

**Ecological quality status evaluation of a monsoonal tropical estuary using benthic indices: comparison via a seasonal approach**

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**Abstract**

The use of biotic indices has garnered attention during the last decade due to its extensive application in evaluating Ecological quality status (EcoQS) of marine waters and estuaries. Three seasonal surveys were conducted in the Kundalika estuary, India to evaluate the ecostatus using five benthic indices and comparing their effectiveness considering the estuarine salinity gradient and seasonality. All indices gave divergent results displaying a wide range of classes (good to bad) across salinity zones and seasons. Comparatively, M-AMBI discriminated the EcoQS suitably than other indices. Hence, a seasonally averaged approach for M-AMBI was proposed to obtain a final mean EcoQS which assigned moderate status to the euhaline and poly-mesohaline zones and poor status to the oligohaline zone. Considering the high degree of spatial heterogeneity and seasonality in the estuary, the monsoon data was found to lower the EcoQS due to natural stress in some cases; the exclusion of the monsoon season resulted in a more valid ecostatus. Therefore, this approach which combines information from the non-monsoon seasons stands out in providing a useful basis for ecological management by scrutinizing responses of macrobenthos. Also, we suggest salinity zone-wise evaluation for more effective classification chiefly in tropical monsoonal estuaries. An effort to establish a final EcoQS was performed; however, future in-depth studies are necessary to ascertain the reliability of the successful biotic index (M-AMBI) in estuaries with different stressors.

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## 1. Introduction

Remarkable advances in research have led to incremental developments in the ecological status assessment of coastal environments by implementing different approaches. Estimation of environmental quality is integral to the planning of management initiatives that can ameliorate the conditions resultant of escalating anthropogenic perturbations, particularly in estuaries. Analysis of benthic macrofaunal composition has become one of the mainstays of environmental quality assessment and has led to a development of varied indicators for assessing the ecological quality status of estuaries (Tweedley et al. 2015). Several studies have demonstrated that the macrobenthos respond appropriately to anthropogenic and natural stress (Simboura and Zenetos 2002; Reiss and Kröncke 2005a; Zettler et al. 2007) as they exhibit low motility, high longevity, cover a range of feeding guilds, exhibit different degrees of tolerance to stress conditions (McLusky and Elliot 2004; Tataranni and Lardicci 2010) and give integrated response to perturbations. Some of the most frequently employed benthic indices like AZTI Marine Biotic Index (AMBI) (Borja et al. 2000) and BENTIX (Simboura and Zenetos 2002) are based on the proportion of sensitive to opportunistic species (Borja et al. 2008). Similarly, indices like the multivariate AMBI (M-AMBI) which is a combination of species richness, Shannon-Weiner Index and AMBI (Muxika et al. 2005) and the Benthic Opportunistic Annelida Amphipod index (BO2A) index (Dauvin and Ruellet 2009) which employs ratios between different taxa have also been employed in many areas (Borja et al. 2012; Sánchez-Moyano et al. 2017).

In transitional waters, apart from strong salinity gradients, a single water body can display variable physico-chemical conditions throughout its length (Bald et al. 2005; Teixeira et al. 2008) displaying a mosaic of habitats. Estuarine organisms are restricted to particular sections of environmental gradients, resulting in well-developed spatial distribution patterns (Chainho et al. 2007). The use of benthic indices has limitations mainly in estuarine ecosystems where there is a paucity of data in basic information on ecological characteristics of soft-bottom macrobenthic communities (Bigot et al. 2008; Muniz et al. 2005), more so in the tropics. Since transitional waters are more complex than other categories of surface waters (Patrício et al. 2012), they must be dealt in a special manner when implementing the scheme of Ecological Quality Status (EcoQS) assessment. Prior to the establishment of EcoQS, the existence of different salinity regimes within a system should be evaluated as salinity is known to greatly influence the composition of benthic communities (McLusky and Elliott 2004; de Paz et al. 2008) that can affect the performance of benthic indices. A major pitfall indicated for the disparity of indices is their inability to distinguish between stresses due

to natural fluctuations and anthropogenic disturbances (Borja and Muxika 2005; Reiss and Kröncke 2005b; Labruno et al. 2006; Chainho et al. 2008). Therefore, type-specific reference conditions should be established since the low salinity inner areas naturally support less diverse fauna compared to the high salinity areas (de Paz et al. 2008). Also, it would be better to summarize the information into a single evaluation, incorporating complexities of biological communities with regard to seasonality and anthropogenic pressures, for ease of communication. Considering that almost all benthic biotic indices were developed for temperate waters, studies related to their efficacy and applicability is also largely concentrated in temperate latitudes (Bigot et al. 2008). While similar methodical assessment of the eco-status of Indian estuaries have recently been initiated (Khan et al. 2014; Sigamani et al. 2015; Feebarani et al. 2016; Mulik et al. 2017), the environmental quality of the major estuaries along the west coast of India is little known despite this coastal segment being exposed to unbridled industrialization and urbanization (Zingde 2002; NIO 2009).

The present area of study was placed within the 40 km long Kundalika estuary, northwest coast of India which presents three existing scenarios with respect to perturbations. The upper estuary receives industrial effluents from an outfall and domestic waste. The middle estuary is subjected to extensive dredging. The lower estuary too is actively dredged and has fishing and cargo jetties with substantial boat and barge movements. The considerable fresh water inputs into the estuary during the monsoon result in substantial flushing out of contaminants (Chowdhury et al. 2017). The anthropogenic and natural perturbations threaten the existing biota and warrant an immediate attention for assessment of ecological status. Bearing in mind that very little information on different biological components of this estuary is available to date; establishing robust biotic indicators for ecological status assessment becomes more challenging. The principle aim of this study was to test the efficiency of five oft-used indices for EcoQS evaluation of the Kundalika estuary taking into account the estuarine salinity gradient and seasonal variability. In view of this, we test the following hypotheses, a) a gradient of improved ecological quality should be observed from the upper to lower estuary; and b) the index should be able to discriminate the accurate EcoQS in the salinity zones considering the seasonal variability.

## **2. Materials and Methods**

### **2.1 Study site**

Kundalika River originates in the Sahyadri mountain range of western India and meets the Arabian Sea after a westerly course of about 65 km. The estuary is shallow with a depth ranging from 1m at the mouth (Revdanda) to 8 m at the upstream (Roha). Tides are semi-diurnal in nature

with the spring range of 3 to 8 m at the mouth decreasing to 1.3 m at 35 km inland (Roha). The region experiences an average rainfall of 3,750 mm and tropical warm, humid and maritime climate throughout the year with a range of 25 °C to 35 °C (Pande and Nayak 2013). The wastewater from diversified industries, amounting to about 10000-15000 m<sup>3</sup> d<sup>-1</sup> after minimal treatment is pumped into the weak tidal zone of the estuary through a point discharge, 30 km upstream from its mouth (NIO 2009). The area around the discharge point progressively accumulates pollutants even though a portion of it travels downstream. The Revdanda port situated at the estuarine mouth is an important fish landing centre of the district and provides shelter for many fishing boats. Apart from the industrial waste, the estuary also receives around 1.4 MLD of domestic waste (NIO 2009).

## 2.2 Sampling Design and laboratory methods

Sampling was carried out seasonally at 10 stations (Fig.1) in the estuarine area characterized by variation in salinity between 0.5 and 35.6. Three sampling surveys were carried out in March, September and December 2014 representing premonsoon, monsoon and postmonsoon seasons respectively. During the monsoon period, stations 1 and 3 could not be sampled due to adverse weather conditions. Bottom water samples were collected in duplicates at low tide. Temperature (°C) was measured in situ using a hand held thermometer, pH with a microprocessor controlled pH meter and salinity by Mohr titration method following Grasshoff et al. (1999). Dissolved oxygen (DO) was fixed immediately after collection of water samples and was measured by Winkler's method (Grasshoff et al. 1999). Additionally, nutrient concentrations (NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N and PO<sub>4</sub><sup>3-</sup>-P) were estimated photometrically and suspended solids (SS) by filtration method using standard protocols (Grasshoff et al. 1999). Surface sediment samples were also collected using a van Veen grab (0.04 m<sup>2</sup>) for the analysis of sediment grain size and total organic carbon (C<sub>org</sub>) content. Grain size analysis was performed using the wet-sieving pipette method (Buchanan 1984). The sediment sub-samples were oven dried at 85°C to a constant weight, prior to homogenization in an agate mortar and pestle to analyze the total organic carbon (TOC) content by TOC analyzer (SHIMADZU SSM-5000A series) and expressed as the percentage of sediment dry weight. For macrofaunal analyses, sediment samples were collected in quadruplicate using a van Veen grab (0.04 m<sup>2</sup>) at each station, sieved through 0.5 mm mesh and the retained material was preserved in 5% buffered formalin solution containing Rose Bengal stain. Macrofaunal specimens were sorted, counted and identified to the lowest possible taxonomic resolution, mostly up to species level with the help of available literature (Day 1967; Brinkhurst 1971; Fauchald 1977; Lincoln 1979; Lyla et al. 1998;

Chapman 2007). Unclassifiable specimens (completely damaged individuals) were omitted from analyses.

### 2.3 Data analysis and Benthic Indices

Large arrays of diversity indices have been used to assess the environmental quality and the effect of disturbances on benthic communities. In the present study, macrofaunal species abundance data were subjected to univariate analysis to estimate the total density (N); number of species (S); species richness,  $d$  (Margalef Index); species evenness,  $J'$  (Pielou's); Simpson's index ( $1-\lambda'$ ); Shannon-Weiner Index,  $H'(\log_2)$  and Hurlbert Index ( $ESn$ ). Multivariate analysis was also carried out using macrobenthic species abundance data to understand the similarity between stations by using the technique of non-metric multidimensional scaling (nMDS) based on the Bray-Curtis similarity matrix. A Principal Component Analysis (PCA) was performed using physico-chemical variables in order to distinguish the abiotic variables responsible for the variability between stations. Ordination of environmental factors was carried out by PCA after normalization of log transformed variables. All the above analyses were performed using the software package PRIMER v.6 (Clarke and Warwick 2001).

Among the various proposed indices, univariate method like Shannon-Weiner Index ( $H'$ ) (Shannon and Weaver 1963), biotic indices such as BO2A (Dauvin and Ruellet 2009), AMBI (Borja et al. 2000, 2007), BENTIX (Simboura and Zenetos 2002) and multimetric M-AMBI (Muxika et al. 2007) were applied in the present study in order to evaluate the ecological status of the estuary. The Shannon-Weiner Index is one of the traditional methods used in benthic ecology, which incorporates species richness as well as equitability and depends on sample size. In the AZTI Marine Biotic Index (AMBI), benthic species were assigned to five ecological groups:

EGI – species sensitive to organic enrichment and present in unpolluted conditions.

EGII – species indifferent to enrichment, present at low densities with non-significant variations.

EGIII – species tolerant to excess organic enrichment, stimulated by slightly unbalanced situations.

EGIV – second order opportunistic species, slightly to pronounced unbalanced situations.

EGV – first order opportunistic species, pronounced unbalanced situations.

AMBI was computed for each individual replicate, averaged for each site and species were assigned to ecological groups based on the species list of v. Nov 2014 (presented in the AMBI software program v.5.0) which is available on the web page <http://www.azti.es>. Among all the

macrofaunal groups, the minor taxa (6%) with higher taxonomic levels (eg. gastropoda, brachyura) were eliminated from the AMBI calculation as per the guidelines given by Borja and Muxika (2005) and this did not affect the assessment. When taxa were not listed in the AMBI species list, they were assigned to the common group of their genus that was present in the list (Borja and Tunberg (2011) and in the absence of group it was classified as not assigned (NA). The multivariate AMBI (M-AMBI) combines Shannon-Weiner diversity, species richness and AMBI values in a factor analysis multivariate approach. For arriving at M-AMBI index, reference conditions for three salinity regimes i.e. euhaline, polyhaline-mesohaline and oligohaline were derived separately by increasing the highest species richness and diversity of all replicates, by 15% (Borja et al. 2012; Cai et al. 2014) for each zone following Borja et al. (2009) and Feebarani et al. (2016). Taking into account the salinity zones, reference conditions to calculate M-AMBI were set as follows: euhaline zone – diversity 4.5, richness 36; poly-mesohaline zone – diversity 4.0, richness 16; oligohaline zone – diversity 1.8, richness 10. BENTIX considers sensitive (GS) and tolerant (GT) taxa based on the reduction of five EG's (as per AMBI) to two EG's i.e. EGI, II as EGI and EGIII, IV, V as EGII for BENTIX. For BENTIX computation, a newly developed Add-in v.09 (beta version) software package was used which is freely downloadable from <http://www.hcmr.gr/listview3.php?id=1195>. For stations 7 and 9 (premonsoon) and St-10 (monsoon) where the number of individuals were <20 ind., BENTIX and BO2A were not calculated due to their operational limits (Çağlar and Albayrak 2012). BO2A was also not calculated for station 8 (monsoon) due to the presence of solitary taxa i.e. pelecypods. These stations were examined carefully while calculating AMBI. Computational details of indices ( $H'$ , BO2A, BENTIX, AMBI AND M-AMBI) and their thresholds are presented in Table 1. Spearman's rank correlation coefficients were used to visualize significant relationships between benthic indices and environmental variables using the STATISTICA 7 software package. The correlation results were considered significant at  $p < 0.05$ .

### **3. Results**

#### *3.1 Habitat characterization and physico-chemical variables*

Based on the bottom water salinity, the estuary could be divided into three zones, corresponding to different classes of the Venice salinity classification (euhaline, poly-mesohaline and oligohaline) as shown in Table 2. Seasonal influence in the form of copious fresh water discharges during monsoon dictated the extent of each salinity zone. As such, some stations changed their assignment to salinity zones in response to the monsoon. Thus, during monsoon, the euhaline zone was restricted up to station 4 (lower estuary), the upper limit of the poly-mesohaline zone

extended up to station 6 and the oligohaline segment stretched from stations 7 to 10 indicating the pronounced impact of monsoonal processes on the salinity regime of the estuary.

The pH indicated marked spatial variations than seasonal with a decreasing trend towards the upper estuary (Table 2). Bottom water temperatures were expectedly lower during the monsoon season. DO was higher in the euhaline zone. In the poly-mesohaline and oligohaline zones, improved DO values were observed during the monsoon season.

$\text{NH}_4^+$ -N was markedly higher in the oligohaline zone during the premonsoon (av  $50 \mu\text{mol l}^{-1}$ ), monsoon (av  $11.5 \mu\text{mol l}^{-1}$ ) and postmonsoon (av  $11.7 \mu\text{mol l}^{-1}$ ) as compared to the other two zones. Also, during premonsoon, increased values of  $\text{NH}_4^+$ -N (av  $11.8 \mu\text{mol l}^{-1}$ ) were observed at the poly-mesohaline zone. Elevated  $\text{NO}_3^-$ -N values were observed in the poly-mesohaline (av  $27.9 \mu\text{mol l}^{-1}$ ) and euhaline zones (av  $12.4 \mu\text{mol l}^{-1}$ ) during premonsoon, whereas maximum  $\text{NO}_2^-$ -N was present in the poly-mesohaline zone during the premonsoon (av  $9.8 \mu\text{mol l}^{-1}$ ) and postmonsoon (av  $14.3 \mu\text{mol l}^{-1}$ ) seasons. SS indicated a decreasing trend from the estuarine mouth to the head of the estuary. The granulometric composition revealed that the sediment was spatially heterogenous, silt being the dominant fraction (33.5–83.9%) followed by the sand (8.9-46.4%). Silt percentage decreased towards the upper estuary and the sand and clay fractions indicated vice-versa. The total organic carbon ( $\text{C}_{\text{org}}$ ) content was high ( $>1.9\%$ ) with minor variations. Results of PCA revealed that the first two axes of the PCA ordination accounted for 51.6 % of total variance with 30 % for PC1 and 21.6 % for PC2 (Fig. 2). The variability along the first axis suggested that the fresh water dominated oligohaline zone had higher sand %,  $\text{PO}_4^{3-}$ -P and  $\text{NH}_4^+$ -N than the other zones with higher salinities. The variability along the second axis was mainly explained by an increase in DO levels and a decrease in parameters such as  $\text{C}_{\text{org}}$ , clay and  $\text{NO}_2^-$ -N in the euhaline zone.

### 3.2 Macrofaunal assemblages and their assignment to ecological groups

Totally, 13260 macrobenthic individuals belonging to 78 diverse taxa were identified from the estuary, of which, polychaetes, amphipods, oligochaetes and pelecypods formed by far the most diverse groups and the rest was comprised of minor groups. Polychaetes dominated the macrobenthos (36 species, 11570 ind.) and contributed numerically up to ~87 % of the population, followed by oligochaetes (5.5 %) and amphipods (3 %). Overall, *Namalycastis ouanaryensis* (36%) and *Paraprionospio patiens* (16.9 %) were the dominant polychaete species. The oligohaline stretch was characterized by the dominant presence of freshwater oligochaetes which peaked at 61.7 % during premonsoon and declined to 1.8 % in postmonsoon. The seasonal comparative macrofaunal densities and dominant constituents of all three salinity zones are presented in Table 3. Spatially, the

macrofaunal density was appreciably higher in the euhaline and oligohaline zones during the postmonsoon season. Reduced macrobenthic abundances were observed during the monsoon across all zones. The majority of the macrofauna identified was ascribed to an ecological group (EG) based on the classification according to the AZTI database. From the total macrobenthic species, 18 species were assigned by us to category of similar genus (23.1%) and 5 taxa were not assigned (6.4%) to any category (Appendix 1). Fig. 3 represents the mean percentage of each ecological group which indicated a preponderance of EG-IV (second order opportunistic species) followed by EG-II (species indifferent to enrichment). The dominance of opportunistic species (EG-IV) was higher at euhaline and oligohaline zones during all seasons owing to the presence of *Cossura coasta* and *N. ouanaryensis* respectively. During premonsoon, EG-V dominated at the oligohaline zone owing to the abundant presence of oligochaetes. The EG-II species were dominant at the poly-mesohaline zone during premonsoon and monsoon seasons, whereas during the postmonsoon the reappearance of other groups besides EG-II species indicated resettlement of macrobenthos.

### 3.3 Univariate and multivariate analyses

In this study, it was observed that species number (S) was highest in the euhaline zone during premonsoon and postmonsoon seasons (Table 4). A distinct trend of decreasing species numbers was seen from outer to the inner estuary. Higher Margalef's species richness (av 2.1) was recorded in the euhaline zone during postmonsoon, followed by premonsoon season (av 1.8) as opposed to the other two zones. Pielou's evenness  $J'$  was low only in the oligohaline zone (0.2) during postmonsoon. Shannon-Weiner diversity ( $H'$ ) displayed highest values (av 2.3) in the euhaline zone during premonsoon and postmonsoon seasons, and lowest values (av 0.5) in the oligohaline zone during postmonsoon. Similarly, the Simpson's dominance index ( $1-\lambda'$ ) was low ( $<0.4$ ) in the oligohaline zone during all three seasons and in the poly-mesohaline zone during premonsoon. The trends followed by Hurlbert index (ES100) was very similar to that of  $H'$ , where higher values were from the euhaline and lower from the oligohaline zones. Significant positive correlations were obtained for S, d,  $1-\lambda'$ ,  $H'$  and ES100 with pH and salinity and negative correlations with  $\text{NH}_4^+\text{-N}$  (Table 5).

The MDS plot (Fig. 4) clearly identified the spatial variation of the sampling stations along the salinity gradient of the estuary (stress=0.1), forming different clusters of stations corresponding to the euhaline, poly-mesohaline and oligohaline zones. The euhaline stations were presented in two clusters and had the highest species diversity. The poly-mesohaline zone stations showed a limited overlap of stations 5 and 7 (postmonsoon) with the other two zones. In the oligohaline cluster,



stations 8 (monsoon) and 9 (premonsoon) were placed away from the other stations as the only macrobenthic group present at these locations were pelecypods.

### 3.4 Ecological quality status assessment

During the study period, the applied biotic indices covered different levels of ecological quality status (EcoQS) across seasons. Fig.5 represents the distribution of stations reflecting the EcoQS as per the five applied indices during the three seasons. Also embedded in each figure is a histogram that depicts the zone wise quality status with respect to seasons for each index. The ecological status assessment revealed that some discrepancies in EcoQS were found along the Kundalika estuary in two aspects: a) estuarine spatial gradient and b) seasonal variations. The extent of the three salinity zones varied seasonally depending on the freshwater input during the three seasons. The euhaline zone was comprised of 4-5 stations and the poly-mesohaline zone was represented by 2-3 stations depending on the season. Conversely, the oligohaline stretch varied widely including 2 (premonsoon) to 4 (monsoon) stations (Table 2). Thus, the oligohaline zone fluctuated more in response to the monsoonal fresh water discharge.

The upper estuarine stations were classified as mostly bad during all three seasons by  $H'$  (Fig.5a) whereas, the stations in the middle estuary were assigned moderate to poor status and the lower estuary stations were in good to poor categories. In salinity zone-wise classification, (as depicted in Histogram Fig. 5a),  $H'$  in the euhaline zone fluctuated from moderate status during premonsoon to poor status during monsoon but regained the moderate status during postmonsoon. Contrastingly, the poly-mesohaline zone indicated improved status from bad (premonsoon) and poor (monsoon) to moderate (postmonsoon). Oligohaline segment was designated poor to bad across all seasons.

Station-wise, BO2A displayed a highly variable range of values from good to bad status without any definite trend (Fig. 5b). Zone wise comparison indicated that in the euhaline and oligohaline zones, BO2A signified an improved status during monsoon as compared to the other two seasons (Histogram Fig. 5b) while the poly-mesohaline was consistently moderate throughout.

BENTIX produced a poor status in most of the stations during postmonsoon season while the other seasons displayed a variable range from high to poor status (Fig. 5c). Similarly, the EcoQS assigned to the euhaline zone by BENTIX ranged from good (monsoon) to poor (postmonsoon) whereas, the poly-mesohaline zone ranged from good (premonsoon and postmonsoon) to moderate

(monsoon). The poor status of oligohaline segment during premonsoon and postmonsoon as obtained by BENTIX turned into moderate status during monsoon (Histogram Fig. 5c).

All stations (except st-5) were classified as moderate by AMBI during postmonsoon and good to poor status during the remaining seasons (Fig. 5d). AMBI calculated for zones across seasons (Histogram Fig. 5d) indicated moderate EcoQS in the oligohaline zone. The EcoQS obtained by AMBI in the euhaline zone was moderate during premonsoon and postmonsoon and poor during the monsoon season. This index also assigned a moderate status to the poly-mesohaline zone during the premonsoon and monsoon seasons which improved to good during postmonsoon.

According to M-AMBI, most of the stations ranged from good to bad, except stations 4 (premonsoon) and 5 (postmonsoon) which presented a high status. The status alternated from good to poor during other seasons (Fig. 5e). In zone wise classification (Histogram Fig. 5e), M-AMBI values classified the euhaline zone as moderate during premonsoon and postmonsoon and poor during monsoon. Likewise, eco-status of poly-mesohaline improved from poor (premonsoon) and moderate (monsoon) to good (postmonsoon). As per M-AMBI, the oligohaline zone maintained a poor status throughout the study except for moderate status during premonsoon. It was also observed during the postmonsoon season, that a better status was assigned by this index to all zones than monsoon indicating the recovery of disturbed macrobenthic assemblages. Surprisingly, none of the biotic indices correlated with any of the environmental variables except the univariate index  $H'$  (Table 5).

### 3.5 Seasonal averaged approach

Table 6 represents the zone wise seasonal averaged index values and corresponding ecostatus for all five applied indices. The agreement between indices on the ecostatus was higher in the euhaline zone (60-80%) and decreased towards the oligohaline zone (40-60%). As the status varied seasonally, an approach was adopted wherein a mean status was derived by averaging the seasonal EcoQS of each index zone wise (Table 6) to arrive at a final status existing in the current study area as suggested by Feebarani et al. (2016). Further, it was also attempted to derive the final mean eco-status by taking the mean of non-monsoonal seasons (premonsoon and postmonsoon). In the euhaline zone, the mean EcoQS of the tested indices achieved by merging all seasons ranged from moderate (BENTIX and AMBI) to poor ( $H'$ , BO2A and M-AMBI). Consequently, when only two seasons (excluding monsoon) were considered, a 'moderate' status was obtained by all indices except BO2A. In the poly-mesohaline zone, the seasonally averaged EcoQS assigned were good (BENTIX), moderate (BO2A, AMBI, M-AMBI) and poor ( $H'$ ). Similarly, in this zone, the EcoQS remained the same for all indices except AMBI when mean of only two seasons (pre and post) were considered. The EcoQS

in the oligohaline zone fluctuated between moderate (AMBI), poor (BO2A, BENTIX, M-AMBI) and bad (*H*). The averaged ecostatus in the oligohaline zone remained unchanged even on the exclusion of monsoon data.

#### **4. Discussion**

The present study indicated that the variability in environmental variables and benthic indices between individual stations and zones can be large thus emphasizing the inherent spatial heterogeneity in estuarine benthic systems (Elliot and O'Reilly 1991; Elliot and Quintino 2007). Besides, the seasonal fluctuations were also evident in these parameters largely due to the dominant monsoonal processes that are known to impact the abiotic and biotic components of Indian estuaries (Sivadas et al. 2011). The benthic indices indicated discrepancies in their assessments of EcoQS reflecting differing status of estuarine environmental conditions.

##### *4.1 Physico-chemical variables and macrobenthic assemblages*

In the present investigation, the salinity displayed clear and consistent spatial and seasonal fluctuations as expected for most tropical estuaries. During monsoon season, the rainfall resulted in more freshwater input into the estuary, making it freshwater dominated and more turbid due to strong influx from upstream. PCA analysis clearly highlighted spatial variations in the study area indicating that the fresh water dominated oligohaline zone also had higher nutrient load than other zones with higher salinities. These changes may be attributed to anthropogenic inputs from the catchment area and outfall location in the vicinity of this zone. The high  $\text{NH}_4^+\text{-N}$  values in the oligohaline zone and to some extent in the poly-mesohaline zone during the premonsoon had reduced appreciably during the monsoon and post monsoon seasons probably due to the excellent flushing of the estuary by abundant freshwater discharge during these periods (Table 2). In fact, Chowdhury et al. (2017) reported that the seasonal changes regulated the nutrient enrichment in the Kundalika estuary by preventing over accumulation during monsoon season (high flushing period).

In the Kundalika estuary, benthic communities appeared to follow a continuum from the euhaline to the oligohaline areas (Chainho et al. 2006) with a clear tendency of decreasing number of taxa from downstream to upstream. Substantial freshwater inputs during monsoonal rains caused defaunation of benthic fauna in the estuary possibly due to the mortality of population attributed to sudden salinity fluctuations and increased turbidity (Alongi 1990; Sivadas et al. 2011). The low macrofaunal density during the monsoon season were followed by increased numbers during postmonsoon season, indicating quick recovery associated with stabilized estuarine conditions as

reported in other Indian estuaries (Harkantra and Rodrigues 2003). Improved abundances observed during postmonsoon may also be attributed to key events such as recruitment (Sivadas et al. 2011). Though the euhaline zone was highly diverse, it was characterised by the presence of anthropogenic stressors favouring EG-IV species in high numbers. On the other hand, in the poly-mesohaline zone, the substantial presence of EG-II species along with EG-IV and EG-V fauna indicated that the zone was only partially affected by the effluents released at the upstream stations. The higher percentage of EG-V in the oligohaline zone during premonsoon was indicative of poor environmental conditions due to various anthropogenic stressors. In the current study, the environmental sensitivity of each zone was judged based on the evaluation of not only the hydro-sedimentological parameters but also considering the expert judgement of the prevailing stressors of each zone. In sites experiencing intermediate levels of disturbance, expert opinion can help facilitate evaluation of the indices performances (Weisberg et al. 2008). The expert judgement in this study is that of the authors who are well-versed in benthic monitoring and have sufficient knowledge on local conditions of the estuary. Hence, it was established that the oligohaline zone was heavily impacted whereas the poly-mesohaline and euhaline zones were moderately impacted (Tables 2 and 4, Fig. 2). Thus, our first hypothesis was proved right as ecological quality was observed to have improved from the upper to lower estuary.

#### 4.2 Zonal EcoQ status assessment:

Several studies have shown that benthic communities experience seasonal changes in abundance, mainly related to recruitment events (Reiss and Kröncke 2005a; Chainho et al. 2007) which greatly affect indices performance that includes pollution sensitive and tolerant species metrics. As salinity has an important role in defining species diversity and abundances which can impact the final EcoQS scores, zone-wise evaluation of EcoQS was considered to be more appropriate (Feebarani et al., 2016).

$H'$  assigned the highly diversified euhaline zone as moderate to poor EcoQS depending upon seasons, which concurred with other applied indices (except BO2A). However,  $H'$  underestimated the poly-mesohaline zone (poor to bad) despite an adequate representation of k-selected individuals like *Nephtys paradoxa*, *Glycinde kameruniana*, *Sternaspis scutata*, *Gammurus* sp. (Table 3) at this area and species belonging to EG-I and EG-II (Fig. 3). This occurred as  $H'$  considered only the species type and abundances ignoring the sensitivity of the species type. Therefore,  $H'$  could not adequately discriminate between the impacted oligohaline and relatively lesser impacted poly-mesohaline zone. Similar inadequacies of  $H'$  have been reported by others (Labruno et al. 2006;

Sivadas et al. 2016) wherein the index value was affected by the natural occurrence of high species densities even when the species were normally associated with unimpacted environments. The  $H'$  appropriately reflected the poor to bad EcoQS of oligohaline zone especially during the premonsoon which was expected due to its negative correlation with the high  $\text{NH}_4^+\text{-N}$  that was present in this zone. In the present study too, it was apparent that  $H'$  varied seasonally following the upheaval in benthic abundances during the monsoon and subsequent resettlement.

The BO2A was severe in classifying the EcoQS of the species-rich euhaline zone which was assigned as mostly poor whereas, the impacted oligohaline zone was mostly classified as bad. Thus, the index failed to discriminate between sites of differential impacts. This is because BO2A index is based on the presence of only opportunistic (Annelida) and sensitive (Amphipoda except for *Jassa* sp.) species and unlike other indices; it does not take into account the other macrobenthic taxa that are also equally important. Therefore, irrespective of the presence of sensitive benthic groups like sipunculids, phoronids, cumaceans and nemerteans, the euhaline zone was incorrectly ranked poor. It was also criticised that sensitivity to pollution for the same taxonomic group (polychaetes, oligochaetes and amphipods) used for BO2A differs from species to species (Afli et al. 2008) and therefore might respond differently to stressors than expected. As it generally happens in an estuary, another drawback of BO2A was that it cannot be calculated for sites which had an inadequate number of individuals (<20 ind.) according to the criteria of Dauvin and Ruellet (2009). For instance, BO2A was not calculated for stations 7, 9 (both premonsoon) and 10 (monsoon) due to insufficient macrobenthic organisms. Similarly, station 8 (monsoon) could not be taken into account as none of the three categories of organisms (polychaetes, oligochaetes and amphipods) were present. Therefore, it impaired the accurate EcoQS of the salinity zones as these stations were not considered.

The BENTIX index overestimated the status at poly-mesohaline zone (mostly 'good') as compared to other indices, though the zone was reasonably impacted by dredging activities and pollutants from the upstream as indicated by the chemistry data. This could be because BENTIX values were not calculated for some stations in this zone due to inadequate numbers of individuals thus leading to the overestimation. Also, the relatively lesser impacted euhaline zone varied extensively from poor (underestimation) to good (overestimation) depending on the seasons. The main cause could be the discrepancy in the level of sensitivity of benthic taxa while assigning to various ecological groups by BENTIX as compared to AMBI (Simboura and Reizopoulou 2008; Simboura and Argyrou 2010). For instance, some amphipod species were assigned to the tolerant

group (EG-II) of BENTIX whereas AMBI assigned these species to the sensitive group (EG-I). BENTIX erroneously categorized many organisms that are considered sensitive by AMBI in the tolerant ecological group viz. *Lumbrinereis latreilli*, *Ampelisca diadema*, *Ampelisca spinipes* and *Erichthonius brasiliensis* leading to underestimation during postmonsoon. Hence, BENTIX was not entirely successful in evaluating the ecological conditions of the Kundalika estuary.

AMBI classified the Kundalika estuary as mostly moderate across zones and seasons with few exceptions. Despite the oligohaline zone being anthropogenically stressed and having the highest percentage of species belonging to EG-IV, a moderate status was assigned by AMBI which overestimated the EcoQS. This was due to lack of sensitivity of AMBI when there is a dominance of one group of species as also observed by Puente and Diaz (2008) which biases the results towards a general classification of better status (Chainho et al. 2008). Also, AMBI robustness is reduced when there are low numbers of taxa in a sample (Afli et al. 2008) which was observed at the oligohaline zone of this estuary. Some authors have highlighted that AMBI overestimates the status due to its lack of sensitivity to discriminate among polluted areas where naturally stressed species (tolerant) occur (Puente et al. 2008; Hutton et al. 2015). Nevertheless, AMBI varied over a narrow range, even when sensitive/opportunistic species did vary in relation to pollution levels which rendered this index ineffective especially in the inner parts of the estuary.

M-AMBI correctly determined the euhaline zone as mostly moderate (premonsoon and postmonsoon) as this zone had activities associated with the fishing port. M-AMBI also appropriately indicated the steady improvement of the poly-mesohaline zone from poor status during premonsoon to good status in postmonsoon. This zone was influenced by the effluents released in the upper oligohaline zone during the premonsoon which got flushed out during the monsoon season leading to better ecological conditions during the postmonsoon. The moderate status of the oligohaline zone during premonsoon, as per M-AMBI, despite the presence of stressors, was probably due to the influence of diversity and species richness values owing to the presence of multiple oligochaete species. However, the index correctly assigned a poor status to this zone during the other seasons due to the disappearance of most oligochaete species. M-AMBI could aptly reflect the EcoQS zonewise as it incorporated AMBI values,  $H'$  and species richness according to the reference conditions set as per the salinity typology. Multimetric indices that integrate diversity measures like M-AMBI may be able to detect gradual changes of benthic community than AMBI, BENTIX or BO2A that are based only on ecological grouping (Kröncke and Reiss 2010). Although

some authors (Sigamani et al. 2015; Khan et al. 2014) recommend using AMBI rather than M-AMBI for defining the EcoQS of Indian waters, M-AMBI index performed better in this estuary.

While M-AMBI was the most applicable among the tested indices, it has the inherent disadvantage of requiring reference conditions which is not easily achievable (Muxika et al 2007; Borja et al. 2012). Generally, reference conditions are derived by one or a combination of the following methods; i) comparison with pristine areas or site with minor disturbances; (ii) historical data and information; (iii) numerical models; and (iv) expert judgement (Muxika et al. 2007; Forchino et al. 2011). Deriving reference conditions for Indian waters is a major challenge due to lack of pristine areas and unavailability of historical data (Sivadas et al. 2016). Therefore, keeping in mind the above-mentioned criteria, in complex habitats such as ours, reference conditions were selected by increasing the highest diversity and richness to 15% for each salinity zone (Cai et al. 2014; Feebarani et al. 2016; Mulik et al. 2017) of the estuary. There is no doubt that zone-wise evaluation implied a better EcoQS, but the level of discrimination based alone as a function of reference conditions of available data may not accurately reflect the estuarine health. Some areas can naturally present low structural parameters (richness and diversity), in which there is a tendency of misclassification even when reference conditions are set/modified (Muxika et al. 2007). In such cases, to avoid ambiguous classifications, expert judgement was used as an indicative to test the harmonization of the results of benthic indices. Thus, the EcoQS determined by M-AMBI for each zone were in tune with the ecostatus assigned by expert judgement supporting the accuracy of M-AMBI in the Kundalika estuary. M-AMBI worked in this estuary better than other indices primarily because it incorporated species diversity values in relation to reference conditions derived for each salinity zone which is very critical for an estuary where seasonal salinity gradient fluctuations were prominent.

In the present study, the main stressor was the high  $\text{NH}_4^+\text{-N}$  in the oligohaline zone which caused reduction in benthic species richness (Belal et al. 2016). The elevated  $\text{NO}_3^-\text{-N}$  in the other two zones may be attributed to the sources in the upper estuary and is considered to be an important stressor for sensitive benthic taxa (Chambers et al. 2012). This could be the reason that no significant association was observed between the biotic indices (except for  $H'$ ) and any single environmental variable. While  $H'$  did give significant correlations with salinity and  $\text{NH}_4^+\text{-N}$ , the index underestimated the ecostatus of the estuary. In our study,  $H'$  and S correlated significantly with salinity, pH and  $\text{NH}_4^+\text{-N}$  suggesting that the inclusion of these measures in the M-AMBI index contributed to its effectiveness. It is also possible that the macrobenthic community composition at

some places may have been influenced by multiple factors or variables other than that investigated (Fitch and Crowe 2010). Therefore, the reliability of indices cannot be determined through correlation analyses alone as many taxa may respond similarly to natural and anthropogenic disturbances.

The indices disagreed on the EcoQS especially in the impacted oligohaline zone. This failure is mainly because different indices, even if based on the same notion, do not interpret the EcoQS information in similar way (Ruellet and Dauvin 2007). The lack of congruence among indices in temperate waters was highlighted by several authors due to the dissimilar scales for thresholds used in the assignment of EcoQS categories (Munari and Mistri 2010; Muniz et al. 2012), spatial variability in benthic communities (Brauko et al. 2015) and differences in design/structure of indices (Spagnolo et al. 2014). Similarly, in tropical systems the low congruity has been attributed to the different methodologies of species classification adopted by various indices (Mulik et al. 2017). Conversely, another study conducted in tropical waters by Sivadas et al. (2016) found a strong agreement between indices. From our study, the disparity among indices was mainly due to: (i) exclusion of some stations due the indices' operational limits; (ii) improper assignment of species to ecological groups; and (iii) structure of indices which uses various methods.

#### *4.3 Seasonal averaged approach: Derivation of mean EcoQS*

Our results showed that the natural variability resulted in gradual shifts in benthic communities which modified the indices. An averaged assessment of indices was derived to arrive at a final mean EcoQS by taking into account each salinity zone and seasonality. Thus, the zonewise performance of seasonally averaged indices is compared below.

*Euhaline stretch:* Considering the different pressures identified in this zone (see section 4.1) and the moderate species diversity, only AMBI and BENTIX correctly assigned moderate mean EcoQS to this zone (Table 6). M-AMBI and *H'* gave a 'poor' mean status due to the inclusion of low values derived for the monsoon season. Exclusion of the monsoon data resulted in assignment of moderate status by these two indices.

*Poly-mesohaline stretch:* This zone was characterised by lesser diversity than euhaline zone and impacted by effluents released in the upper estuary (section 4.1). BENTIX and AMBI overestimated this zone by assigning an average good ecostatus while *H'* underestimated by assigning a poor status (Table 6). M-AMBI particularly displayed a gradual improvement from poor (premonsoon) to moderate (monsoon) and to good (postmonsoon) status as the diversity increased and assigned a final



moderate status. A final mean 'moderate' status obtained by M-AMBI was in accordance with the conditions prevailing there.

*Oligohaline stretch:* Despite this zone being under highest typical disturbed conditions, AMBI overestimated this zone as moderately degraded which might be due to the presence of higher species diversity and richness (of opportunistic species). Conversely, BO2A and  $H'$  underestimated the zone as 'bad'. A 'poor' status was assigned by M-AMBI and BENTIX thus adequately reflecting the EcoQS of that zone which was characterized by numerous anthropogenic activities.

The monsoon regime in the Indian subcontinent is known to influence the macrobenthic assemblage structure by defaunation, migration and spawning of species. Thereafter, the recovery of benthic communities occurs during the non-monsoon periods when conditions are stabilised which favour the recruitment and resettlement of macrobenthic species (Gaonkar et al. 2013). The macrobenthic diversity and abundances were lower during monsoon at all zones in the Kundalika estuary similar to that reported in other monsoonal estuaries of the Indian coast (Sivadas et al. 2011; Feebarani et al. 2016). Therefore, benthic indices that are derived for the monsoon season may be poor owing to natural perturbations than anthropogenic. A perusal of Table 6 indicated that inclusion of monsoon data either lowered the ecostatus (due to natural processes) or maintained the status quo independent of the applied index. Kröncke and Reiss (2010) suggested that data collected after natural events should be excluded for ecological assessments as it can significantly reduce the EcoQS. As the monsoon season is the primary recruitment period for the benthic community, species abundance data will show fluctuations ultimately reflecting in the indices (Sivadas et al. 2016). Therefore, we suggest that valid inferences could be drawn by excluding the monsoon data. In the Kundalika estuary, premonsoon season presented the most stressed scenario due to accumulation of contaminants whereas better environmental conditions prevailed during postmonsoon due to the flushing out of contaminants during the monsoon. For this reason, we propose that it is best to take the mean of premonsoon and postmonsoon to give a better interpretation of an index which highly depends on species diversity and richness. Hence, we recommend seasonal approach which could allow the annual assessment by addressing the worst case and best case scenarios occurring in estuaries subjected to natural and anthropogenic perturbations. Considering the premonsoon and postmonsoon data, amongst the tested indices, M-AMBI succeeded most in producing an ecologically relevant classification of the estuary. The multivariate index, thus, was observed to have fulfilled the criteria of the second hypothesis i.e. to discriminate the accurate EcoQS in the salinity zones considering the seasonal variability.

#### 4.4 Applicability of benthic indices in tropical monsoonal estuaries

The chosen five benthic indices are the most commonly used, based on their robustness and sensitivity, at least in temperate estuaries (Madeiros et al. 2012; Borja et al. 2015; Sánchez-Moyano et al. 2017). As there is no assurance that indices initially developed for temperate areas can assess the estuarine condition on the same scale for tropics, five indices were tested for their effectiveness in the tropical monsoonal Kundalika estuary. It was noted that the typically impoverished macrobenthic assemblages of the estuary interfered with the final EcoQS scores obtained by various methods. As  $H'$  depends more on species types and abundances, rather than sensitivity, its applicability in a tropical monsoonal estuary like Kundalika was error-prone due to the naturally low occurrence of macrobenthos which reduced further during the monsoon. Univariate indices are, in general, good indicators of change in the community structure but their use as an indicator of benthic health can be distorted in estuaries characterised with a natural low diversity (Puente et al. 2008). BO2A and BENTIX were also constrained by low macrobenthic abundances that prevented derivation of values in at some stations affecting final EcoQS. The reduction in the abundances of opportunistic polychaetes in some zones during the monsoon led to the overestimation of BO2A despite very low numbers of amphipods. Thus, the application of BO2A failed in this study, though this index was designed specifically for transitional waters. Reasons for poor performance of BENTIX also included the wrong assignment of species to ecological groups which was highlighted by Sánchez-Moyano et al. (2017) limiting its use in estuaries. It may also be noted that in this investigation, the performance of indices like BENTIX,  $H'$  and BO2A were hampered due to low number of individuals which is typical of estuaries. So therefore, these indices may be applied with caution in an estuarine milieu.

The implementation of AZTI indices (AMBI and M-AMBI) have been tested in tropical coastal waters in general (Bigot et al. 2008; Sivaraj et al. 2014) and estuaries in particular (Khan et al. 2014; Sivadas et al. 2016; Mulik et al. 2017). In Indian estuaries, Khan et al. (2014) and Sigamani et al. (2015) established the suitability of AMBI index in assessing the estuarine EcoQS based on appraisal of individual stations without considering the salinity zones. Conversely, the conclusions of this study was based on the salinity zones following Feebarani et al. (2016) and Mulik et al. (2017) who evaluated the EcoQS of the Cochin and Ulhas estuaries on the west coast of India. Feebarani et al. (2016) assessed the Cochin estuary by considering both the spatial and seasonal variability and a mean EcoQS was obtained considering all three seasons. In contrast, in the Kundalika estuary, a more meaningful EcoQS status was obtained by the exclusion of monsoon data.

## 5. Conclusions and recommendations

Our survey permits an evaluation of the current ecological conditions in the Kundalika estuary, based on a clear distinction between salinity stretches that seems to be a promising approach to assess the EcoQS over time. The M-AMBI index which is a triad of metrics incorporating AMBI, species diversity and richness appeared to perform best in this study in distinguishing areas subjected to various degrees of impact. It can be concluded that, despite the low resolution of M-AMBI at some zones, the seasonal approach yielded better trends in EcoQS classification: the euhaline zone presented a moderate status along with the poly-mesohaline zone and for the oligohaline zone a degraded 'poor' status was obtained. But other applied indices were unable to properly differentiate EcoQS due to various constraints posed by each index. However, derivation of appropriate reference conditions for M-AMBI can be a difficult task and need to be addressed as per local conditions and available data.

The study suggests that the zone-wise evaluation provided a better integrated view of EcoQS, rather than evaluating the status of individual stations which added to the complexity and increased variability. Caution may be exercised while including data of low reliability sites for interpretation of EcoQS in estuaries. As all indices are influenced by species diversity and abundance that is salinity dependant, avoidance of monsoonal data is recommended to prevent possible underestimation. Coherent results can be evolved best by merging data from the non-monsoonal seasons while evaluating tropical monsoonal estuaries. Additionally, the seasonal averaged approach needs to be incorporated into future decision-making processes aimed at effective restoration and conservation management considering expert's opinion, management objectives and available capital budget. More studies are required to be undertaken in differently stressed estuaries on a seasonal scale to underpin the most effective benthic biotic index.

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	<b>Shannon-Weiner</b>	<b>BO2A</b>	<b>BENTIX</b>	<b>AMBI</b>	<b>M-AMBI</b>
<b>Calculation</b>	$H' = -\sum p_i \log_2 p_i$	$BO2A = \log \{(f_{oa}/f_{sa} + 1) + 1\}$	$BENTIX = [6x\%(GI+GIII) + 2x\%(GIII+GIV+GV)]/100$	$AMBI = [0(\%GI) + 1.5(\%GII) + 3(\%GIII) + 4.5(\%GIV) + 6(\%GV)] / 100$	Factor analysis (FA) and discriminant analysis (DA) based on $S$ , $H'$ and AMBI
<b>Eco QS</b>	<b>Range</b>				
High	>4.0	0.0 – 0.02452	4.5 – 6	0.0 – 1.2	>0.77
Good	3.0 – 4.0	0.02452 – 0.13002	3.5 – 4.5	1.2 – 3.3	0.53 – 0.77
Moderate	2.0 – 3.0	0.13002 – 0.19884	2.5 – 3.5	3.3 – 5.0	0.38 – 0.53
Poor	1.0 – 2.0	0.19884 – 0.25512	2.0 – 2.5	5.0 – 6.0	0.20 – 0.38
Bad	0.0 – 1.0	> 0.25512	0.0	6.0 – 7.0	<0.2

**Table 1** Calculation and threshold limits used for ecological status of each index.

$p_i$  – relative abundance of the  $i$ th species,  $f_{oa}$  – opportunistic annelid (polychaeta and clitellata) frequency,  $f_{sa}$  – amphipod frequency (except *Jassa* sp.), GI to GV – ecological groups (see text),  $S$  - species richness and  $H'$  - Shannon–Wiener diversity values.

References for thresholds:  $H'$  - Chainho et al. (2007), BO2A - De-la-Ossa-Carretero and Dauvin (2010), BENTIX - Simboura and Zenetos (2002), AMBI - Borja et al. (2000, 2007), and M-AMBI - Muxika et al. (2007).

Table 2 Average and standard deviation of environmental parameters across seasons according to salinity zones in the Kundalika estuary.

Zones	Euhaline			Poly-mesohaline			Oligohaline		
	Pre	Mon	Post	Pre	Mon	Post	Pre	Mon	Post
Stations	1-5	2-4*	1-4	6-8	5-6	5-7	9-10	7-10	8-10
Salinity	35.0 ± 1.0	31.3 ± 1.6	34.7 ± 0.8	22.4 ± 3.8	18.9 ± 3.7	21.3 ± 6.5	0.6 ± 0.6	1.3 ± 2.5	1.9 ± 3.0
pH	8.2 ± 0.0	8.0 ± 0.1	8.3 ± 0.1	7.9 ± 0.2	7.9 ± 0.1	7.9 ± 0.2	7.5 ± 0.1	7.5 ± 0.2	7.3 ± 0.2
WT (°C)	28.7 ± 0.5	26.1 ± 1.3	28.8 ± 0.6	29.1 ± 0.3	27.4 ± 0.3	28.5 ± 1.1	28.3 ± 0.4	28.2 ± 0.5	29.0 ± 0.1
DO (mg l <sup>-1</sup> )	6.8 ± 0.4	5.9 ± 1.2	6.1 ± 0.2	4.1 ± 1.1	5.6 ± 0.0	4.9 ± 1.2	4.0 ± 2.2	5.9 ± 0.5	4.5 ± 1.9
NH <sub>4</sub> <sup>+</sup> -N (µmol l <sup>-1</sup> )	2.6 ± 1.0	1.8 ± 0.9	0.8 ± 0.1	11.8 ± 8.2	3.6 ± 1.4	1.5 ± 1.1	50.0 ± 4.9	11.5 ± 3.3	11.7 ± 2.8
NO <sub>3</sub> <sup>-</sup> -N (µmol l <sup>-1</sup> )	12.4 ± 4.9	3.3 ± 3.3	1.8 ± 2.5	27.9 ± 12.0	4.4 ± 2.8	5.6 ± 2.3	5.6 ± 0.1	4.3 ± 2.3	1.9 ± 0.8
NO <sub>2</sub> <sup>-</sup> -N (µmol l <sup>-1</sup> )	1.9 ± 2.3	0.9 ± 0.4	0.8 ± 0.7	9.8 ± 6.0	1.0 ± 0.1	14.3 ± 8.2	1.2 ± 1.0	1.7 ± 2.5	6.2 ± 7.6
PO <sub>4</sub> <sup>3-</sup> -P (µmol l <sup>-1</sup> )	1.2 ± 0.2	2.5 ± 1.4	1.1 ± 0.2	1.8 ± 0.3	2.7 ± 0.7	1.8 ± 0.5	3.6 ± 2.5	1.8 ± 0.4	1.8 ± 0.5
SS (mg l <sup>-1</sup> )	35.9 ± 9.9	231.5 ± 48.8	134 ± 63.0	52.7 ± 26.4	142.5 ± 23.3	92.3 ± 20.5	27.8 ± 11.7	24.8 ± 15.3	84.7 ± 13.1
Sediment type	Silty	Silty	Silty	Silty	Sandy-silt	Silty	Sandy-silt	Silty-clay	Sandy-silt
Sand (%)	14.1 ± 9.7	13.1 ± 18.5	8.9 ± 14.5	19.1 ± 16.2	34.3 ± 16.5	12.0 ± 2.8	46.4 ± 50.3	26.6 ± 46.3	34.3 ± 41.3
Silt (%)	79.4 ± 10.5	69 ± 38.3	83.9 ± 14.2	55.1 ± 16.8	39.7 ± 2.4	59.0 ± 22.1	51.6 ± 50.3	33.5 ± 21.1	40.7 ± 22.9
Clay (%)	6.4 ± 1.8	18 ± 19.8	7.2 ± 1.0	25.8 ± 21.9	26 ± 14.1	29.0 ± 19.7	2.0 ± 0.0	39.9 ± 25.4	25.0 ± 21.8
C <sub>org</sub> (%)	2.2 ± 0.3	2.2 ± 0.1	2.1 ± 0.3	2.0 ± 0.2	2.1 ± 0.8	2.2 ± 0.5	2.7 ± 0.1	1.9 ± 1.0	2.0 ± 1.1

\*Stations 1 and 3 were not sampled during monsoon season; Pre=premonsoon, Mon=monsoon, Post=postmonsoon

Table 3 Species density and dominant taxa along the salinity gradient in the Kundalika estuary.

	<b>Euhaline</b>	<b>Poly-mesohaline</b>	<b>Oligohaline</b>
<i>Premonsoon</i>			
<b>Density range</b>	6 – 406 ind m <sup>-2</sup>	6 – 238 ind m <sup>-2</sup>	6 – 325 ind m <sup>-2</sup>
<b>Total density</b>	2001 ind m <sup>-2</sup>	400 ind m <sup>-2</sup>	963 ind m <sup>-2</sup>
<b>Dominant taxa</b>	<i>Paraprionospio patiens</i> (20.3%) <b>P</b> <i>Parheteromastus tenuis</i> (13.1%) <b>P</b> <i>Cossura coasta</i> (11.6%) <b>P</b>	<i>Nephtys paradoxa</i> (59.4%) <b>P</b> <i>Namalycastis ouanaryensis</i> (21.9%) <b>P</b> <i>Glycinde kameruniana</i> (12.5%) <b>P</b>	<i>Namalycastis ouanaryensis</i> (33.7%) <b>P</b> <i>Tubificoides pseudogaster</i> (18.2%) <b>O</b> <i>Tubificoides</i> sp. (14.3%) <b>O</b>
<i>Monsoon</i>			
<b>Density range</b>	6 – 144 ind m <sup>-2</sup>	6 – 94 ind m <sup>-2</sup>	6 – 63 ind m <sup>-2</sup>
<b>Total density</b>	263 ind m <sup>-2</sup>	244 ind m <sup>-2</sup>	200 ind m <sup>-2</sup>
<b>Dominant taxa</b>	<i>Cossura coasta</i> (54.7%) <b>P</b> <i>Glycera capitata</i> (28.5%) <b>P</b>	<i>Nephtys paradoxa</i> (38.4%) <b>P</b> <i>Sternaspis scutata</i> (15.4%) <b>P</b> <i>Lumbriculidae</i> (12.8%) <b>O</b> <i>Capitella capitata</i> (10.2%) <b>P</b> <i>Parheteromastus tenuis</i> (10.2%) <b>P</b>	<i>Namalycastis ouanaryensis</i> (34.4%) <b>P</b> <i>Abra</i> sp. (31.3%) <b>Pe</b> <i>Nephtys paradoxa</i> (21.9%) <b>Pe</b>
<i>Postmonsoon</i>			
<b>Density range</b>	6 – 1831 ind m <sup>-2</sup>	6 – 50 ind m <sup>-2</sup>	6 – 4125 ind m <sup>-2</sup>
<b>Total density</b>	4488 ind m <sup>-2</sup>	263 ind m <sup>-2</sup>	4438 ind m <sup>-2</sup>
<b>Dominant taxa</b>	<i>Paraprionospio patiens</i> (40.8%) <b>P</b> <i>Cossura coasta</i> (35%) <b>P</b>	<i>Namalycastis ouanaryensis</i> (28.5%) <b>P</b> <i>Nephtys paradoxa</i> (9.5%) <b>P</b> <i>Notomastus</i> sp. (9.5%) <b>P</b> <i>Gammurus</i> sp. (9.5%) <b>A</b>	<i>Namalycastis ouanaryensis</i> (92.9%) <b>P</b>
<b>TOTAL (ind m<sup>-2</sup>)</b>	<b>6752</b>	<b>907</b>	<b>5601</b>

Dominant species are listed with their respective contributions to total density composition and the taxa are indicated in bold (A – Amphipoda, O – Oligochaeta, P – Polychaeta, Pe – Pelecypoda).

Table 4 Range and average (between brackets) of the univariate analyses in the study area: abundance (N); species number (S); Margalef's species richness (d); Pielou's species evenness (J'); Hurlbert index (ES100); Shannon-Weiner diversity (H') and Simpson's dominance Index (1- $\lambda'$ ).

Zones	Seasons	S	N	d	J'	ES100	H'	1- $\lambda'$
<b>Euhaline</b>	Pre	1-32 (12)	56-1332 (400)	0-4.3 (1.8)	0.8-0.9 (0.8)	1.0-21.1 (9.5)	0.0-3.7 (2.3)	0.0-0.9 (0.7)
	Mon	4	94-169 (131)	0.6-0.7 (0.6)	0.5-0.8 (0.7)	4	1.0-1.6 (1.3)	0.4-0.6 (0.5)
	Post	6-27 (15)	88-3457 (1122)	0.9-3.9 (2.1)	0.4-0.8 (0.6)	6.0-21.0 (10.5)	1.5-3.7 (2.3)	0.5-0.9 (0.7)
<b>Poly-mesohaline</b>	Pre	1-5 (4)	13-313 (133)	0-0.9 (0.5)	0.6-0.7 (0.6)	1-5 (3.6)	0.0-1.6 (1.0)	0.0-0.5 (0.3)
	Mon	4-7 (6)	100-144 (122)	0.6-1.3 (1.0)	0.8-0.9 (0.8)	4-7 (5.5)	1.5-2.4 (2.0)	0.6-0.8 (0.7)
	Post	5-6 (5)	69-106 (88)	0.9-1.2 (1.0)	0.8-0.9 (0.9)	5-6 (5.5)	1.9-2.4 (2.2)	0.7-0.8 (0.7)
<b>Oligohaline</b>	Pre	1-10 (6)	19-944 (481)	0-1.3 (0.7)	0.8	1-8.7 (4.9)	0.0-2.7 (1.3)	0.0-0.8 (0.4)
	Mon	1-3 (2)	13-81 (50)	0-0.5 (0.2)	0.7-0.8 (0.7)	1-3 (2)	0.0-1.3 (0.7)	0.0-0.6 (0.3)
	Post	3-5 (4)	1069-2075 (1479)	0.3-0.6 (0.4)	0.1-0.4 (0.2)	2.2-4.6 (3.2)	0.2-0.9 (0.5)	0.1-0.3 (0.2)

Pre=premonsoon, Mon=monsoon, Post=postmonsoon

Table 5 Spearman correlation matrix between univariate indices and environmental variables. Only values of statistically significant correlations ( $p < 0.05$ ) are presented.

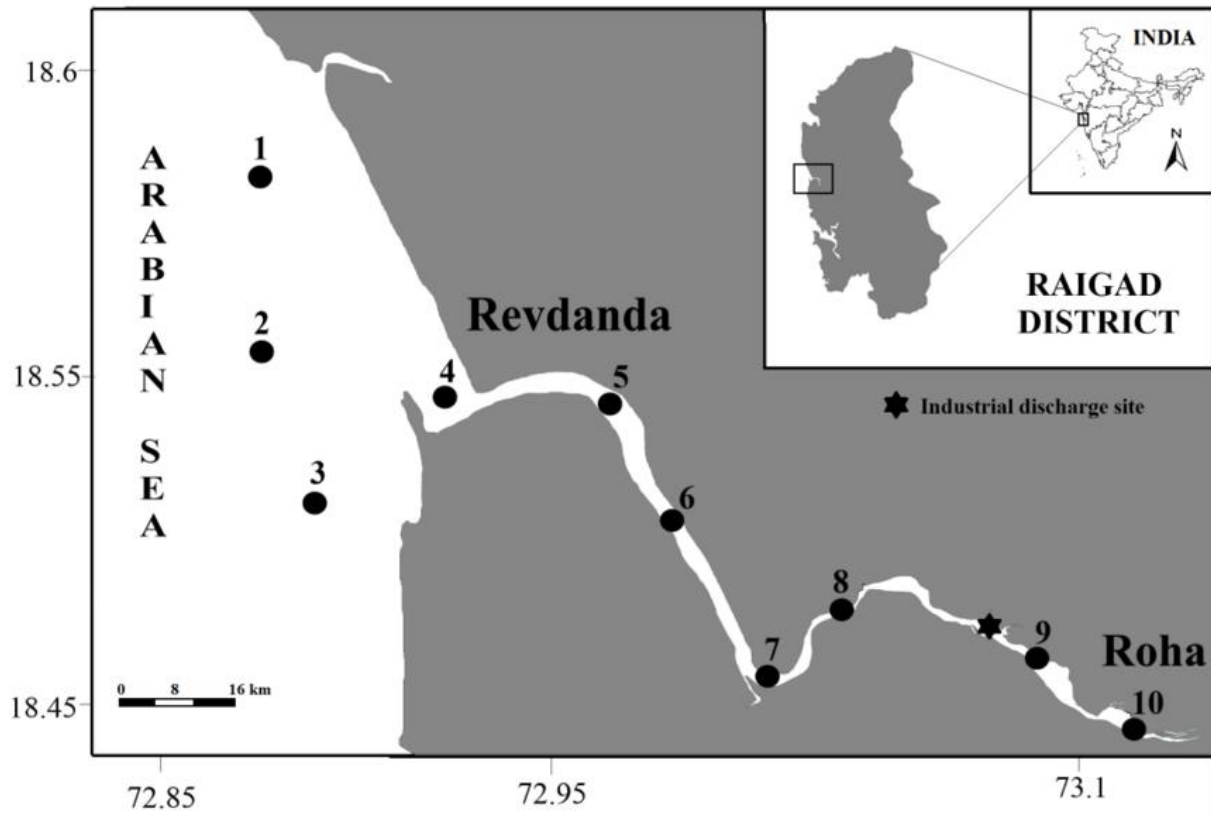
	<b>pH</b>	<b>Salinity</b>	<b>PO<sub>4</sub><sup>3-</sup>-P (<math>\mu\text{mol l}^{-1}</math>)</b>	<b>NO<sub>3</sub><sup>-</sup>-N (<math>\mu\text{mol l}^{-1}</math>)</b>	<b>NH<sub>4</sub><sup>+</sup>-N (<math>\mu\text{mol l}^{-1}</math>)</b>	<b>SS (<math>\text{mg l}^{-1}</math>)</b>	<b>Silt (%)</b>
<b>S</b>	0.5415	0.4958			-0.4507		
<b>N</b>						0.3799	
<b>d</b>	0.6054	0.5573	-0.3872		-0.5080		0.3771
<b>J'</b>				0.4053			
<b>ES(100)</b>	0.5630	0.5246			-0.4816		
<b>H'</b>	0.500	0.4748			-0.4746		
<b>1-<math>\lambda'</math></b>	0.4507	0.4172			-0.4658		

Table 6 Comparative mean status of benthic indices (H', BO2A, BENTIX, AMBI and M-AMBI) according to salinity zones and seasons. (Pre=premonsoon, Mon=monsoon, Post=postmonsoon, mod=moderate).

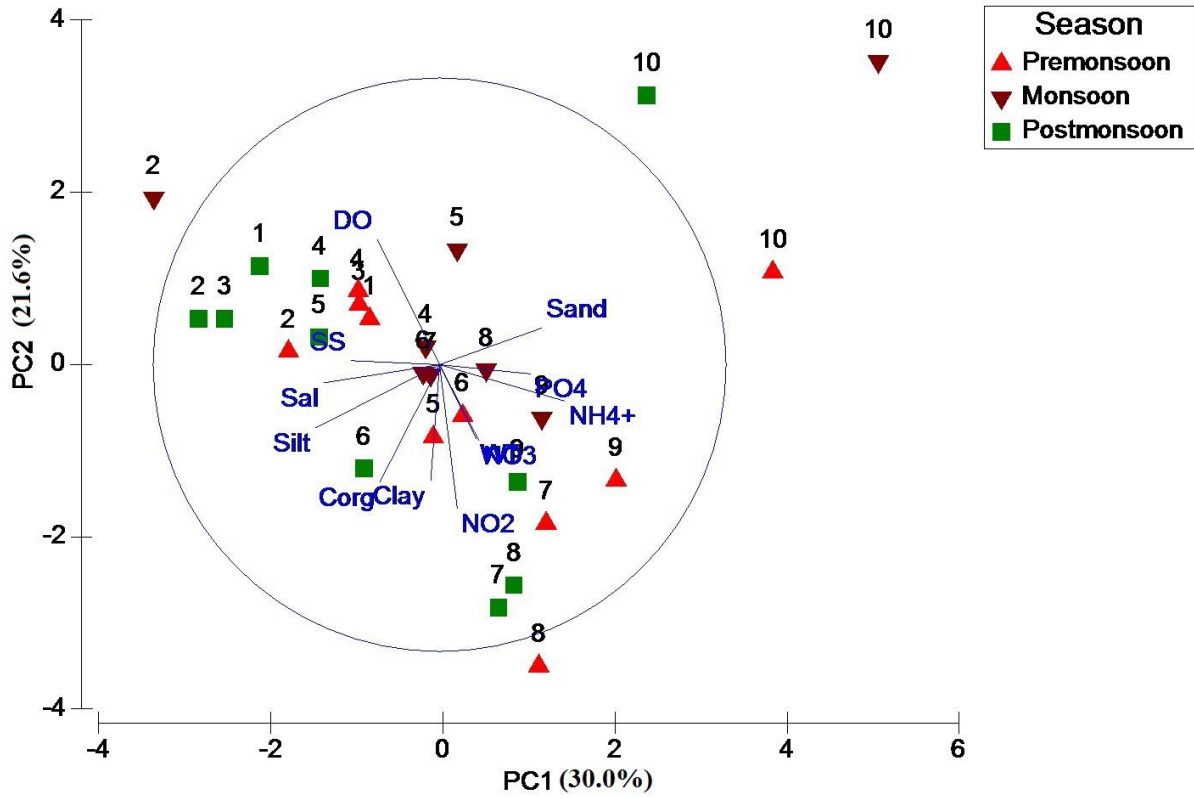
Zones	Seasons	H'	BO2A	BENTIX	AMBI	M-AMBI
<b>Euhaline</b>	Pre	2.31 (mod)	0.21 (poor)	2.88 (mod)	4.22 (mod)	0.46 (mod)
	Mon	1.34 (poor)	0.15 (mod)	3.74 (good)	5.01 (poor)	0.20 (poor)
	Post	2.25 (mod)	0.24 (poor)	2.38 (poor)	4.11 (mod)	0.45 (mod)
	<b>Mean (all seasons)</b>	<b>1.97 (poor)</b>	<b>0.20 (poor)</b>	<b>2.99 (mod)</b>	<b>4.44 (mod)</b>	<b>0.37 (poor)</b>
	<b>Mean (Pre &amp; Post)</b>	<b>2.27 (mod)</b>	<b>0.23 (poor)</b>	<b>2.63 (mod)</b>	<b>4.16 (mod)</b>	<b>0.46 (mod)</b>
<b>Poly-mesohaline</b>	Pre	0.98 (bad)	0.14 (mod)	4.18 (good)	3.46 (mod)	0.35 (poor)
	Mon	1.95 (poor)	0.14 (mod)	3.47 (mod)	3.54 (mod)	0.47 (mod)
	Post	2.17 (mod)	0.15 (mod)	3.72 (good)	3.12 (good)	0.57 (good)
	<b>Mean (all seasons)</b>	<b>1.70 (poor)</b>	<b>0.15 (mod)</b>	<b>3.79 (good)</b>	<b>3.38 (mod)</b>	<b>0.46 (mod)</b>
	<b>Mean (Pre &amp; Post)</b>	<b>1.58 (poor)</b>	<b>0.15 (mod)</b>	<b>3.95 (good)</b>	<b>3.29 (good)</b>	<b>0.46 (mod)</b>
<b>Oligohaline</b>	Pre	1.33 (poor)	0.30 (bad)	2.25 (poor)	4.37 (mod)	0.42 (mod)
	Mon	0.67 (bad)	0.19 (mod)	2.94 (mod)	4.14 (mod)	0.33 (poor)
	Post	0.50 (bad)	0.29 (bad)	2.07 (poor)	4.45 (mod)	0.33 (poor)
	<b>Mean (all seasons)</b>	<b>0.83 (bad)</b>	<b>0.24 (poor)</b>	<b>2.42 (poor)</b>	<b>4.32 (mod)</b>	<b>0.36 (poor)</b>
	<b>Mean (Pre &amp; Post)</b>	<b>0.92 (bad)</b>	<b>0.29 (bad)</b>	<b>2.16 (poor)</b>	<b>4.41 (mod)</b>	<b>0.38 (poor)</b>



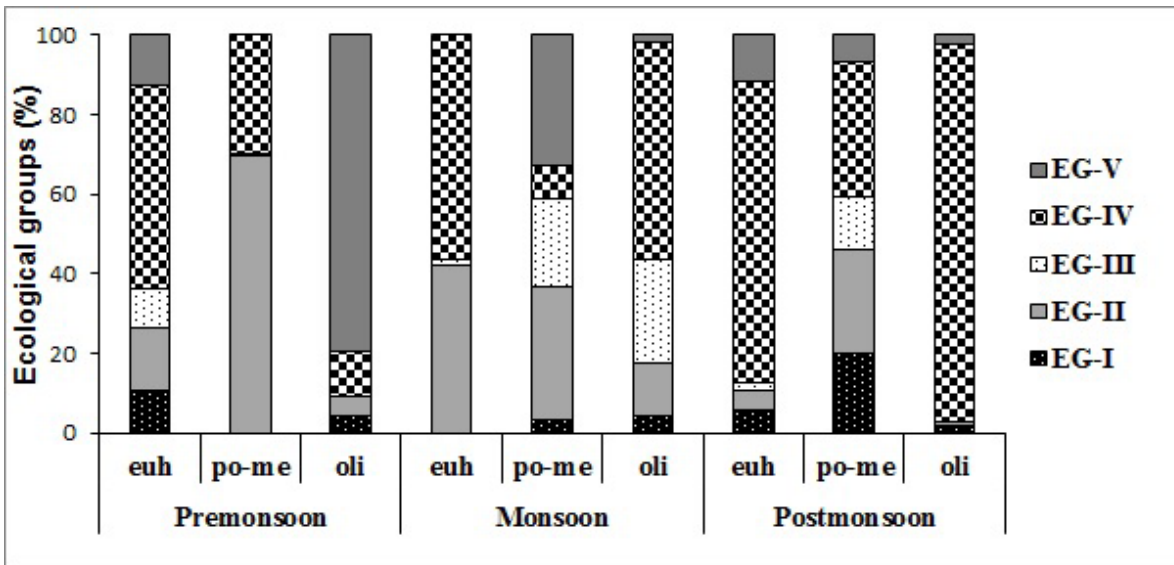
Figures



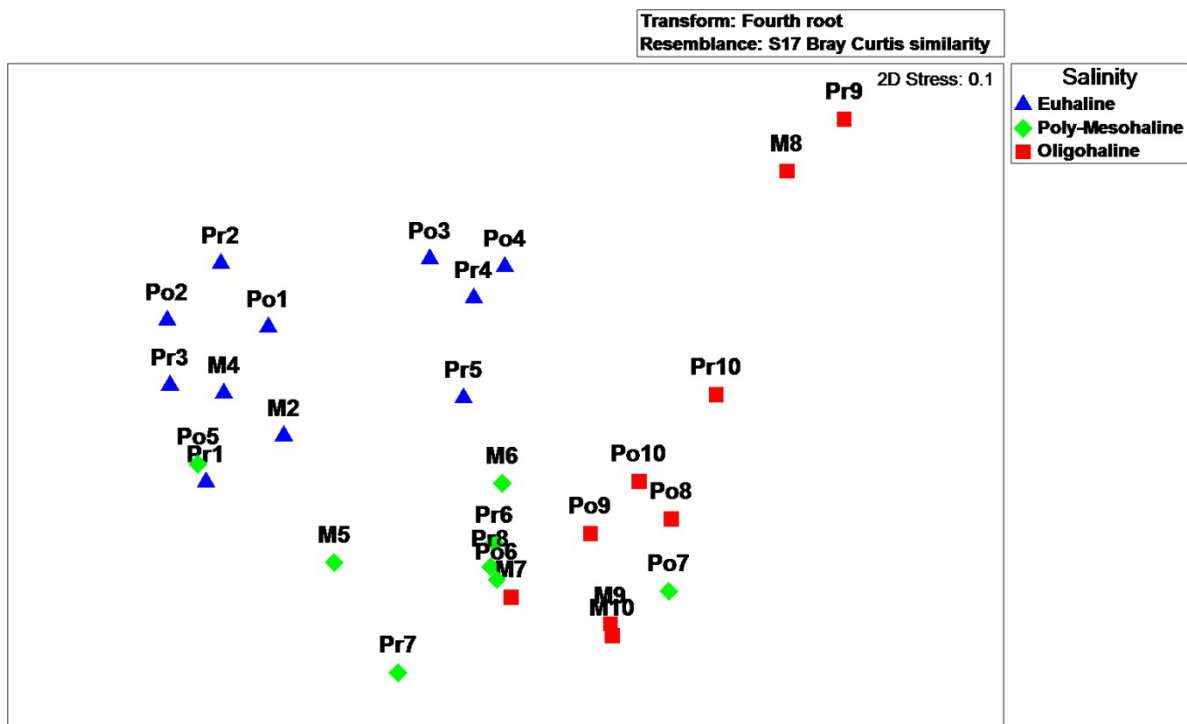
**Fig 1** Study area with station locations.



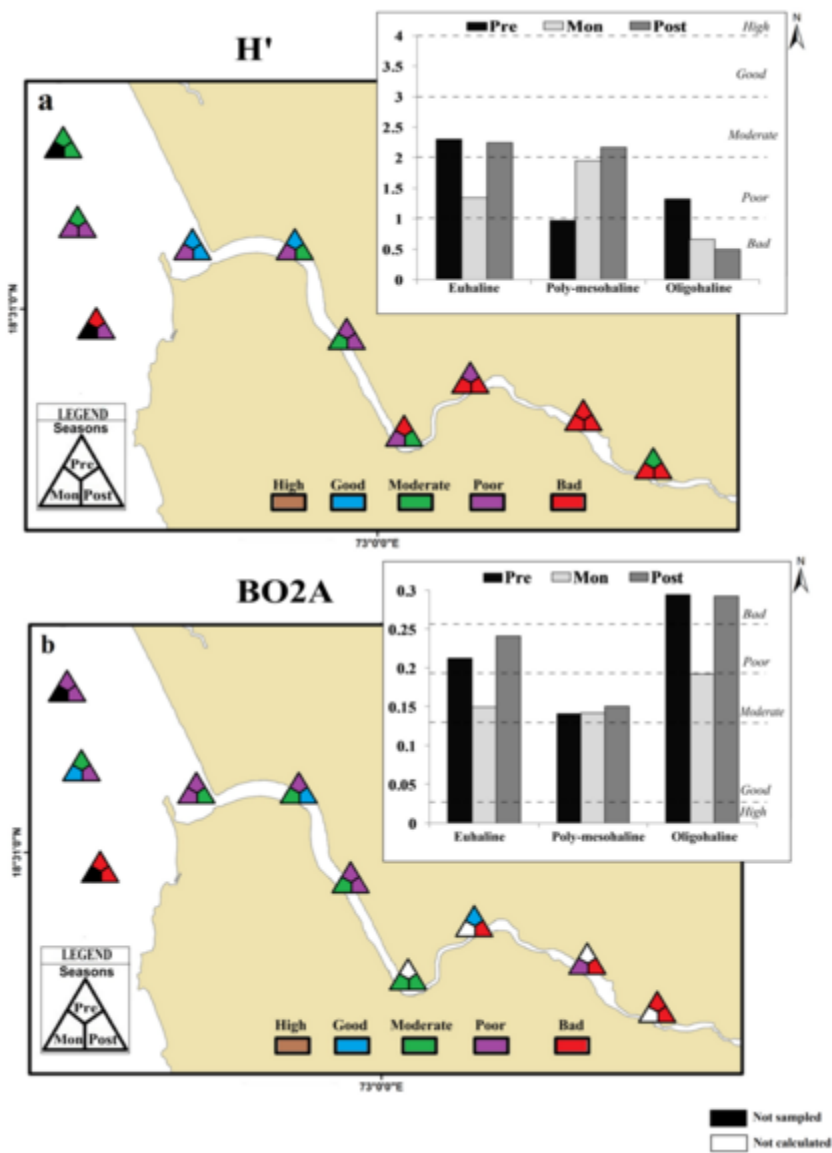
**Fig 2** Principal component analysis (PCA) ordination of the environmental variables of the Kundalika estuary during the three seasons. The points indicate scores and vectors indicate loadings.



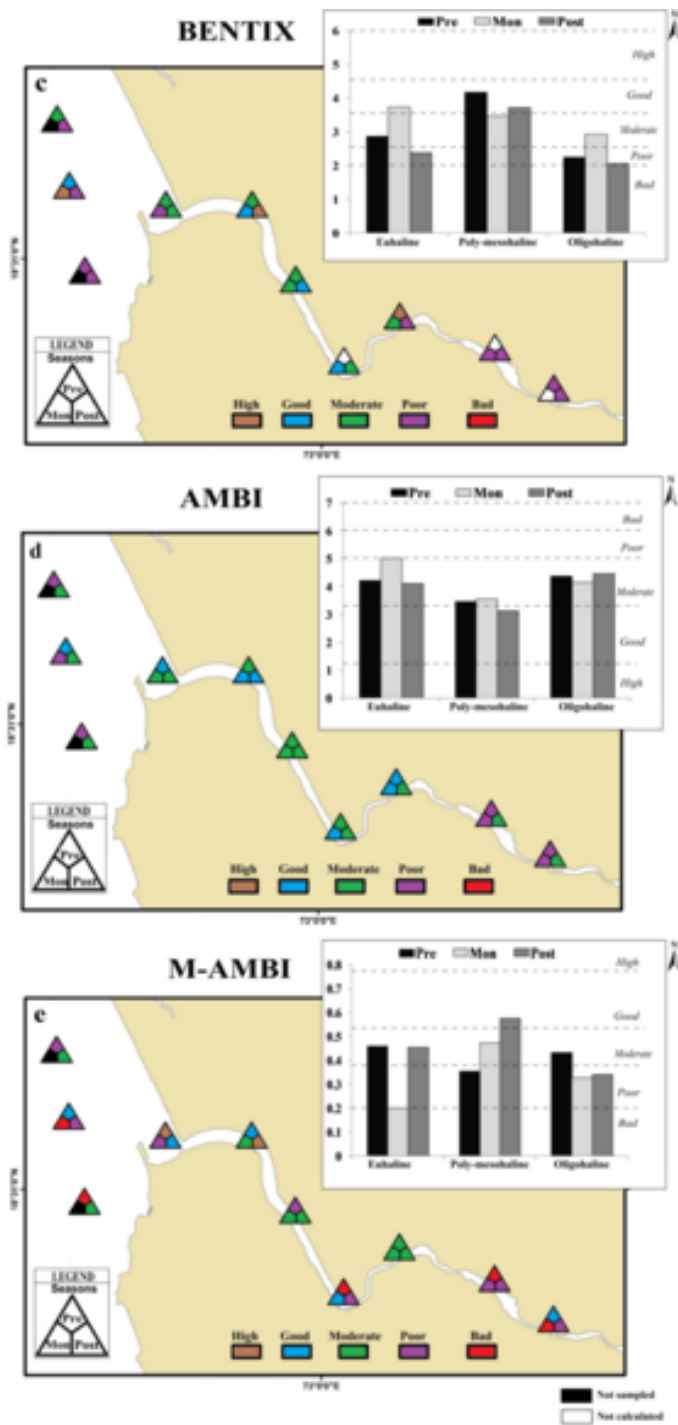
**Fig 3** Percentage of ecological groups of AMBI (EG: I – V) along the salinity zones across seasons. Zones: euh=euhaline, po-me= poly-mesohaline, oli=oligohaline; EG I-V (see text)



**Fig 4** MDS ordination of stations based on species abundance data. Stations are labelled in accordance with seasons (Premonsoon=Pr, Monsoon=M, Postmonsoon=Po)



**Fig 5** Spatial distribution of the ecological quality classification obtained for each index across stations and seasons. Embedded histogram represents quality status across the three zones. **5A.** (a) *H'*, (b) *BO2A*. **5B.** (c) *BENTIX*, (d) *AMBI*, (e) *M-AMBI*



5B. (c) BENTIX, (d) AMBI, (e) M-AMBI

**Appendix 1: List of species present in the study area and their Ecological groups (EG) according to AMBI species list. Assigned EG for the study are given in parentheses.**

<b>POLYCHAETA</b>	<b>EG</b>
<i>Paleanotus chrysolepis</i> (Schmarda, 1861)	I
<i>Phyllodoce malmgreni</i> (Gravier, 1900)	II
<i>Sigambra parva</i> (Day, 1963)	III
<i>Namalycastis ouanaryensis</i> (Saint-Joseph, 1901)	(IV)
<i>Dendronereis arborifera</i> (Peters, 1854)	(IV)
<i>Neanthes glandicincta</i> (Southern, 1921)	I
<i>Nereis</i> sp. (Linnaeus, 1758)	III
<i>Leonnates decipiens</i> (Fauvel, 1929)	(III)
<i>Nephtys</i> sp. (Cuvier, 1817)	II
<i>Aglaophamus dibranchis</i> (Aglaophamus, 1877)	II
<i>Nephtys paradoxa</i> (Malm, 1874)	II
<i>Glycera capitata</i> (Orsted, 1843)	II
<i>Glycinde kameruniana</i> (Augener, 1918)	(II)
<i>Glycinde capensis</i> (Day, 1960)	(II)
<i>Goniada maculate</i> (Orsted, 1843)	II
<i>Diopatra neopolitana capensis</i> (Day, 1960)	(I)
<i>Lumbrineris meteorana</i> (Augener, 1931)	(II)
<i>Lumbrineris aberrans</i> (Day, 1963)	(II)
<i>Lumbrineris latreilli</i> (Audoin and Milne Edwards, 1834)	II
<i>Prionospio</i> sp. (Malmgren, 1867)	NA
<i>Prionospio sexoculata</i> (Augener, 1918)	(IV)
<i>Paraprionospio</i> sp. (Caullery, 1914)	IV
<i>Paraprionospio patiens</i> (Yokoyama, 2007)	(IV)
<i>Magelona cincta</i> (Ehlers, 1908)	I
<i>Tharyx</i> sp. (Webster and Benedict, 1887)	IV
<i>Aphelochaeta filibranchia</i> (Day, 1961)	(IV)
<i>Caulleriella acicula</i> (Day, 1961)	(IV)
<i>Heterospio</i> sp. (Ehlers, 1874)	II
<i>Cossura coasta</i> (Kitamori, 1960)	IV
<i>Cossura longocirrata</i> (Webster and Bendict, 1887)	IV
<i>Capitella capitata</i> (Fabricius, 1780)	V
<i>Notomastus</i> sp. (M. Sars, 1851)	III
<i>Parheteromastus tenuis</i> (Monro, 1937)	V
<b>MALDANIDAE</b> (Malmgren, 1867)	I
<i>Sternaspis scutata</i> (Ranzani, 1817)	III
<i>Isolda pulchella</i> (Muller inGrube, 1858)	III
<i>Ditrupa arietina</i> (O.F.Muller, 1776)	I
<b>AMPHIPODA</b>	
<i>Ampelisca</i> sp. (Kroyer, 1842)	I
<i>Ampelisca abdita</i> (Mills, 1964)	III
<i>Ampelisca diadema</i> (Costa, 1853)	II

## Appendix 1 (contd...)

<b>AMPHIPODA</b>	<b>EG</b>
<i>Ampelisca spinipes</i> (Boeck, 1861)	I
<i>Ampelisca spinifer</i> (Reid, 1951)	I
<i>Paramicrodeutopus schmitti</i> (Shoemaker, 1942)	II
<i>Lembos</i> sp. (Bate, 1857)	I
<i>Autonoe longipes</i> (Liljeborg, 1852)	I
<i>Pontogeneia</i> sp. (Kohlm, 1975)	(II)
<i>Gammarus</i> sp. (Fabricius, 1775)	I
ISAEIDAE (Dana, 1852)	NA
<i>Photis longicaudata</i> (Bate and Westwood, 1862)	I
<i>Cheiriphotis megacheles</i> (Giles, 1885)	(I)
<i>Microprotopus</i> sp. (Norman, 1867)	I
<i>Microprotopus maculatus</i> (Norman, 1867)	I
<i>Erichthonius brasiliensis</i> (Dana, 1853)	I
<i>Corophium</i> sp. (Latreille, 1806)	III
PODOCERIDAE (Leach, 1814)	NA
<i>Dyopedos</i> sp. (Bate, 1857)	III
<i>Dulichia</i> sp. (Kroyer, 1845)	III
<i>Caprella</i> sp. (Lamarck, 1801)	II
<b>OLIGOCHAETA</b>	
<i>Dero</i> sp. (Oken, 1815)	II
<i>Dero digitata</i> (Muller, 1773)	II
<i>Pristina</i> sp. (Ehrenberg, 1828)	V
<i>Tubificoides</i> sp. (Lastockin, 1937)	V
<i>Tubificoides pseudogaster</i> (Baker, 1980)	V
<i>Limnodrilus</i> sp. (Claparede, 1862)	V
ENCHYTRAEIDAE (Vejdovsky, 1879)	V
LUMBRICULIDAE (Vejdovsky, 1879)	(V)
<b>PELECYPODA</b>	
SOLECURTIDAE (d'Orbigny, 1846)	NA
<i>Venerupis</i> sp. (Lamarck, 1818)	I
<i>Barbatia virescens</i> (Reeve, 1844)	(I)
<i>Meretrix</i> sp. (Lamarck, 1799)	(I)
LATERNULIDAE (Hedley, 1818)	NA
<i>Laternula</i> sp. (Roding, 1798)	I
<i>Abra</i> sp. (Lamarck, 1818)	III
<b>OTHER GROUPS</b>	
<i>Pagurapseudopsis gymnophobia</i> (Barnard, 1935)	(III)
Cumacea (Kroyer, 1846)	I
Nemertea (Schultze, 1851)	III
Sipuncula (Rafinesque, 1814)	I
Phoronida (Hetschek, 1888)	I



**Appendix 2: Ecological quality status (EcoQS) and M-AMBI coefficients derived for each station in the Kundalika estuary. Pr= premonsoon, M= monsoon, Po= postmonsoon.**

Zones	Stations	AMBI	Diversity	Richness	X	Y	Z	M-AMBI	Status
Euhaline	Pr1	5.3438	2.2359	5	0.69394	0.94878	0.38342	0.28468	Poor
	Pr2	2.6518	2.4226	7	0.24397	1.3292	0.50382	0.56335	Good
	Pr3	5.75	0	1	-1.9126	-2.0456	-1.8361	0.032197	Bad
	Pr4	3.3073	3.9173	31	2.4351	1.9203	1.9347	0.8493	High
	Pr5	4.029	3.3333	13	0.66145	0.65812	0.82805	0.56712	Good
	M2	5.8295	1.0958	3	-1.3867	-1.7312	-1.1973	0.13095	Bad
	M4	4.1853	0.77048	4	0.98897	0.47506	0.90075	0.26416	Poor
	Po1	3.9375	2.064	6	0.30446	0.16084	0.06736	0.40842	Moderate
	Po2	3.9583	1.5088	6	0.51008	0.01896	0.37371	0.3624	Poor
	Po3	4.2182	1.7493	19	0.49708	0.21066	0.11847	0.4807	Moderate
Po4	4.321	2.9806	18	0.83717	0.46117	0.72484	0.55976	Good	
Poly-mesohaline	Pr6	4.161	1.58	5	-0.5463	0.53585	-0.0317	0.38308	Poor
	Pr7	4.25	0	1	-2.4233	-1.2774	0.05833	0.15247	Bad
	Pr8	1.982	1.35	5	0.16017	0.96094	0.01881	0.52561	Moderate
	M5	2.604	1.49	4	-0.3913	0.47505	-0.0387	0.46911	Moderate
	M6	4.484	2.41	7	0.32795	0.41721	-0.027	0.47445	Moderate
	Po5	2.417	3.49	14	2.8684	1.8428	0.18007	0.87607	High
	Po6	3.962	2.2	6	0.13217	0.17145	0.04003	0.47315	Moderate
	Po7	4.442	1.99	4	0.49358	0.71517	0.10527	0.37507	Poor
Oligohaline	Pr9	3.5	0	1	1.3464	-0.2291	0.93945	0.19906	Bad
	Pr10	5.727	1.5919	9	-2.2579	0.50284	-1.3834	0.66529	Good
	M7	3.1875	1.2957	3	0.51194	0.93198	0.72594	0.5505	Good
	M8	2.0625	0.65002	2	0.30762	-1.4597	0.00948	0.45837	Moderate
	M9	5.5625	0.72193	2	0.53044	0.91684	0.22075	0.24549	Poor
	M10	5.75	0	1	1.5352	1.3136	1.1393	0.053018	Bad
	Po8	4.3852	0.8623	6	-0.8181	-0.0762	0.40849	0.49697	Moderate
	Po9	4.5743	0.30174	2	0.86145	0.37495	0.60449	0.2261	Poor
	Po10	4.4008	0.24558	4	0.34647	0.20388	0.46854	0.29978	Poor