# Ecological Habitat Quality Assessment Of A Tropical Estuary Using Macrobenthic Functional Characteristics And Biotic Indices

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## Abstract

Seasonal variation in the macrobenthic functional characteristics, such as trophic structure and secondary production were studied, along with their structural characteristics such as density, biomass, and community distribution in Cochin estuary (CE), a tropical monsoonal estuary along the Southwest coast of India during 2018-19. The biotic indices, AMBI (AZTI's Marine Biotic Index) and M-AMBI (Multivariate-AMBI) were used to assess the ecological quality of the study area by using the sensitivity of macrobenthic fauna to disturbances. A distinct temporal variation was evident in the macrobenthic structural characteristics, wherein high density, biomass, and species richness were observed during the post-monsoon. Polycheate species were dominant during pre-monsoon (Prionospio cirrifera) and monsoon (Mediomastus sp), while gastropod species, Stenothyra perpumila was dominant during post-monsoon. The trophic structure analysis revealed the dominance of deposit-feeding polychaetes during the pre-monsoon in association with the fine sediments laden with high organic carbon. In contrast, during post-monsoon, the polychaete trophic groups were more or less evenly distributed in the sandy substratum. The higher density of suspension feeders and herbivorous-grazers observed during the post-monsoon indicated improved habitat quality compared to the pre-monsoon dominated by the detritivores. The macrobenthic secondary production was also high during the post-monsoon, contributed by a diverse assemblage of mollusks, crustaceans, and polychaetes having size >0.7 mm. The biotic indices also showed an evident increase in the habitat quality of the estuary from pre-monsoon to post-monsoon (AMBI moderately disturbed to undisturbed; M-AMBI- poor-moderate-good to good-high conditions), similar to the results of benthic structural and functional characteristics. The study highlights the significance of the utility of benthic functional characteristics while assessing the habitat quality of an ecosystem

Keywords: Macrobenthos; Trophic structure; Secondary production; Cochin estuary; AMBI&M-AMBI

## Introduction

Estuaries, though complex and dynamic in their hydrographic and ecological attributes, form one of the most productive and bio-diverse ecosystems in the world (Chapman and Wang 2001). They provide a wide variety of ecosystem services by linking the adjacent terrestrial, freshwater, and marine systems (Elliott and Whitfield 2011). These ecosystems remarkable for the strong spatiotemporal variability in their physico-chemical attributes generated by the tidal and fluvial forcing often create a challenging environment for the inhabitance of many aquatic organisms. Thus, the estuarine residents subjected to the intense environmental gradients in their habitats, along with their biotic and abiotic interactions often form distinct community assemblages (Cadotte et al. 2013). The species that can adapt to the environmental fluctuations will benefit and sustain their population corresponding to the habitat changes while others get eliminated (Elliott and Whitfield 2011).

Macrobenthos are vital components of estuarine and coastal habitats and have a crucial role in many of the ecological processes, such as secondary production, bioturbation and nutrient cycling, pollution metabolism, and mineralization (Snelgrove 1998). As they are sediment inhabitants with limited mobility, they are used as bioindicators for assessing the environmental quality of various habitats. Many macrobenthic organisms graze on microflora and form major prey item for higher epibenthic predators and demersal fishes, thus forming a crucial energy link between the primary producers and demersal fishes (Sturdivant et al. 2013). The spatio-temporal variability in macrobenthic structural properties such as density, biomass, and diversity are widely used in ecological studies to understand the environmental changes in various ecosystems corresponding to natural and anthropogenic perturbations (Joydas et al. 2011: Yan et al. 2017; Rehitha et al. 2017; Dauvin et al. 2020; Mulik et al. 2020). In addition to the structural characteristics, functional characteristics such as trophic structure and secondary production, can also be used as potential tools for assessing environmental variability occurring in these diverse ecosystems (Gaudêncio and Cabral 2007; Dolbeth et al., 2012; Rodil et al. 2019).

The macrobenthic trophic structure provides information on the distribution of organisms based on their different feeding types and helps in the elucidation of the benthic/pelagic coupling processes and ecosystem functioning (Rakocinski et al. 1997; Cari et al. 2020). The trophic structure of benthic communities in ecosystems is subjected to changes in accordance with various natural and anthropogenic stressors, such as climate change, acidification, eutrophication, industrial development, and urbanization (Koch and Wolff 2002; Gaudencio and Cabral 2007; Kroeker et al. 2011; Carstensen et al. 2014; Hossain et al. 2019) and hence can be used as a sensitive indicator of benthic community responses. The secondary production is the building up of heterotrophic biomass

in the course of time (Benke and Huryn 2007), and is considered as an indicator of the trophic capacity, health, and functioning of different ecosystems (Dolbeth et al. 2012). It accounts for the cumulative responses of a population to stresses from various living and nonliving ecosystem components and gives information on material cycling and energy flow in ecosystems (Benke and Huryn 2010). Ecosystem models suggest that a significant amount of energy produced in the aquatic ecosystems is channeled via benthos, which ultimately supports the commercially important fish species (Pedersen et al. 2008; Fuhrmann et al. 2015). Thus, benthic secondary production comprises a vital component of the energy flow and organic matter cycling in aquatic ecosystems (Brey 1990). As macrobenthic fauna functions as a critical member in the estuarine food webs, their production estimates can give significant insights into the environmental variations occurring in the ecosystems compared to the benthic static measures such as density, biomass, and diversity.

The AZTI's Marine Biotic Index (AMBI) (Borja et al. 2000, 2007) and multivariate-AMBI (M-AMBI) (Borja et al. 2004; Muxika et al. 2007), are the biotic indices developed for European waters, based on the sensitivity of macrobenthos to disturbances for assessing the ecological status of coastal marine ecosystems. Though it was used in temperate waters earlier, studies conducted in the tropical waters used these indices successfully in assessing ecological status (Bigot et al. 2008; Khan et al. 2014; Feebarani et al. 2016; Joydas et al. 2017). Depended on the setting up of reference conditions to these indices makes it more reliable for the interpretation of ecological status of different geographic regions (Borja et al., 2012). Studies used the indices successfully in evaluating both natural and human induced disturbances worldwide (Bigot et al. 2006, 2008; Borja and Tunberg 2011; Feebarani et al. 2016).

Cochin estuary is a monsoonal tropical estuary, located in the southwest coast of India, and it is well-known for its high biological productivity and biodiversity (Qasim 2003). The estuary is perpetually influenced by the freshwater inputs from seven major rivers and also by the tidal incursion of the seawater from the Arabian Sea, thus creating a prominent gradient in the salinity distribution, in turn supporting the growth and sustenance of diverse flora and fauna (Madhu et al. 2007). The anthropogenic interventions started in the estuary by the second half of the 19th century in turn making it a sink of large amounts of organic and inorganic effluents from several anthropogenic sources (Balachandran et al. 2005). These long-term and continuous anthropogenic effluent discharges into the estuary resulted in organic enrichments, eventually leading to deterioration of its habitat quality (Martin et al. 2011). These environmental alterations get reflected in the biotic components inhabiting the estuary, especially in its benthic inhabitants because of their proximity and close interaction with the substratum.

Even though, studies pertaining to the structural as well as functional characteristics of macrobenthic fauna as a tool for a comprehensive evaluation of the ecosystem functioning has gained a wide acceptance in recent years (Dolbeth et al. 2003, 2012; Wong et al. 2011; MacDonald et al. 2012; Bolam et al. 2014; Rodil et al. 2019), such studies are quite few in the tropical estuaries (Magalhães and Barros 2010; Bissoli and Bernardino 2018). In the CE, ample studies have been conducted on various aspects of the macrobenthic community such as its distribution and community structure (Pillai 1977; Devi et al. 1991; Nair et al. 1983; Noyel et al. 2020), ecology from different environments (Ambika Devi and Pillai 1990; Aravindakshan et al. 1992; Kumar and Antony 1993; Rehitha et al. 2019a), and community structure in relation to pollution for the last few decades (Unnithan et al. 1975; Remani et al. 1983; Martin et al. 2011; Feebarani et al. 2016; Rehitha et al. 2017). However, studies pertaining to the assessment of benthic habitat quality using macrobenthic functional characteristics did not receive much research attention in the CE till date. Furthermore, no relevant information is available in the CE pertaining to the macrobenthic secondary production so far. Therefore, the present study was specifically aimed 1) to gather information on the ecology and variability of macrobenthic trophic structure and the secondary production in the CE along with the macrobenthic structural properties and also 2) to evaluate the benthic habitat quality using macrobenthic functional characteristics and biotic indices such as AMBI and M-AMBI.

## **Materials and Methods**

## Study area

The Cochin estuary (CE) is a tropical micro-tidal eutrophic estuary (Lat. 9° 30′–10° 10′ N and Long.76° 15′–76° 25′ E), situated along the south-west coast of India parallel to the Indian coastline (Qasim 2003). The two major inlets to the Arabian Sea; Cochin the widest (width 450 m) and Azhikode (width 250 m), enables regular seawater incursion into the CE, thus providing the characteristics of a tropical estuary (Qasim 2003). The estuary receives enormous quantity of freshwater from seven rivers, especially during the southwest monsoon period (June to September), of which two rivers (Periyar and Chalakudy) empties to the northern limb of the CE and the remaining five rivers (Muvattupuzha, Pamba, Manimala, Meenachil, and Achancoil) debouches to the southern limb of the estuary. The estimated annual freshwater influx of the CE to the Arabian Sea is 22,000 × 10<sup>6</sup> m<sup>3</sup> (Revichandran et al. 2012). The annual precipitation in and around the regions of the CE is ~ 320 cm, out of which ~ 60–70% occurs during the southwest monsoon period which controls the seasonal hydrography and biotic distribution of the estuary (Qasim 2003; Madhu et al. 2010).

## Sampling strategy and laboratory analysis

For the study, seasonal sampling was conducted in the CE during 2018 to 2019 (Fig. 1). The pre-monsoon 2018 (hereafter PreM) was characterized by warm environmental conditions with less rainfall (av. 193.2 mm), the post-monsoon 2018 (hereafter PostM) was moderately warm with intermediate rainfall (av. 246.9 mm) and the monsoon 2019 (hereafter MonS) with heavy rainfall (av. 478.5 mm). The samples for PostM were collected after the cessation period of the Kerala flood event, happened in connection with the southwest monsoon 2018. The samples for the MonS period were collected after the excessive rainfall occurred during the south-west monsoon of 2019. The samples were collected from seven locations in the CE, from the inlet towards the northern and southern upstream locations respectively, characterized by higher freshwater influence (oligohaline-0.5-5). Stations 2 and 3 were located in the northern mesohaline region (5-18 salinity) and stations 5 and 6 were positioned in the southern mesohaline region. Station 4 was fixed in the inlet region (polyhaline-18-30), which was continuously influenced by the tidal incursion from the Arabian Sea.

The bottom water samples were collected using a Niskin sampler (5 L capacity Hydro-Bios, Germany). The bottom water temperature was recorded using a portable Sea-Bird CTD (CTD 19 plus, SB Electronics). Salinity was measured using a Digi Auto Salinometer (Model TSK, accuracy  $\pm 0.001$ ). For the estimation of the dissolved oxygen (DO) concentrations, bottom water samples were collected in glass bottles (125 mL) carefully, without air bubbles and which were fixed onboard and subsequently analyzed in the laboratory following Winkler's titration method (Grasshoff et al. 1983). In order to estimate the suspended particulate matter (SPM), the water samples (250 mL) were filtered through a pre-weighed and pre-combusted (80 °C) millipore membrane filters (nominal pore size 0.45 µm). The concentration of the suspended matter was measured from the differences in the weight of the filter paper before and after drying (APHA 2005). For the estimation of chlorophyll a (chl-a), the bottom water sample (1 L) was filtered onto GF/F filter paper (Whatman, nominal pore size 0.7 µm) and extracted using 10 ml of 90% acetone. The fluorescence of the sample was measured using a Fluorometer (Model 7200, Turner Designs) before and after acidification (Parsons 1984). The inorganic nutrients (nitrite, nitrate, phosphate, and silicate) were analyzed using a Skalar Segmented Flow Auto Analyzer (Model SA-1050), according to the method of Grassholf et al. (1983). The sediment samples for analyzing the sediment texture, composition and macrobenthic fauna were collected using a Van-Veen grab of area 0.05 m<sup>2</sup>. Dried sediment samples (dried in a hot air oven at 60 °C) were subjected to textural analysis according to the method of Krumbein and

Pettijohn (1938) and the organic carbon content was estimated by the wet oxidation method (El Wakeel and Riley 1957).

The collected sediment samples were washed onboard through a sieve with mesh size of 0.3mm and subsequently preserved in 4% formalin Rose-Bengal mixture (Birkett and McIntyre 1971). In the laboratory, organisms were size-fractionated by passing through a series of mesh sieves of different sizes 2.8, 2, 1.4, 1, 0.71, 0.5, 0.3 mm (Edgar 1990a; Rakocinski and Zapfe 2005). The macrobenthic fauna retained in each sieve fraction were sorted into a different taxon (polychaetes, molluscs, crustaceans etc.) and were preserved simultaneously in separate labeled vials. The taxonomic identification of the macrobenthic fauna was carried out to the lowest possible levels using standard taxonomic manuals and published literature and updated using the website World Register of Marine Species (Fauvel 1953; Day 1967; Gosner 1971; Fauchald 1977; Beesley et al. 2000; WoRMS Editorial Board 2020).

The trophic structure of the macrobenthic fauna in the study area was analyzed by identifying the feeding guilds of the dominant group, polychaetes, and other macrobenthic taxa with the help of published literature (Fauchald and Jumars 1979; McDonald 2010; Jumars et al. 2015; WoRMS Editorial Board 2020). Polychaete feeding guilds were assigned based on their feeding mode, the motility pattern, and the morphological structure used in feeding. The other macrobenthic taxa were categorized into different feeding groups according to their diet and feeding mode as Carnivorous (CR), Herbivorous (HR), Omnivorous (OM), and Deposit feeder (ingests sediment; DE), Detritus feeder (ingests particular matter only, without sediment; DT), Suspension/Filter feeder (strains particles from the water, SU), Predator (eats live animals only; PR), Scavenger (carrion only; SC), and Grazer (feeds by scraping, either on algae or sessile animals, GR).

The macrobenthic density was estimated as numerical abundance per m<sup>2</sup> and the wet weight was measured as biomass, which was blotted before weighing and expressed as  $g.m^2$ . The samples were wet weighed for each taxonomic group within each sieve size fractions for analyzing the taxonwise biomass. Macrobenthic biomass of each taxon (Polychaeta, Oligochaeta, Amphipoda, Isopoda, Bivalvia, Gastropoda, and Others) was converted into ash-free dry weight (mg AFDW m<sup>-2</sup>) using the taxon-wise conversion factors of Ricciardi and Bourget (1998). The secondary production for each taxon retained in each sieve size was calculated using the general equations of Edgar (1990a), which relates daily macrobenthic production P (g day<sup>-1</sup>) to ash-free dry weight B ( $\mu$ g) and water temperature T (°C), (P) = 0.0049×B<sup>0.80</sup>×T<sup>0.89</sup>. The total production potential for each grab sample was calculated by aggregating the taxon-size-fraction production values (Rakocinski and Zapfe 2005). Daily production values were scaled up to a square meter basis.

## **Statistical analysis**

For assessing the significance of seasonal variation of the abiotic and biotic variables (PreM, MonS, and PostM), one-way analysis of variance (ANOVA) was carried out. Multiple comparisons were conducted using Tukey's post hoc test (using GraphPad prism 5) on the datasets where significant variations were observed (Day and Quinn 1989). Multivariate analysis was carried out on the square root transformed data of the secondary production of macrobenthos in PRIMER 6 (version 6.1.5) (Clarke and Warwick 2001). The Bray–Curtis similarity index and group average linkage were used for the cluster analysis and non-metric multidimensional scaling (NMDS) (Clarke and Warwick 1994). One-way analysis of similarity (ANOSIM, with 999 permutations), which tests for the differences between the groups of (multivariate) samples from different locations based on the production from different sieve fractions, was carried out to understand the differences between seasons based on macrobenthic production. The Redundancy analysis (RDA) for elucidating the relationships of trophic structure and secondary production with the environmental parameters was performed using CANOCO software (version 4.5).

The benthic indices such as the AZTI's Marine Biotic Index (AMBI; Borja et al. 2000), and the multivariate-AMBI (M-AMBI; Muxika et al. 2007) were used to assess the quality of the environment by translating the community composition into an ecological quality classification (Borja et al. 2000, Muxika et al. 2007; Feebarani et al. 2016). Based on the sensitivity of macrobenthic organisms to an increasing gradient of stress, ranging from the most sensitive species (ecological group I) to the most opportunistic species (ecological group V), they were classified into five ecological groups according to AZTI Laboratory (http://www.azti.es). The percentage of unassigned taxa after updating the species assignment had a mean value of 1.9. A modified version of AMBI, M-AMBI (Muxika et al. 2007), which is a combination of Shannon diversity, species richness and AMBI, was also derived using AMBI software. Since, reference conditions are a prerequisite for deriving M-AMBI values as the salinity gradient can influence species richness and diversity; reference conditions were derived for each salinity zone separately (Feebarani et al. 2016). Also, the study area has been impacted by multiple anthropogenic activities, their species diversity and richness values could not be adapted to set the reference conditions. Therefore, the reference conditions for evaluating M-AMBI index was derived by increasing the highest species diversity and richness values of all stations in each salinity zone by 15% following Cai et al. (2014) and adapting the azoic conditions (diversity and richness equal to 0 and AMBI equal to 6) for the bad status.

### Results

# **Environmental parameters**

The bottom water temperature varied between 28.1 and 32.4 °C in the study area, with a slightly higher temperature during PreM (Table 1). Salinity varied seasonally with high salinity during PreM (av. 21.6 ± 5) than MonS (av. 11.3 ± 5) and PostM (av. 15.7± 7.6). The dissolved oxygen (DO) distribution showed a prominent seasonal variation with conspicuously higher DO during PostM compared to other seasons (Table 1 and 3). The suspended particulate matter (SPM) varied between 14.8 and 109.8 mg/L, with high concentration during MonS and low during PostM. The nitrate varied between 1.47 and 30.13  $\mu$ M with high concentration during MonS and low during PreM. In contrast, the nitrite showed higher values during PreM and lower values during MonS. The phosphate varied between 0.15 and 2.33  $\mu$ mol in the study region with high concentrations during MonS. On the other hand, the silicate concentration varied between 12.01 and 91.9  $\mu$ M with higher values during PostM (av. 57.5 ± 23.2  $\mu$ M). The chl-*a* ranged from 0.18 to 27.4 mg/m<sup>3</sup> with higher concentration during PreM compared to other seasons (Table 1).

In the estuary, the percentage of sand varied from 0.68 to 86.60 during the study period, with evidently higher percentage during PostM (Table 1). Silt fraction ranged between 1.73 and 90.1%, with high percentage during MonS (av.  $46.2 \pm 33.1$  %) and low during PostM (av.  $29.3 \pm 14.6$  %). Clay fraction varied between 8.75 and 58.60% with higher clay content during PreM (av.  $48.0 \pm 9.0$ %) and lower during MonS (av.  $13.2 \pm 2.9$ %) respectively. Silty clay substratum dominated during PreM, while the substratum was sandy silt during MonS and silty sand during PostM. High sand percentage was observed in the upstream locations (St.1&7) during MonS and in the inlet (St. 4) throughout the study period. High silt content was observed in the southern stations (St. 5&6) during MonS, while high clay fraction in the stations at the southern limb of the estuary (St. 5, 6&7) during PreM. Sediment organic carbon varied between 9.32 and 37.65 mg/g, with higher concentration during PreM compared to the other periods. High organic carbon was present at the southern stations (St. 5 & 6) where fine sediment fractions dominated.

## Macrobenthic density and biomass

The macrobenthic biomass varied between 0.41 and 95.4 gm<sup>-2</sup> during the study period, with higher biomass observed during PostM (av.  $49.5 \pm 28.1 \text{gm}^{-2}$ ) compared to MonS (av.  $4.96 \pm 6.7 \text{ gm}^{-2}$ ), and PreM (av.  $4.4 \pm 3.1 \text{ gm}^{-2}$ ) (Table 2). Spatially, the inlet showed higher biomass throughout the study period. The macrobenthic density varied between 80 and 24360 ind.m<sup>-2</sup> in the CE. The density was noticeably higher during PostM (av.  $13124\pm6063$  ind.m<sup>-2</sup>) compared to PreM (av.  $1460 \pm 1843$ )

ind.m<sup>-2</sup>) and MonS (av. 266±228 ind.m<sup>-2</sup>). During PreM, high macrobenthic density was observed at the southern upstream station and low density at the northern upstream location. During MonS, higher density was observed at the northern upstream location and lower at the second northern location. The macrobenthic density and biomass exhibited significant seasonal variation (p< 0.0001) in the estuary (Table 3). The macrobenthic diversity was high during MonS, while richness and number of species were high during PostM (Table 2).

#### Macrobenthic community structure

The macrobenthic community of the CE consisted of 13 faunal groups during the study period, of which polychaetes, oligochaetes, amphipods, tanaidaceans, bivalves and decapods were the common faunal groups observed during all the seasons. During PreM, polychaetes and oligochaetes constituted 96% of the total density, when other faunal groups represented <2% only (Fig. 2). During MonS, along with polychaetes (av. 66%), amphipods, bivalves, tanaidaceans, nemerteans, cumaceans, oligochaetes and gastropods were observed in the macrobenthic community. While during PostM, gastropods (av. 80%) formed the dominant group. In the CE, 8 faunal groups represented the macrobenthic community during PreM, 9 during MonS, and 13 during PostM.

A total of 67 macrobenthic taxa were identified during the study period. Similar to the macrobenthic density and biomass, the number of taxa was also high during PostM with 48 taxa, whereas only 26 taxa were observed during MonS, and 23 during PreM respectively. *Prionospio cirrifera*, *Prionospio cirrobranchiata*, *Cirratulus filiformis*, *Sigambra parva*, *Mediomastus* sp, *Nereis* sp, *Cossura coasta*, and *Nephtys oligobranchia* were the major polychaete species during PreM. During MonS, *Mediomastus* sp, *Dendronereis estuarina*, *Diopatra neapolitana*, *Sigambra parva*, and *Prionospio cirrifera* formed the predominant species. The gastropod *Stenothyra perpumila* dominated during PostM followed by polychaetes such as *Mediomastus* sp, *Diopatra neapolitana*, *Prionospio cirrifera*, *Cirratulus cirratus*, and *Nephtys oligobranchia*. *Oligochaeta*, *Americorophium triaenonyx*, *Victoriopisa chilkensis*, *Cheriophotis megacheles*, *Apseudus chilkensis*, *Nemertea*, *Bodotria* sp, *Arcuatula senhousi*a were the other macrobenthic taxa observed in the estuary during the study period (Supplementary Table S1).

#### Macrobenthic trophic structure

During the study period, polychaetes were organized into 10 different feeding guilds such as CMJ (carnivorous, motile, jawed), CDJ (carnivorous, discretely motile, jawed), HMJ (herbivorous motile jawed), SDT (surface deposit-feeding, discretely motile, tentaculate), SST (surface deposit-feeding, motile, tentaculate), BMX (subsurface

deposit-feeding, motile, with other structures, usually eversible sac-like pharynges), CMX (carnivorous, motile, with other structures, usually eversible sac-like pharynges), SDJ (surface deposit-feeding, discretely motile, jawed), and FDT (filter-feeding, discretely motile, tentaculate). During PreM, the SDT was the dominant feeding guild (av. 52.5%), while BMX was the dominant guild during MonS (av. 23.9%) and PostM (av. 4.5%). Thus, the surface deposit-feeding polychaetes (SDF) were dominated during PreM (constituted by the guilds, SDT, SST, and SMT) in the estuary, followed by carnivores (CR-consists of the guilds CMJ, CMJ/CDJ, and CDJ). During MonS, surface and subsurface feeders (SSDF) were the dominant trophic groups, followed by carnivores. By contrast, during PostM, all the trophic groups were more or less evenly distributed (Fig. 3a).

The remaining macrobenthic fauna (without polychaetes) were grouped into 6 different feeding guilds such as omnivorous-detritivores (OM-DT), omnivorous-suspension feeders (OM-SU), omnivorous-detritivores/predators/scavengers (OM-DT/PR/SC), carnivorous-predators (CR-PR), carnivorous-scavengers (CR-SC), and herbivorous-grazers (HR-GR). During PreM, OM-DT (av. 9.02%) and OM-DT/PR/SC (av. 1.77%) were dominated; while during MonS, OM-DT (av. 18.3%) and OM-SU (12.9%) were the dominant feeding groups. During PostM, HR-GR (80.8%) dominated in the estuary, followed by OM-SU (6.2%). Hence the omnivores were found to be the dominant groups during PreM (10.8%) and MonS (30.7%), whereas herbivores were dominated during PostM (75.9%) (Fig. 3b).

## Macrobenthic secondary production

Macrobenthic secondary production in the CE varied between 0.014 and 0.802 g m<sup>-2</sup> d<sup>-1</sup>, with a distinct seasonal variation (Table 1). The secondary production was higher during PostM (av. 0.389  $\pm$  0.21 g m<sup>-2</sup> d<sup>-1</sup>) compared to MonS (av. 0.094  $\pm$  0.112 g m<sup>-2</sup> d<sup>-1</sup>), and PreM (av. 0.095  $\pm$  0.06 g m<sup>-2</sup> d<sup>-1</sup>) in the estuary (Fig. 4). Macrobenthic production was high for the sieve mesh size 0.5 mm during PreM, while high production was observed for the sieve mesh size 0.7 mm during MonS and PostM. Spatially, the inlet location showed higher macrobenthic production, irrespective of seasons. Considering the size categorized macrobenthic taxon wise production in each sieve size, the production in the larger sieves (> 2.8 mm and 2 mm) were contributed by decapod crustaceans, benthic fish, and large polychaetes during PreM. On the other hand, decapod crustaceans, bivalve, and gastropod mollusks, and polychaetes were the major contributors during MonS. During PostM, decapod crustaceans and bivalve mollusks were contributed mostly to the production in large sieve fractions. The macrobenthic production in the inlet region was found to be contributed by large sieve fractions almost throughout the study period. The macrobenthic secondary production was mainly contributed by polychaetes during PreM and MonS while gastropods were the chief contributor during PostM.

The MDS analysis of the macrobenthic production contributed by different sieve fractions to the total secondary production exhibited a distinct temporal variability (Global R-0.375, p-0.003) in the study area (Fig. 5). Furthermore, the ANOSIM analysis also exhibited significant variations between PreM and PostM (Global R-0.541, p-0.001), and MonS and PostM (Global R-0.569, p-0.001).

#### **AMBI and M-AMBI**

Using the AMBI analysis, among the 67 taxa, 62 were fit into five ecological groups based on the AZTI classification, and remaining 5 taxa were not included in the species list for analysis. The list of identified species along with the assigned ecological groups is given in the Supplementary Table S2 (according to the species list of AMBI, December 2020). Using AMBI 0 to 12.5% of species grouped under EG I, 2.7- 26.3 % in EG II, 9.9-83.3% in EG III, 0-72.9 % in EG IV, and 0-27.8 % were in EG V during PreM. During MonS, 0-50 %, 0-27.8 %, 38.9-100%, 0-20 %, 0-16.7 % of the species were grouped into EG I, EG II, EG III, EG IV, and EG V, respectively. While during PostM, 36.7-96.4 %, 0.8-6.2 %, 0.4-47.5 %, 0-8.5 %, and 0-2% of the species were belonged to EG I, EG II, EG III, EG IV and EG V, respectively (Fig. 6a). The AMBI values ranged from 2.6 to 4.4 during PreM, 1.35 to 3.3 during MonS, and 0.11 to 1.96 during PostM. Hence the AMBI values revealed slightly disturbed to moderately disturbed conditions during PreM while slightly disturbed conditions were observed during MonS. During PostM, AMBI values showed undisturbed conditions in the estuary except at station 4, inlet (slightly disturbed condition) (Fig. 6b). The M-AMBI values ranged from 0.41-0.785 in the oligohaline region, 0.216-0.698 in the mesohaline regions, and 0.396-0.859 in the polyhaline regions. The M-AMBI values ranged from 0.374-0.547 during PreM, 0.216-0.786 during MonS, and 0.560-0.859 during PostM, indicating the habitat quality status ranging from poor-moderate-good conditions, poor-moderate-good-high, and good-high conditions respectively in the estuary (Fig. 6c).

## Influence with the environmental parameters

The RDA analysis carried out to understand the influence of environmental variables on macrobenthic trophic groups indicated a clear-cut seasonal variation (Fig. 7). During PreM, suspension feeders (OM-SU) exhibited a positive correlation with chl-*a* and SPM, while during MonS positive correlation was found with Chl-*a* only. During PostM, OM-SU were positively correlated with salinity, sand, SPM, and DO. Detritivores (OM-DT) showed a positive relationship

with organic carbon and clay during PreM, with clay during MonS and with salinity, sand, and SPM during PostM. Herbivorous grazers (HR-GR) were found to be positively correlated with chl-*a*. The results of redundancy analysis exhibited variability in secondary production in association with the changes occurring in the environmental parameters (Fig. 8). Secondary production exhibited a positive relationship with the benthic biomass throughout the period. During PreM, it showed a positive correlation with the fine sediment texture (silt and clay), organic carbon, nutrients, and dissolved oxygen. During MonS, it was correlated with sandy texture, temperature, and salinity. During PostM, it was influenced by DO, salinity, sandy texture, and temperature. SPM and Chl-*a* were closely correlated during PreM. Pearson correlation analysis showed a positive relation of AMBI index with clay, temperature, and negative relation with sand. M-AMBI showed a positive relation with biomass, and secondary production, and negative relation with organic carbon. Macrobenthic density positively correlated with DO and silicate (Supplementary Table S3).

## Discussion

The dynamic and complex nature of the estuarine habitats endowed with high biological production and sustaining a wide variety of fauna and flora makes them unique, yet ecologically significant coastal habitats globally. In recent years, estuaries around the world are subjected to immense ecological degradation as a result of the several natural and anthropogenic activities occurring along its watersheds. These degradation activities bring about drastic changes both in its ecosystem structure and functioning (Elliott et al. 2007), which eventually causes remarkable consequences on the biological compartments. The hydrography of the CE is greatly influenced by the fluvial discharges contributed by the monsoonal rainfall and associated river runoff, and also by the regular intrusion of saline water from the adjacent Arabian Sea through tidal influxes (Qasim 2003). The extensive anthropogenic activities occurring in and around the CE, influence the estuarine inhabitants to some extent. Devi et al. (1991) reported changes in the benthic community of the CE due to the influence of industrial activities. Proliferation of opportunistic polychaetes was observed in the northern and central estuary as a result of organic enrichments (Martin et al. 2011). Recent studies reported shift in the benthic fauna due to the continuous dredging carried out as a part of the developmental activities in the CE (Rehitha et al. 2017; Noyel et al. 2020). The present study, thus, addresses the assessment of environmental habitat quality, using macrobenthic structural and functional characteristics, along with benthic biotic indices in the CE based on a seasonal scale data.

The influence of southwest monsoonal precipitation on the estuarine hydrography was evident throughout the study region, with marked changes in many of the hydrographic attributes of the CE. Freshwater conditions prevailed in the upstream stations whereas mesohaline conditions were observed in other locations except at inlet during monsoon. As the freshwater input got reduced, a gradual increase in salinity was observed during post-monsoon period in the estuary. The present study has documented a marked seasonal variation in the sediment texture in the CE. The substratum of the CE is mostly comprised of the sediments carried from the rivers as well by those transported from sea to the estuary through tidal incursion (Gopinathan and Qasim 1971; Veerayya and Murty 1974). During monsoon, the upstream sampling locations received more sand through the increased river discharges associated with monsoon, whereas the predominance of sand in the inlet region can be linked to its marine origin. So, runoff from rivers, sediment erosion and accumulation, tidal action and complicated flow patterns created by several canals and islands are the reason for the spatio-temporal variability of sediment texture (Noyel et al. 2010; Rehitha et al. 2019b; Pandiyarajan et al. 2021). In the present study, a distinct seasonal variation was observed in the macrobenthic distribution with high macrobenthic density and biomass during post-monsoon. In tropical estuaries, a substantial increase in macrobenthic density is observed after the heavy monsoonal rains, indicating a faster recovery of the biota upon attainment of stabilized environmental conditions (Harkantra and Rodrigues 2003; Sivadas et al. 2011). The present study also witnessed higher macrobenthic density, biomass, and species diversity during post-monsoon in the CE, subsequent to the heavy rainfall and associated flood event (2018). The marked decline in inorganic nutrients and heavy metal concentrations in the CE after the flood was substantiated by the previous report of Vineetha et al. (2020). The extensive removal of the accumulated nutrients and contaminants from the estuary by the freshwater influxes concurrent to monsoon might have improved the estuarine habitat quality and made the environment more habitable for the increased settlement and proliferation of the diverse macrobenthic community during post-monsoon. In addition, turbulent waters found to facilitate the wide dispersal of both larvae and adults of the sedentary macrobenthic organisms (Dobbs &Vozarik 1983; Hernández-Arana et al. 2003). Similar to previous studies, a monsoonal decrease in macrobenthic density was also evident in the study region as a result of the marked salinity variations and the unstable substratum (Sivadas et al. 2011; Rehitha et al. 2019a,b). The lowest macrobenthic biomass observed during pre-monsoon with the proliferation of small-sized macrobenthic organisms indicates a stressed environment impacted by higher organic loads and other anthropogenic disturbances.

The macrobenthic community composition also exhibited evident seasonal variation with the dominance of polychaetes and oligochaetes (together 96%) during pre-monsoon, polychaetes, and amphipods (together 78%) during monsoon, and gastropods and polychaetes (together 88%) during post-monsoon. The polychaetes, equipped with diverse feeding guilds are found to possess high

stability and adaptability to different habitats (Fauchald andJumars 1979; Simboura et al. 2000). Polychaetes and oligochaetes, which often dominate in organic rich fine sediments, were observed in the clayey sediments of pre-monsoon. Similar to earlier studies in the CE, though polychaetes dominated the macrobenthic community (Devi and Venugopal 1989; Kumar 1993; Noyel et al. 2020), a shift in the benthic community structure was noticed in the present study during postmonsoon. The number of macrobenthic taxa observed was also high in the coarse substratum of the post-monsoon compared to the fine sediments of pre-monsoon, indicating the marked influence of substratum on the macrobenthic community distribution. Similar to the macrobenthic community distribution, a distinct seasonal variation in species composition was also evident, with the dominance of surface deposit feeding Prionospio cirrifera (39%) during pre-monsoon, the subsurface deposit feeding Mediomastus sp (18.2 %), during monsoon, and Stenothyra perpumila (76%), the herbivorous grazer during post-monsoon. Prionospio cirrifera is considered tolerant to disturbances, and an increase in their population was observed in regions with organic enrichments or pollution (Simboura and Zenetos 2002). Earlier studies also reported the dominance of this spionid polychaete in fine grained sediments (Jayaraj et al. 2008; Noyel et al. 2020). Rivero et al. (2005), considered *Mediomastus* sp as indicators of areas with intermediate disturbances. Americorophium triaenonyx was the major species observed among amphipods during monsoon. The species is able to tolerate wide ranges of salinity and hence exhibit wide distribution in the CE (Nair et al. 1983). Hence the major environmental factors found to affect the distribution of macrobenthic communities were salinity, and sediment characteristics which was found to be similar to earlier studies (Jayaraj et al. 2008; Rehitha et al. 2019). The gastropod S. perpumila was abundant throughout the estuarine stations during post-monsoon, but their absence at the inlet indicates their preference for medium saline waters (5-18). Instead of S. perpumila, another molluscan Arcuatula senhousia was found in high abundance at the inlet during post-monsoon. This species, considered to be a marine invasive species (Sreedhar 1991) is a suspension-feeder, can achieve considerable densities in both intertidal and subtidal soft sediments of estuaries (Kikuchi and Péres 1977; Crooks 1992), forming a thick mat over the bottom, thereby preventing the growth of other benthic organisms. Nishijima et al. (2013) have reported the dominance of this species after the cessation of a flood event in Ohta River Estuary, Japan.

Variation in the macrobenthic trophic structure is known to occur concurrently to subtle shifts in the benthic habitat quality resulting from several natural and anthropogenic activities (Rakocinski et al. 1997). In the present study, the trophic structure analysis of the macrobenthic fauna was carried out by assessing the feeding guilds of polychaetes and that of the other non-polychaete macrobenthic taxa separately. The analysis of polychaete feeding guild revealed a clear seasonality with the dominance of surface deposit-feeding polychaetes during pre-monsoon (73%), subsurface (39%), and surface deposit feeders (39%) during monsoon, and more or less even distribution of all the trophic groups during post-monsoon (SSDF-36%, SDF-32% & CR-31%). The finer sediments usually entrap more organic carbon, and the present study observed fine sediments with high organic content during pre-monsoon. Martin et al. (2011) pointed out an increased organic enrichment in the CE and consequent increase of opportunistic species during pre-monsoon. Studies also observed the dominance of deposit-feeding organisms in fine sediments with high organic content, whereas carnivores dominant in coarse sediments with low organic content (Paiva 1993; Venturini and Tommasi 2004; Gaudencio and Cabral 2007). Previous studies have reported a more balanced distribution of feeding types in the coarse substratum (Dolbeth et al. 2009; Mcdonald et al. 2012). Coarse substratum offers more microhabitats, oxygenation and easy mobility for organisms; hence the existence of coarse substratum and better habitat quality of the post-monsoon might have supported the existence of diverse macrobenthic taxa. Being buried in the sediments, the sub-surface deposit feeders can utilize the organic matter more efficiently than the surface feeders and hence are found to dominate in the disturbed monsoonal conditions.

The analysis of the other macrobenthic feeding guilds revealed a dominance of omnivorousdetritivores (74%) during pre-monsoon, which was similar to the results of polychaete feeding guilds. Detritus form a major energy source for the macrobenthic communities in estuaries. However, high density of detritus feeders is an indication of a stressed environment (Beukema 1991; Hossain 2018; Rehitha et al. 2019b). The positive correlation of detritivores with organic carbon evident in the RDA plot during pre-monsoon indicates their environmental preferences. The CE receives enormous volumes of organic loads through unprocessed industrial ( $104 \times 10^3 \text{ m}^3 \text{ dav}^{-1}$ ) and domestic  $(0.26 \times 10^3 \text{ m}^3 \text{ day}^{-1})$  sewage from the Cochin city and from several other anthropogenic sources (Balachandran et al. 2005). Therefore, the estuary acts as a sink of nutrients leading to organic enrichments (Martin et al. 2011, 2013), which might impart deleterious effects to the biotic components of the estuary. In the present study also, a high concentration of organic carbon was observed during pre-monsoon, compared to the monsoon and post-monsoon periods. Spatially, higher organic carbon was observed in the southern mesohaline locations during pre-monsoon and post-monsoon. During monsoon, the organic carbon was high in the northern and southern mesohaline locations where fine sediments dominated. The finer sediments accumulated more organic carbon compared to the coarser sediments.

With the commencement of the monsoon and subsequent dilution of the estuary, a decrease in organic carbon was observed both in monsoon and post-monsoon. The monsoonal river runoff also contributed to an increase in the sand and silt sediment fractions into the estuary. With the increase in the suspended particulate matter during monsoon, an increase in the abundance of omnivorous-suspension feeders (36%) was also noticed along with omnivorous-detritivores (48%). The suspension feeders exhibited a positive relation with chlorophyll-*a*, and SPM, in multivariate analysis, thus indicating their food preferences. During post-monsoon, a shift in the trophic group was observed with the dominance of herbivorous-grazers (87%) in the estuary, together with omnivorous-suspension feeders. The increased density of grazers and suspension feeders in the estuary pointed towards the prevalence of an improved environmental quality compared to premonsoon. Antonio et al. (2012) have shown that particulate organic matter (POM) brought by the rivers serves as an important energy source for the estuarine benthic fauna when there is a high river influx. Thus, the suitable substratum and food availability form determinants for modulating the distribution of different trophic groups in the CE.

The organic matter of terrestrial origin usually contains refractory compounds such as cellulose and lignin, which is hard for most aquatic consumers to digest and assimilate, and hence only those organisms with specific digestive enzymes are able to break glycosidic bonds of cellulose into oligosaccharides and utilize these sources of energy (Cividanes et al. 2002; Sakamoto et al. 2007). According to Antonio et al. (2010), estuarine molluscs can digest cellulose and assimilate river-derived particulate matter as a potential energy source. The dominance of gastropod molluscs during post-monsoon indicated their better capability in the utilization of the river derived organic matter. The RDA analysis has shown a positive relationship between the density of herbivorous grazers and chl a points the view. The majority of the suspension feeders depend on autotrophic food sources whereas the deposit feeders ingest sediment-associated heterotrophic food sources. Thus, the dominance of deposit feeders during pre-monsoon in the CE indicates the prevalence of heterotrophic mode of nutrition during pre-monsoon compared to post-monsoon. Furthermore, an increase in the density of suspension feeders also points towards the prevalence of better water quality, as they often avoid turbid waters to prevent clogging of their feeding structures. The positive relation of gastropod density with the DO thus affirms the above mentioned statement. Similar to the results of macrobenthic community structure, trophic structure analysis also exhibited spatiotemporal variation, and it revealed a more balanced distribution of trophic groups during postmonsoon, which also substantiates the use of trophic groups in assessing the habitat quality of the ecosystem.

A distinct seasonal variation in macrobenthic secondary production was observed in the CE, with high production during post-monsoon and low during pre-monsoon. Secondary production is the amount of material available to subsequent trophic levels after the resource utilization by the macrobenthic fauna (Dolbeth et al. 2005; Buzzelli et al. 2007). Therefore, during post-monsoon, the amount of materials available to the higher trophic levels was high, compared to other seasons. Climatic events, such as above-average rainfall contributed to flushing of the tropical aquatic systems are considered to improve the environmental scenario of the ecosystem after their incidences (Bouvy et al. 1999; Jackson et al. 2001; Costa et al. 2017). High secondary production observed for the sieve mesh size 0.7 mm during monsoon and post-monsoon indicates the contribution of largesized organisms to the production. In contrast, the small-sized organisms contributed to the production during pre-monsoon (sieve size 0.5 mm). The high dominance of small-sized organisms in a benthic community may indicate habitat instability (Schwinghamer 1983). A diverse community of molluscs, crustaceans, and polychaetes contributed to the secondary productivity during postmonsoon, whereas polychaetes formed the predominant contributor to macrobenthic secondary productivity during pre-monsoon. Spatially, the inlet showed high secondary production throughout the study period, where the sandy substratum was dominated. The structural biodiversity of the hardbottom habitats are temporally more stable (in terms of length, abundance, biomass) than the structural biodiversity of the soft sediments (Rodil et al. 2020). The sediment texture showed the dominance of finer fractions (clay) during pre-monsoon, while coarser (sand) fractions dominated during monsoon and post-monsoon. In the present study, the benthic production was observed to vary in accordance with the substratum changes and it was further substantiated by the results of RDA analysis. Sandy substratum supports more production than muddy bottoms. Similar to the macrobenthic community structure with diverse benthic inhabitants in the post-monsoon, the secondary production also reflects a similar scenario with high production during post-monsoon.

Previous studies in Indian estuaries have effectively established the utility of AZTI's biotic indices (AMBI and M-AMBI) in ecological quality assessments (Khan et al. 2014; Sigamani et al. 2015; Feebarani et al. 2016; Mulik et al. 2017; Jayachandran et al. 2020; Pandey et al. 2021). Similar to the studies of Feebarani et al. (2016), Mulik et al. (2017), and Dias et al. (2018), the present study also evaluated the ecological quality of the CE based on salinity zones. The indices also showed similar results as exhibited by the macrobenthic structural and functional properties such as community composition, trophic structure, and secondary production. The AMBI values revealed slightly disturbed to moderately disturbed conditions in the CE during pre-monsoon; slightly disturbed conditions in monsoon and slightly disturbed to undisturbed conditions during post-

monsoon in the estuary. Species tolerant to disturbances and second order opportunists (ecological group-III-57% and EG-IV-23.9% respectively) were dominant during pre-monsoon, 59.8% and 19% of species were belongs to EG-III and EG I (sensitive species to disturbance) during monsoon, while EG-I (84%) was dominant during post-monsoon along with EG-III (9%). Although highly diverse fauna observed in the inlet during post-monsoon, the observed higher percentage of EG-III species indicated slightly disturbed status by AMBI (47.5%). The positive correlation of AMBI with clay and negative relation with sand indicated a decrease in the habitat quality with the increase in fine sediment fractions. The M-AMBI indicated a habitat quality status ranging from poor-moderate-good conditions during pre-monsoon, poor-moderate-good-high conditions during monsoon, and goodhigh conditions during post-monsoon in the estuary. The negative correlation of M-AMBI with organic carbon also indicated the decrease in benthic habitat quality with an increase in organic carbon content. Similar to earlier studies (Borja et al. 2008; Borja and Tunberg 2011; Feebarani et al. 2016; Equbal et al. 2018; Jayachandran et al. 2020), the present study also proved the efficiency of AMBI and M-AMBI in evaluating the seasonal changes effectively, though these indices have established to detect various anthropogenic stresses from different geographical areas (Borja et al. 2000, 2003; Muxika et al. 2007; Bigot et al. 2008; Joydas et al. 2017; Rehitha et al. 2021). Therefore, a better benthic habitat quality condition was observed in the CE during the monsoon and post monsoon subsequent to the excessive monsoonal precipitation, owing to the dilution of the contaminants and increase of oxygen. Whereas, slightly disturbed to moderately disturbed conditions revealed by AMBI and poor-moderate-good conditions exhibited by M-AMBI are indicative of the stressed conditions in the CE during pre-monsoon. Feebarani et al. (2016) also observed an improvement of the benthic community status in most degraded sites of the CE after monsoon, using these biotic indices. Hence, along with the community structure, the functional characteristics of the benthic community can also be effectively used for assessing the habitat quality, and the results of the present study were in good agreement with the results of marine biotic indices used.

## Conclusion

The present study evidenced seasonal variation in community structure, trophic structure and secondary production of macrobenthic fauna in the Cochin estuary. An increase in benthic diversity and biomass was observed during post-monsoon along with a seasonal shift in the macrobenthic taxa composition. Surface deposit feeding polychaetes and omnivorous-detritivores (non-polychaete) dominated in the fine substratum of pre-monsoon. Sub-surface deposit feeding polychaetes and omnivorous-suspension feeders increased during monsoon owing to the food availability. On the other hand, an even distribution of polychaetes trophic groups with an increased density of

suspension feeders and herbivorous-grazers (non-polychaete) observed during post-monsoon. Shift of trophic groups from heterotrophic consumers of pre-monsoon to autototrophic consumers of postmonsoon indicates better water quality during post-monsoon, after the monsoonal flood. The relatively higher secondary production during post-monsoon contributed by organisms of size> 0.7mm points towards higher organic material available for the sustenance of subsequent trophic levels in the benthic food chain. The biotic indices also (AMBI and M-AMBI) exhibited a better habitat quality of the CE during post-monsoon as indicated by the macrobenthic structural and functional properties. While, poor-moderate-good ecological status of pre-monsoon indicate the estuary was considerably affected by anthropogenic pressures. The present study, therefore, put forth the significant elucidation of benthic functional characteristics (i.e., trophic structure, secondary production) in the CE in a comprehensive manner, which can provide a proper understanding of the changes in the habitat quality, resulting from both natural and anthropogenic disturbances.

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SI	Parameters	Seasons			
NO		PreM	MonS	PostM	
1	Temperature (°C)	31.4±0.9	29.8±0.5	28.8±0.99	
2	Salinity	$21.6 \pm \! 5.0$	11.3±11.3	13.4±5.8	
3	DO (ml/L)	3.2±1.1	3.5±1.3	5.2±0.5	
4	SPM (mg/L)	43.7±32.8	62.4±33.1	28.8±7.3	
5	Nitrate (µmol)	3.3±0.9	17.8±9.7	3.8±3.9	
6	Nitrite (µmol)	$0.8\pm0.4$	0.5±0.2	0.56±0.2	
7	Phosphate (µmol)	$0.7 \pm 0.4$	1.3±0.7	$0.96 \pm 0.5$	
8	Silicate (µmol)	15.4±2.9	25.0±13.1	57.5±23.2	
9	Chlorophyll-a	$10.9 \pm 8.2$	3.2±1.4	$1.4{\pm}1.5$	
	$(mg/m^3)$				
10	Sand (%)	21.2±18.7	40.5±33.5	51.1±20.4	
11	Silt (%)	30.9±13.3	46.2±33.1	29.3±14.6	
12	Clay (%)	48.0±9.0	13.2±2.9	19.5±6.6	
13	OC (%)	26.0±9.4	19.6±10.6	19.2±6.6	

**Table 1** Seasonal variation of physicochemical parameters in the Cochin estuary; (DO- Dissolvedoxygen, SPM-Suspended particulate matter, OC- Organic Carbon)

 Table 2
 Seasonal variation of macrobenthic species density, biomass and diversity indices

SI	Parameters		Seasons			
NO		PreM	MonS	PostM		
1	Macrobenthic density (ind.m <sup>-2</sup> )	1460±1843	266±228	13124±6063		
2	Macrobenthic biomass (g,m <sup>-2</sup> )	4.39±3.10	4.96±6.71	49.49±28.01		
3	Shannon Wiener diversity H'(log2)	1.91±0.6	2.3±1.07	$1.04{\pm}1.2$		
4	Species Richness (d)	0.94±0.31	1.01±0.6	$1.25\pm0.95$		
5	Total number of species (S)	$7.42 \pm 2.51$	6.71±3.8	12.7±8.90		

SI	Parameters	Seasonal		Tukey's	Spatial	
NO		P value	F	Multiple	Р	F
			value	Comparison	value	value
				Test		
1	Temperature (°C)	<	16.10	MonS-PostM	0.6944	0.6439
		0.0001				
2	Salinity	0.0578	3.353	-	0.0544	2.772
3	DO (ml/L)	0.0035	7.869	PreM- MonS	0.8020	0.4948
4	Nitrite (µmol)	0.1235	2.355	-	0.6544	0.7001
5	Nitrate (µmol)	0.0003	12.81	<u>PreM – PostM</u>	0.8243	0.4630
6	Phosphate (µmol)	0.1361	2.232	-	0.7308	0.5935
7	Silicate µmol)	0.0002	14.18	<u>PreM – MonS</u>	0.8409	0.4390
8	SPM (mg/L)	0.0965	2.670	-	0.9488	0.2553
9	Sand (%)	0.1054	2.557	-	0.0411	3.026
10	Silt (%)	0.3142	1.236	-	0.0290	3.356
11	Clay (%)	<	54.61	$\underline{MonS - PostM}$	0.9711	0.2004
		0.0001				
12	OC (mg/g)	0.3149	1.233	-	0.0975	2.264
13	Chlorophyll-a (mg/m <sup>3</sup> )	0.0043	7.496	$\underline{MonS - PostM}$	0.6873	0.6538
14	Macrobenthic density	<	26.36	PreM -MonS	0.9544	0.2428
	(ind.m <sup>-2</sup> )	0.0001				
15	Macrobenthic biomass	<	16.75	PreM -MonS	0.8462	0.4311
	(g.m <sup>-2</sup> )	0.0001				
16	Secondary production (g	0.0011	10.09	PreM -MonS	0.5094	0.9201
	$m^{-2} d^{-1}$ )					

**Table 3** The results of ANOVA and Post hoc test for the abiotic and biotic parameters; (DO-Dissolved oxygen, SPM-Suspended particulate matter, OC- Organic Carbon).

Significant values are highlighted in Bold; the seasons which are not significantly different are underlined.



Fig. 1 Map showing the study locations in the Cochin estuary



Fig. 2 Seasonal distribution of major macrobenthic groups in the Cochin estuary



**Fig. 3** Seasonal variation of macrobenthic trophic structure in the Cochin estuary; **a**)-trophic structure of polychaetes during pre-monsoon, monsoon and post-monsoon, **b**) - trophic structure of other macrobenthic groups during pre-monsoon, monsoon and post-monsoon.



**Fig. 4** Variation of sieve-wise macrobenthic secondary production in the Cochin estuary during **a**) pre-monsoon, **b**) monsoon and **c**) post-monsoon; **d**) seasonal variation of total secondary production.



Fig. 5 The nMDS plot depicting of the seasonal variation of macrobenthic production from different sieve fractions



**Fig.6** Variation of AMBI and M-AMBI indices in the Cochin estuary; **a**) percentage contribution of species to different ecological groups **b**) spatio-temporal variation of AMBI index and **c**) spatio-temporal variation of M-AMBI index.



**Fig.7** The RDA plot showing the relation of macrobenthic trophic groups with environmental variables during **a**) pre-monsoon, **b**) monsoon and **c**) post-monsoon.



**Fig. 8** The RDA plot showing the relation of macrobenthic production with environmental variables during **a**) pre-monsoon, **b**) monsoon and **c**) post-monsoon respectively.

# **Figure Legends**

Fig. 1 Map showing the study locations in the Cochin estuary

Fig. 2 Seasonal distribution of major macrobenthic groups in the Cochin estuary

**Fig. 3** Seasonal variation of macrobenthic trophic structure in the Cochin estuary; **a**)-trophic structure of polychaetes during pre-monsoon, monsoon and post-monsoon, **b**) - trophic structure of other macrobenthic groups during pre-monsoon, monsoon and post-monsoon.

**Fig. 4** Variation of sieve-wise macrobenthic secondary production in the Cochin estuary during **a**) pre-monsoon, **b**) monsoon and **c**) post-monsoon; **d**) seasonal variation of total secondary production.

**Fig. 5** The nMDS plot depicting of the seasonal variation of macrobenthic production from different sieve fractions

**Fig.6** Variation of AMBI and M-AMBI indices in the Cochin estuary; **a**) percentage contribution of species to different ecological groups **b**) spatio-temporal variation of AMBI index and **c**) spatio-temporal variation of M-AMBI index.

**Fig.7** The RDA plot showing the relation of macrobenthic trophic groups with environmental variables during **a**) pre-monsoon, **b**) monsoon and **c**) post-monsoon.

**Fig. 8** The RDA plot showing the relation of macrobenthic production with environmental variables during **a**) pre-monsoon, **b**) monsoon and **c**) post-monsoon respectively.

Supplementary Table S1: Seasonal data of macrobenthic species density (Ind.m<sup>-2</sup>).

Supplementary Table S2: The list of identified species assigned to different ecological groups.