospheric CO<sub>2</sub> and the productivity of sediments (Hader et al., 2007). The tides in the study area are also mixed type, ranging ~2.3 m during spring and 1.5 m during the neap tide (Manoj and Unnikrishnan 2009). The habitat at Siridao, being a shallow tidal mudflat, gets exposed to sunlight for a longer duration during low tide when compared to Chicalim. This might reduce the photosynthetic oxygen production. The Naval Hydrographic Chart of 2003 shows a well-developed mudflat that gets entirely exposed during the low tide. These could be the probable reasons for periodic anoxic conditions as indicated by the TOC:TS ratio at Siridao compared to Chicalim and can be attributed to habitat characteristics. However, such a condition (periodic anoxia in the sediment) may not impact the abundance of *M. casta* as it is a suspension feeder and can obtain dissolved oxygen from seawater during the high tide. Whereas, at Chicalim, low TOC:TS ratio during PreM indicate periodic anoxic condition, where anoxic condition during MON and POM seasons making the habitat favourable for the deposit feeders. It has been reported that the breeding season commences in September, and peak spawning season is from October to December (POM) (Mohite and Mohite, 2009) and during this season, the sediment in Chicalim being oxic seems to be optimum for breeding and/or spawning of bivalves.

The productivity of the benthic system has been described earlier using sedimentary PRT content (Danovaro et al. 1999; Dell'Anno et al. 2002), and PRTs are most important source of nitrogen for benthic invertebrates (Danovaro et al. 1993; Fabiano et al. 1995). PRTs showed strong negative correlation with sand ( $r = -0.792$ ;  $p < 0.001$ ) and positive correlation with silt ( $r = 0.779$ ;  $p < 0.001$ ) and clay ( $r = 0.434$ ;  $p < 0.034$ ) suggesting an important role of sediment grain size in the accumulation of PRT. The dominance of PRT at Chicalim during PreM (Fig. 4f) indicates fresh OM, which could be contributed either by benthic primary production (Fabiano et al. 1995) or deposition of phytoplankton from the water column (Pusceddu et al. 2003). Higher PRT content during PreM will benefit *P. malabarica*, as their peak somatic period is from February to April. Low protein content during POM coinciding with higher abundance can be attributed to assimilation by the bivalves as they attain the harvestable size during this season.

The chlorophyll-*a* in the surface sediment was almost similar at both the sites, but the content of PRT was high at Chicalim can be attributed to the accumulation of large amounts of OM derived from the water column phytoplankton. Diatoms are an important component of the microphytobenthos population, which account for nearly 50% of primary production in the estuarine benthic environments (Underwood and Kromkamp 1999). The annual microphytobenthos production in the Zuari-Mandovi estuarine system was found to range from 34.6 – 52.7 g C m<sup>-2</sup> yr<sup>-1</sup> (Ansari and Parulekar 1994). Krishna Kumari et al. (2002) reported high primary production and abundance of phytoplankton cells in the water column during PreM in the Zuari estuary. Chicalim is influenced by different anthropogenic activities such as shipbuilding workshops and yards, barge cleaning, repairing, etc., whereas Siridao is relatively pristine when compared to Chicalim (Khandeparker et al. 2017). Studies have pointed out high sediment PRT content in the industrial and anthropogenically influenced sites in the estuarine and coastal systems (Cotano and Villate 2006; Muniz et al. 2011; Venturini et al. 2012; Salas et al. 2015). Relatively high (~7 times higher) PRT concentration at Chicalim than Siridao sediment suggests anthropogenic inputs, and the results are also supported by MALDI-TOF Mass spectrum analysis. However, irrespective of the source, higher availability of protein in the sediment in the case of Chicalim will benefit the deposit-feeding benthic invertebrates. The input of terrestrially derived materials in the Chicalim sediment is also supported by the predominance of CHO during MON and POM. This was also indicated by TOC/TN ratio, which has been used to discriminate the OM sources into the marine and terrestrial derived matter (Meyers 1994). The sedimentary OM characterized by low TOC/TN ratios (4-10) indicates a contribution from bacteria and plankton, whereas high TOC/TN ratios (>20) suggests input from terrestrial plant material (Meyers 1994). The minimum TOC/TN ratio during PreM and maximum during POM at Chicalim, indicate the contribution of OM from both marine and terrestrial origin to the sediments during PreM and further increase in terrestrial OM inputs during MON and POM. The surface run-off through the river basin during MON acts as a source of terrestrial materials in the estuarine sediment. The contribution of CHO to the labile organic matter (LOM) at Siridao also increased during MON (41.6%) compared to PreM (30.7%), indicating the input of terrestrial matter. However, relatively high CHO (~10 folds) content at Chicalim attributed to semi-enclosed nature and such a condition would support *P. malabarica* (Table 3a).

During all the seasons, the PRT/CHO ratio was more than 1 at Siridao, and it was maximum during POM followed by PreM and MON. However, at Chicalim, the PRT/CHO ratio was high during PreM (1.76), and it was less than 1 during MON and POM. The PRT/CHO ratio has been used to differentiate the fresh or aged OM (Danovaro et al. 1993) in the sediment. PRT/CHO ratio >1



indicates newly or recently generated OM and the ratio  $<1$  suggests the presence of non-living or aged detrital material (Danovaro et al. 1993). This indicates that at Siridao, the OM was newly generated, whereas, at Chicalim, it was degraded-detrital and heterotrophic in nature. Low ratios also suggest their utilization as indicated by the higher abundance of *P. malabarica*. This was also supported by observations carried out at Dona Paula Bay, which is situated at the Zuari estuarine mouth, as sedimented particulate matter flux in the sediment trap was relatively fresh during summer, and detritus rich during the winter season (Bhaskar et al. 2000). The high percentage of TVC (viable heterotrophic bacteria) to TBC during MON compared to non-monsoon seasons at both the sites suggests their role in allochthonous OM processing. Studies on the water column primary production and bacterial carbon demand from the station located near Chicalim revealed a shift from an autotrophic system during PreM to the heterotrophy during MON and POM seasons (De Souza et al., 2003). A recent study by Eswaran and Khandeparker (2017) reported a high rate of bacterial production as well as the change in the bacterial community in the surface water in the inner region of this estuary during MON, which was found to be fuelled by the allochthonous material of riverine origin. These observations also support net heterotrophy during MON season in the Zuari estuary. The Chicalim being a low energy area, will benefit the deposition of OM of allochthonous origin, a condition favourable for the growth of *P. malabarica*.

Lipids (LPD) are useful to indicate the detrital input derived from the water column and benthic production (Muniz et al. 2011), and LPD content has been used to determine the nutritional quality of sedimentary OM (Gremare et al. 2002). Although the PRT, CHO, and LPD were high in Chicalim sediment, the percentage contribution of LPD to the LOM was high at Siridao (on an average 23%) when compared to Chicalim (10.6%) indicating nutritionally rich detrital input at Siridao (Table 3a). This probably helps the larger (harvestable size 21-37 cm) filter feeding *M. Casta* while siphoning the seawater from just above the sediment surface during ebb and flood tide. However, how much of this is actually available to the bivalves needs further validation. High PRT/CHO and LPD/CHO ratios at Siridao compared to Chicalim indicate labile and nutritionally rich OM. However, lower ratios during MON suggest lower productivity. Previous studies on the carbon and nitrogen isotopic composition of suspended particles from the Zuari estuary indicated terrestrial derived OM during southwest monsoon (SWM) and in situ produced OM during non-monsoon seasons (Bardhan et al. 2015; Shynu et al. 2015). The high flushing rate, high turbidity in the water column leading to lower light intensity were responsible for low autochthonous production during the period of SWM (Bardhan et al. 2015). The contribution of LOM to the TOM decreased during MON and POM seasons as compared to PreM, indicating an increase in the refractory OM in

the sediments of Chicalim and Siridao. Recent study on TOC/TN ratio at the Zuari estuary also reported mixed input of marine and terrestrial OM at Chicalim (C/N ratio: 16.56) and autochthonous OM at Bambolim (C/N ratio: 8.7) as well as Agacaim (C/N ratio: 10.2), the stations located on the either side of Siridao along the northern bank of Zuari estuary (Gardade and Khandeparker 2017). Such autochthonous OM could be derived from microphytobenthos, which in turn benefit the suspension feeders.

Thus, relatively high content of elemental and biochemical components at Chicalim as compared to Siridao could be attributed to the semi-enclosed nature, sediment grain size, and the influence of anthropogenic input. It seems that the degraded-detrital and aged OM of low quality accumulated in Chicalim sediment are available for *P. malabarica* as compared to recently generated fresh OM with better quality and high nutritional value found in Siridao sediment which possibly helps the suspension feeders during the high water.

#### **Sources of fatty acids in the sediments**

The study region is influenced by the tides, waves, and winds causing re-suspension of sediment and the churning of water, which could be attributed to the patchy distribution of FAs in the sediment core. The sources of organic matter were identified based on the FA biomarkers (Table 5). The short-chain saturated FAs (C12 - C20) are the indicators of phytoplankton in the marine environment (Carrie et al. 1998; Hu et al. 2006; Guo et al. 2019), however, long-chain FAs (C22 – C30) are indicators of vascular plants (Wakeham and Beier 1991; Meyers 1997; Meziane and Tsuchiya 2000; Dunn et al. 2008; Guo et al. 2019). The dominance of SCFAs at Chicalim and Siridao suggested input of phytodetritus in the sediment core, as SCFAs are less susceptible to microbial degradation and indicate deposition of partially degraded aged OM in sediments (Dunn et al. 2008). Sediment trap studies at the Dona Paula Bay have revealed ~61% of particulate matter of primary production gets mineralized in the water column and remaining ~39% reach to the sediment (Bhaskar et al. 2000). Further, large amount of sedimented carbon is either consumed (98%) at the sediment-water interface or re-suspended before burying into the sediment. A recent study on lipid biomarkers from the Pearl River estuary reported that phytoplankton derived labile OM undergoes microbial degradation during sinking through the water column as well as in the surface of the sediment; however, terrestrial OM which is refractory in nature gets enriched in the sediments (Guo et al. 2019). The vascular plant-specific FAs (C22:0 or C24:0) were dominant during the PreM at Siridao and during MON and POM seasons at Chicalim, indicating deposition of plant material, which will add to the quality of food available for the deposit feeders. The banks of Mandovi-Zuari Rivers and the

connecting canal (Cumbarjua) between these estuaries are lined with mangroves, and other terrestrial plants and Cumbarjua canal supplies additional water and sediment to the Zuari estuary during MON and ebb tides (Dessai et al. 2009). Wafar et al. (1997) reported the maximum litter fall of mangroves during the PreM and POM seasons in the Mandovi-Zuari estuarine systems. Though mangrove vegetation is found near Siridao, the material derived from the mangroves is expected to be exported out to other regions of the estuary due to the hydrodynamics operating at Siridao, resulting in lower content of LCFA during the MON and POM seasons compared to the PreM. The PUFAs, C18:2 $\omega$ 6 and C18:3 $\omega$ 3 have been used as biomarkers of mangroves, which are found in the leaves of mangrove plants (Meziane et al. 2007). The detection of LCFAs and PUFAs confirmed the contribution of mangrove-derived OM in the sediments of the Zuari estuary, and this might benefit the benthic organisms. These PUFAs have also been used as biomarkers for green macroalgae, and their presence indicates fresh detritus inputs to the sediment (Carrie et al. 1998; Meziane and Tsuchiya 2000). The long-chain PUFA, C22:6 $\omega$ 3 and C20:5 $\omega$ 3 have been used as typical dinoflagellates and diatom biomarkers, respectively (Volkman et al. 1980, 1989; Carrie et al. 1988; Zimmerman and Canuel 2001). Although previous study has reported presence of these PUFAs in the surface sediments of the Zuari estuary (Harji 2011), these PUFAs were not detected in the present study indicating their degradation either by bacteria or its utilization by the higher organisms via grazing (Carrie et al. 1998; Hu et al. 2006; Dunn et al. 2008).

The MUFAs are commonly found in algae, zooplankton, and bacteria. The FA, C22:1 $\omega$ 9 is used as a specific biomarker of zooplankton (Venturini et al. 2012) and was found at both the stations during the PreM and the FA, C24:1 $\omega$ 9, a zooplankton biomarker (Carrie et al. 1998) was reported at Chicalim during PreM and MON seasons. The benthic zooplankton, Mysids, and Cumaceans were reported from the Mandovi-Zuari estuarine system (Padmavati and Goswami 1996), which are mostly benthic, living on the surface of the sediments or even burrow into the sediments and also found swimming in the water column (Porter 2016). These are generally omnivorous and can feed on algae, detritus, and zooplankton. The Cumaceans, feed on microorganisms and organic material from the sediment and can compete for the food from the sediment with *P. malabarica*. The reduction in salinity due to a large amount of freshwater input into the estuary during the SWM may cause death and decay of the zooplankton, adding to the organic pool.

The studies on the microbial community from the surface sediment of Chicalim revealed the dominance of specific group, i.e. *Gammaproteobacteria* at Chicalim (91%) than Siridao (55.9%)

indicating less diversity at the contaminated site than relatively pristine site (Khandeparker et al. 2017). The *Gammaproteobacteria* have been reported to play a significant role in the sulfur cycling as well as in the degradation of OM which is derived from phytoplankton in the marine sediments (Asami et al. 2005; Gihring et al. 2009; de Moraes et al. 2014) resulting in readily available food for the deposit feeders.

### **Seasonal variations in the diet of bivalves**

Bivalves are filter feeders and are generally found in the estuarine sediments (Harkantra 1975; Nair et al. 1984; Parulekar et al. 1984; Alexander et al. 1993; Narasimham and Laxmilatha 1996). On examination of the gut content of *P. malabarica*, granules of sediment particles as well as microphytobenthos were observed. Whereas, *M. casta* take their food from the water column by filtering the seawater and such filter-feeding habit is responsible for the accumulation of particles inside their body (Al-Mossawi et al. 1983). The abundance of these bivalves in Siridao (*M. casta*) and Chicalim (*P. malabarica*) also coincided with the chlorophyll-*a* in the sediment, further pointing towards the importance of food from the sediment for these bivalves. Though *Paphiamalabaricais* dominant at Chicalim, their abundance was higher than *M. Casta* which are dominant at Siridao. It can be noted that the harvestable size of *P. Malabarica* is smaller (19-30mm) than the harvestable size (21-37mm) of *M. casta*. This indicates that the density of bivalves depends upon the space available for their growth, and will result in competition for food. Since *M. casta* is a suspension feeder and can filter their food, mainly phytoplankton from the water column, it might experience comparatively lower competition for food when compared to *P. malabarica*.

The FAs from the tissues of the bivalves varied with the seasons, and some were species-specific. During PreM, the FA, C20:4 $\omega$ 6 was dominant (40.5%) in the tissue of *P. malabarica*. This FA is found dominant in the diatoms along with C20:5 $\omega$ 3, C14:0, C16:1 $\omega$ 7, and are used as biomarkers of diatoms (Volkman et al. 1980; Carrie et al. 1998). Other diatom-specific FAs including C16:1 $\omega$ 7 (4.82%), C14:0 (3.51%), and dinoflagellates specific FA, C18:1 $\omega$ 9 (2.7%) also found in the tissues of *P. Malabarica* which might have contributed from the food. It has been reported that the diatoms and dinoflagellates form a major component in the diet of bivalves (Alfaro et al. 2006; Bachok et al. 2009; Wang et al. 2015). The diatoms are the dominant contributors to the phytoplankton community in the water column of this estuary, followed by dinoflagellates and a minor contribution from silicoflagellates and blue-green algae (Krishna Kumari et al. 2002; Patil and Anil 2011). Thus, *P. Malabarica* being a facultative deposit feeder, the presence of diatom-specific FAs in their tissues can be attributed to their feeding on both sediment and water column

phytoplankton and their assimilation. The availability of dead and degraded phytoplankton deposited in the sediment and the microphytobenthos as food for *P. Malabarica* cannot be ruled out and requires further studies to specifically characterize the source of food. It has been reported that the FA, C20:4 $\omega$ 6 plays an important role in the maturation of gametes of bivalves (Soudant et al. 1996; Bachok et al. 2009). The gametogenesis and gonad developmental activities of *P. Malabarica* are high during PreM (Mohite and Mohite 2009). Nagvenkar and Jagtap (2013) found high content of proteins and lipids during PreM season in the *P. malabarica* from the Zuari estuary and suggested their role in the maturation of gametes. Thus, the presence of FA, C20:4 $\omega$ 6 in *P. Malabarica* during PreM season would provide energy required for gametogenesis. Earlier studies have reported that terrestrial plants also act as source of food for the bivalves (Bachok et al. 2009; Wang et al. 2015) however, their contribution was low (<4%) in their tissues. The present observations corroborate with these findings, and content of LCFA (C24:0) in *P. Malabarica* was low. TOC/TN ratio (17.5) indicated a mixed contribution of marine and terrestrial derived OM in the surface sediment of Chicalim. This also supports the presence of FAs derived from the diatoms, dinoflagellates, and higher plants in the tissues of *P. malabarica*.

At Siridao, the presence of short-chain saturated FAs (C12+C14+C16+C20; 38.4%) and dinoflagellate specific FA (C18:1 $\omega$ 9; 4.01%) suggests phytoplankton derived material as an important source of food for *M. Casta* during PreM. It has been reported that bivalve has diverse filtering activity which is found to be influenced by the concentration of planktons, their size and shape as well as nutritive values (Rajesh et al. 2001; Arapov et al. 2010). The high contribution of dinoflagellates than diatoms was also reported in the diet of filter-feeding bivalves *Austrovenus stutchburyi* and *Paphies australis* from the Matapouri estuary (northern New Zealand) (Alfaro et al. 2006) and it was attributed to the pelagic nature of dinoflagellates. Occurrence and dominance of diatoms and dinoflagellates varied with space and time in the water column of the Zuari estuary (Krishna Kumari et al. 2002; Patil and Anil 2008, 2011). Alkawri and Ramaiah (2010) found high abundance of dinoflagellates at low salinity estuarine station, i.e. off Siridao. The occurrences of diatom and dinoflagellate blooms were reported (Patil and Anil 2008, 2011) in the nearby region (~2-3 km) of the present study site (Chicalim). Though this phenomenon was found more prominent during the monsoon period, the diatom blooms are also reported during the non-monsoon seasons. The termination of blooms leads to the deposition of planktons to the sediment contributing to the sediment organic pool. Tamelander and Heiskanen (2004) reported that diatoms with intact cells settle faster in the coastal Baltic Sea whereas, vegetative cells of dinoflagellates undergo disintegration process in the water column leading to the formation of slower

sinking phytodetritus during a spring bloom. Sara (2006) pointed out that hydrodynamic conditions of the regions have an impact on the origin and quality of OM available for the bivalves (mussels) while studying a lagoon which is open to flow and closed pond. It was observed that the mussels in the closed pond could be able to use *in loco* produced OM by means of wind-driven resuspension, whereas in lagoon, the benthic and pelagic habitats were uncoupled with each habitat having distinct dynamics and production (Sara 2006). Similar are the conditions in Chicalim (actively coupled benthic and pelagic habitats) and Siridao (with uncoupled benthic and pelagic habitats). Thus the OM available for the bivalves will have a profound influence on the secondary production. Sediment texture analysis also revealed that Siridao is hydrodynamically more energetic than Chicalim and the impact of this was observed on the content of OM at both the study sites. The content of sedimentary chlorophyll-*a*, although was almost similar at both the sites, the PRT content was relatively high at Chicalim (PRT 4.15 mg g<sup>-1</sup>; PRT/CHO 2.12; Chl*a* 2.11 µg g<sup>-1</sup>) than Siridao (PRT 0.43 mg g<sup>-1</sup>; PRT/CHO 1.34; Chl*a* 1.73 µg g<sup>-1</sup>), suggesting accumulation of planktonic particles from the water column. The diverse flux of food particles, species-specific feeding nature, and hydrodynamic condition of the study sites could be the possible reasons for the difference in the composition of food particles in the diet of *P. malabarica* and *M. casta*.

The MON season was characterized by the dominance of bacteria and mangrove-derived FAs over diatoms and dinoflagellates in the tissues of both the bivalves. It has been reported that mangrove detritus enriched with bacteria act as one of the important food sources for the suspension-feeding bivalve, *Geloinacoaxans* in the subtropical mangrove forest, Okinawa, Japan (Bachok et al. 2003). However, the two bivalves in the present study show differential feeding habits, benefits of such nutrition will differ from species to species along with the habitats. The consumption of mangrove-derived detritus by the filter-feeding bivalves (*Austrovenus stutchburyi* and *Paphies australis*) was also reported from the Matapouri estuary of northern New Zealand (Alfaro et al. 2006). Zuari estuary is characterized by high run-off (100 to 400 m<sup>3</sup> s<sup>-1</sup>) during SWM and relatively low (< 10 m<sup>3</sup> s<sup>-1</sup>) during the dry period (Shetye and Murty 1987). The surface run-off through catchment area and mangrove vegetation during MON precipitation could transport large amount of terrestrial materials into the estuarine area. The mangrove litterfall from the Mandovi-Zuari estuarine system acts as an important source of particulate and dissolved matter for the microbial food chain (Wafar et al. 1997). Chicalim being hydrodynamically less energetic, the sedimentation of particles associated bacteria would favour the deposit feeders. The high contribution of viable heterotrophic bacteria to the TBC and low PRT/CHO (<1) ratio in the surface sediment during MON at both the

sites suggests role of bacteria in the processing of allochthonous OM and the presence of degraded-detrital OM.

During POM season, high (38.6%) content of short-chain saturated FAs indicate the presence of partially degraded materials in the tissues of *P. malabarica*. Diatom specific FA(C16:1 $\omega$ 7;3.51%)was also reported in *P. Malabarica* during this season, but its contribution was low compared to PreM. However, diet of *M. casta* showed a dominance of bacteria-specific FA (C17:0; 4.42%) followed by dinoflagellate(C18:1 $\omega$ 9; 1.73%) and diatom(C16:1 $\omega$ 7; 1.11%)specific FA pointing towards the capture of food from the water column rather than from the sediment. The ratio of PRT/CHO showed detrital-heterotrophic nature of sedimentary OMat Chicalim (PRT/CHO: 1.17) and newly generated fresh OM at Siridao (PRT/CHO: 4.89). The high content of TOC/TN ratio (23.6)in the sediments of Chicalim also suggested input of allochthonous matter during this season. The seasonal changes in the source of food could be the reason for the variations in the food composition of bivalves,which in turn could be responsible for seasonal variation in the abundance of bivalves. Bachok et al. (2009) reported seasonality in the food sources of *Quidnipaguspalatum*,a bivalve collected from the intertidal flat (Tomigusuku) of Okinawa Island, Japan with dominance of vascular plants and bacteria during warm season, macroalgae and phytoplankton during cold and diatoms during rainy season and these food components were derived from the sediment and the water column.Wang et al. (2015) revealed that the availability of food in the system and species-specific feeding habits of the bivalves are the major factors for the seasonal changes in their diet composition, as observed in the present study.

### **Factors influencing the partitioning of bivalves in the Zuari estuary**

The bivalve *P.malabarica*is found dominant in the silty-sandy sediment of Chicalim, and *M. casta*in the sand dominated sediment of Siridao. The distribution of these species was reported earlier in the estuaries of Goa (Nagvenkar 2014;Parulekar et al. 1984). Earlier studies have reported the occurrence of these bivalves(*Paphiaspp.* and *Meretrix spp.*)along the Kali estuary, Karnataka (Alagarswamy and Narasimham 1973; Nair et al. 1984) and Ashtamudi estuary, Kerala (Appukuttan et al. 2002).

The RDA analysis depicting the relationship between biotic and abiotic variables with respect to the abundance of bivalves indicated that the sedimentary PRT content was one of the determining ( $p<0.05$ ) factors in governing the distribution of these bivalves in their respective habitats. The bivalve *P.malabarica*,present in Chicalim found to be influenced by the high content of biochemical parameters,organic carbon, sediment with silt and clay, and higher sediment chlorophyll-*a*. The

sediment with higher percentage of fine particles hold higher content of food material, and such habitat is suitable for facultative deposit-feeding bivalves. However, *M. Casta* was mainly influenced by sandy sediment and chlorophyll-*a* from the water column, and this can be attributed to its suspension-feeding habit suggesting the utilization of food particles directly from the water column. It has been reported that bivalves change their feeding behaviour with respect to sediment type, water flow, and hydrological conditions (Olafsson 1986). The bivalve, *Macomabalthica* from exposed sandy habitat preferred suspension-feeding mode and bivalves from stagnant, sheltered, muddy sand sediment showed deposit-feeding habits (Olafsson 1986). Suspension feeding was mainly observed during the high amount of suspended materials, and deposit-feeding was a response of a decrease in the supply of these food particles.

Alexander et al. (1993) reported that the distribution and burrowing of bivalves are dependent upon the sediment characteristics and shell morphology and ornamentations. The morphological features of these two bivalves are comparatively distinct. The shell of *P. malabarica* is slightly inflated, triangularly ovate with a concentric sculpture on the surface, and the anterior and posterior margins are narrowly rounded. However, the shell of *M. casta* is thick, smooth, devoid of any sculpture, and triangularly ovate (Narasimham and Laxmilatha 1996). The bivalves with smooth and flattened shells get a chance to escape from the wash-out event of the high energy waves, and currents during flood tide and thick shell facilitate them to cope up with high-temperature exposure for a longer duration of ebb tide period (Soon et al., 2017). Thus, *M. casta* with the smooth shell may be well adapted to the sandy substratum at Siridao, which may help in easy burrowing into the tightly packed sediment to avoid predation, and as this region gets exposed for a longer duration during low tide, the thick shell of *M. casta* is advantageous. Bivalve, *M. Casta* being suspension feeder, also gets a chance to use suspended particles during incoming high tide water as reflected in the composition of the diet, which showed the dominance of dinoflagellates, mangrove litter, and bacteria. However, *P. malabarica* used OM derived from diatoms as the major food source along with terrestrial materials, dinoflagellates and bacteria. Dinoflagellates are mostly found as pelagic phytoplankton and are directly available to the suspension feeders (Alfaro et al. 2006), as observed in the case of *M. Casta* and the RDA output also showed relation between *M. casta* population and water column chlorophyll-*a*. The sediment chlorophyll-*a* content was almost similar at both the sites, but, high content of OM at Chicalim compared to Siridao suggests the accumulation of either fresh or detritus planktonic matter from the water column which could be available for deposit-feeding *P. malabarica*. Siridao being shallow mudflat showed low accumulation of OM in the sediment from the water column. It seems that sediment characteristics driven by a complex interplay of physical processes



and seasons resulting in variation in the quality and quantity of food determine the fate of these organisms.

### **Summary:**

The two economically important bivalves, *Paphia malabarica* and *Meretrix casta* inhabit the Zuari estuary. A clear partitioning in their population has been observed as *Paphia malabarica* (Chemnitz, 1782) inhabit the southern bank (Chicalim), whereas *Meretrix casta* (Gmelin, 1791) inhabit the northern bank (Siridao) of the Zuari estuary. *Paphia malabarica* an exclusive inhabitant of Chicalim with silty-sandy sediment, whereas, *M. casta*, exclusive to Siridao, which is dominated by sand. The population of *P. malabarica* facilitated by the semi-enclosed nature of the habitat at Chicalim with high amount of degraded and aged sediment organic carbon, high chlorophyll-*a*, elemental, and biochemical components. Whereas, Siridao which experience high impact of tidal currents, low sediment organic carbon, and high water column chlorophyll-*a* facilitated *M. casta*. The fatty acids in the tissues of *P. malabarica* indicate their ability to source the food from the sediment and water column, whereas, *M. casta* can source their food from the water column. Chicalim can be considered as an actively coupled benthic-pelagic habitat, and Siridao as an uncoupled habitat. Thus, the diverse flux of food particles, nature and quality of food, species-specific feeding ecology, and local hydrodynamics operating at these habitats could be the determining factors in the partitioning of the bivalves. Since, habitats act as breeding grounds for the bivalves, they can be demarcated for population replenishment during the spat fall for successful recruitment of these commercially important bivalves and controlled harvest methods can be employed for conservation and management of the natural stocks.

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### Captions for figures

**Figure 1.** Map showing location of sampling stations in the Zuari estuary.

**Figure2.** Seasonal and vertical distribution of sediment texture at the study sites (a) Chicalim (b) Siridao in the Zuari estuary. (Note: PreM: Pre monsoon; MON: Monsoon; POM: Post monsoon).

**Figure3.** Seasonal variations in the abundance of bivalves and sediment chlorophyll-*a* at Chicalim and Siridao, Zuari estuary (Note: PreM: Pre monsoon, MON: Monsoon, POM: Post monsoon). Asterisk (\*) and dashed line indicate significant differences among the seasons. (Significance levels - \*\*\* $p < 0.004$ , \*\* $p < 0.017$ , \* $p < 0.043$ )

**Figure 4.** Seasonal and vertical distribution pattern of sedimentary parameters at Chicalim, Zuari estuary. (Note: TVC: Total Viable count; TBC: Total Bacterial Count; NA: Sample not Available; PreM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon). Asterisk (\*) indicate significant difference. Horizontal dashed line indicate significant differences among the seasons and vertical dashed line indicate significant differences among depths. (Significance levels - \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ )

**Figure 5.** Seasonal and vertical distribution pattern of sedimentary parameters at the Siridao, Zuari estuary. (Note: TVC: Total Viable Count; TBC: Total Bacterial Count; PreM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon). Asterisk (\*) indicate significant differences. Horizontal dashed line indicate significant differences among the seasons and vertical dashed line indicate significant differences among depths. (Significance levels - \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.002$ , \*\* $p < 0.024$ ).

**Figure6.** Redundancy analysis (RDA) triplot depicting relation between biotic and abiotic variables during different seasons in the Zuari estuary. Arrows in red indicate abiotic variables and blue indicate species variables. Lambda is eigenvalue explained by the abiotic variables. Variable with  $p < 0.05$  is highlighted in bold.

### Captions for tables

**Table 1:** Results of two-way ANOVA of biogeochemical parameters, sediment texture, bacterial and bivalve abundance in the surface sediments (0-5 cm) at Chicalim and Siridao, Zuari estuary.

**Table 2:** Results of two-way ANOVA of biogeochemical parameters and bacterial abundance in the sediment core (0-20 cm) collected from Chicalim and Siridao, Zuari estuary.

**Table 3a:** Seasonal variations in the mean ( $\pm$  SD) concentration of elemental and biochemical components of the sediments at the study sites along with two-way ANOVA results.

**Table 3b:** Seasonal variations of proteins analysed from surface sediments of Chicalim and Siridao through MALDI TOF/TOF Mass spectrum analysis.

**Table 4:** Seasonal variations in the composition and area (%) of fatty acids (FAs) in the sediment core (0-20 cm) and bivalve tissues from Chicalim and Siridao, Zuari estuary.

**Table 5:** Fatty acid biomarkers used to identify the sources of organic matter.

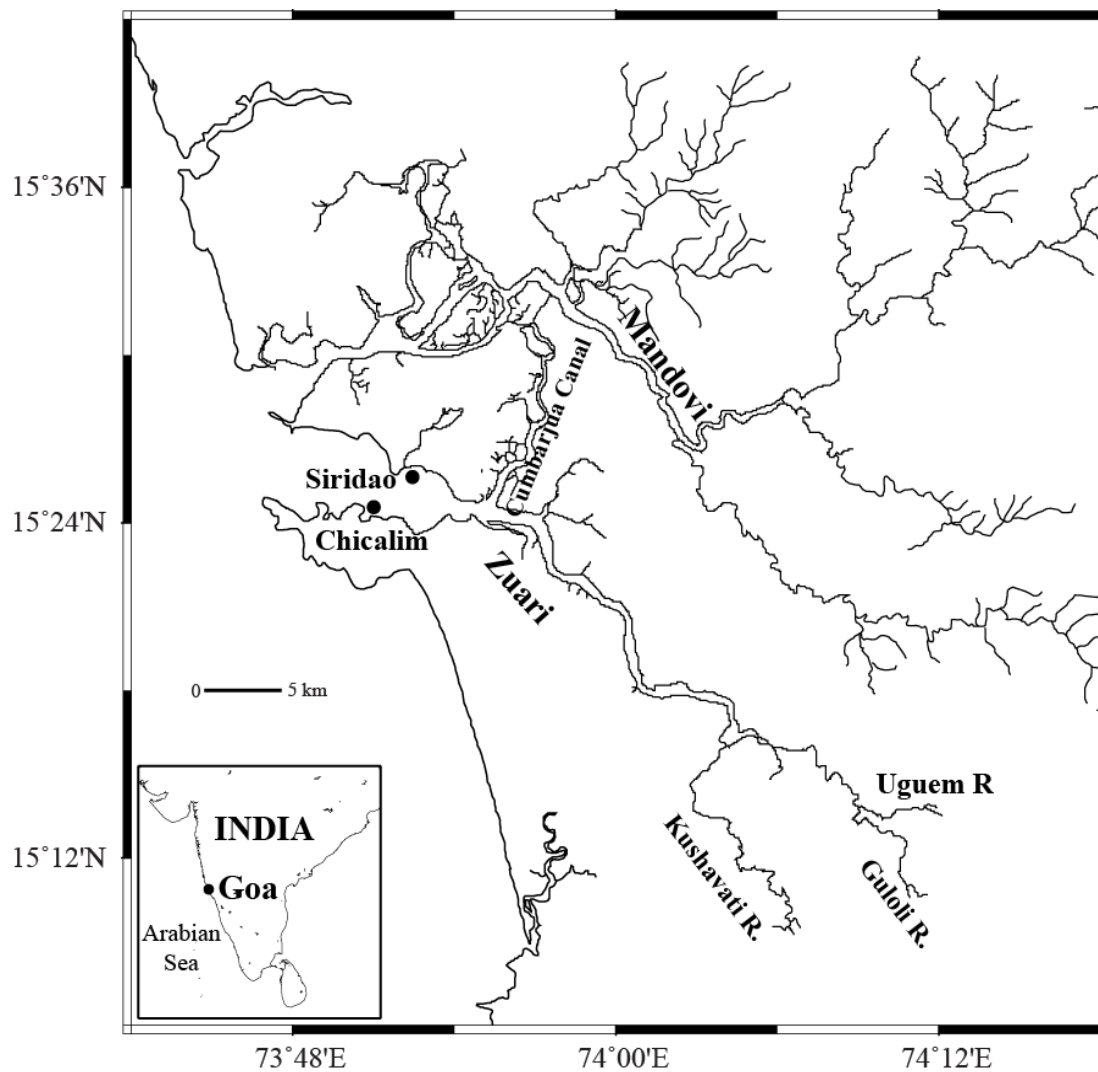


Fig. 1 Map showing location of sampling stations in the Zuari estuary.

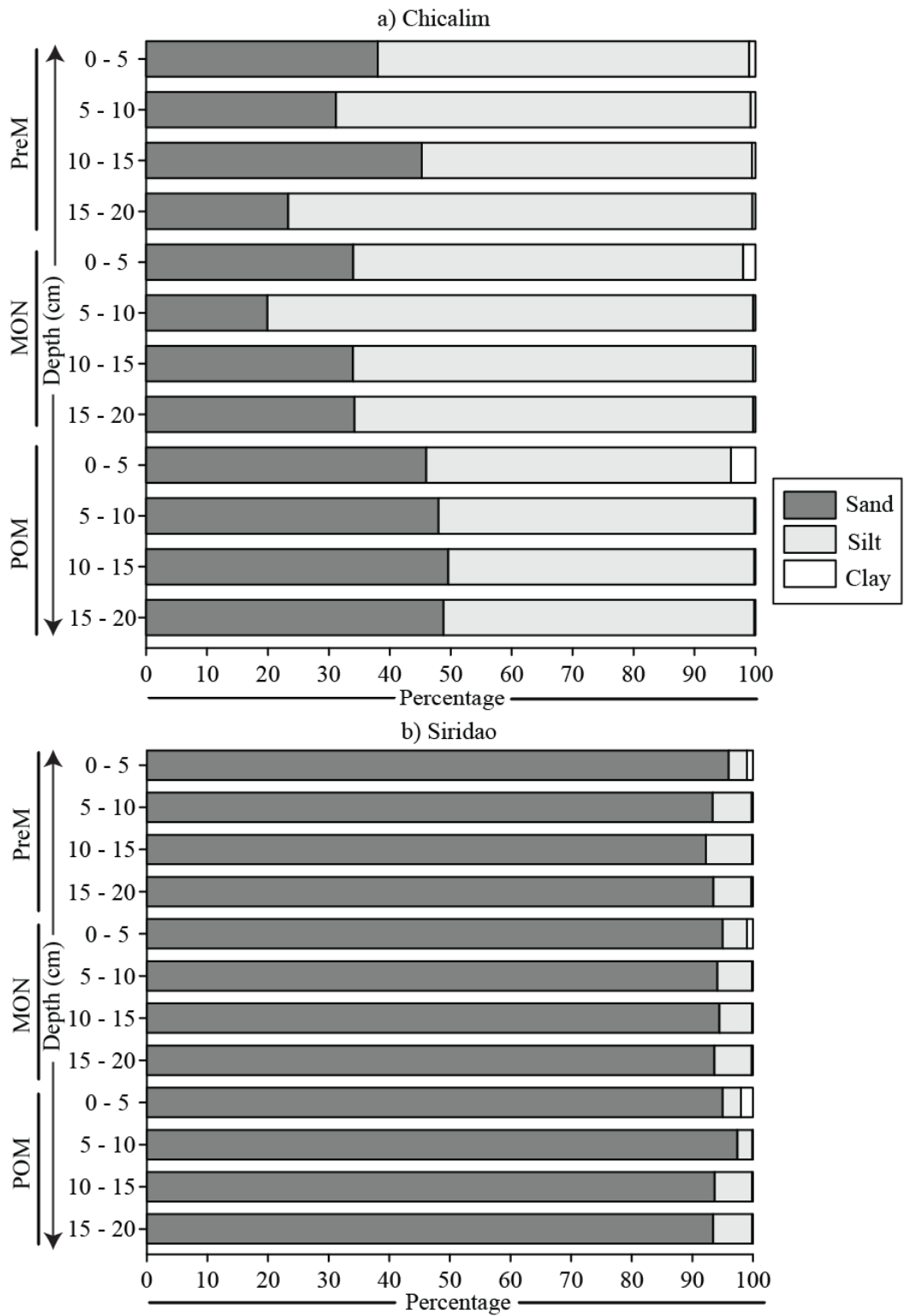


Fig. 2 Seasonal and vertical distribution of sediment texture at the study sites (a) Chicalim (b) Siridao in the Zuari estuary. (Note: PreM: Pre monsoon; MON: Monsoon; POM: Post monsoon).

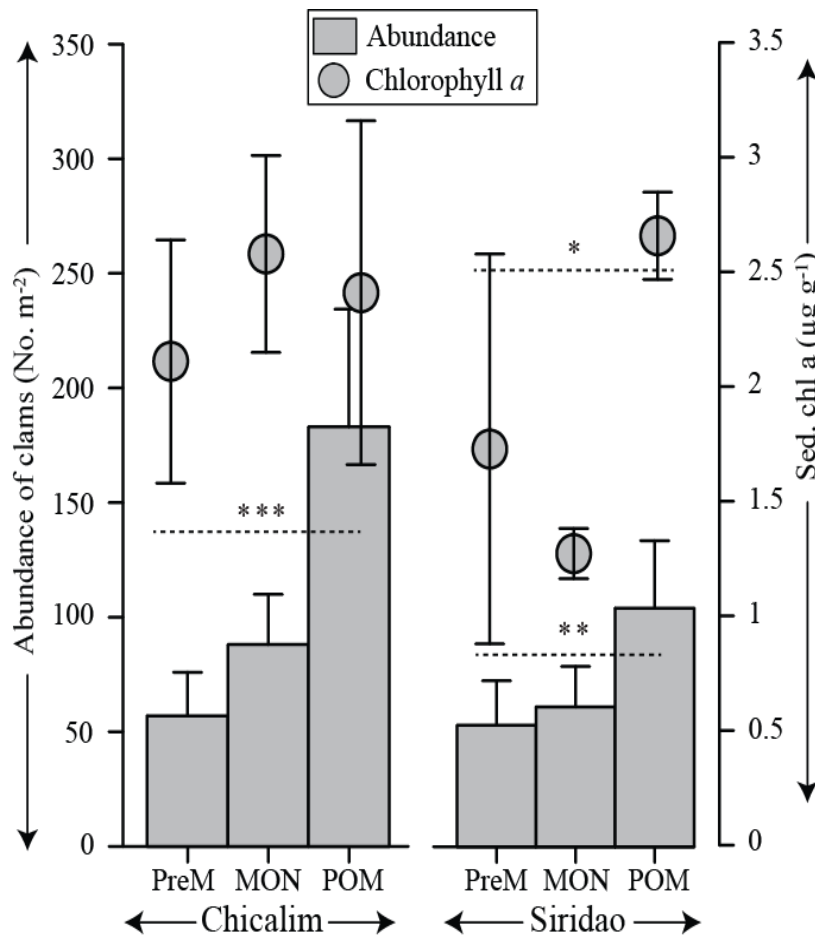


Fig. 3 Seasonal variations in the abundance of bivalves and sedimentary chlorophyll-*a* at Chicalim and Siridao, Zuari estuary (Note: PreM: Pre-monsoon, MON: Monsoon, POM: Post-monsoon). Asterisk (\*) and dashed line indicate significant differences among the seasons. (Significance levels - \*\*\*  $p < 0.004$ , \*\*  $p < 0.017$ , \*  $p < 0.043$ )

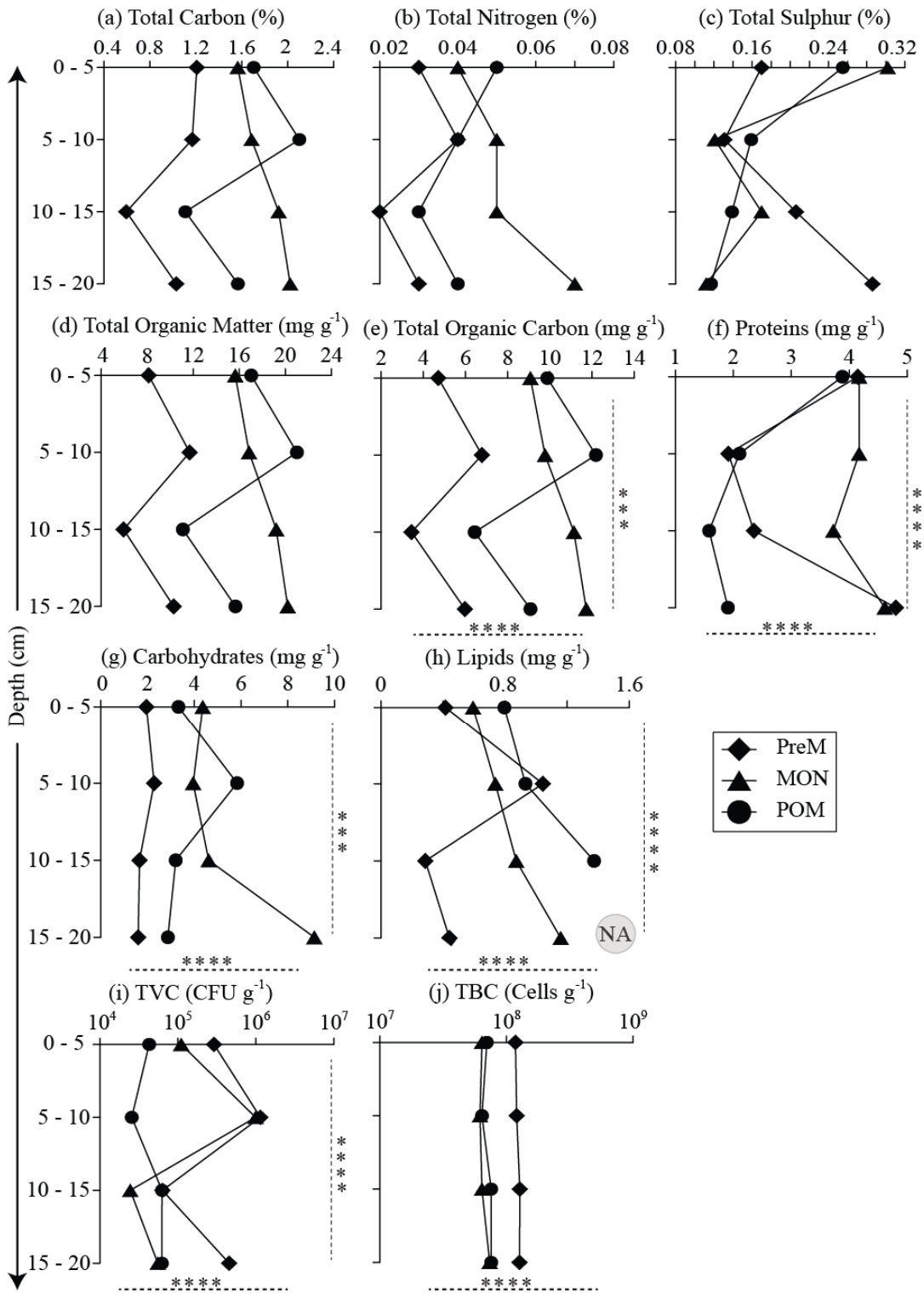


Fig. 4 Seasonal and vertical distribution pattern of sedimentary parameters at Chicalim, Zuari estuary. (Note: TVC: Total Viable count; TBC: Total Bacterial Count; NA: Sample not Available; PreM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon). Asterisk (\*) indicate significant difference. Horizontal dashed line indicate significant differences among the seasons and vertical dashed line indicate significant differences among depths. (Significance levels - \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ )



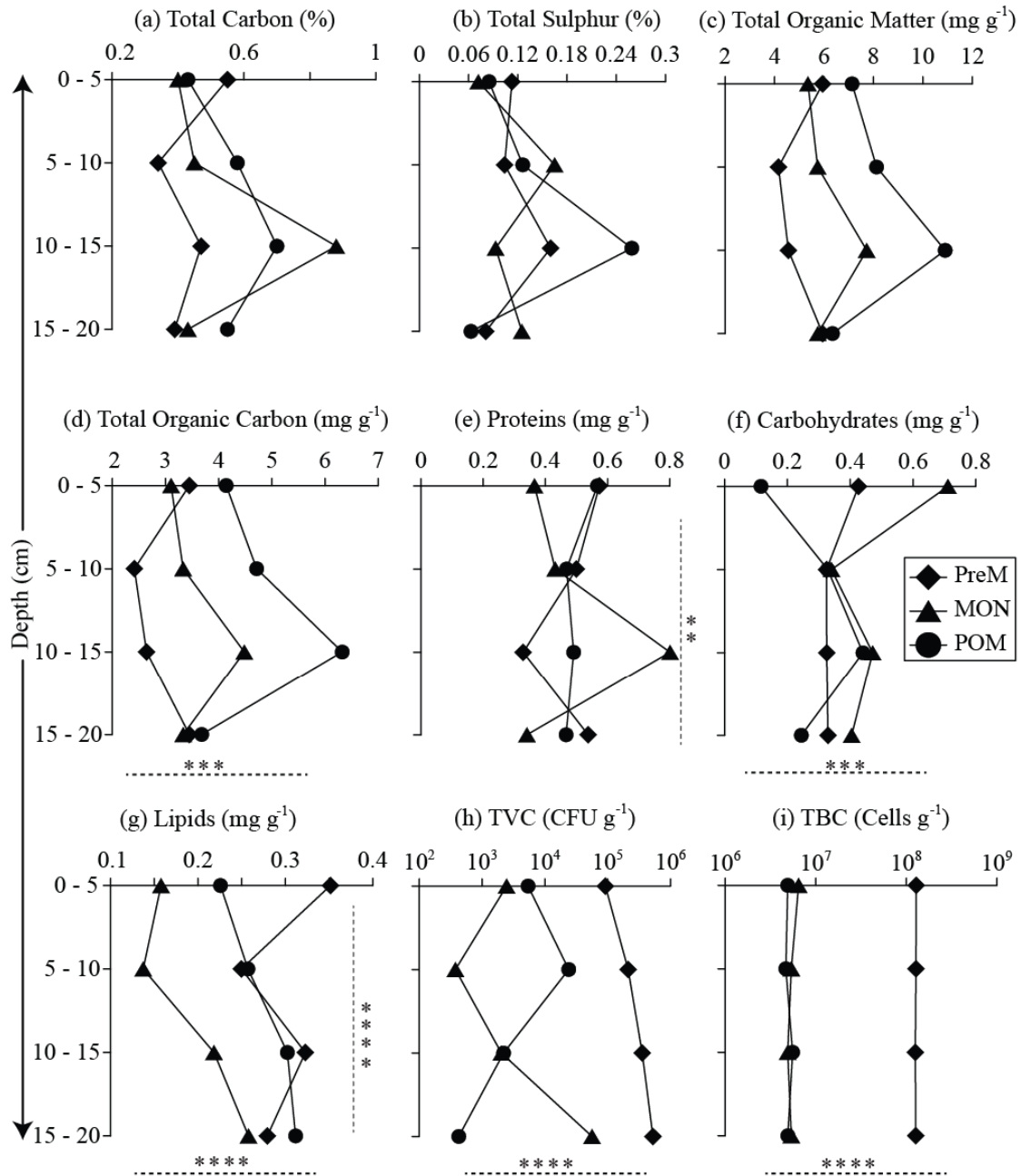


Fig. 5 Seasonal and vertical distribution pattern of sedimentary parameters at the Siridao, Zuari estuary. (Note: TVC: Total Viable Count; TBC: Total Bacterial Count; PreM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon). Asterisk (\*) indicate significant differences. Horizontal dashed line indicate significant differences among the seasons and vertical dashed line indicate significant differences among depths. (Significance levels - \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.002$ , \*\* $p < 0.024$ ).

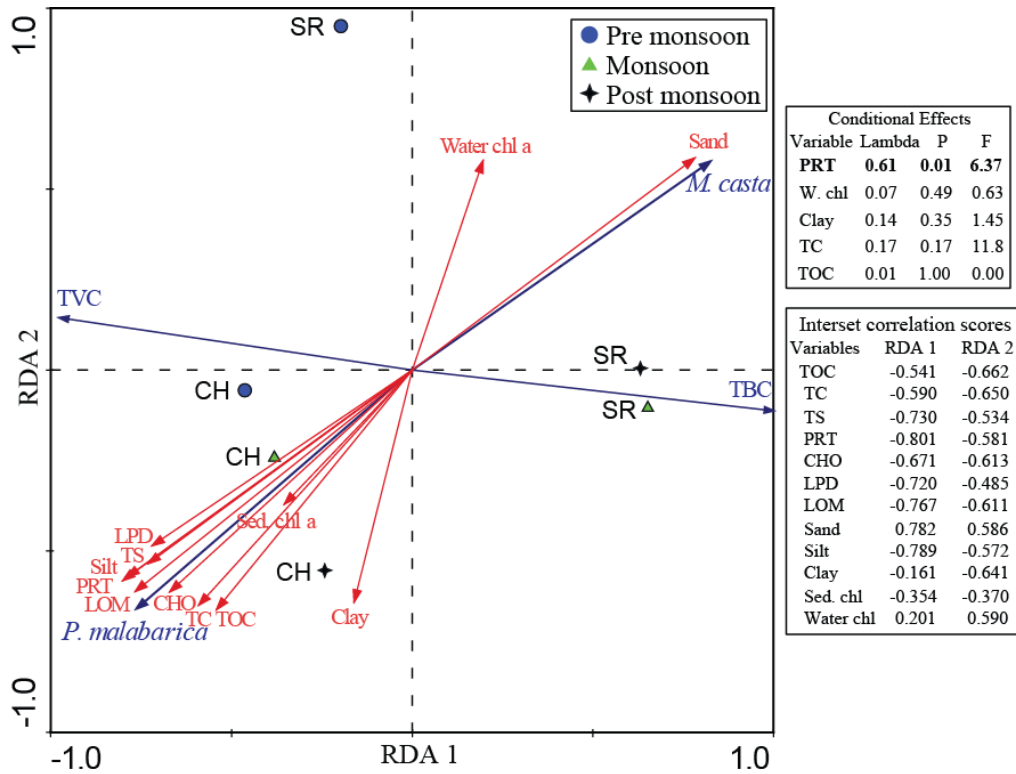


Fig. 6. Redundancy analysis (RDA) triplot depicting relation between biotic and abiotic variables during different seasons in the Zuari estuary. Arrows in red indicate abiotic variables and blue indicate species variables. Lambda is eigenvalue explained by the abiotic variables. Variable with  $p \leq 0.05$  highlighted in bold. (CH: Chicalim; SR: Siridao; PRT: Proteins; LPD: Lipids; CHO: Carbohydrates; LOM: Labile Organic Matter; TC: Total Carbon; TOC: Total Organic Carbon; TS: Total Sulfur; TBC: Total Bacterial Counts; TVC: Total Viable Counts)

**Table 1:** Results of two-way ANOVA of biogeochemical parameters, sediment texture, bacterial and bivalve abundance in the surface sediments (0-5 cm) at Chicalim and Siridao, Zuari estuary.

Factors	Parameters	df	MS	F	p value
<b>Chicalim</b>					
Seasons	TOC	2	0.011	17.216	<b>0.023</b>
	PRT	2	0.001	0.627	0.592
	CHO	2	0.031	22.549	<b>0.016</b>
	LPD	2	0.006	18.200	<b>0.021</b>
	LOM	2	0.005	9.414	0.051
	TVC	2	0.349	235.045	<b>0.001</b>
	TBC	2	0.040	36.754	<b>0.008</b>
	Sand	2	0.015	33.500	<b>0.009</b>
	Silt	2	0.008	8.357	0.059
	Clay	2	0.064	0.997	0.465
	Sed. Chl a	2	0.001	1.147	0.427
	<i>Paphia malabarica</i>	2	0.123	60.648	<b>0.004</b>
<b>Siridao</b>					
Seasons	TOC	2	0.001	0.822	0.519
	PRT	2	0.001	1.058	0.449
	CHO	2	0.011	39.941	<b>0.007</b>
	LPD	2	0.002	37.333	<b>0.008</b>
	LOM	2	0.002	11.700	<b>0.038</b>
	TVC	2	1.387	101.710	<b>0.002</b>
	TBC	2	0.092	56.214	<b>0.004</b>
	Sand	2	0.000	4.500	0.125
	Silt	2	0.028	3.537	0.162
	Clay	2	0.029	3.769	0.152
	Sed. Chl a	2	0.018	10.762	<b>0.043</b>
	<i>Meretrix casta</i>	2	0.046	21.543	<b>0.017</b>
Stations (Chicalim*Siridao)	TOC	1	0.041	59.756	<b>0.0001</b>
	PRT	1	0.811	640.421	<b>0.0001</b>
	CHO	1	0.725	879.040	<b>0.0001</b>
	LPD	1	0.033	172.565	<b>0.0001</b>
	LOM	1	1.159	2.53+03	<b>0.0001</b>
	TVC	1	3.152	417.007	<b>0.0001</b>
	TBC	1	0.005	3.834	0.098
	Sand	1	0.426	1.82+03	<b>0.0001</b>
	Silt	1	3.597	822.189	<b>0.0001</b>
	Clay	1	0.180	5.029	0.066
	Sed. Chl a	1	0.003	2.674	0.153
	Bivalve	1	0.057	27.446	<b>0.002</b>
Seasons*Stations (Sites - Chicalim & Siridao)	TOC	2	0.005	7.402	<b>0.024</b>
	PRT	2	0.001	1.125	0.385
	CHO	2	0.029	35.525	<b>0.0001</b>
	LPD	2	0.006	32.870	<b>0.001</b>
	LOM	2	0.01	21.07	<b>0.002</b>
	TVC	2	0.347	45.936	<b>0.0001</b>
	TBC	2	0.010	7.387	<b>0.024</b>
	Sand	2	0.007	31	<b>0.001</b>
	Silt	2	0.014	3.126	0.117
	Clay	2	0.010	0.281	0.764
	Sed. Chl a	2	0.011	9.363	<b>0.014</b>
	Bivalve	2	0.010	4.570	0.062

(Note: Bold value indicates significant p values; df: degree of freedom; MS: mean square; TOC: Total Organic Carbon; PRT: Proteins; CHO: Carbohydrates; LPD: Lipids; LOM: Labile Organic Matter; TVC: Total Viable Count; TBC: Total Bacterial Count)

**Table 2:** Results of two-way ANOVA of biogeochemical parameters and bacterial abundance in the sediment core (0-20 cm) collected from Chicalim and Siridao, Zuari estuary.

Factors	Parameters	df	MS	F	p value
<b>Chicalim</b>					
Seasons	TOC	2	0.038	78.374	<b>0.0001</b>
	PRT	2	0.080	59.083	<b>0.0001</b>
	CHO	2	0.230	227.764	<b>0.0001</b>
	LPD	2	0.016	33.243	<b>0.0001</b>
	LOM	2	0.115	164.304	<b>0.0001</b>
	TVC	2	1.397	316.064	<b>0.0001</b>
	TBC	2	0.174	124.396	<b>0.0001</b>
Depth	TOC	3	0.005	10.026	<b>0.001</b>
	PRT	3	0.038	28.169	<b>0.0001</b>
	CHO	3	0.010	9.636	<b>0.002</b>
	LPD	3	0.015	31.707	<b>0.0001</b>
	LOM	3	0.008	11.976	<b>0.001</b>
	TVC	3	0.671	151.684	<b>0.0001</b>
	TBC	3	0.004	2.687	0.094
Seasons * Depth	TOC	6	0.003	6.026	<b>0.004</b>
	PRT	6	0.021	15.635	<b>0.0001</b>
	CHO	6	0.030	29.368	<b>0.0001</b>
	LPD	6	0.029	60.768	<b>0.0001</b>
	LOM	6	0.024	34.685	<b>0.0001</b>
	TVC	6	0.441	99.838	<b>0.0001</b>
	TBC	6	0.001	0.428	0.847
<b>Siridao</b>					
Seasons	TOC	2	0.005	11.252	<b>0.002</b>
	PRT	2	0.001	0.952	0.413
	CHO	2	0.009	11.639	<b>0.002</b>
	LPD	2	0.003	72.444	<b>0.0001</b>
	LOM	2	0.003	4.917	<b>0.028</b>
	TVC	2	10.085	111.638	<b>0.0001</b>
	TBC	2	0.397	257.070	<b>0.0001</b>
Depth	TOC	3	0.001	2.628	0.098
	PRT	3	0.003	4.539	<b>0.024</b>
	CHO	3	0.000	0.326	0.806
	LPD	3	0.001	24.111	<b>0.0001</b>
	LOM	3	0.002	3.335	0.056
	TVC	3	0.119	1.314	0.315
	TBC	3	0.001	0.793	0.521
Seasons * Depth	TOC	6	0.001	2.483	0.085
	PRT	6	0.002	3.847	<b>0.022</b>
	CHO	6	0.003	3.143	<b>0.043</b>
	LPD	6	0.000	9.333	<b>0.001</b>
	LOM	6	0.002	3.711	<b>0.025</b>
	TVC	6	1.649	18.253	<b>0.0001</b>
	TBC	6	0.003	1.850	0.172

(Note: Bold value indicates significant p values; df: degree of freedom; MS: mean square; refer Table 1 for abbreviations).

**Table 3a:** Seasonal variations in the mean ( $\pm$  SD) concentration of elemental and biochemical components of the sediments at the study sites along with two-way ANOVA results.

	Chicalim			ANOVA p value			Siridao			ANOVA p value		
	PreM	MON	POM	S	D	S*D	PreM	MON	POM	S	D	S*D
TC (%)	1.00 $\pm$ 0.28	1.80 $\pm$ 0.21	1.62 $\pm$ 0.41	-	-	-	0.44 $\pm$ 0.09	0.54 $\pm$ 0.23	0.57 $\pm$ 0.11	-	-	-
TN (%)	0.03 $\pm$ 0.01	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	-	-	-	BDL	BDL	BDL	-	-	-
TS (%)	0.20 $\pm$ 0.07	0.18 $\pm$ 0.09	0.17 $\pm$ 0.06	-	-	-	0.11 $\pm$ 0.03	0.11 $\pm$ 0.04	0.13 $\pm$ 0.09	-	-	-
TOM (mg g <sup>-1</sup> )	9.02 $\pm$ 2.52	18.0 $\pm$ 2.10	16.2 $\pm$ 4.09	-	-	-	5.15 $\pm$ 0.93	6.14 $\pm$ 1.07	8.13 $\pm$ 1.99	-	-	-
TOC (mg g <sup>-1</sup> )	5.23 $\pm$ 1.46	10.4 $\pm$ 1.22	9.40 $\pm$ 2.37	<b>0.0001</b>	<b>0.001</b>	<b>0.004</b>	2.99 $\pm$ 0.54	3.56 $\pm$ 0.62	4.71 $\pm$ 1.15	<b>0.002</b>	0.098	0.085
PRT (mg g <sup>-1</sup> )	3.30 $\pm$ 1.39	4.17 $\pm$ 0.36	2.37 $\pm$ 1.03	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	0.49 $\pm$ 0.11	0.48 $\pm$ 0.21	0.50 $\pm$ 0.05	0.413	<b>0.024</b>	<b>0.022</b>
CHO (mg g <sup>-1</sup> )	1.88 $\pm$ 0.31	5.52 $\pm$ 2.43	3.81 $\pm$ 1.36	<b>0.0001</b>	<b>0.002</b>	<b>0.0001</b>	0.35 $\pm$ 0.05	0.48 $\pm$ 0.16	0.28 $\pm$ 0.14	<b>0.002</b>	0.806	<b>0.043</b>
LPD (mg g <sup>-1</sup> )	0.55 $\pm$ 0.34	0.84 $\pm$ 0.24	1.03 $\pm$ 0.30	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	0.30 $\pm$ 0.05	0.19 $\pm$ 0.06	0.27 $\pm$ 0.04	<b>0.0001</b>	<b>0.0001</b>	<b>0.001</b>
LOM (mg g <sup>-1</sup> )	5.73	10.5	7.21	<b>0.0001</b>	<b>0.001</b>	<b>0.0001</b>	1.14	1.16	1.06	<b>0.028</b>	0.056	<b>0.025</b>
TOC/TN	17.5 $\pm$ 1.77	20.3 $\pm$ 2.76	23.6 $\pm$ 4.74	-	-	-	NA	NA	NA	-	-	-
PRT/CHO	1.76	0.76	0.62	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	1.38	1.00	1.76	<b>0.0001</b>	0.136	<b>0.001</b>
LPD/CHO	0.29	0.15	0.27	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	0.85	0.40	0.97	<b>0.0001</b>	0.094	<b>0.004</b>
PRT: LOM (%)	57.7	39.6	32.8	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	43	41.8	47.2	<b>0.033</b>	0.087	<b>0.014</b>
CHO: LOM (%)	32.8	52.4	52.9	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	30.7	41.6	26.8	<b>0.0001</b>	0.461	<b>0.003</b>
LPD: LOM (%)	9.5	8.0	14.3	<b>0.004</b>	<b>0.0001</b>	<b>0.0001</b>	26.3	16.6	26.0	<b>0.0001</b>	<b>0.006</b>	<b>0.038</b>
TVC ( $\times 10^5$ CFU g <sup>-1</sup> )	4.88 $\pm$ 4.65	2.96 $\pm$ 4.67	0.48 $\pm$ 0.18	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	2.96 $\pm$ 1.87	0.15 $\pm$ 0.27	0.08 $\pm$ 0.11	<b>0.0001</b>	0.315	<b>0.0001</b>
TBC ( $\times 10^8$ Cells g <sup>-1</sup> )	1.23 $\pm$ 0.05	0.66 $\pm$ 0.05	0.71 $\pm$ 0.06	<b>0.0001</b>	0.094	0.847	1.27 $\pm$ 0.01	0.06 $\pm$ 0.01	0.05 $\pm$ 0.04	<b>0.0001</b>	0.521	0.172
TVC:TBC	0.40	0.45	0.07	-	-	-	0.23	0.28	0.16	-	-	-
Bivalve density (No m <sup>-2</sup> )	57 $\pm$ 19	88 $\pm$ 22	183 $\pm$ 51	<b>0.004</b>	-	-	53 $\pm$ 19	61 $\pm$ 18	104 $\pm$ 29	<b>0.017</b>	-	-

(Note: TC: Total Carbon; TN: Total Nitrogen; TS: Total Sulphur; TOM: Total Organic Matter; TOC: Total Organic Carbon; PRT: Proteins; CHO: Carbohydrates; LPD: Lipids; LOM: Labile Organic Matter; TVC: Total Viable Count; TBC: Total Bacterial Count; BDL – Below Detection Limit; NA: Not Applicable; PreM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon; S: Seasons; D: Depths; Bold values indicate significant p values)

**Table 3b:** Name of some proteins identified by MALDI TOF/TOF-MS, their sources and functions.

Source	Peptide ion	Name of protein	Source organisms	Function
SR-PreM	1336.343	50S ribosomal protein L7/L12	<i>Azorhizobium caulinodans</i>	Protein synthesis (Gudkov, 1997; Smit et al., 2012)
SR-MON	1008.757	Putative quercetin 2,3-dioxygenase PA1205	<i>Pseudomonas aeruginosa</i>	Flavonoid metabolism (Pillai and Swarup, 2002)
SR-POM	768.645	Amino acid acetyl transferase	<i>Yarrowia lipolytica</i>	Acetylation (Smit et al., 2012)
SR-POM	936.739	Serine hydroxymethyl transferase	<i>Mycoplasma genitalium</i>	Amino acid metabolism (Lin et al., 2015)
CH-PreM	877.395	Glutaminase	<i>Alkaliphilus oremlandii</i>	Amino acid metabolism (Smit et al., 2012)
CH-PreM	666.199	Heavy metal-dependent transcription regulator	<i>Rhizobium meliloti</i>	Transcriptional regulator (Reeve et al., 2002)
CH-PreM	877.395	Nucleoid associated protein ybaB	<i>Salmonella paratyphiA</i>	Organization and compaction of DNA (Dame, 2005)
CH-MON	954.310	Phosphoglycerate kinase	<i>Leptospira borgpetersenii</i>	Glycolysis (Lin et al., 2015)
CH-POM	877.188	Regulator of microtubule dynamics protein-2	<i>Macaca fascicularis</i>	Membrane protein (Riederer et al., 1997)
CH-POM	954.319	Uncharacterized protein ORF 47	<i>Ostereid herpesvirus1</i>	<i>Ostereid herpesvirus1</i> involved in mass mortality of oyster <i>Crassostrea gigas</i> (Renault et al., 2014)

(Note: SR: Siridao; CH: Chicalim; PreM: Pre-monsoon; MON: Monsoon; POM: Post-monsoon).

**Table 4:** Seasonal variations in the composition and area percentage of fatty acids (FAs) in the sediment core and bivalve tissues collected from the Chicalim and Siridao located in the Zuari estuary.

Depth (cm)	Pre-monsoon				Monsoon				Post-monsoon			
	Chicalim		Siridao		Chicalim		Siridao		Chicalim		Siridao	
	FA	Area %	FA	Area %	FA	Area %	FA	Area %	FA	Area %	FA	Area %
0 - 5	C18:0	42.6	C12:0	15.2	C14:0	9.32	C12:0	27.8	C12:0	1.65	C12:0	13.2
	C18:2 $\omega$ 6	19.8	C15:0	9.01	C20:0	12.7	C15:0	9.94	C16:0	12.5	C15:0	6.16
	C22:0	4.19	C16:0	15.7	C24:0	31.8	C16:0	5.14	C20:0	6.78	C16:0	12.1
	C22:1 $\omega$ 9	19.9	C18:0	6.71	C24:1 $\omega$ 9	10.8	C18:0	3.01	C22:0	3.85		
5 - 10	C12:0	0.90	C16:0	0.30	C12:0	1.54	C12:0	14.9	C12:0	0.29	C12:0	0.75
	C16:0	2.22	C18:2 $\omega$ 6	4.55	C15:0	11.3	C16:0	3.76	C13:0	0.88	C16:0	3.24
	C18:0	64.3	C18:3 $\omega$ 3	0.69	C16:0	4.71	C17:0	5.18	C15:0	0.17	C18:0	11.8
	C18:2 $\omega$ 6	31.1	C22:0	38.8			C18:0	2.87	C20:0	0.48	C22:0	4.54
			C22:1 $\omega$ 9	21.0					C22:0	80.2		
		C23:0	19.8									
10 - 15	C12:0	9.67	C12:0	4.38	C12:0	13.7	C12:0	11.9	C12:0	0.49	C12:0	3.06
	C15:0	6.40	C16:0	10.5	C14:0	12.3	C15:0	11.4	C15:0	8.44	C17:0	0.88
	C16:0	2.86	C17:0	13.9	C15:0	12.9	C16:0	1.44	C16:0	2.44	C18:0	9.88
			C18:3 $\omega$ 3	4.39	C18:0	2.34	C18:0	2.93	C18:0	6.22	C20:0	4.01
										C21:0	7.53	
15 - 20	C12:0	19.3	C12:0	1.80	C12:0	0.29	C12:0	1.02	C12:0	10.7	C12:0	6.53
	C14:0	9.52	C14:0	1.18	C15:0	1.96	C16:0	0.46	C15:0	1.56	C16:0	8.19
	C23:0	10.6	C16:0	3.06	C16:0	13.8	C17:0	1.20	C16:0	7.75	C17:0	5.42
	C24:0	22.3	C17:0	9.67	C18:0	3.98	C22:0	6.65	C18:2 $\omega$ 6	3.77	C18:0	7.52
	C24:1 $\omega$ 9	10.4	C24:0	40.9	C24:0	2.81					C20:0	6.02
Bivalve	PM		MC		PM		MC		PM		MC	
	C14:0	3.51	C12:0	23.5	C12:0	9.01	C12:0	13.4	C12:0	11.2	C12:0	6.90
	C16:0	22.6	C14:0	1.78	C15:0	3.54	C17:0	5.82	C16:0	14.9	C16:0	10.3
	C16:1 $\omega$ 7	4.82	C16:0	11.8	C16:1 $\omega$ 7	2.34	C18:1 $\omega$ 9	2.71	C16:1 $\omega$ 7	3.51	C16:1 $\omega$ 7	1.11
	C18:0	6.53	C18:1 $\omega$ 9	4.01	C18:2 $\omega$ 6	2.95	C18:3 $\omega$ 6	2.70	C18:0	8.20	C17:0	4.42
	C18:1 $\omega$ 9	2.70	C20:0	1.39					C20:0	4.20	C18:1 $\omega$ 9	1.73
	C20:4 $\omega$ 6	40.5										
	C24:0	1.66										

(Note: PM: *Paphia malabarica*; MC: *Meretrix casta*)

**Table 5:** Fatty acid biomarkers used to identify the sources of the organic matter.

Fatty acids (FAs)	Sources of FAs	References
C15:0, iC15:0, aC15:0, C17:0, iC17:0, aC17:0, C18:1 $\omega$ 7	Bacteria	Volkman et al., 1989; Canuel and Martens, 1993; Rajendran et al., 1995 Carrie et al., 1998; Meziane and Tsuchiya, 2000; Zhukova, 2005
C14:0, C16:1 $\omega$ 7, C20:4 $\omega$ 6, C20:5 $\omega$ 3	Diatoms	Volkman et al., 1980, 1989; Carrie et al., 1998; Meziane and Tsuchiya, 2000
C18:1 $\omega$ 9, C18:4 $\omega$ 3, C22:6 $\omega$ 3	Dinoflagellates	Carrie et al., 1998; Zimmerman and Canuel, 2001
C20:1 $\omega$ 9, C22:1 $\omega$ 9, C24:1 $\omega$ 9	Zooplankton	Carrie et al., 1998; Venturini et al., 2012
C16:4 $\omega$ 3, C18:2 $\omega$ 6, C18:3 $\omega$ 3, C18:3 $\omega$ 6	Macroalgae	Volkman et al., 1989; Meziane and Tsuchiya, 2000
C18:3 $\omega$ 3, C18:2 $\omega$ 6	Mangroves	Bachok et al., 2003; Meziane et al., 2007
Short chain fatty acids (C12:0 - C20:0)	Phytoplankton	Meyers, 1997; Hu et al., 2006; Dunn et al., 2008; Guo et al., 2019
Long chain fatty acids (C22:0 - C30:0)	Higher plants	Meyers, 1997; Meziane and Tsuchiya, 2000; Dunn et al., 2008; Guo et al., 2019

(Note: i: iso; a: anteiso)