

**Factors structuring spatio-temporal dynamics of macrobenthic communities of three differently modified tropical estuaries**

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**Abstract**

Tropical estuaries of industrialized northwest coast of India, subject to seasonal and multifarious anthropogenic interventions, are poorly studied. Three estuaries, Ulhas, Amba and Savitri were investigated seasonally to identify the principal factors among natural and anthropic stressors that shaped spatio-temporal macrobenthic patterns. The macrobenthic community structure and chemical parameters differed significantly between estuaries, zones and seasons. Multivariate dispersions were significant between the estuaries whereas for the zones and seasons, significant variability was nonexistent. Multivariate regression analysis indicated that both natural and anthropogenic drivers influenced the spatio-temporal variability of macrobenthos of Ulhas and Amba. In Savitri, no anthropogenic factor was significantly influential. Salinity explained a greater proportion of the variability of macrobenthic structure than other factors in all estuaries. The pollution tolerant species responded largely to salinity changes and were observed to inhabit specific salinity zones. Thus, the spatio-temporal patterns of the estuarine macrobenthos were primarily dictated by the salinity.

**Keywords:** Macrobenthos; Spatio-temporal variability; Salinity; DistLM; Tropical estuaries; India.

## 1. Introduction

Estuaries are naturally stressed and extremely variable ecosystems that are simultaneously exposed to high degrees of anthropogenic stress (Elliott and Quintino 2007). The ecological processes of estuaries are dynamic due to the inherent plasticity of the physico-chemical processes that vary in short spatio-temporal scales. The complexity of estuarine ecology and the drivers of biotic assemblages are often the subject of many investigations. Globally, estuaries have become the fulcrum of urban civilizations and industrial clusters resulting in divergent anthropogenic pressures impacting the estuarine environmental quality. Increasing human settlements as well as burgeoning industrial establishment along the estuarine banks have resulted in mangrove destruction, dredging, industrial and sewage disposal, which are some common issues plaguing most estuaries (Kennish 2002; Smith et al. 2003). These activities often result in the deterioration of the estuarine environmental quality that modifies the composition of resident biotic assemblages by way of reduced species richness and abundances.

It is often hard to discriminate between natural or human-induced pressures that influence the aquatic life of the estuaries due to the complexity of the estuarine ecosystem (Elliott and Quintino 2007; Dauvin and Ruellet 2009; Chapman et al. 2013). Many studies have attempted to understand the phenomenon of "Estuarine Quality paradox", a concept defined by Dauvin (2007) to describe the estuarine conundrum. It is also commonly observed in estuaries that the upper zone that is marked by low salinity is often the most stressed by pollutants (Ysebaert et al. 1993; Dias et al. 2018). Thus, it is pertinent to decipher, in estuaries subjected to salinity variations and anthropogenic pollution concomitantly, the major drivers that structure the resident biotic communities. An understanding of the causal factors shaping estuarine biological patterns is essential to derive meaningful management initiatives aimed at maintaining estuarine ecological balance. The non-availability of baseline data and knowledge gaps in the understanding of the key predictors of biotic distribution patterns are major hindrances for the formulation and implementation of conservation measures in estuarine ecosystems.

Macrobenthos play a major role in the estuarine processes at the sediment-water interface such as detritus recycling, nutrient cycling and facilitate energy flow to higher trophic levels (Carvalho et al. 2007; Wildsmith et al. 2011). Macrobenthos often reflect the biological integrity of an ecosystem as they respond to natural and/or human induced stress on account of being sedentary (Veiga et al. 2016). Spatial and temporal distribution patterns of macrobenthic communities are known to differ as per abiotic factors such as salinity, temperature, depth, dissolved oxygen, sediment types (Herman et al. 1999; Fujii 2007; Lu et al. 2008) and assorted human activities such as

industry, urban sewage discharge, farming, fishing, agriculture (Nalesso et al. 2005; Ferrando and Mendez 2011). Besides the high degree of spatial heterogeneity along the estuarine gradient, the seasonal fluctuations within a system also alter the macrobenthic community structure (Sivadas et al. 2011; Gaonkar et al. 2013). The west coast of India receives the heaviest rainfall in the Indian subcontinent due to the orography of the region (Suprit et al. 2012). The strong flushing during monsoon is known to modify the hydrological parameters that in turn affect the macrobenthic community structure and cause natural stress in the estuarine environment (Feebarani et al. 2016). The mortality and recruitment of macroinvertebrates during different seasons result in continuous seasonal variation in their abundance and composition. Hence, it is imperative to consider the seasonality for the chosen estuaries that are subjected to both the natural and anthropogenic perturbations.

Tropical estuaries, particularly in developing countries, are amongst the most polluted as their banks are often overpopulated and utilised for setting up various industrial establishments (Feebarani et al. 2016). This has resulted in indiscriminate disposal of various effluents, often untreated, into the upper zones with weak tidal action leading to the accumulation of pollutants (Araujo et al. 2017). Likewise, estuaries of the Indian subcontinent are also recipients of various anthropogenic discharges, some of them even developing hypoxic conditions. Past studies have indicated that macrobenthic abundances of Indian estuaries were mostly correlated with natural parameters like salinity, pH, water temperature, DO etc. (Chandran et al. 1982; Nair et al. 1983; Khan 2003; Mahapatro et al. 2011; Hemalatha et al. 2014; Kutty et al. 2016). However, considering the increasing levels of anthropogenic inputs into these estuaries, it can be expected that the natural gradient distribution patterns of estuarine macrobenthos can be modified by the anthropogenic factors (Sivadas et al. 2011). Generally, in estuaries with slight anthropogenic pressures, the environmental alterations are not strong enough to exclude the species that are sensitive to pollution. Alternatively, in estuaries subjected to a greater extent of anthropogenic stress, species are influenced by pollutants, leading to the colonization of opportunistic species (Medeiros et al. 2016) throughout the estuarine gradient. Stress in marine communities is generally manifested as distinct changes in multivariate dispersion of species data. Higher variability in samples collected from anthropogenically stressed areas as compared to reference sites have been reported (Warwick and Clarke 1993).

The present study was focused on three tropical estuaries, Ulhas, Amba and Savitri located on the northwest Indian coast. While the available information on the chemical parameters of Ulhas (Menon and Mahajan 2010; Ram et al. 2003; Ram et al. 2009; Rathod and Patil 2009; Mishra et al.

2007), Amba (Pande and Nayak 2013; Ingole et al. 1989; Ram et al. 2009) and Savitri (Lianthuamluaia et al. 2013; Chavan and Jawale 2013) is substantial, the biodiversity of these estuarine ecosystems is yet to be investigated thoroughly. Some information is published regarding plankton (Ulhas; Borkar et al. 2002), fish diversity (Amba; Paulinose et al. 2004) and foraminiferans (Savitri; Pachkhande et al. 2014). Though some degree of macrobenthic data is available for the Ulhas (Mathew and Govindan 1995; Athalye et al. 2003; Mulik et al. 2017), no such data exists for Amba and Savitri estuaries. A comprehensive water quality assessment report (NIO 2009, 2018) indicated that Ulhas estuary was severely polluted while Amba and Savitri were also impacted by various stressors, though to a lesser extent. The Ulhas is in the proximity of the heavily urbanized and industrialized Thane-Mumbai metropolis and receives a significant amount of domestic sewage and industrial effluents apart from terrestrial and agricultural runoff (Athalye et al. 2003). Hypoxic and even anoxic episodes are known to prevail during certain tidal phases in the upper stretches of the estuary (NIO 2013). The Amba estuary receives treated effluents from a petrochemical complex (Ram et al. 2009) and is also subject to dredging. Majority of industrial units located near the upper estuarine zone of Savitri are chemical plants whose combined effluents are released in the oligohaline zone (NIO 2009, 2018). Apart from the myriad anthropogenic interventions, these estuaries also experience natural stress from the annual monsoonal (June-September) rainfall. It is characteristic of Indian estuaries to receive copious freshwater discharge during the seasonal rainfall period that often surpasses the total capacity of the estuaries resulting in fresh water domination (Sarma et al. 2011). Considering that the three estuaries were subject to voluminous monsoonal discharge and varying levels and types of anthropogenic stress, it was, therefore, of interest to investigate the primary factors that dictated the spatio-temporal dynamics of the estuarine benthic communities.

The present study had two primary research objectives i) to discern the spatio-temporal variability in environmental variables and macrobenthic assemblages of three differently stressed monsoonal estuaries and ii) to identify the primary factors among the natural and anthropogenic stressors that majorly determined the macrobenthic community structure of each estuary by employing multivariate statistical approaches. The major questions postulated were (1) whether the expected spatial variation in macrobenthic species composition (along the natural salinity gradient) was maintained in the three estuaries irrespective of their pollution degree? and (2) what were the major drivers of macrobenthic responses in the estuaries considering their spatio-temporal variations? We hypothesize that the expected gradient patterns in species diversity (as dictated by the salinity regime) are modified by the anthropic stressors of these estuaries. It is possible that the

major influencing abiotic factor of macrobenthic spatio-temporal variability could be anthropogenic rather than the natural variables in the studied estuaries.

## **2. Materials and Methods**

### **2.1 Study area**

The study was carried out in three estuarine systems of Thane (Ulhas) and Raigad (Amba and Savitri) districts of Maharashtra (Fig. 1). All the estuaries originate in the western slopes of Sahyadrian ranges and drain into the Arabian Sea. The Ulhas has a catchment area of about 4637 km<sup>2</sup>. Following the westerly course of about 100 km, it meets the Arabian Sea at Vasai (19° 16'N and 72° 45'E). The estuarine area receives a freshwater discharge of 146 m<sup>3</sup>.s<sup>-1</sup> (Central Water Commission; CWC Nasik). The flushing time of the estuary varies between 73 and 211 tidal cycles during the dry season leading to the possibility of a buildup of pollutants in the inner estuary (Ram et al. 2009). Average annual rainfall is about 2943 mm. The estuary receives 425 Million Litres per Day (MLD) of domestic sewage through various point discharges along its entire length (NIO 2018).

The Amba has a catchment area of about 420 km<sup>2</sup> and meanders along a length of over 140 km before opening into the Mumbai harbour (18° 50' N and 72° 54' E). The estuarine area receives a freshwater discharge of 96 m<sup>3</sup>.s<sup>-1</sup> (Velamala et al. 2016). The estuary is shallow with an average depth of 3 m. The flushing time of the estuary during dry season varies from 6 to 26 tidal cycles depending on the tide (Dinesh Kumar et al. 1997). Average annual rainfall is about 2100 mm. The Savitri has a catchment area of about 2,262 km<sup>2</sup> and meets the sea at Bankot (17° 59' N and 73° 02'E). The estuarine area receives a freshwater discharge of 264.5 m<sup>3</sup>.s<sup>-1</sup> CWC Nasik). The flushing time of the Savitri during dry season varies from 28 to 111 tidal cycles depending on the tide (NIO 1994). Average annual rainfall is about 3560 mm. The upper and middle estuary receives 7.5 MLD of domestic sewage (NIO 2009).

### **2.2 Sampling programme**

The sampling campaign was carried out seasonally at twelve (U1-U12), thirteen (A1-A13) and eight (S1-S8) subtidal sites of Ulhas, Amba and Savitri estuary respectively (Fig.1). An industrial discharge outfall is present in the upstream of Ulhas (U10) and Savitri (S6) and the lower reaches of Amba estuary (A6). Sampling sites were selected considering the different salinity zones, from mouth to the uppermost estuarine stretch (sites with higher number were placed at increasing distance from the estuarine mouth). Each sampling site was sampled during the three seasons i.e. pre-monsoon (May), monsoon (September) and post-monsoon (November/ December) of 2013. Quadruplicate sediment samples were collected at each site during each period using a van Veen

grab of 0.04 m<sup>2</sup> bite area for the study of macrobenthos. Additionally, sediment samples were collected in duplicate for the analysis of texture, organic carbon (C<sub>org</sub>) and total petroleum hydrocarbons (PHc-sediment). Concurrent with the macrobenthic sampling, bottom water samples were collected in duplicate at each site during the ebb tide using Niskin samplers for the analyses of various physicochemical parameters like water temperature (WT), pH, suspended solids (SS), salinity, dissolved oxygen (DO), phosphate-phosphorus (PO<sub>4</sub><sup>3-</sup>-P), nitrite-nitrogen (NO<sub>2</sub><sup>-</sup>-N), nitrate-nitrogen (NO<sub>3</sub><sup>-</sup>-N) and ammonia-nitrogen (NH<sub>4</sub><sup>+</sup>-N).

### 2.3 Analytical procedures

Macrofaunal samples once collected, each replicate sample was carefully washed and processed through 0.5 mm mesh sieve and retained organisms were fixed in 5% formalin mixed with Rose Bengal. In the laboratory, organisms were sorted, identified to the lowest possible taxonomic level (mostly species), enumerated and expressed as ind. m<sup>-2</sup>. The species-level identification was done as per the available identification keys. Water samples were preserved in ice immediately upon collection and were analyzed in the laboratory. The temperature was recorded in situ using a digital thermometer and pH measurement was carried out using a Cyber Scan (Model pH 510) pH meter. Salinity, SS, DO and nutrients were analyzed using standard methods (Grasshoff et al. 1999). PHc in sediment was measured with a fluorescence spectrophotometer (LS 3B Perkin Elmer) (IOC-UNESCO 1982). Sediment texture was analyzed by combined sieving and pipette method (Buchanan 1984) and organic carbon (C<sub>org</sub>) by titration method (Walkey and Black 1934).

### 2.4 Statistical analyses

The statistical program, PRIMER v6 (Clarke and Gorley 2006) and Permutational Multivariate Analyses of Variance (PERMANOVA) + for PRIMER (Anderson et al. 2008) was employed to carry out multivariate analyses of macrobenthic species abundance data and environmental data. The species abundance data matrix was fourth-root transformed to downweight the influence of numerically dominant taxa, which were used to build a similarity matrix using Bray-Curtis similarity coefficient. The spatial distribution of benthic assemblages of the three estuaries was visualized by using non-metric multidimensional scaling (nMDS). A 3-way Permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was applied to the similarity matrix of site replicates, in order to assess the differences in macrobenthic communities using a model with three factors, i) estuary (fixed, three levels; Ulhas, Amba, Savitri), ii) zone (fixed, four levels; euhaline, polyhaline, mesohaline, oligohaline) and iii) season (fixed, three levels; pre-monsoon, monsoon, post-monsoon). Also, pair-wise comparisons were made as significant

interactions were observed for the factors used. As PERMANOVA test can show significant differences between groups, but does not distinguish between a difference due to location (factor effects) or dispersion (variance), homogeneity of multivariate dispersion was tested with Permutational Analysis of Multivariate Dispersion (PERMDISP) (Anderson 2006), using distances among centroids calculated both between estuaries, zones, seasons and for the zone x season interaction. For the PERMANOVA and PERMDISP tests, a total of 9999 unrestricted permutations of raw data were used. As the environmental data had different measurement scales, the data was normalized (Clarke and Gorley 2006). Principal Component Analysis (PCA) was conducted to visualize the distribution pattern of site centroids of all three estuaries, based on environmental variables. Using the similar model employed for macrobenthic data, a 3-way PERMANOVA was applied to the environmental data, in order to assess the spatio-temporal differences in abiotic characteristics. The DistLM (Distance-based Linear Models) routine was used to assess the relative contribution of abiotic parameters to the variability observed in the macrobenthic community structure (Anderson et al. 2008) of each estuary. The DistLM routine used the BEST selection procedure to select the best possible combination of predictor variables based on the AICc (Akaike's Information Criteria corrected) criterion (Burnham and Anderson 2004). BEST is a procedure that examines the value of the selection criterion for all the possible combinations of predictor variables that could significantly explain the variation in the macrobenthic community structure (Clarke and Gorley 2006; Anderson et al. 2008). The overall 3 best models were given in the output. Distance-based Redundancy Analysis (dbRDA) was performed to provide a visual representation of the macrobenthic community fitted to the significant predictor variables in the multi-dimensional space (Li et al. 2017). The dbRDA plot is an ordination that overlays vectors for the environmental variables on the spatial arrangement of samples, with the length and direction of a vector suggestive of the degree of the correlation between the variable and the portrayed sample arrangement (Anderson et al. 2008).

### **3. Results**

#### **3.1. Structure of macrobenthic assemblages**

Overall, 189 macrobenthic taxa were identified from all three estuaries during the three seasonal surveys, the maximum being observed at Amba (118), followed by Ulhas (86) and Savitri (72) (Appendix 1). Lower number of taxa was recorded from Ulhas (12) and Savitri (16) during the monsoon season. Polychaeta was the most dominant group of macrobenthos in all the estuaries; Ulhas (83.4%), Amba (64.7%) and Savitri (63%). Insecta (4%), Amphipoda (10.9%) and Bivalvia (12.7%) was the second dominant group in Ulhas, Amba and Savitri respectively. The contribution

of other 14 groups to the total macrofauna was merely <5%. Of these, some groups were restricted to a particular estuary, for instance, Nematoda, Hirudinea, Ostracoda, and Pycnogonida were limited to Ulhas (0.01-2.6%) while Nemertea, Echiura, Anthozoa, Sipuncula, Stomatopoda, and Hydrozoa were present only at Amba (0.04-0.3%). The occurrence of Polycladida was recorded at Savitri alone (0.04%).

PERMANOVA indicated that the macrobenthic community structure differed significantly between the estuaries ( $F= 10.7$ ;  $P = 0.0001$ ), zones ( $F= 8.8$ ;  $P = 0.0001$ ) and seasons ( $F=4.0$ ;  $P = 0.0001$ ). Moreover, all the interactions were also significant. Thus, it was imperative to perform further pair-wise comparisons that indicated significant differences between all possible pairs of estuaries, zones and seasons (Table 1). This was corroborated by the MDS plot (Fig. 2), which demonstrated that the spatial variations in the macrobenthic community corresponded with the different salinity zones (euhaline, polyhaline, mesohaline and oligohaline) of the traditionally used ‘Venice System’ of classification. The euhaline and oligohaline clusters of the three estuaries were very well demarcated whereas some overlap was observed in the polyhaline and mesohaline groups. Also, the MDS graph indicated that the macrobenthic composition of the three estuaries was mostly different. The PERMDISP revealed a significant difference in the dispersions between the three estuaries which was attributable to the greater average dispersion in Savitri despite the fact that this estuary was anthropogenically less impacted than the other two estuaries. Though the multivariate community structure varied significantly between zones and seasons (PERMANOVA), no significant differences were observed in the multivariate dispersion across zones ( $F = 2.84$ ,  $P = 0.09$ ), seasons ( $F = 2.39$ ,  $P = 0.14$ ) and in zone x season interaction ( $F = 0.80$ ,  $P = 0.91$ ).

The dominant taxonomic composition of each estuary across the zones varied significantly and many of the recorded species were estuary specific. Overall, the macrobenthic abundance of Ulhas was dominated by *Namalycastis ouanaryensis* (43.4%), *Streblospio gynobranchiata* (27.1%) and *Namalycastis indica* (10%); Amba by *N. ouanaryensis* (21.5%), *Nephtys hystricis* (15%); and Savitri by *Paraprionospio cordifolia* (26.4%), *Barnea birmanica* (8.6%) and *Trochochaeta* sp. (6.1%). The euhaline zone of Ulhas was dominated by *Mesopodopsis orientalis* (pre-monsoon) and *Corophium* sp. (post-monsoon), Amba by *N. hystricis* (pre-monsoon) and *Paraprionospio cristata* (post-monsoon) while Savitri by *B. birmanica* (pre-monsoon) and *P. cordifolia* (post-monsoon). The opportunistic polychaete, *S. gynobranchiata* was present exclusively in Ulhas where the species was restricted to the polyhaline (pre-monsoon) and mesohaline (post-monsoon) zones of the estuary. Azoic conditions prevailed near the outfall in Ulhas (U8 and U9) during the pre-monsoon. As this situation was remedied during the monsoon, the zone was repopulated by a high abundance of



opportunistic nereids (*N. ouanaryensis*; 76.3% and *N. indica*; 22.8%). *N. ouanaryensis* was the dominant taxa at all the salinity zones of the Ulhas during monsoon and was abundant in the low saline upper stretches during post-monsoon too. This species was observed to be dominant in the oligohaline zones of Amba during all three seasons. The oligohaline zone of Ulhas was populated by Chironomidae during the pre-monsoon season. *N. hystricis* was present abundantly in the polyhaline zone during all three seasons. The poly-mesohaline zone of Savitri was populated by oligochaetes (*Aulophorus furcatus* and *Limnodrilus hoffmeisteri*), spionids (*Prionospio cirrifera*) and nereids (*Dendronereis arborifera*). *Cheiriphotis megacheles*, *Melita dentata*, *Magelona cincta* and *Trochochaeta* sp. were present exclusively in the euhaline zones while *Prionospio cirrifera* occupied only the mesohaline and oligohaline zones. The oligohaline zone of Savitri was populated only by the oligochaete, *Aulophorus furcatus* during the pre-monsoon.

### 3.2. Characterization of the abiotic environment

For abiotic factors, results of PERMANOVA demonstrated that there were significant differences between estuaries ( $F=12.7$ ,  $P=0.0001$ ), zones ( $F=5.5$ ,  $P=0.0001$ ) and seasons ( $F=5.0$ ,  $P=0.0001$ ). Pair-wise comparisons indicated significant differences among pairs of estuaries, zones and seasons (except between monsoon and post-monsoon). The primary two principle components (PC1 and PC2) together explained 50.6% of the total variability (Fig. 3). The first principle component axis (PC1) discriminated sites mainly based on  $\text{NO}_3^-$ -N,  $\text{PO}_4^{3-}$ -P,  $\text{C}_{\text{org}}$  and  $\text{NO}_2^-$ -N while the second axis (PC2) grouped the sites as per sand,  $\text{NH}_4^+$ -N, PHc and silt. The PCA plot indicated that the abiotic parameters controlling the distribution of sites of each of the estuary were different. For instance, Ulhas which had significant anthropogenic activities was mainly characterized by nutrients ( $\text{PO}_4^{3-}$ -P,  $\text{NO}_2^-$ -N,  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N) and PHc. Amba was well oxygenated while Savitri was influenced by sandy substratum.

The spatio-temporal variability in hydro-sedimentological parameters of the three estuaries, for all sites and seasons, is displayed in Fig. S1a and Fig. S1b (Supplementary material; SM). The bottom water temperatures in all estuaries were expectedly higher during the pre-monsoon. Markedly lower pH ( $7.2\pm 0.2$ ) during monsoon was observed in Savitri. A decreasing trend in the SS was evident from the estuarine mouth to the oligohaline zone of all the three estuaries. However, Amba recorded consistently high SS values irrespective of seasons. Majority of sites displayed low SS values during the monsoon. Salinity showed spatial as well as temporal variations as expected for most of the tropical estuaries. During the monsoon season, all three estuaries were predominantly oligohaline having very low salinity i.e.  $< 2$  in Ulhas (sites 3-12), Amba (sites 9-13) and Savitri (sites 4-8) indicating the marked monsoonal impact on the salinity regime. During pre-monsoon, the

euhaline zone in Amba ( $32.6\pm 6.4$ ) extended up to the upper estuary signifying the predominant marine influence on the estuary as compared to the other two estuaries. The extent of each salinity zone varied seasonally depending on the quantum of the freshwater discharge and tidal influence. Some zones were absent in these estuaries depending on the seasons. Thus, the polyhaline zone during the monsoon in Ulhas, oligohaline zone during pre-monsoon in Amba and mesohaline zone during the pre-monsoon and post-monsoon seasons in Savitri were not established.

In Ulhas, considerably lower DO values were recorded at the poly-mesohaline zone ( $2.9 \pm 0.5$  mg l<sup>-1</sup>) and outfall site (U10; 3.2 mg l<sup>-1</sup>) during the pre-monsoon whereas hypoxic conditions were observed at U8 (1.3 mg l<sup>-1</sup>) and U10 (0.4 mg l<sup>-1</sup>) during the post-monsoon. Near hypoxic conditions were prevalent at the mesohaline zone of Ulhas during the post-monsoon ( $2.1\pm 0.1$  mg l<sup>-1</sup>). The other two estuaries were relatively well oxygenated throughout the study period except at the discharge point of Savitri where DO was significantly low (S6; 2.4 mg l<sup>-1</sup>) during the pre-monsoon. Seasonally, the concentration of nutrients (except NH<sub>4</sub><sup>+</sup>-N) was lowest during the monsoon in all the estuaries. The maximum NO<sub>2</sub><sup>-</sup>-N levels were observed in the poly-mesohaline zone of Ulhas ( $>11.0$  μmol l<sup>-1</sup>) and the outfall of Savitri (9.5-13.8 μmol l<sup>-1</sup>) during the pre-monsoon and post-monsoon seasons. The concentration of NO<sub>3</sub><sup>-</sup>-N was markedly high throughout the Ulhas estuary with maximum value recorded at U3 during both pre-monsoon (84.5 μmol l<sup>-1</sup>) and post-monsoon (128.3 μmol l<sup>-1</sup>). In Amba, reasonably higher values of NO<sub>3</sub><sup>-</sup>-N were observed during pre-monsoon ( $20.6\pm 11.0$  μmol l<sup>-1</sup>) and post-monsoon ( $14.4\pm 5.4$  μmol l<sup>-1</sup>). Conversely, high values of NO<sub>3</sub><sup>-</sup>-N ( $>10$  μmol l<sup>-1</sup>) were observed only in the poly-mesohaline zone of Savitri during both pre-monsoon and post-monsoon season. The sites near the outfall (U8 and U9) present in Ulhas displayed high values of NH<sub>4</sub><sup>+</sup>-N during all three sampling periods. Moreover, a majority of the sites (67%) showed high values of NH<sub>4</sub><sup>+</sup>-N (12.1 μmol l<sup>-1</sup> - 96.5 μmol l<sup>-1</sup>), particularly in the post-monsoon, indicating the deteriorated state of a major part of the Ulhas estuary. Amba and Savitri had relatively lower concentration of NH<sub>4</sub><sup>+</sup>-N. The PHc-sediment values were low in all estuaries across all sites ( $\leq 10.5$  μg g<sup>-1</sup>) and seasons. The textural analyses (Fig. S2, SM) revealed that the sediments were predominantly silty in Ulhas (54.9-91.3%) and Amba (51.3-93.7%) irrespective of seasons. The sediments were spatially assorted with sand being the major component in most of the sites of Savitri followed by silt. The C<sub>org</sub> content in sediment was  $\geq 2.1$  % in the meso-oligohaline sites of Ulhas. The C<sub>org</sub> of Amba ranged from 1.3% to 2.9% with minor spatial and seasonal variations. In Savitri, high values of C<sub>org</sub> ( $\geq 2.0$  %) were consistently recorded at S6 (outfall) and S8 (most upstream location).

### 3.3 Abiotic and biotic interactions

The results of DistLM, using AICc (Best) as the selection criterion for three estuaries are summarized in Table 2. The marginal tests performed, indicated that different predictor variables could explain a considerable portion of the variation in macrobenthic communities of Ulhas (5.3-12.8%), Amba (4.9-12.4%) and Savitri (6.3-6.9%) at a significance level of  $p < 0.05$ . Among the significant predictor variables, salinity was detected to be the most powerful predictor for a single variable model that accounted for 12.8% (Ulhas), 12.4% (Amba) and 6.9% (Savitri) of the variance in the macrobenthic community structure. For Ulhas estuary, two explanatory variables (salinity and  $\text{NO}_2^-$ -N) accounted for 21.6% of the variance in the overall best model solution (smallest AICc coefficient; 291.5) (Table 2). The analyses indicated that salinity alone provided the overall Best solution for Amba (AICc; 317.8) and Savitri (AICc; 203.1). The first two dbRDA axes of Ulhas explained 100% of the fitted variation and 21.6% of the total variation in the structure of the macrobenthic assemblages (Fig. 4). On the other hand, dbRDA axes of both the Amba and Savitri explained 100% of the fitted variation and 12.4% (Amba) and 6.9% (Savitri) of the total variation. The first dbRDA axis of Ulhas was mainly defined by salinity while the second axis was related to  $\text{NO}_2^-$ -N. In Amba and Savitri, the first dbRDA axis correlated positively with salinity. The dbRDA plots showed that among all the investigated parameters, salinity was the most important factor that was responsible for spatio-temporal variations in macrobenthic communities of all the three estuaries.

### 4. Discussion

The identification of the key abiotic variables that shape spatio-temporal patterns of macrobenthic fauna is difficult, particularly in estuaries exposed to human interventions (Dethier and Schoch 2005; Sánchez-Moyano et al. 2010). This study examined the macrobenthic and environmental data to identify the primary drivers responsible for the macrobenthic community patterns in the three estuaries, each being exposed to diverse natural and anthropogenic pressures. The MDS and PERMANOVA analyses clearly indicated that the macrobenthic community structural patterns were dictated by the salinity gradient in all three estuaries irrespective of the quantum of anthropogenic perturbations. Anthropogenically stressed sites were clustered within their salinity zones indicating that these areas were inhabited by tolerant organisms with specific salinity preferences. Significant PERMANOVA and insignificant PERMDISP results for the factors, zones and seasons indicated that the groups differed due to the location effects. If anthropogenic effects were more prominent than the natural forcing, it would have been reflected in terms of increases or decreases in the multivariate dispersion of macrobenthic data at the stressed zones vis-à-vis the

unimpacted zones (Warwick and Clarke 1993; Chapman et al. 1995). However, in this study differences in dispersion for the zones and seasons were not significant (as indicated by PERMDISP).

The three estuaries were significantly different in terms of their benthic and abiotic variables, possibly due to the exposure to varying natural and anthropogenic factors. The Ulhas was mostly stressed with eutrophic conditions (low DO and high nutrients) prevailing at the poly-mesohaline zone and the outfall (U10) situated in the oligohaline zone. High  $\text{NH}_4^+\text{-N}$  concentrations at the sites near the outfall (U8 and U9) indicated the impact of industrial effluents (Athalye et al. 2003; Karikari et al. 2006) that led to the complete absence of macrobenthos at these sites during pre-monsoon. Similarly, hypoxic conditions observed at U8 and U10 during the post-monsoon resulted in a near complete absence of macrobenthos with only the solitary presence of an opportunistic polychaete i.e. *N. ouanaryensis*. The tidally influenced periodic occurrence of low oxygen ( $<2.9 \text{ mg l}^{-1}$ ) at the middle estuarine sites of Ulhas (U4-U7) resulted in the proliferation of the opportunist spionid, *Streblospio gynobranchiata* in this zone. This overwhelming presence of *S. gynobranchiata*, which thrived in the existing eutrophic conditions (as per the correlations obtained), was indicative of the stressed state of this estuarine region. Similar observations were made by Ergen et al. (2006) and Çinar et al. (2012) in the Izmir Bay, where *S. gynobranchiata* occurred abundantly in the polluted inner region of the bay. The lower oligohaline sites of Ulhas was characterized by low DO and high  $\text{NH}_4^+\text{-N}$  signifying anthropogenic stress while the upper oligohaline zone was well oxygenated with low  $\text{NH}_4^+\text{-N}$ . However, the species numbers were low throughout the oligohaline zone during the non-monsoon seasons indicating that the low salinity of the zone adversely influenced the macrobenthic diversity (Ysebaert et al. 2003; Dauvin 2008) even in sites relatively free of anthropogenic stress.

The entire Amba estuary was marine dominated (Paulinose et al. 2004) as it had a salinity of nearly 35 at the mouth region which was more or less maintained till the upper estuary during pre-monsoon. One possible reason could be the dredging activity at the inlet that increases the tidal inflow to the estuary leading to a rising incursion of high saline water to the upstream (Velamala et al. 2016). High concentration of  $\text{NO}_3^-\text{-N}$  throughout the estuary in all seasons except monsoon indicated that the estuary was under environmental stress. In the Amba, macrobenthic species declined rapidly in the oligohaline zone, where the salinity dropped abruptly reflecting the influence of salinity on macrobenthic communities. Similar to the Ulhas and Amba, Savitri too indicated some anthropogenic stress though at a much lesser extent. Elevated levels of nutrients ( $\text{NO}_3^-\text{-N}$  and  $\text{NO}_2^-\text{-N}$ ) at the poly-mesohaline zone of the Savitri were probably associated with the sewage outfall and

industrial effluents. However, relatively speciose macrobenthic communities were present at this zone during the non monsoonal periods indicating that the macrobenthic structure was unaffected by nutrient peaks in this zone.

The importance of natural and human-induced factors in determining the distribution patterns of estuarine macrobenthic fauna has long been documented and several studies have investigated the association between macrobenthic assemblages and both the variables (Mucha et al. 2003; Nunes et al. 2008; Sánchez-Moyano et al. 2010; Ourives et al. 2011). Results of the multivariate regression analysis, DistLM (marginal test), indicated that both the natural and anthropogenic drivers influenced the composition and variability of macrobenthic community of Ulhas and Amba. In Savitri, only natural factors (pH, SS and salinity) significantly contributed to the variability of macrobenthos. DistLM models indicated that salinity explained a greater proportion of macrobenthic structure than the other factors for all three estuaries (Table 2, Fig. 4). The pre-eminence of the natural factors in influencing macrobenthic structure is well documented in several other estuarine macrobenthic studies (Inglis and Kross 2000; Teske and Wooldridge 2003; Ritter et al. 2005; Hampel et al. 2009; Silva et al. 2011; Yan et al. 2017). Most of the studies conducted in both the temperate and tropical waters have identified salinity as the primary factor that controlled macrobenthic distribution patterns (Palmer et al. 2002; Ysebaert et al. 2003; Chainho et al. 2006; Mariano and Barros 2015; Medeiros et al. 2016). The pronounced spatial patterns along the salinity gradient (presented in the MDS plot Fig. 2) indicated that salinity majorly influenced similarity/dissimilarity of macrobenthic species between the estuarine sites. Conversely, there are studies that have documented that other natural variables like sediment texture were more important than salinity in shaping the benthic biotic distribution in estuaries (Peeters et al. 2000; Teske and Wooldridge 2003). The influence of anthropogenic factors on the macrobenthos was most evident in Ulhas where eutrophication in the poly-mesohaline zone led to poor or even complete absence of organisms. However, in the other two estuaries, the anthropogenic impacts were localized or even not that evident.

A substantial drop in salinity during the monsoon was the hallmark of all the three tropical estuaries. The strong monsoonal rainfall along with the freshwater input by the rivers causes the seasonality in salinity of the estuaries along the west coast of India (Vinita et al. 2015). The strong flushing during monsoon is known to modify the hydrological parameters such as water temperature, pH, DO, salinity and cause natural stress in the estuarine environment (Feebarani et al. 2016). Tropical estuaries receive high river runoff during monsoon that aids in diluting, flushing and then transporting the contaminants out of the estuary and refining these water bodies (VishnuRadhan et al. 2015). Accordingly, nutrient concentrations ( $\text{PO}_4^{3-}\text{-P}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ ) were the lowest during the

monsoon in all the estuaries signifying efficient flushing of these water bodies. Even though the monsoon season witnessed lower levels of contaminants, a weakened macrobenthos community structure was observed during this period. For instance, benthic taxa were reduced from 39 (pre-monsoon) to 12 (monsoon) in Ulhas, 76 (pre-monsoon) to 51 (monsoon) in Amba and 36 (pre-monsoon) to 16 (monsoon) in Savitri (Appendix 1). However, during the post-monsoon when the fresh water discharge reduced, the recovery of benthic fauna was noticed due to the increased salinity of the estuarine systems. The macrobenthic community structure is susceptible to both the salinity changes and the magnitude of freshwater discharge to the estuaries (Rutger and Wing 2006; Palmer et al. 2011). A substantial decline in number of taxa in all the three estuaries during the monsoon signified the impact of the abrupt reduction in salinity on the resident macrobenthos. A similar observation of low macrobenthic diversity during the monsoon has been reported from other monsoonal estuaries of the Indian coast (Sivadas et al. 2011; Feebarani et al. 2016). The macrobenthic communities during this period were entirely dominated by opportunistic nereids like *N. ouanaryensis* (Ulhas and Amba) and *Dendroneries arborifera* (Savitri) that are known for their unique potential to withstand the low salinity conditions (Athalye and Gokhale 1991; Anaero-Nweke 2013).

The three different estuaries could be distinguished on the basis of the specificity of estuarine fauna and some taxa were associated with a particular salinity range. For example, the chironomids were present only at Ulhas and Savitri while they were totally absent in Amba. This could be attributed to the fact that Amba experiences less variation in salinity owing to the low freshwater discharge as compared to the other two estuaries. The macrobenthic taxa such as Nemertea, Stomatopoda, Echiura, Sipuncula, Anthozoa and Hydrozoa, which are predominantly marine (Daly et al. 2007; Struck et al. 2007; Sundberg and Gibson 2008; Wortham 2009), were present only in the tide dominated Amba estuary (Dinesh Kumar et al. 1997). Although each estuary had its own characteristics with some degree of discrimination, similar taxa were also present in the three systems. In estuarine systems found in the similar biogeographical region, probably the same species would inhabit similar estuarine zone (Barros et al. 2012). In the current study, similar patterns of macrofaunal distribution were observed in the oligohaline zones of the three estuaries. The freshwater dominated areas of all three estuaries had a sizable population of Nereididae and Oligochaeta, which are typical inhabitants of this zone (Glasby 1999; Trichkova et al. 2013).

The peculiarities in the distribution of major taxa that were observed along the salinity gradient are discussed further as potential marker species for describing the natural conditions of the three systems. The euhaline zone across the three estuaries was characterized by the presence of high

salinity tolerant species such as *M. orientalis*, *Corophium* sp., *B. birmanica*, *C. megacheles*, *M. dentata*, *M. cincta* and *Trochochaeta* sp., *P. cordifolia*, *N. hystricis*, *P. cristata* (Rao and Sarma 1979; Snowden and Ekweozor 1990; Bortone 2005; Hanamura et al. 2008; Esquete et al. 2011; Bochert and Zettler 2012). Though *N. hystricis* dominated the euhaline zone, they are also known to tolerate lower salinities as well (Snowden and Ekweozor 1990) and therefore were present in the polyhaline zone of Amba and Savitri. Poly-mesohaline species such as *S. gynobranchiata*, *N. hystricis*, *D. arborifera* and *L. hoffmeisteri* (Sarma and Rao 1982; Ergen et al. 2006; Rodriguez et al. 2006) occupied the middle estuarine zone. Taxa with affinity for low salinity, such as Chironomids, *N. ouanaryensis* and *A. furcatus* (Glasby 1999; Reed 2003; Ragi and Jaya 2014), were found to occur in the oligohaline zone. While the predominant presence of opportunistic species was registered in all the three estuaries, they too indicated a preference for particular salinity ranges. For instance, *Cossura coasta*, *Mediomastus capensis* (Muniz et al. 2005; Jaleel et al. 2014) and spionids (*P. cordifolia* and *P. cristata*) (Dean 2008) dominated the euhaline zone. *S. gynobranchiata*, *N. ouanaryensis*, *D. arborifera*, *L. hoffmeisteri* (Sarma and Rao. 1982; Çinar et al. 2012; Kang et al. 2017) were prevalent in the poly-mesohaline zone and the oligohaline zone was dominated by *N. ouanaryensis* and *D. arborifera* (Sarma and Rao 1982; Sevi Sawestri 2012). It is important to note that while the above mentioned species were tolerant to different kinds of perturbations, their presence was limited to only certain salinity zones of these estuaries, thus answering our first question. This indicated that the salinity preferences of macrobenthic species often overruled their responses to the anthropic stressors.

It may be possible to differentiate the impacts of anthropogenic pressures on the macrobenthic assemblages in estuaries, apart from natural parameters, where human interventions are highly pronounced (Rubal et al. 2014) as exemplified by Ulhas. The estuaries that were investigated in this study were exposed to varied anthropogenic activities resulting in low DO, high nutrients and SS. In fact, parts of the Ulhas were periodically hypoxic. The estuarine macrobenthos of each estuary was influenced by a distinctive assortment of natural and anthropogenic factors. The spatio-temporal patterns of the estuarine macrobenthos were primarily dictated by the salinity regime, thus answering our second question. However, depending on the estuary anthropogenic factors were also responsible for macrobenthic community structure. The study presents valuable insight into the complex ecological processes that control the biotic elements in the three ecologically and economically important estuaries where multiple human pressures are also at play. This work also provides a suitable template for future monitoring studies to measure ecological alterations that may result due to natural or anthropogenic factors.

## Supplementary material (SM)

Appendix 1

Fig. S1a

Fig. S1b

Fig. S2

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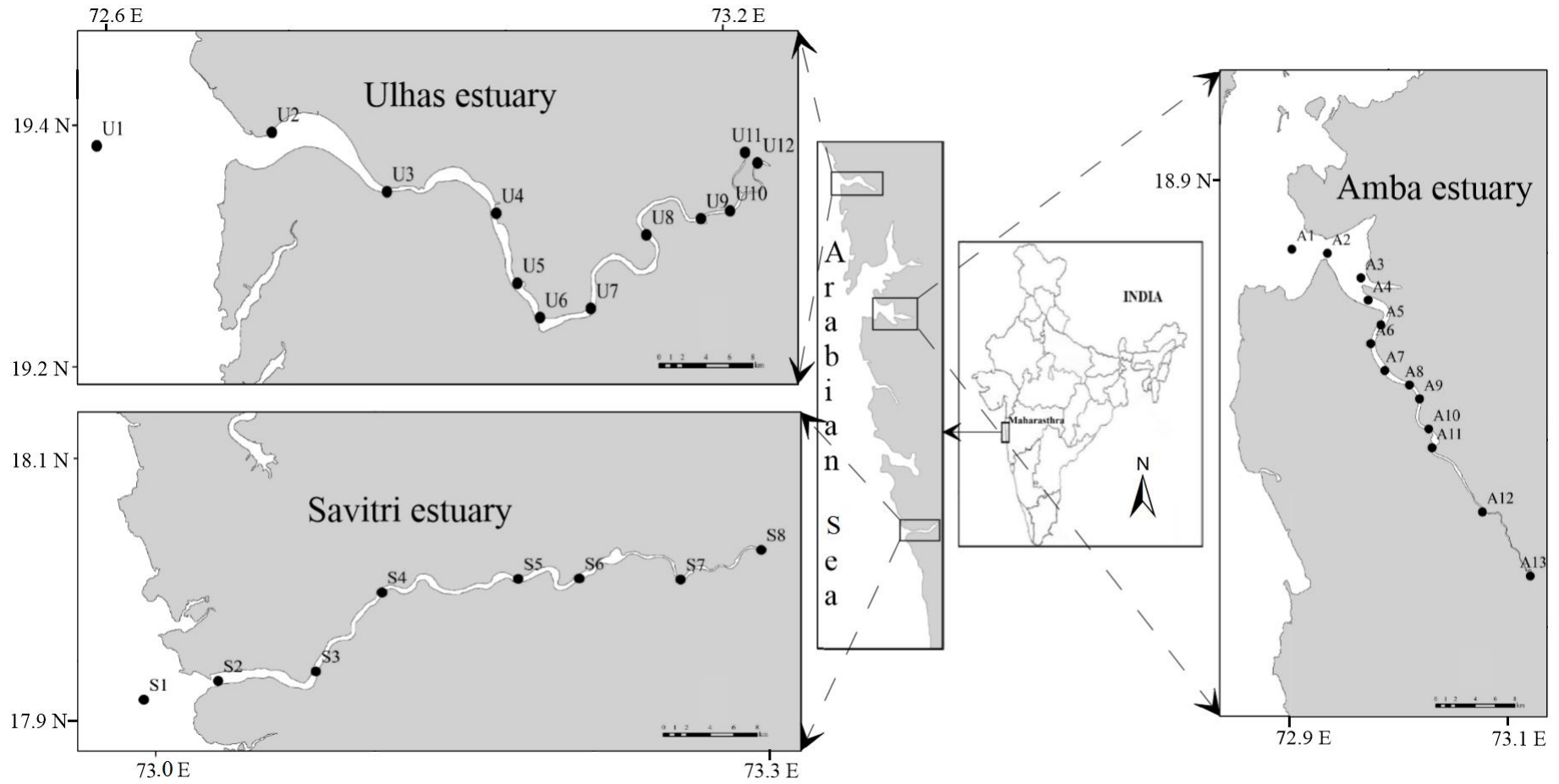
**Table 1:** Results of 3-way Permutational multivariate analysis of variance (PERMANOVA) testing the differences in macrobenthic assemblages and environmental variables using estuary (Es), zone (Zo) and season (Se) as factors. (U = Ulhas, A = Amba, S = Savitri, E = Euhaline, P = Polyhaline, M = Mesohaline, O = Oligohaline, Pre = Pre-monsoon, Mon = Monsoon and Post = Post-monsoon). Significant p- values are shown in bold.

Biotic variables					Abiotic variables			
Source	df	MS	Psuedo-F	<i>p</i> (perm)	df	MS	Psuedo-F	<i>p</i> (perm)
Es	2	31366	10.7	<b>0.0001</b>	2	83.6	12.7	<b>0.0001</b>
Zo	3	25937	8.8	<b>0.0001</b>	3	35.8	5.5	<b>0.0001</b>
Se	2	11836	4.0	<b>0.0001</b>	2	32.9	5.0	<b>0.0001</b>
Es x Zo	6	16198	5.5	<b>0.0001</b>	6	10.0	1.5	<b>0.0320</b>
Es x Se	4	12308	4.2	<b>0.0001</b>	4	12.2	1.9	<b>0.0119</b>
Zo x Se	6	13412	4.6	<b>0.0001</b>	6	6.4	0.9	0.4918
Es x Zo x Se	8	12211	4.2	<b>0.0001</b>	8	7.0	1.1	0.3539
Res	345	2933.8			67	6.6		
Tot	375				98			
Pair-wise comparisons								
	Estuary							
		Groups	<i>t</i>	<i>p</i>		<i>t</i>	<i>p</i>	
		U, A	3.2938	<b>0.0001</b>		3.0741	<b>0.0001</b>	
		U, S	3.6848	<b>0.0001</b>		3.3711	<b>0.0001</b>	
		A, S	2.6485	<b>0.0001</b>		4.1030	<b>0.0001</b>	
	Zones							
		E, P	3.1242	<b>0.0001</b>		1.9946	<b>0.0044</b>	
		E, O	3.7186	<b>0.0001</b>		2.9642	<b>0.0001</b>	
		E, M	2.4782	<b>0.0001</b>		2.4157	<b>0.0002</b>	
		P, O	2.9906	<b>0.0001</b>		2.5826	<b>0.0001</b>	
		P, M	2.1610	<b>0.0001</b>		1.9643	<b>0.0057</b>	
		O, M	1.6366	<b>0.0015</b>		1.6649	<b>0.0228</b>	
	Seasons							
		Pre, Mon	1.5384	<b>0.0052</b>		2.6073	<b>0.0001</b>	
		Pre, Post	2.2578	<b>0.0001</b>		2.5410	<b>0.0001</b>	
		Mon, Post	2.3023	<b>0.0001</b>		1.3272	0.1122	

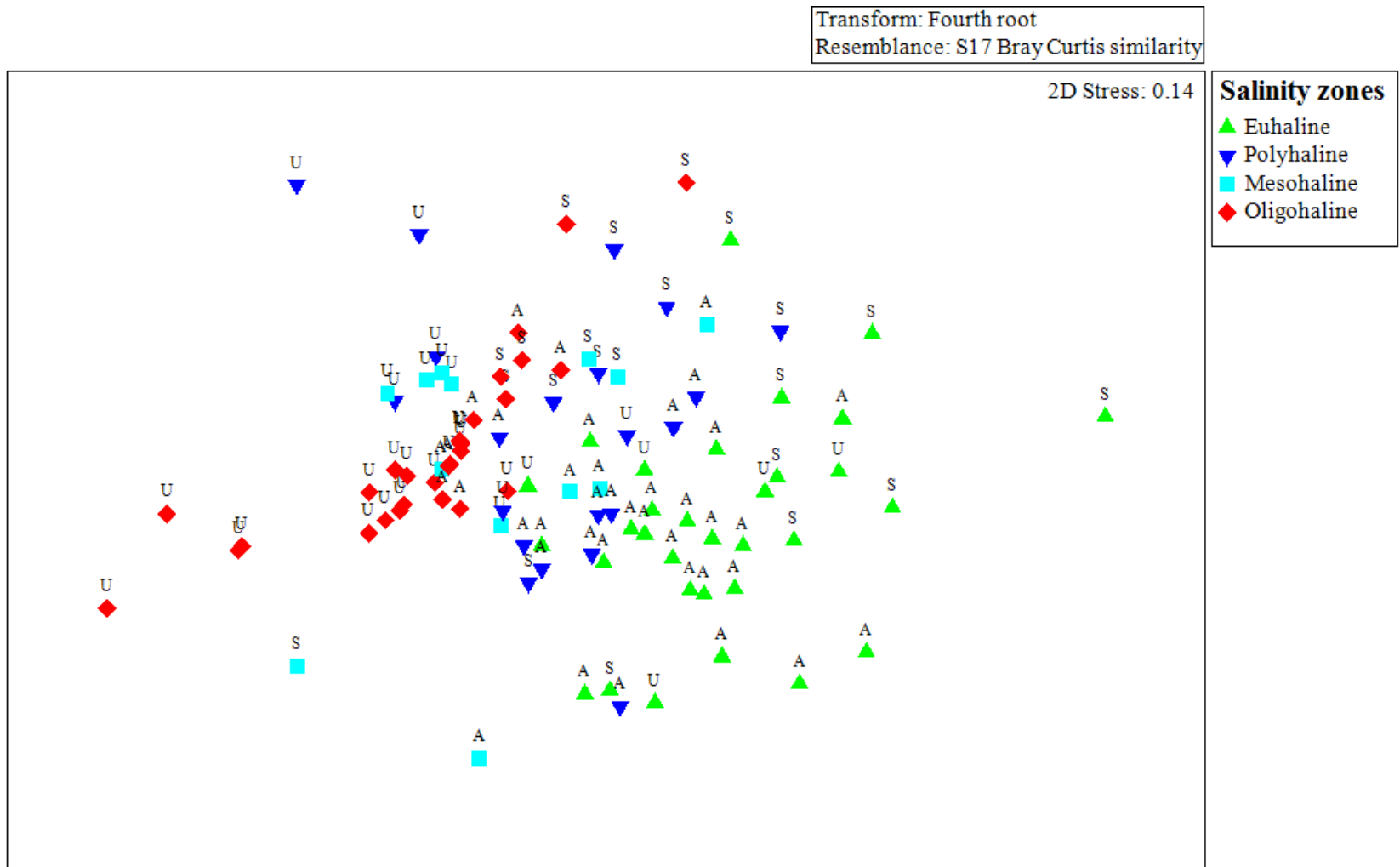
**Table 2:** Results of DistLM (Distance based linear model) multiple regression model for Ulhas, Amba and Savitri estuaries. Marginal test and overall best solutions using the BEST selection procedure and the Akaike Information Criteria corrected (AICc) criterion (Anderson et al., 2008). Only significant results (<0.05) in marginal tests are shown. Overall best models are shown in bold.

Ulhas				Amba				Savitri			
Variables	Pseudo-F	P	%Prop.	Variables	Pseudo-F	P	%Prop.	Variables	Pseudo-F	P	%Prop.
WT	1.98	0.03	5.5	WT	2.91	0.001	7.3	pH	1.57	0.04	6.7
SS	4.04	0.001	10.6	pH	2.16	0.009	5.5	SS	1.49	0.05	6.3
Salinity	5.01	0.001	12.8	SS	3.01	0.0004	7.5	Salinity	1.64	0.02	6.9
DO	2.46	0.004	6.8	Salinity	5.24	0.0002	12.4				
PO <sub>4</sub> <sup>3-</sup> -P	2.28	0.01	6.3	PO <sub>4</sub> <sup>3-</sup> -P	2.33	0.004	5.9				
NO <sub>2</sub> <sup>-</sup> -N	3.68	0.002	9.8	NO <sub>3</sub> <sup>-</sup> -N	4.88	0.0001	11.7				
NO <sub>3</sub> <sup>-</sup> -N	1.89	0.04	5.3	PHc	1.90	0.02	4.9				
NH <sub>4</sub> <sup>+</sup> -N	2.10	0.03	5.8	Clay	2.37	0.005	6.0				
PHc	2.25	0.01	6.2								
Overall best solutions	<b>AICc</b>	<b>R<sup>2</sup></b>	<b>Variables</b>	<b>AICc</b>	<b>R<sup>2</sup></b>	<b>Variables</b>	<b>AICc</b>	<b>R<sup>2</sup></b>	<b>Variables</b>		
	<b>291.48</b>	<b>0.2161</b>	<b>Salinity, NO<sub>2</sub><sup>-</sup>-N</b>	<b>317.79</b>	<b>0.12399</b>	<b>Salinity</b>	<b>203.05</b>	<b>0.06939</b>	<b>Salinity</b>		
	291.63	0.26652	WT, Salinity, NO <sub>2</sub> <sup>-</sup> -N	318.01	0.17046	Salinity, Clay	203.11	0.06672	pH		
	291.76	0.31728	WT, pH, Salinity, NO <sub>2</sub> <sup>-</sup> -N	318.04	0.16991	WT, Salinity	203.2	0.06331	SS		

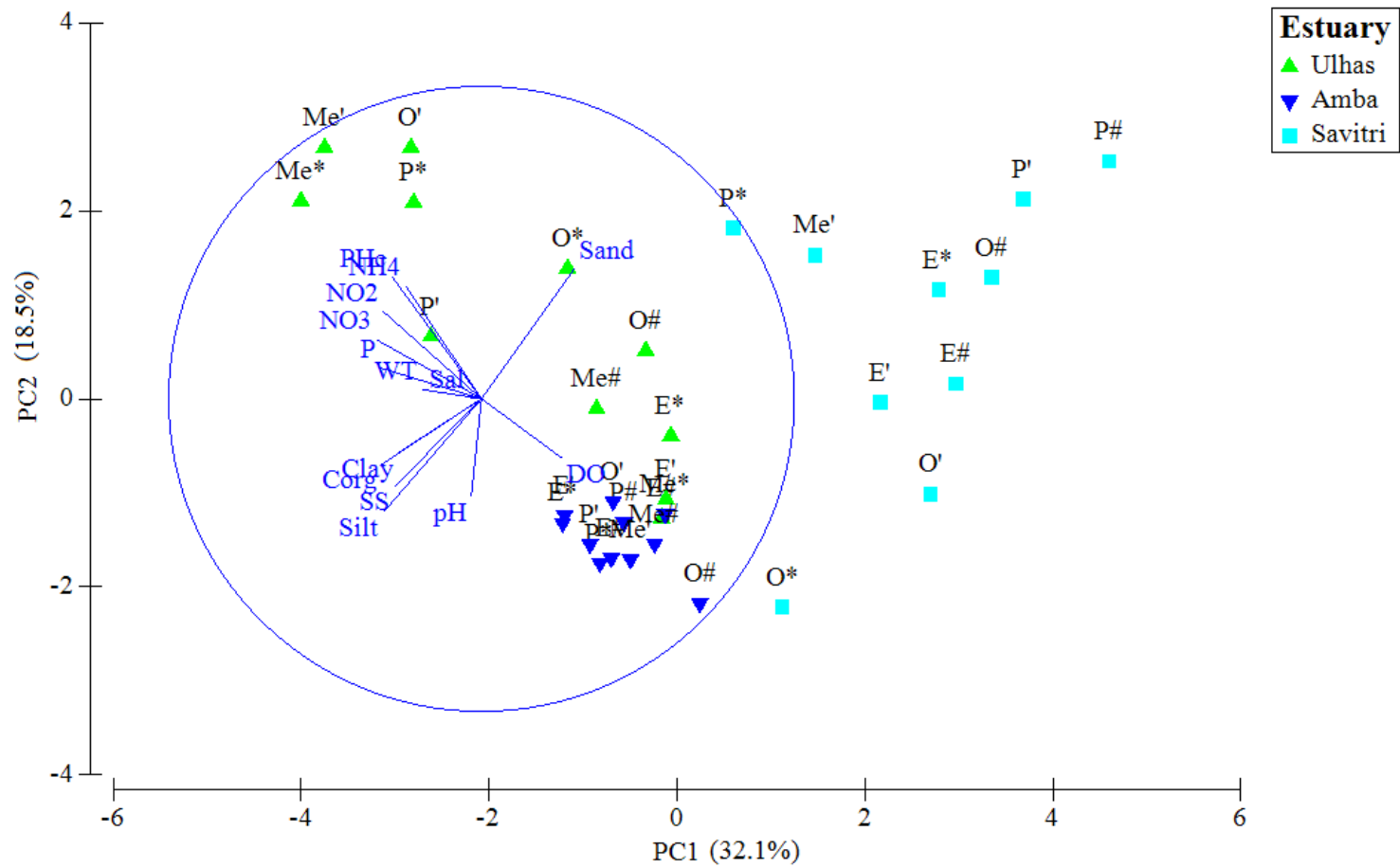
(WT=Water Temperature, SS=Suspended Solids, Sal=Salinity, DO=Dissolved Oxygen, PO<sub>4</sub><sup>3-</sup>-P=Phosphate-Phosphorus, NO<sub>2</sub><sup>-</sup>-N=Nitrite-Nitrogen, NO<sub>3</sub><sup>-</sup>-N=Nitrate-Nitrogen, NH<sub>4</sub><sup>+</sup>-N=Ammonia-Nitrogen, PHc=Petroleum Hydrocarbons)



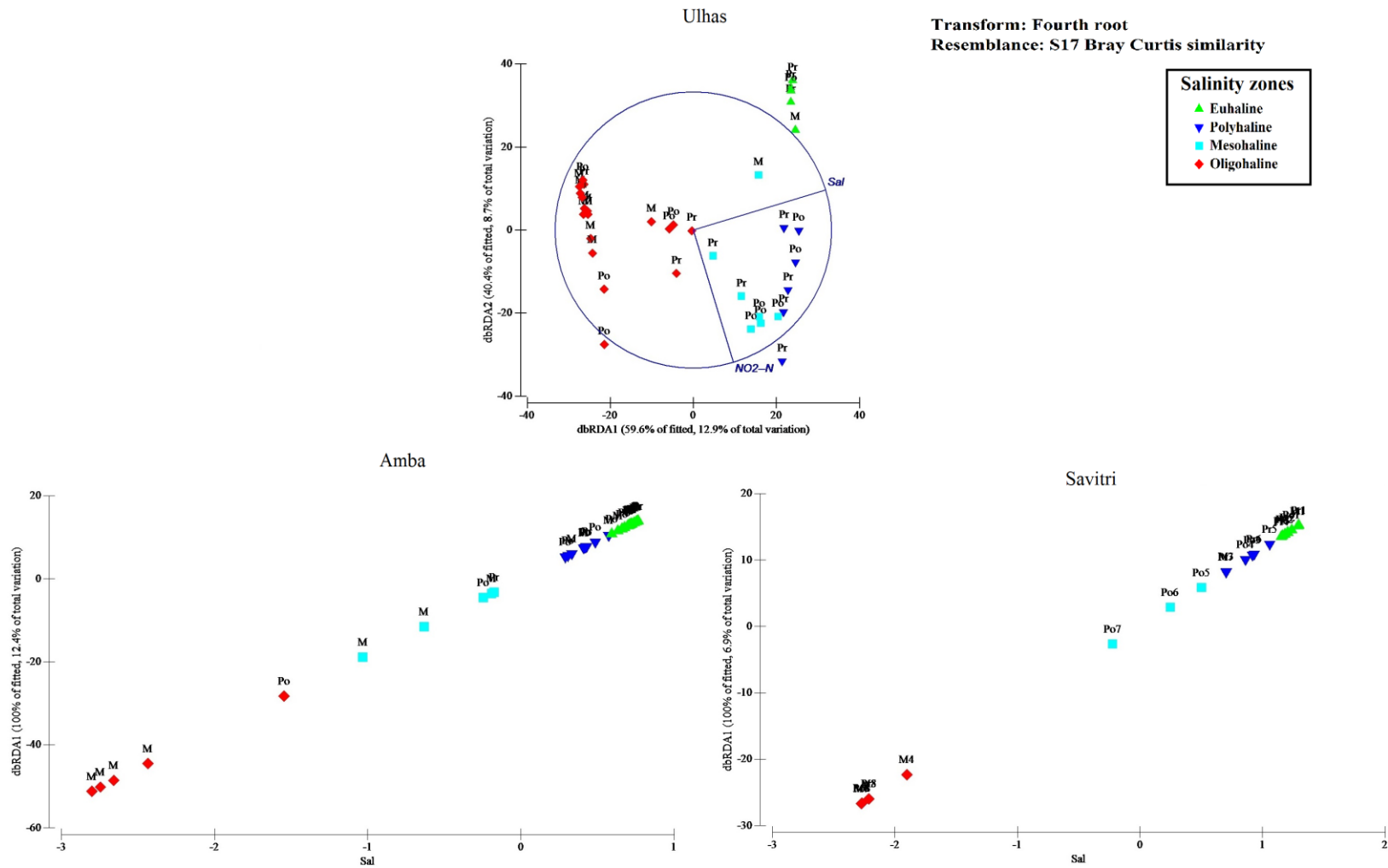
**Figure 1:** Map of the sampled sites (black dots) of each estuary (Ulhas, Amba and Savitri)



**Figure 2:** Non-metric Multidimensional scaling (n-MDS) ordination of macrobenthic species abundance data of the three estuaries (U=Ulhas, A=Amba and S=Savitri)



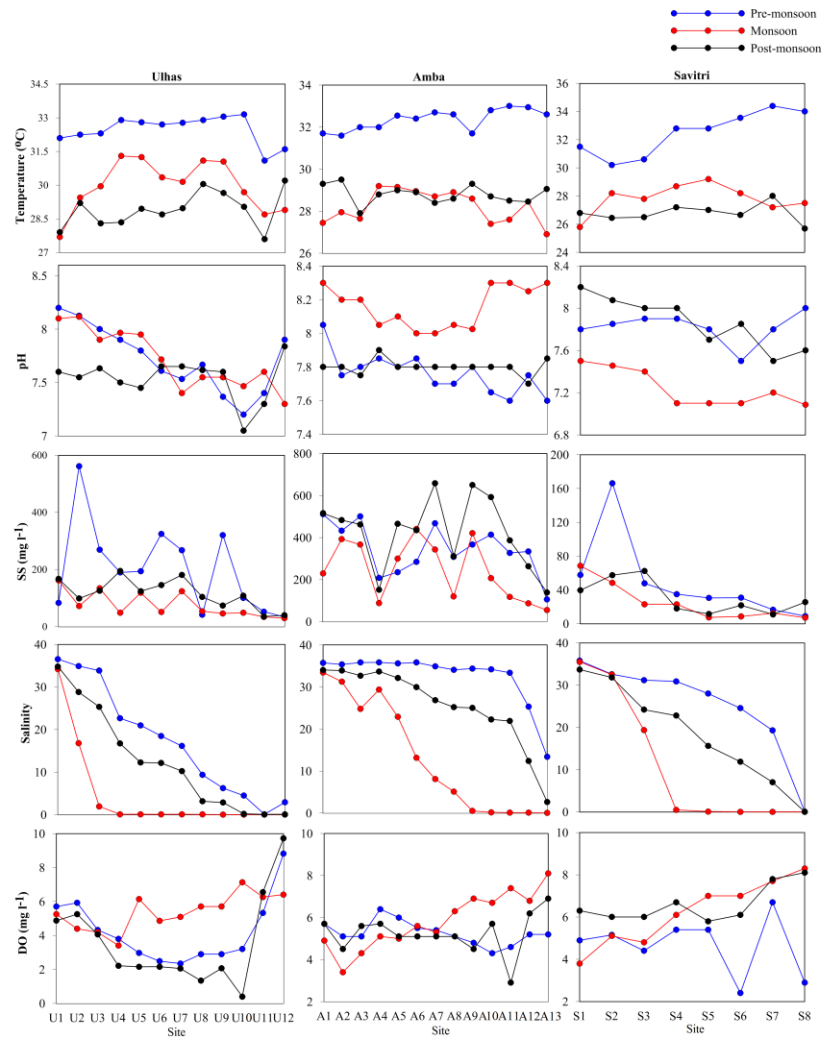
**Figure 3:** Two-dimensional Principal Component Analysis (PCA) ordination of the environmental variables of the three estuaries during the entire study period (\* = Pre-monsoon, # = Monsoon, ' = Post-monsoon, E = Euhaline, P = Polyhaline, Me = Mesohaline and O = Oligohaline)



**Figure 4:** Two-dimensional distance-based redundancy analysis (dbRDA) ordination based on the best set of abiotic variables (using BEST as selection procedure and AICc as selection criterion) and macrobenthic data from the three estuaries Pre-monsoon = Pr, Monsoon = M, Post-monsoon = Po, Sal = Salinity and  $\text{NO}_2^-$ -N = Nitrite-Nitrogen)

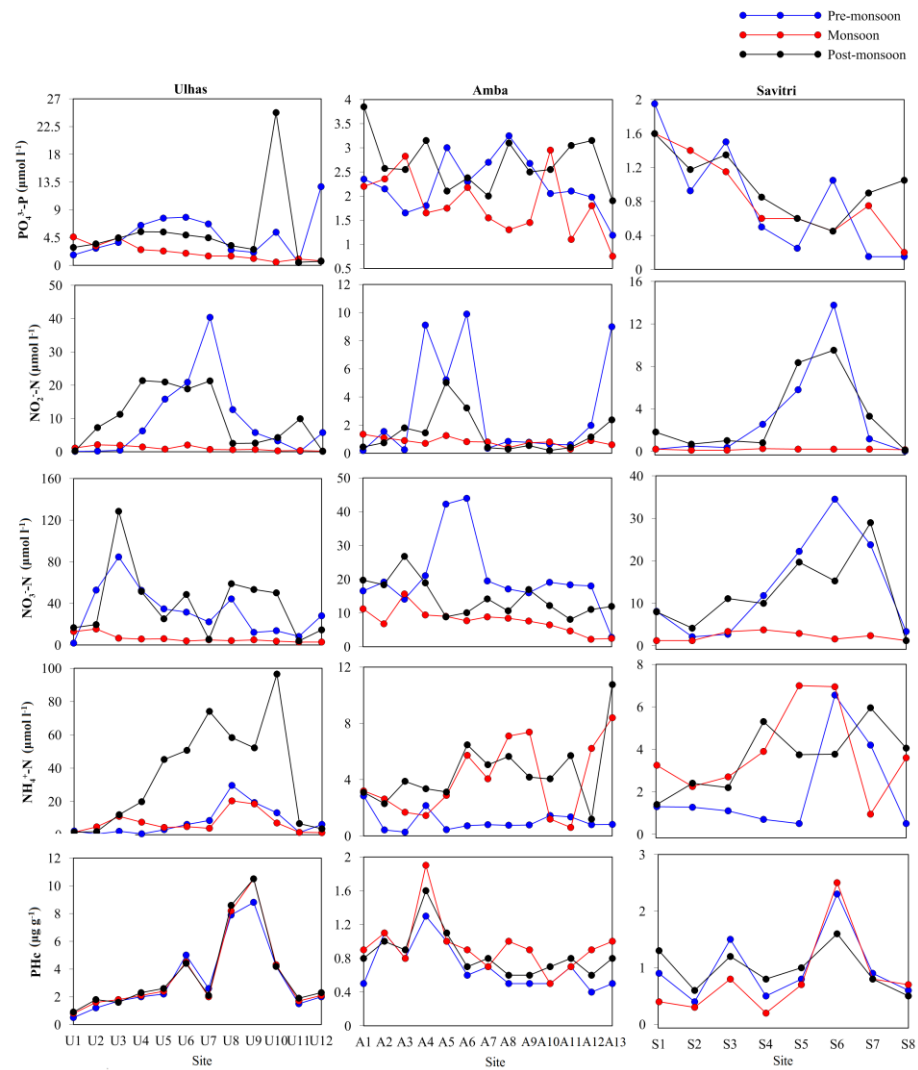
### Captions for Supplementary figures

- Fig.S1a Spatio-temporal distribution of water variables (water temperature, pH, suspended solids, salinity and dissolved oxygen) in the three estuaries.
- Fig.S2b Spatio-temporal distribution of nutrients (phosphate-phosphorous;  $\text{PO}_4^{3-}\text{-P}$ , nitrite-nitrogen;  $\text{NO}_2^- \text{-N}$ , nitrate-nitrogen;  $\text{NO}_3^- \text{-N}$ , ammonia-nitrogen;  $\text{NH}_4^+ \text{-N}$ ) and PHc-sediment in the three estuaries.
- Fig.S3 Spatio-temporal distribution of sediment variables (sand %, silt %, clay % and  $\text{C}_{\text{org}}$  %) in the three estuaries.



**Fig. S1a**





**Fig. S1b**

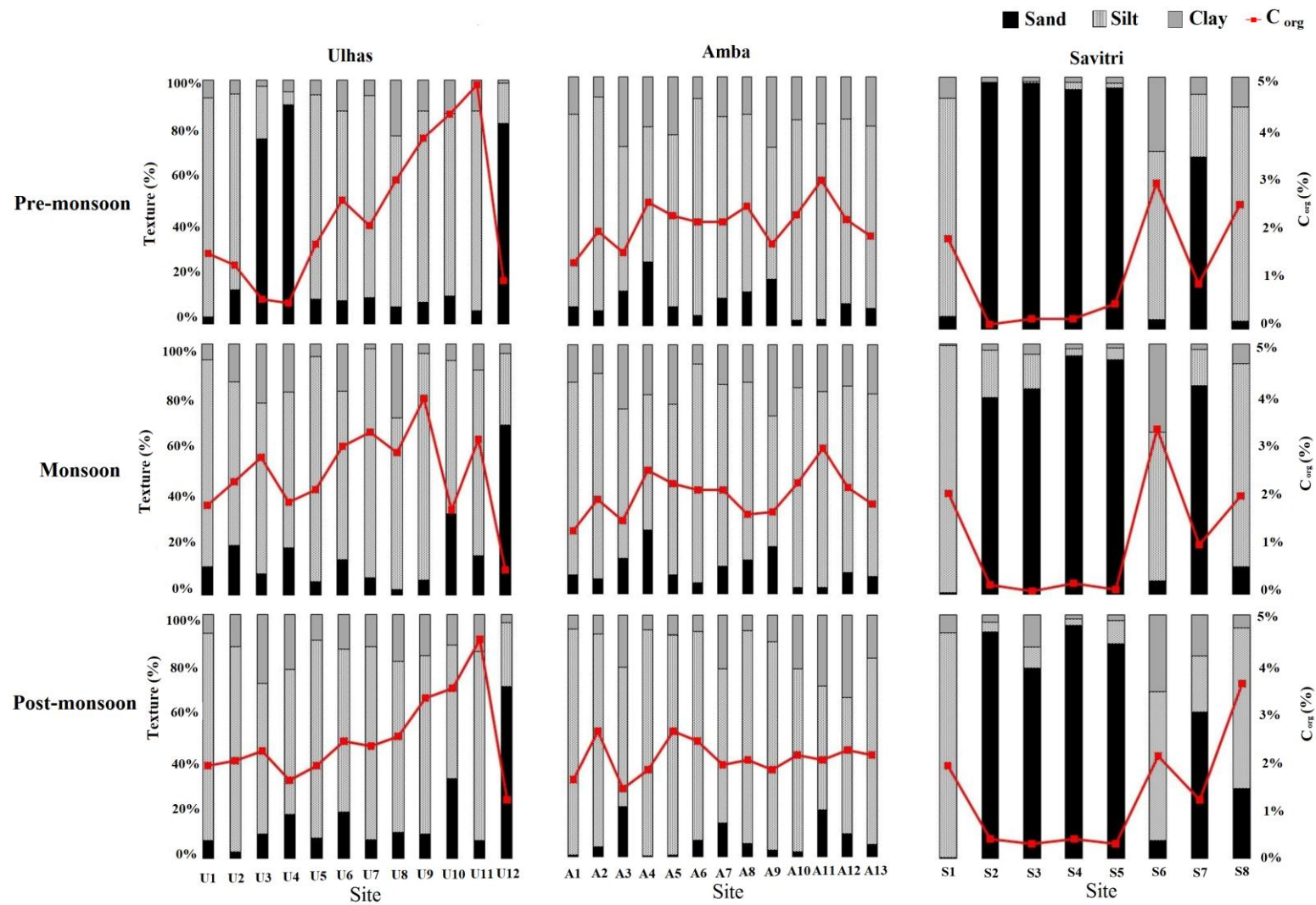


Fig. S2