Influence of hydrological factors on bacterial community structure in a tropical monsoonal estuary in India

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Abstract
In the present study, we analyzed variations in bacterial community structure along a salinity gradient in a tropical monsoonal estuary (Cochin estuary [CE]), on the southwest coast of India, using Illumina next-generation sequencing (NGS). Water samples were collected from eight different locations thrice a year to assess the variability in the bacterial community structure and to determine the physico-chemical factors influencing the bacterial diversity. Proteobacteria was the most dominant phyla in the estuary followed by Bacteroidetes, Cyanobacteria, Actinobacteria, and Firmicutes. Statistical analysis indicated significant variations in bacterial communities between freshwater and mesohaline and euryhaline regions, as well as between the monsoon (wet) and nonmonsoon (dry) periods. The abundance of Betaproteobacteria was higher in the freshwater regions, while Alphaproteobacteria and Epsilonproteobacteria were more abundant in mesohaline and euryhaline regions of the estuary. Gammaproteobacteria was more abundant in regions with high nutrient concentrations. Various bacterial genera indicating the presence of fecal contamination and eutrophication were detected. Corrplot based on Pearson correlation analysis demonstrated the important physico-chemical variables (temperature, salinity, dissolved oxygen, and inorganic nutrients) that influence the distribution of dominant phyla, class, and genera. The observed spatio-temporal variations in bacterial community structure in the CE were governed by regional variations in anthropogenic inputs and seasonal variations in monsoonal rainfall and tidal influx.

Keywords Cochin estuary · Bacterial diversity · Next-generation sequencing (NGS) · Estuaries · Proteobacteria · Monsoonal estuaries

Introduction
Marine microbial communities are vital to global biogeochemical cycles of carbon, nitrogen, sulfur, and phosphorous. They are the engines of every ecosystem and constitute massive biomass, diversity, and activity in the global oceans (Graham et al. 2016). Hence, it is extremely important to understand the microbial community structure to appreciate how ecosystems function and to recognize factors that control microbial communities. But even with modern tools (high-throughput amplicon sequencing, metagenomics, and metatranscriptomics), it is cumbersome to determine microbial community structure and map its variations in space and time. Physico-chemical factors directly affect microbial diversity and community composition, and variations in microbial community structure affect ecosystem functioning. Extensive studies have been carried out to reveal the bacterial diversity and community structure in many marine and lacustrine ecosystems (Bowman et al. 2003; Acinas et al. 2004; Heijts et al. 2008; Bobrova et al. 2016; Jeffries et al. 2016). However, only a few studies have addressed bacterial diversity from monsoonal estuaries (Crump et al. 2004; Bernhard et al. 2005; Khandeparker et al. 2017; Eswaran and Khandeparker 2019). Very little is known about the complex factors influencing bacterial community composition or the effects these communities have on estuarine ecosystems (Dolan 2005; Teira et al. 2008).

Estuaries make up some of the most complex and dynamic aquatic ecosystems due to freshwater influence from rivers...
and tidal influence from the seas. Mostly, the terrigenous riverine inputs together with the tidal mixing processes characterize the estuarine environments. CE is a highly dynamic tropical microtidal monsoonal estuary (Shivaprasad et al. 2013). The biodiversity in monsoonal estuaries is strongly influenced by monsoonal rains and riverine influx in addition to the estuarine variabilities in physical, chemical, and biological factors due to tidal influx (Qasim 2003). The average monsoonal rainfall in the CE is 2038 mm (CWC data, 2016). The river influx from six major rivers amounts to 20,000 mm³/year, and the annual precipitation varied between 630 and 916 mm (Revichandran et al. 2012). During the monsoon (wet period), the riverine influx brings freshwater which accounts for 60–70% of the total annual river discharge to the system. The domestic sewage and industrial effluents dumped into the estuary results in nutrient enrichment in the CE (Madhu et al. 2007). During the dry period (nonmonsoonal months), the tidal influx is more pronounced due to reduced freshwater influx and precipitation (Madhu et al. 2007; Srinivas et al. 2003). Due to the variation in the monsoonal rains, tidal influx, riverine inputs, and the associated pollutants, the water quality of the ecosystem and the associated bacterial community diversity is affected (Vajravelu et al. 2018).

The bacterial community diversity in the CE and the impact of physico-chemical parameters on the distribution of these communities concerning monsoonal rains have not been studied so far. It is important to study the spatial and seasonal patterns in bacterial community structure as it reflects the selection mechanisms exerted by the dynamic environment on bacterial groups with specific functions and properties. Though microbial biogeography is addressed in many environments in recent years, principles that govern microbial distribution remain poorly understood (Thompson et al. 2017; Nemergut et al. 2011). Furthermore, metagenomic analysis of bacterial communities from estuarine environments found that salinity is the most important factor influencing bacterial composition in estuarine environments compared to other physico-chemical factors (Crump et al. 2004; Dong et al. 2004; Wu et al. 2019; Herfort et al. 2017). We hypothesized that the distribution of different bacterial groups in the CE could be a function of spatial gradients and seasonal variabilities in salinity and monsoonal rains.

Materials and methods

Station description and sampling details

The CE is a complex shallow estuary with an average depth of 4 m (Fig. 1). Six major rivers, the Pamba, Achancovil, Manimala, Meenachil, Periyar, and Muvattupuzha, along with their tributaries and several canals bring large volumes of freshwater into the CE. The saline water from the neighboring Arabian Sea enters the CE through the two inlets—one at Cochin and the other at Azhikode (Fig. 1). During the peak southwest monsoon (June–September), the rivers transport an enormous amount of freshwater into the CE, which transforms it almost entirely into a freshwater lake except near the two inlet regions. While in the dry period (nonmonsoonal months, October–May), the riverine influxes gradually decrease, allowing salinity to build up in the estuary (Qasim 2003; Jythibabu et al. 2006).

The water samples were collected from 8 distinct stations along the estuary for three months (August, November, and February in 2015–2016) (Fig. 1). The 8 stations were distinct with respect to inputs and outputs from the river and tides. Station 1 was located far upstream of the Periyar River, while Station 2 runs through the Industrial Belt of the city. Station 3 is the region where the Periyar River empties into the estuary, and Station 4 or Kochi inlet is where the estuary meets the Arabian Sea. Stations 5, 6, and 7 are located further downstream of Kochi, which receives a lot of sewage wastes from the urban population, similar to S3. Station 8 is situated beyond the Thanneermukkam tidal saltwater barrage near rice paddy plantations.

Water (5 L) samples were collected from each station in sterilized 1-L glass bottles, immediately placed on ice, and shielded from sunlight. At each station, the salinity, water temperature, and pH were recorded. The subsamples (triplicate) were collected to reduce the sampling variability at each station during the study period. Upon returning to the lab, the samples were filtered through 0.2-μm filters (0.47-mm diameter, Millipore, USA) using a sterilized vacuum filtration apparatus. Once filtration was complete, the filters were stored at ~80°C until further processing.

Environmental parameters

Temperature and salinity were measured using a conductivity temperature density profiler (CTD, SBE, Seabird 19). The inorganic nutrients (nitrate, nitrite, phosphate, ammonia, and silicate) were estimated spectrophotometrically (Shimadzu UV 1800) using standard protocols (Grasshoff and Ehrhardt, 1983). Dissolved oxygen (DO) content was determined following Winkler’s titration method (Grasshoff 1983).

Enumeration of total plate count (TPC)

Total plate count was employed to enumerate the viable bacteria in the water sample. Briefly, the water samples were serially diluted using 0.85% physiological saline and plated onto nutrient agar (NA). The plates were then incubated under room temperature for 24 h. The colonies that developed on NA plates were enumerated, and TPC was expressed as a number of colony-forming units (CFU)/ml for a water sample.
Extraction of DNA from water samples

DNA was extracted from bacteria concentrated on 0.2-μm filters (Millipore, USA). Under aseptic conditions, the frozen filters were thawed and cut into small pieces using sterilized scissors. The total DNA was extracted using the PowerSoil DNA isolation kit following the manufacturer’s instructions (Qiagen, USA) (Cao et al. 2013). The DNA (triplicate) from each sampling station was pooled and sent to GenePath Dx (Pune, India) for library construction and next-generation sequencing.

Amplicon library construction

The DNA samples were first quantified using a broad range Qubit system (Life Technologies, CA, USA), and the average concentration was 10 ng/μl. Library construction involved two PCR reactions. The first reaction ligated two 20 base pair (bp) proprietary tags on either end of the targeted V3–V4 regions using 16S Illumina primers F: 5′-CCTACGGGNGGCWGGCAG-3′ and R: 5′-GACTACHVGGGTATCTAATCC-3′ (Jasna et al. 2020, Parvathi et al. 2019), with the initial amount of 20 ng of DNA. Cycle conditions included an initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 60°C for 20 s, 72°C for 20 s, and then a final extension at 72°C for 10 min (Klindworth et al. 2013). The amplified products of 400–600 bp were visualized by gel electrophoresis, diluted 1:10 using 10 mM Tris-HCl (pH 8.0), and used as templates for the second PCR. This indexing PCR was completed using QuantiTect MultiPlex PCR kit (Qiagen, Germany). Both forward and reverse indexing primers contained a 100 bp tag, including the adapter and unique barcode sequences, and were used at 200 nM. Cycling conditions included initial
denaturation of 95°C for 15 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 63°C for 45 s, and extension at 72°C for 90 s. The amplified products were quantified using a Qubit broad range system (Life Technologies, CA, USA) (Parvathi et al. 2019; Ramanan et al. 2016).

**Illumina sequencing**

PCR products were molar normalized, pooled into a single tube, and purified to a minimum of 300 bp using PureLink PCR Purification kit (Invitrogen, CA, USA). The purified, pooled sample was then diluted to 4 nM final concentration using resuspension buffer (RSB, Illumina, CA, USA). The sample was denatured for 5 mins and neutralized using 0.2 N NaOH and HT1 buffer, respectively (Illumina, CA, USA). It was then pooled with other libraries prepared for NGS in a ratio depending on amplicon size/total panel size, desired sequencing depth, and number of samples pooled in each sub-library. Pooled libraries were further diluted down to a final 15 pM and spiked with 5% PhiX (Illumina, CA, USA) as a control and diversity enhancer. Samples were then loaded into an Illumina MiSeq v3 cartridge (Illumina, CA, USA) and ran in a 2 × 300 mode on an Illumina MiSeq next-generation sequencer (Illumina, CA, USA) (Parvathi et al. 2019; Ramanan et al. 2016).

**Initial processing of sequence reads**

The bcl2fastq Conversion Software embedded in the MiSeq was used for demultiplexing. The quality reads (> Q30) were filtered out using the automated FASTQ Tool Kit application on Illumina BaseSpace Labs website for downstream analysis. Within the application, TagCleaner software was used to remove the adapter sequences.

**Data analysis**

The QIIME preprocessing application (version 1.0.0, Illumina BaseSpace) was used for the analysis of raw sequence data (Caporaso et al. 2010). Preprocessing included demultiplexing, quality filtering, operational taxonomic units (OTUs) picking, and taxonomic assignment using Greengenes. QIIME outputs were used to create a Biological Observation Matrix file. Before succeeding analysis, OTUs abundance was normalized. QIIME software (version 1.7.0) was used to analyze alpha diversity and richness. The data were normalized and transformed by the Bray–Curtis method. Permutational multivariate analysis of variance (PERMANOVA) was performed to understand the significant variations in species abundance. A correlation matrix or correlogram was generated for analyzing the relationship between environmental factors and dominant phyla, class, and genus using the corrrplot R package, version 0.84 (Wei and Simko 2017). Canonical correspondence analysis (CCA), a multivariate method, was used to elucidate the relationships between biological and environmental variables (CANOCO 4.5) (Lepš and Šmilauer 2003). A Monte Carlo test was used to determine the significance of each axis and to evaluate the influence of the environmental variables upon the overall distribution of bacterial species and their distribution at each site and sampling season (Salles et al. 2004; Sapp et al. 2007). Similarity percentage breakdown (SIMPER) was used to calculate the partition of the average Bray–Curtis dissimilarity between different clusters into components from different genera (Clarke 1993). This allowed the identification of genera that are the most important in creating observed patterns in similarity.

**Nucleotide sequence accession number**

Paired-end Illumina sequence data from this study was submitted to the National Center for Biotechnology Information Sequence Read Archive (SRA) under bio project number PRJNA595444.

**Results**

**Environmental parameters**

Based on the climatology of the study area, seasons have traditionally been classified into monsoon (June to September), post-monsoon (October to January), and pre-monsoon (February to May) (Menon et al. 2000). Maximum rainfall, accounting for 60–65% of the total annual rainfall in the study area, was received from June to September (wet period, with an average rainfall of 528.75 mm); whereas the dry period, subclassified into dry periods I and II, received an average rainfall of 151.75 mm and 133 mm, respectively (Fig. S1). Salinity was the most fluctuating variable in the CE ranging from 0 to 28. The entire estuary was freshwater dominated during the wet period, except at the inlet station, S4, which sustained a salinity of 16. With the retreat of the southwest monsoon, the riverine influx reduced by 45%, resulting in salinity stratification in the estuary with euryhaline salinity at the inlet (Table 1); freshwater conditions in stations S1, S2, S7, and S8; and mesohaline salinities in stations S3, S5, and S6 (Table 1). The major inorganic nutrients were high during the sampling period in the CE (Table 1).

**Total plate count, taxonomic richness, and α-diversity of the prokaryotic community**

The total plate count (TPC) of bacteria ranged from 0.22 to 5.98 × 10⁵ CFU/ml (Fig. S2). The bacterial abundance was
higher in dry period II and especially in the euryhaline region of the estuary. The bacterial community diversity was highest in dry period I compared to other periods (Table 2). The overall species coverage of the samples was highest (≥ 68.6%) for dry period I, followed by wet period (≥ 66.68%) and dry period II (≥ 63.19%). The Good’s average was high (0.99), indicating that most of the bacterial diversity was attained by sequencing. However, Shannon diversity, Chao richness, and Simpson diversity indices, which represent abundance and evenness of the species distribution, varied slightly with sampling period and with stations (Table 2). There was a significant difference in α-diversity between the dry and wet seasons at all stations (Kruskal–Wallis, *p* < 0.05), and bacterial diversity significantly differed both spatially and temporally (Kruskal–Wallis, *p* < 0.05).

### Seasonal and spatial variations in bacterial diversity at phylum and class level

The relative abundances of different phyla in the eight locations from three different sampling periods (24 samples) are shown in Fig. 2. Total bacterial diversity was distributed among 32 different phyla, with Proteobacteria, Bacteriodetes, Firmicutes, Actinobacteria, Cyanobacteria, and Verrucomicrobiae accounting for more than > 95% of the total OTUs in all the samples (Fig. 2). Phylum Proteobacteria was dominated by Alphaproteobacteria, Gammaproteobacteria, and Betaproteobacteria, with Betaproteobacteria being more abundant in the freshwater regions and Alphaproteobacteria in the meso and euryhaline regions of the estuary. Betaproteobacteria was the most dominant class throughout the freshwater-dominated estuary during the wet period in all stations except S4 (Fig. S3).

### Bacterial diversity at generic level

A total of 975 genera contributing to 60 to 75% of total generic diversity was considered and represented in Fig. 3. The most dominant genera were *Sanguibacter*, *Saccharopolyspora*, *Prochlorococcus*, *Arcobacter*, and *Ruegeria*, with significant spatial variations in abundance. The dominant genus was distinctly different in the freshwater,
mesohaline, and euryhaline regions of the estuary. The most dominant genera in the freshwater regions were Sanguibacter, Saccharopolyspora, Demequina, Chthoniobacter, Bifidobacterium, Paucibacter, Flavobacterium, Limnohabitans, Chitinophaga, Acinetobacter, Oxalobacter, Prochlorococcus, and so on. In mesohaline regions, dominant genera included Microcystis, Prochlorococcus, Agromyces, Vibrio, Clostridium, Prevotella, Enterobacter, Ruminococcus, Lachnospira, and Arcobacter were most dominant in the euryhaline regions of the estuary. Many bacterial genera in the mesohaline regions were also present in the euryhaline regions. However, their percentage abundance varied in these two regions of the estuary. Pollution indicators such as Bacteroides, Microcystis, Agromyces, Vibrio, Clostridium, Prevotella, Enterobacter, Ruminococcus, Lachnospira, and Pseudomonas were detected.

### Statistical analysis

Nonmetric multidimensional scaling (NMDS) analysis revealed two clusters of bacterial communities during the wet period (Fig. 4). During the wet period, the entire estuary could be considered as a freshwater lake and hence formed two clusters: S4 in one cluster and all other stations in another cluster. During dry months, the bacterial communities were clustered into three: euryhaline stations (S4, salinity ≥ 25), mesohaline stations (S3, S5, and S6, salinity = 5–20), and freshwater stations comprising stations S1, S2, and S8.
Fig. 2 Phylum level taxonomic distribution of major and minor bacterial communities from eight sampling locations during a wet period and b dry period I and c dry period II in the Cochin estuary (CE).

Fig. 3 Genus level distribution of dominant bacterial communities, from eight sampling locations during a wet period and b dry period I and c dry period II in the Cochin estuary (CE).
Based on SIMPER analysis, the average similarity within the cluster was 73%, and the degree of dissimilarity between freshwater and mesohaline clusters was 40%. Dry period I yielded three clusters with an average similarity of 75% in the freshwater cluster. The average dissimilarity between freshwater and mesohaline stations was 32% and that between freshwater and euryhaline stations was 38%. The average dissimilarity between mesohaline and euryhaline stations was 26%. NMDS revealed two clusters in dry period II. The average dissimilarity between freshwater and mesohaline stations was 42% and that between mesohaline and euryhaline stations was 21% in dry period II.
CCA analysis of the phylum, class, and generic level diversity showed distinct spatial and seasonal patterns in the distribution of bacterial communities (Fig. S4). The distribution of bacterial communities at each station was influenced by different environmental parameters, such as DO, silicate, and nitrate in the freshwater regions; high inorganic nutrients such as ammonia, phosphate, and nitrite in the mesohaline regions; and salinity in the euryhaline station, S4. Correlogram based on Pearson correlation analysis revealed the influence of physico-chemical parameters on the distribution of dominant phyla, class, and genus (Fig. 5). Proteobacteria, Cyanobacteria, and Alphaproteobacteria showed a positive correlation with nitrite and ammonia. Betaproteobacteria showed a positive correlation with silicate, DO, nitrate, and temperature (Fig. 5). Salinity and other physico-chemical factors played an important role in determining the distribution of Cyanobacteria, Gammaproteobacteria, Alphaproteobacteria, and dominant bacterial genera in the estuary (Fig. 6).

The number of shared and unique OTUs at the genus level in dry and wet periods is indicated in the Venn diagram (Fig. S5). Comparative analysis showed that ~1621 OTUs were shared during all the seasons, 172 OTUs were unique to the wet period and 375 OTUs to dry period I and 87 OTUs to dry period II. PERMANOVA analysis demonstrated that bacterial community structure significantly varied temporally during wet and dry seasons (pseudo $F = 3.342, p = 0.002$) and spatially (pseudo $F = 1.615, p = 0.016$).

**Discussion**

Estuaries, being dynamic mixing zones of the ocean and freshwater masses, are characterized by steep spatial and temporal gradients of physical, chemical, and biological parameters. Hence, it is essential to fathom the impact of these gradients on the local bacterial community, their metabolism, and the water quality in an estuarine system. Salinity has been demonstrated as an important environmental factor structuring bacterial communities in coastal ecosystems (Ortega-Retuerta et al. 2013; Liu et al. 2015; Herlemann et al. 2016). Though CE is composed of similar aquatic microbial phyla found within other aquatic environments (Eswaran and Khandeparker 2019; Meziti et al. 2016; Savio et al. 2015), it hosted variations in the relative abundance of bacterial communities with changes in estuarine hydrography and pollutants. The estuarine bacterial genera fell into three distinct categories: Euryhaline/marine (salinity > 20), mesohaline/brackish (salinity = 5–20), and freshwater (salinity ≤ 5) (see station details and description for seasonal hydrography in the CE) (Fig. 4). Previous studies in the CE have demonstrated the existence of three distinct zones based on salinity variations during dry months and two zones during wet monsoonal months and have indicated unique biological responses to these gradients (Parvathi et al. 2015; Jasna et al. 2017). In addition to the variations imparted by salinity influx, there are regional variations in the input of industrial wastes, agricultural wastes, and sewage inputs, especially from

![Fig. 5 Correlogram showing the correlations between the dominant phylum and class with environmental variables during a wet period and b dry period I and c dry period II in the Cochin estuary (CE). The Pearson correlation coefficients in the correlogram plot are colored based on the value and on the degree of association among the variables. Red and blue colors represent significant negative correlations and positive correlations. Darker color represents stronger correlations.](50587)
many nonpoint sources. Large inputs of fresh organic matter into the estuary from riverine inputs and other nonpoint sources impact heterotrophic production (Jasna et al. 2017) and bacterial diversity. We detected fluctuations in bacterial richness within the three different salinity regimes of the estuary (Table 2). This indicates that factors other than salinity influenced bacterial richness. Freshwater regions of the estuary detected the highest and lowest richness during the dry period. We assume that variations in the lability of organic matter in these regions might be responsible for the proliferation of several adapted bacterial taxa (Bunse et al. 2016). The results of this study corroborated with recent studies on distinct bacterial communities in estuarine environments of Delaware Bay, Chesapeake Bay, Columbia River estuary, and Baltic Sea (Herfort et al. 2017; Herlemann et al. 2011; Hugerth et al. 2015).

The dominance of Proteobacteria in the present study was in concurrence with reports from other tropical estuaries (Bouvier and del Giorgio 2002; Ghosh and Bhadury 2019; Ortmann and Santos 2016; Eswaran and Khandeparker 2019) and also from coastal waters of India (Sachithanandam et al. 2020; Parvathi et al. 2019). The high abundance of Betaproteobacteria and Alphaproteobacteria in our study corroborated with previous studies from other estuarine regions (Sekiguchi et al. 2002). While Betaproteobacteria was found in high proportions in the freshwater regions of the estuary, Alphaproteobacteria, Gammaproteobacteria, and Epsilonbacteria were more dominant in the mesohaline and euryhaline regions of the estuary during dry seasons. Betaproteobacteria was present in high proportions at salinities below 4 and Alphaproteobacteria at salinities above 13. This shows that salinity transitions play a...
significant role in the abundance and distribution of Beta and Alphaproteobacteria. However, the mechanisms causing changes in bacterial community composition at different salinities are currently unclear. Bacteria have remarkable versatility to adapt to different trophic conditions, and hence, environmental variabilities directly reflect upon the distribution and abundance of bacterial communities. Few studies in the CE have linked salinity to differences in the key metabolic capabilities of bacteria (Thottathil et al., 2008a).

The dominance of Proteobacteria, Cyanobacteria, Alphaproteobacteria, and Epsilonproteobacteria was high in regions with high concentrations of ammonia and nitrite. Mesohaline regions were characterized by high concentrations of ammonia, nitrite, and phosphate. The mesohaline regions of the estuary received a high amount of agriculture wastes, industrial effluents, and sewage from the urban population through many non-point sources. These regions lie between the inlet and the freshwater region/Vembanad Lake, which makes these regions less dynamic with low flushing rates during the dry season with no rainfall and less riverine influx (Jasna et al. 2017). Dissolved organic carbon is also more (340 ± 108 μM to 193 ± 102 μM) in the mesohaline regions compared to freshwater regions (< 200 μM) of the estuary (Gupta et al. 2009). The mesohaline regions support high bacterial respiration (Jasna et al. 2017), indicating that these regions have a heavy nutrient load which in turn supports the growth and dominance of Gammaproteobacteria, Alphaproteobacteria, and Epsilonproteobacteria. Gammaproteobacteria were also abundant in the river mouth station, S1 during the wet season, indicating that this group also takes advantage of allochthonous material loadings and nutrient-enriched conditions. In addition to these, variations in other biotic factors, such as high phytoplankton (Madhu et al. 2007), grazing (Sooria et al. 2015), and viral lysis (Jasna et al. 2017), can also shape bacterial community composition in the CE. Hence, the observed variations in bacterial community composition patterns may be a result of a multitude of factors influenced by trophic conditions, anthropogenic inputs, riverine inputs, and tidal influx.

The presence of some genera indicated the extent of pollution in this estuarine environment. Some strains of genera Sanguibacter, Bifidobacterium, and Oxalobacter are known to colonize the animal gastrointestinal tract and in runens of animals such as cattle. They are also isolated from marine and freshwater environments (Garrity et al. 2005). The most dominant genus in mesohaline regions was Microcystis which include members that can produce neurotoxins and hepatotoxins, such as microcystin and cyanopeptolin. The presence of these pathogens in the CE indicates the presence of sewage or fecal contamination from other animal sources. Bacteroides are an important indicator group which is exclusive to warm-blooded animals and constitute a larger portion of pollution indicator bacteria in CE, especially during the dry period. Human- and animal-associated Bacteroides markers have been extensively used for fecal identification studies (Harwood et al. 2014). In the present study, Bacteroides showed a significant positive correlation with nitrate and ammonia and did not show any correlation with salinity. Anthropogenic nutrient over-enrichment (eutrophication) and other factors including expansion of intensive agriculture, rapid industrialization, and urbanization enhance the occurrence of microcystin-producing harmful algal blooms in most regions and thus poses deleterious effects on human health (Garrity et al. 2020). Bacteria involved in fecal contamination such as Bacteroides, Clostridium, Faecalibacterium, Enterococcus, Pseudomonas, Vibrio, Prevotella, Enterobacter, Klebsiella, and Campylobacter are also detected in CE (Boehm and Sassoubre 2014).

Several genera in the CE could be considered as indicators of eutrophication, such as those involved in ammonia oxidation, nitrite reduction, and N2O reduction. Genera such as Paracoccus, Comamonas, Nitrosomonas, and Nitrobacter are involved in the nitrogen cycle and were more abundant in the mesohaline regions of the estuary (Wang et al. 2014; Kim et al. 2015). Dissolve inorganic nitrogen is high in CE with a higher nitrogen fixation rate (Bhavya et al. 2016). These regions receive 7–11 times high organic matter as lateral input as compared to the inputs through rivers (Thottathil et al. 2008b; Gupta et al. 2009). The input of industrial and domestic waste discharges as a result of large human settlement and industrial growth, and agriculture has resulted in excess nutrients in the system. The high abundance of Prochlorococcus marinus and Synechococcus can be considered as an indicator of the trophic status of CE (Rajaneesh et al. 2015). The trophic index scores (TRIX units) showed that CE is highly eutrophic (Martin et al. 2012; Hershey et al. 2019). Hence, it is important to introduce proper management measures to impede the nutrient loading from various non-points to protect the health of this estuary.

**Conclusion**

Our study demonstrated that the seasonal and spatial variations in bacterial community structure are largely influenced by salinity and inorganic nutrients such as nitrite, nitrate, ammonia, phosphate, and silicate in CE. Spatial variations in bacterial community structure were also influenced by regional variations in anthropogenic inputs to a large extent. Regional variations in anthropogenic inputs, especially in the mesohaline regions, imparted by restricted flow in the
CE, had a greater impact in shaping the bacterial community structure during dry periods. Our study demonstrates how bacterial community structure changes along an estuarine gradient during different seasons and the physico-chemical conditions that drive bacterial community shifts. Proteobacteria was the most dominant bacterial phylum, mainly composed of the classes Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. Various genera indicative of eutrophication, fecal contamination, and sewage pollution were identified in this study. Our study suggests that monitoring the presence of important bacterial groups could serve as an appropriate indicator of ecosystem health and pollution. This estuarine system experiences elevated stress from human activity, and increased knowledge of factors that shape its microbiology is crucial.

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Availability of data and materials The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contribution AP conceptualized the work, made funding arrangements, data analysis, and prepared the manuscript. MC: conceptualized the work, made funding arrangements, and prepared the manuscript. NP and NG carried out the Illumina MiSeq sequencing.

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Declarations

Ethics approval No need for ethics approval since this work does not include research on identifiable human material or data.

Consent to participate All the authors agree to participate in this manuscript.

Consent for publication The manuscript has written consent from all the authors and from the institution.

Conflict of interest The authors declare no competing interests.

References

Acinas SG, Klepac-Ceraj V, Hunt DE, Pharino C, Ceraj J, Distel DL, Polz MF (2004) Fine-scale phylogenetic architecture of a complex bacterial community. Nature 430:551–554. https://doi.org/10.1038/nature02649

Bernhard AE, Donn T, Griblin AE, Stahl DA (2005) Loss of diversity of ammonia-oxidizing bacteria correlates with increasing salinity in an estuary system. Environ Microbiol 7:1289–1297. https://doi.org/10.1111/j.1462-2920.2005.00808.x

Bhavya PS, Kumar S, Gupta GVM, Sudheesh V, Sadhama KV, Varrier DS, Dhanya KR, Saravanan N (2016) Nitrogen uptake dynamics in a tropical estuarine estuary (Cochin, India) and adjacent coastal waters. Estuar Coasts 39(1):54–67. https://doi.org/10.1007/s12237-015-9982-y

Bobrova O, Kristoffersen JB, Oulas A, Ivantsyva V (2016) Metagenomic 16s rRNA investigation of microbial communities in the Black Sea estuaries in south-west of Ukraine. Acta Biochim Pol 63(2):315–319. https://doi.org/10.18388/abp.2015_1145

Boehm AB, Sassoubre LM. (2014). Enterococci as indicators of environmental fecal contamination. Enterococci: from commensals to leading causes of drug resistant infection [Internet].

Bouverit TC, del Giorgio PA (2002) Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol Oceanogr 47:453–470. https://doi.org/10.4319/lo.2002.47.2.0453

Bowman JP, McCammon SA, Gibson JA, Robertson L, Nichols PD (2003) Prokaryotic metabolic activity and community structure in Antarctic continental shelf sediments. Appl Environ Microbiol 69: 2448–2462. https://doi.org/10.1128/AEM.69.5.2448-2462.2003

Bunce C, Bertos-Fortis M, Sassenhagen I, Sildever S, Sjöqvist C, Godhe A, Gross S, Kremp A, Lips I, Lundholm N, Rengefors K, Selborn J, Pinhasi J, Legrand C (2016) Spatio-temporal interdependence of bacteria and phytoplankton during a Baltic Sea spring bloom. Front Microbiol 7:517. https://doi.org/10.3389/fmicb.2016.00517

Cao Y, Van De Werthorst LC, Dubinsky EA, Badgley BD, Sadowsky MJ, Andersen GL, Griffith JF, Holden PA (2013) Evaluation of molecular community analysis methods for discerning fecal sources and human waste. Water Res 47:6862–6872. https://doi.org/10.1016/j.watres.2013.02.061

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttenhower C, Kelley ST, Knights D, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nature Meth 7(5):335–336. https://doi.org/10.1038/nmeth.f.303

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Australian Journal Ecol 18(1):117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Crump BC, Hopkinson CS, Sogin ML, Hobbie JE (2004) Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time. Appl Environ Microbiol 70:1494–1505. https://doi.org/10.1128/AEM.70.3.1494-1505.2004

Dolan JR (2005) An introduction to the biogeography of aquatic microbes. Aquat Microb Ecol 41:39–48. https://doi.org/10.3354/ame041039

Dong L, Su J, Wong LA, Cao Z, Chen JC (2004) Seasonal variation and dynamics of the Pearl River plume. Cont Shelf Res 24:1761–1777. https://doi.org/10.1016/j.csr.2004.06.006

Eswaran R, Khedeparker L (2019) Seasonal variation in β-glucosidase-producing culturable bacterial diversity in a monsoon-influenced
Cochin estuary. India Ecol Res 30(1):85–92. https://doi.org/10.1007/s11284-014-1214-6
Parvathil A, Jasna V, Aswathy NK, Nathan VK, Aparna S, Balachandran KK (2019) Microbial diversity in a coastal environment with co-existing upwelling and mud-banks along the south west coast of India. Mol Biol Rep 46:3113–3127. https://doi.org/10.1007/s11033-019-04766-y
Qasim SZ. (2003). Indian estuaries. Allied publishers.
Rajaneesh KM, Smita M, Anil AC, Sawant SS (2015) Synechococcus as an indicator of trophic status in the Cochin backwaters, west coast of India. Ecol Indic 55:118–130. https://doi.org/10.1016/j.ecolind.2015.02.033
Ramanan V, Kelkar K, Ranade S, Gangodkar P, Gogate N, Patil K, Ragte-Wathare T, Agarwal M, Phadke ND (2016) The clinical utility of a custom-developed targeted next-generation sequencing assay for detection of mutations associated with Philadelphia-negative chronic myeloproliferative neoplasms: two case examples with CALR exon 9 mutations. Int J 1:29
Revichandran C, Srinivas K, Muraleedharan KR, Rafeeq M, Amaravayal Ramanan V, Kelkar K, Ranade S, Gangodkar P, Gogate N, Patil K, Ragte-Wathare T, Agarwal M, Phadke ND (2016) The clinical utility of a custom-developed targeted next-generation sequencing assay for detection of mutations associated with Philadelphia-negative chronic myeloproliferative neoplasms: two case examples with CALR exon 9 mutations. Int J 1:29
Revichandran C, Srinivas K, Muraleedharan KR, Rafeeq M, Amaravayal Ramanan V, Kelkar K, Ranade S, Gangodkar P, Gogate N, Patil K, Ragte-Wathare T, Agarwal M, Phadke ND (2016) The clinical utility of a custom-developed targeted next-generation sequencing assay for detection of mutations associated with Philadelphia-negative chronic myeloproliferative neoplasms: two case examples with CALR exon 9 mutations. Int J 1:29
Revichandran C, Srinivas K, Muraleedharan KR, Rafeeq M, Amaravayal S, Vijayakumar K, Jayalakshmy KV (2012) Environmental set-up and tidal propagation in a tropical estuary with dual connection to the sea (SW Coast of India). Environ Earth Sci 66(4):1031–1042. https://doi.org/10.1007/s12665-011-1309-0
Sachithananandam V, Saravanane N, Chandrasekar K, Karthick P, Lalitha P, Elangovan SS, Sudhakar M (2020) Microbial diversity from the continental shelf regions of the Eastern Arabian Sea: a metagenomic approach. Saudi journal biol. Sci 27(8):2065–2075. https://doi.org/10.1016/j.sjbs.2020.06.011
Salles JF, Van Veen JA, Van Elsas JD (2004) Multivariate analyses of Burkholderia species in soil: effect of crop and land use history. Appl Environ Microb 70(7):4012–4020. https://doi.org/10.1128/AEM.70.7.4012-4020.2004
Sapp M, Schwaderer AS, Wiltshire KH, Hoppe HG, Gerds G, Wichels A (2007) Species-specific bacterial communities in the phycosphere of microalgae? Microb Ecol 53(4):683–699. https://doi.org/10.1007/s00248-006-9162-5
Savio D, Sinclair L, Izaj UZ, Parajka J, Reischer GH, Studler P, Blaschke AP, Blöschi G, Mach RL, Kirschner AKT, Famlleiter AH, Eiler A (2015) Bacterial diversity along a 2600 km river continuum. Environ microbial 17(12):4994–5007. https://doi.org/10.1111/1462-2920.12886
Sekiguchi H, Watanabe M, Nakahara T, Xu B, Uchiyama H (2002) Succession of bacterial community structure along the Changjiang River determined by denaturing gradient gel electrophoresis and clone library analysis. Appl Environ Microbiol 68:5142–5150. https://doi.org/10.1128/AEM.68.10.5142-5150.2002
Shivaprasad A, Vinita J, Revichandran C, Reny PD, Deepak MP, Muraleedharan KR, NaveenKumar KR (2013) Seasonal stratification and property distributions in a tropical estuary (Cochin estuary, west coast, India). Hydrol Earth Syst Sci 17:187–199. https://doi.org/10.5194/hess-17-187-2013
Sooria PM, Jothybabu R, Anusha A, Vineetha G, Vinita J, Lallu KR et al (2015) Plankton food web and its seasonal dynamics in a large monsoonal estuary (Cochin backwaters, India) - significance of mesohaline region. Environ Monit Assess 187:1–22. https://doi.org/10.1007/s10661-015-4656-6
Srinivas K, Revichandran C, Maheswaran PA, Asharaf TT, Murukesh N. (2003) Propagation of tides in the Cochin estuarine system, southwest coast of India
Teira E, Gasol JM, Aranguren-Gassis M, Fernández A, González J, Lekunberri I, Álvarez-Salgado XA (2008) Linkages between bacterioplankton community composition, heterotrophic carbon cycling and environmental conditions in a highly dynamic coastal ecosystem. Environ Microbiol 10:906–917. https://doi.org/10.1111/j.1462-2920.2007.01509.x
Thompson LR, Williams GJ, Haroon MF, Shibli A, Larsen P, Shorestein J et al (2017) Metagenomic covariation along densely sampled environmental gradients in the Red Sea. ISME J 11(1):138–151. https://doi.org/10.1038/s41396-015.01438
Thottatlh SD, Balachandran KK, Gupta GVM, Madhu NV, Nair S (2008a) Influence of allochthonous input on autotrophic–heterotrophic switch-over in shallow waters of a tropical estuary (Cochin Estuary). India Estur Coast Shelf Sci 78(3):551–562. https://doi.org/10.1016/j.ecss.2008.01.018
Thottatlh SD, Balachandran KK, Jayalakshmy KV, Gupta GVM, Nair S (2008b) Tidal switch on metabolic activity: salinity induced responses on bacterioplankton metabolic capabilities in a tropical estuary. Estaur Coast Shelf Sci 78(4):665–673. https://doi.org/10.1016/j.ecss.2008.02.002
Vajravelu M, Martin Y, Ayyappan S, Mayakrishnan M (2018) Seasonal influence of physico-chemical parameters on phytoplankton diversity, community structure and abundance at Parangipettai coastal waters, Bay of Bengal, south east coast of India. Oceanologia 60(2):114–127. https://doi.org/10.1016/j.oceano.2017.08.003
Wang Z, Zhang XX, Lu X, Liu B, Li Y, Long C, Li A (2014) Abundance and diversity of bacterial nitrifiers and denitrifiers and their functional genes in tannery wastewater treatment plants revealed by high-throughput sequencing. PLoS One 9(11):e113603. https://doi.org/10.1371/journal.pone.0113603
Wei T, Simko V (2017). R package “corrplot”: visualization of a correlation matrix (Version 0.84)
Wu DM, Dai QP, Liu XX, Fan YP, Wang JX (2019) Comparison of bacterial community structure and potential functions in hypoxic and non-hypoxic zones of the Changjiang estuary. PLoS One 14(6):e0217431. https://doi.org/10.1371/journal.pone.0217431

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