

## **Can the ecological status of three differentially impacted monsoonal tropical estuaries in NW India, be adequately assessed by a common estuarine benthic index?**

**Jyoti Mulik, Soniya Sukumaran\*, Heidy Q. Dias**

CSIR-National Institute of Oceanography, Regional Centre, Andheri (W), Mumbai 400 053, India

\*Corresponding author. Tel.: +91 22 2635 9605; fax: +91 22 2636 4627.

E-mail address: [soniya@nio.org](mailto:soniya@nio.org) (Soniya Sukumaran).

### **Abstract**

Effective management measures for improving rapidly deteriorating estuaries around the world are hugely dependent on accurate evaluation of the ecological status, which is a challenge in itself considering the Estuarine Quality Paradox. Several Benthic Biotic Indices (BBIs) have been tested in estuaries in different bio-geographical zones with no concurrence whatsoever about the best approach in evaluating the ecological status. An often suggested approach is to apply several indices concurrently to gauge the overall status. However, this method typically results in more complexities due to the multiple contradicting conclusions derived by different indices besides increasing the computational load and the requirement of data matrices. Therefore, it would be reasonable to validate available BBIs in various estuaries of a biogeographical zone and select a common estuarine benthic index that can respond effectively to diverse stressors, considering the natural estuarine gradients. Towards this goal, five most frequently employed BBIs viz. AZTI's Marine Biotic Index (AMBI), Multivariate AZTI's Marine Biotic Index (M-AMBI), BENTIX, Benthic Opportunistic Polychaete Amphipoda index (BOPA) and Benthic Opportunistic Oligochaete Amphipoda index (BO2A) were applied in the three differently stressed estuaries (Ulhas, Amba and Savitri) of NW India during the three major seasons (premonsoon, monsoon and postmonsoon). It was aimed to test if any of the applied BBIs could appropriately determine the ecological status across salinity zones of all the estuaries and thus can act as a common estuarine index. While the indices generally concurred in high salinity zones of the heavily polluted Ulhas, discrepancies in ecological status were observed in other zones and estuaries, mostly erroneous when compared to the pre-determined status. As monsoonal index values appeared to have been influenced by the disturbance due to fresh water inflow during this season, the average of the two non-monsoonal seasons was considered for deriving the final status by all indices. Among the BBIs, AMBI exhibited stronger response to pressure gradients and was most successful in discriminating the correct ecological status of most of the salinity zones across the three estuaries. Thus, this index could reasonably be considered for ecological status evaluation of estuaries of NW India irrespective of the diverse anthropogenic stressors and macrobenthic composition. It is recommended that this conclusion be reaffirmed by repeating similar investigations in other monsoonal tropical estuaries of this zone.

**Keywords:** Benthic biotic indices; Macrobenthos; Salinity zones; Estuarine; AMBI; India

## **Introduction**

Incremental industrialization and urbanization during the last few decades have considerably affected estuarine and coastal marine ecosystems necessitating regular assessment of ecological health and adoption of management interventions (Lotze, 2010; Ryu et al., 2016; Ni et al., 2019). Recently, several sets of legislation worldwide such as Oceans Act 2000 and Clean Water Act (1972 amended in 1977) in USA, Australia and Canada, Water Framework Directive (WFD, 2000/ 60/EC) or Marine Strategy Framework Directive (MSFD, 2008/56/EC) in Europe and National Water Act in South Africa, etc. have been developed in order to determine the ecological integrity of the estuarine and coastal waters. Among the various biological quality descriptors, macrofaunal communities are often considered as powerful bio-indicators, because of their rapid responses to multiple kinds of disturbances (Dauer, 1993; Mistri et al., 2017; Pitacco et al., 2018). Accordingly, several Benthic Biotic Indices (BBIs) based on the Pearson and Rosenberg (1978) paradigm have been developed to assess the health of estuarine and marine waters under different legislation worldwide (Borja et al., 2008a; Borja et al., 2015).

Estuaries are unique systems harbouring communities with high productivity and comparatively low biodiversity. These assemblages, besides being exposed to natural spatio-temporal variations in physico-chemical characteristics, are also vulnerable to various anthropic stressors. Thus, present day estuaries are highly altered ecosystems requiring frequent surveillance and immediate management intercessions (Wetzel et al., 2012). Several methodologies have been devised and tested to estimate the biological integrity of estuaries worldwide. BBIs, such as AMBI (Borja et al., 2000), M-AMBI (Muxika et al., 2007), BENTIX (Simboura and Zenetos, 2002), BOPA (Dauvin and Ruellet, 2007) and BO2A (Dauvin and Ruellet, 2009) are based on the sensitivity of macrobenthic species to an environmental pressure gradient. These indices have been employed successfully in temperate (Dauvin et al., 2012; Nebra et al., 2014) as well as tropical/sub-tropical estuaries (Borja and Tunberg, 2011; Khan et al., 2014; Sigamani et al., 2015; Feebarani et al., 2016; Mulik et al., 2017; Rehitha et al., 2017; Dias et al., 2018).

Although multiple BBIs are in use for the assessment of estuarine waters, presently there is no agreement regarding the best approach (Muniz et al., 2012). Intercalibration of different methodologies of benthic ecological assessment within the WFD framework have been attempted to achieve consistency of results (Borja et al, 2007, 2009). On the contrary, there is a school of thought that considers indices to be specific to certain stressors, communities or sites (Pinto et al., 2009). Macrobenthic communities belonging to different geographical areas will be dissimilar owing to

diverse environmental conditions that could prevent the universal application of any one index. Also, these indices may respond differently to diverse natural and anthropogenic perturbations (Muniz et al., 2012). Several studies have found indices to be susceptible to natural environmental gradients such as salinity (Fleischer and Zettler, 2009), sediment type and locality (Blanchet et al., 2008, Teixeira et al., 2008, Pelletier et al., 2018). The susceptibility of indices to seasonality, particularly in estuaries, has also been observed (Chainho et al., 2007; Sivadas et al., 2016; Mulik et al., 2020a). Hence, discrimination of an accurate ecological status of estuaries is highly dependent on the critical choice of the BBI to be employed.

The selection of an index is a major concern as various indices frequently present conflicting classifications of ecological status for the same study area (Borja et al., 2007 and references therein). The output of benthic indices may vary in diverse geographical areas as each index was primarily designed for a specific environmental setting (Salas et al., 2006). Hence, to choose the appropriate index for the ecological status evaluation of particular sites, comparisons of different indices and their response to the environmental pressures are required (Wetzel et al., 2012). Studies have recommended application of several indices to get a better evaluation of the ecological status of estuaries considering that the benthic communities are complex and geographically disparate (Salas et al., 2006; Wetzel et al., 2012) and that indices were originally developed for specific stressors (Afli et al., 2008). However this approach has its own inherent disadvantages in regular environmental studies. Estimation of multiple indices will require different types of data matrices and computations resulting in increased time and cost overruns. Long term comparisons of ecological status of an estuary during different years or even between estuaries within ecoregion would be complex if a medley of indices were applied. The surfeit of information resulting from such a method could render the comprehension and communication of the ecological health difficult to the end user thus defeating the very purpose of the indices i.e. synoptic representation of complex ecosystem data. Moreover, multiple indices more often than not are known to result in assignment of contradictory ecological status to the same area rendering decision making cumbersome or unsatisfactory (Blanchet et al., 2008, Wetzel et al., 2012). Thus, it would be convenient to identify and apply a single estuarine index that can effectively respond to multiple stressors and evaluate the correct ecological status of estuaries located within the same large-scale geographical region, considering its natural variability (Pelletier et al., 2018). This should facilitate robust monitoring programmes especially confronted with present day budgetary constraints.

The estuaries of the north-west coast of India originate in the western slopes of Sahyadrian ranges and drain into the Arabian Sea after following a westerly course. These estuaries are subject to pronounced natural variations due to the distinct monsoon season resulting in significant freshwater inflow and flushing (Rao et al., 2019). Almost all estuaries along this coast have been impacted by various anthropogenic activities and the resultant deteriorating ecology has raised concerns in the local administration and general public. To identify a commonly applicable benthic index to evaluate the estuarine ecological status in this geographical area, three estuaries were chosen i.e. Ulhas, Amba and Savitri (Fig. 1). The Ulhas is highly polluted from a variety of sources such as untreated domestic sewage (511 MLD; NIO, 2018), industrial effluents (46.5 MLD; NIO, 2016), agricultural and terrestrial runoff (NIO, 2018) due to its proximity to the urbanized and industrialized Thane-Mumbai metropolis (Athalye et al., 2003). Low oxygen conditions are known to prevail in the poly-mesohaline stretch of the estuary during certain tidal phases (NIO, 2013). A 'poor' to 'bad' ecological quality status in the middle and upstream zones of the Ulhas to an improved status in the downstream region is already reported (Mulik et al., 2017). The Amba and Savitri estuaries too receive domestic effluents in the oligohaline zone but to a lesser extent than Ulhas. Amba receives treated industrial effluents (4.5 MLD) at lower estuary (NIO, 2018). In Savitri, combined treated industrial effluents (6.0 MLD) are released in the meso-oligohaline zone (NIO, 2018). Apart from the industrial effluents, the meso-oligohaline zone of the estuary receives 7.5 MLD of domestic sewage (NIO, 2018). All three estuaries are also subject to dredging albeit to varying levels.

Consistent macrobenthic and hydro-sedimentological data for the three estuaries were acquired during 2013 for the three major seasons i.e. premonsoon, monsoon and postmonsoon to investigate the spatio-temporal variability of the macrobenthic communities and the major factors influencing the distribution patterns (Mulik et al., 2020b). The major observations of this study were that (i) the hydro-sedimentological characteristics and macrobenthic assemblages were significantly different between the three estuaries, zones and seasons due to their exposure to varied natural and anthropic stressors (ii) the benthic distribution patterns of all three estuaries were primarily dictated by the salinity changes and (iii) while anthropogenic factors too exerted influence on community dynamics in the Ulhas and Amba, no such factor was discernible in the case of Savitri. Thus, these primary datasets provided us the appropriate template to test our hypothesis that a common estuarine benthic index for a geographical region should i) respond effectively to diverse anthropogenic stressors and ii) give consistent results irrespective of dissimilar macrobenthic assemblages and across salinity gradients of the estuaries located in that particular zone. Towards this end, a set of five most effective and successful estuarine benthic indices (Pinto et al., 2009, Borja et al., 2019)

were tested in the three estuaries to identify a possible common estuarine index. Hence, the objective of this study was to ascertain which among the five benthic indices applied, could describe the correct ecological status in all the three estuaries that can be considered as a common estuarine index.

## **2. Materials and Methods**

### **2.1 Study sites**

The estuaries along the NW Indian coast receive substantial fresh water discharges during the monsoon season that extends from June to September. The Ulhas trails the westerly course of about 100 km and meets the Arabian Sea at Vasai (19° 16'N and 72° 45'E). The estuary receives an average freshwater discharge of about 146 m<sup>3</sup>·s<sup>-1</sup> (Central Water Commission; CWC Nasik). The flushing time of the Ulhas varies between 73 and 211 tidal cycles during the non-monsoonal seasons leading to the risk of an increase of pollutants in the inner estuary (Ram et al., 2009). Average annual rainfall of the Ulhas is about 2943 mm. The Amba runs along a stretch of over 140 km before opening into the Mumbai harbour (18° 50'N and 72° 54'E). The estuary receives an average freshwater discharge of 96 m<sup>3</sup>·s<sup>-1</sup> (Velamala et al., 2016). The flushing time of the Amba varies from 6 to 26 tidal cycles depending on the tide during non-monsoonal seasons (Dinesh Kumar et al., 1997). Average annual rainfall of Amba is about 2100 mm. The Savitri connects to the Arabian Sea at Bankot (17° 59'N and 73° 02'E). The estuary receives an average freshwater discharge of 264.5 m<sup>3</sup> s<sup>-1</sup> (CWC Nasik). The flushing time of the Savitri varies from 28 to 111 tidal cycles depending on the tide during non-monsoonal seasons (NIO, 1994). Average annual rainfall of Savitri is about 3560 mm.

### **2.2 Sample collection and laboratory analysis**

The survey was conducted at twelve (U1-U12), thirteen (A1-A13) and eight (S1-S8) subtidal stations of Ulhas, Amba and Savitri respectively covering the estuarine zone (Fig.1) during three consecutive seasons i.e. premonsoon (May), monsoon (September) and postmonsoon (November/December) of 2013. Bottom water samples were collected in duplicate at each station during the ebb tide by using the Niskin bottles. These samples were stored in ice immediately after collection and were taken to the laboratory where these were analysed for salinity, dissolved oxygen (DO), 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). Sediment samples were collected in duplicate for the analysis of texture and organic carbon (C<sub>org</sub>). Salinity, DO and nutrients were analyzed using standard methods (Grasshoff et al., 1999). Sediment texture was

analyzed by a combined sieving and pipette method (Buchanan, 1984) and organic carbon ( $C_{org}$ ) by titration method (Walkey and Black, 1934).

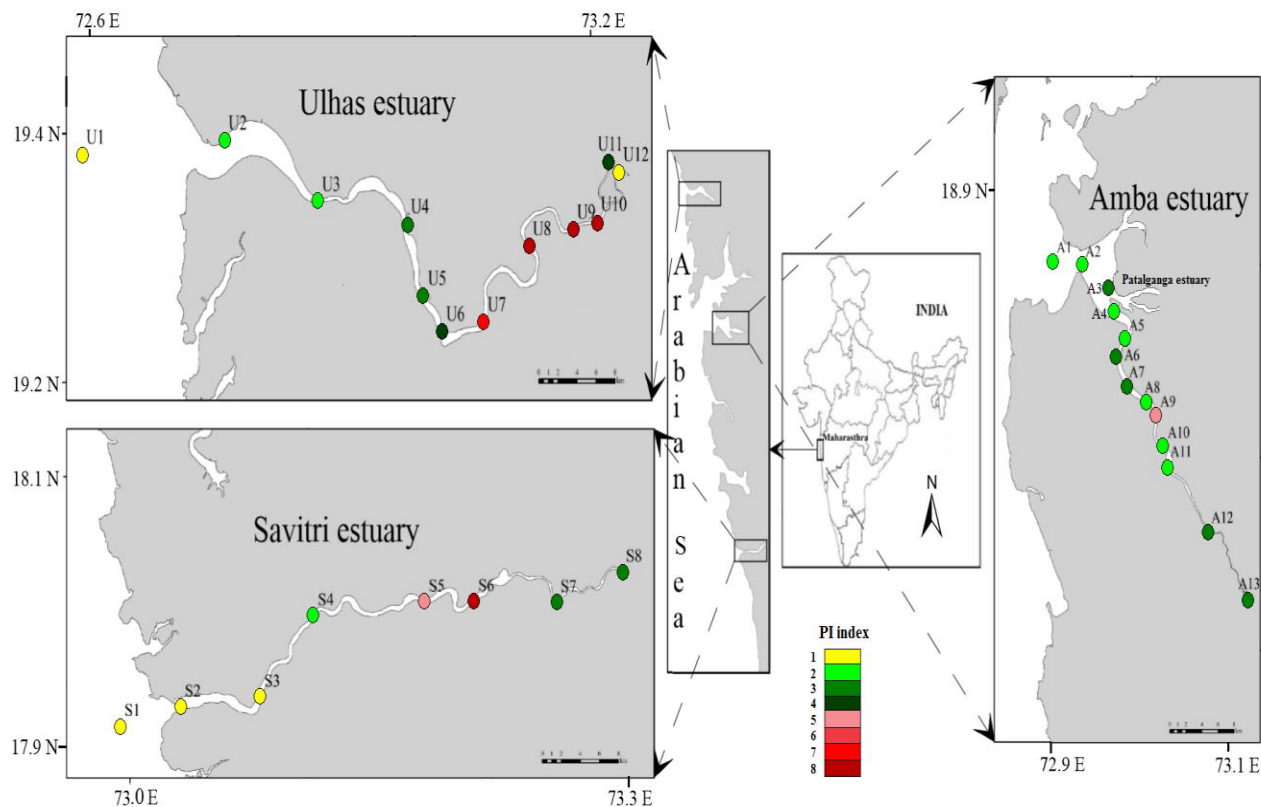


Fig. 1 Map with the three estuarine ecosystem (Ulhas, Amba and Savitri) and the sampling stations with different colours representing the intensity of human stress for each estuary by means of PI (Pressure Index)

For the study of macrobenthos, four replicates of sediment samples were collected at each station during each season using a van Veen grab of  $0.04 \text{ m}^2$  bite area. The macrobenthic samples upon collection were carefully washed and sieved through a  $0.5 \text{ mm}$  mesh sieve. The organisms retained after sieving were fixed in 5% formalin mixed with Rose Bengal. In the laboratory, organisms were then sorted, identified to the lowest possible taxonomic level (mostly species), counted and expressed as number of individuals per square meter ( $\text{ind. m}^{-2}$ ). The species-level identification was done following the available identification keys (Day, 1967; Lyla et al., 1998).

The anthropogenic pressures for each sampling station of all the three estuaries were quantified on a scale of 0 to 3 (0: no pressure, 1: low, 2: medium and 3: high) based upon the best professional judgment following the approach proposed by Aubry and Elliott (2006). A Pressure Index (PI) was calculated as the total of partial pressures for each station.

### 2.3 Computation of ecological indices

Five BBIs were calculated using the macrobenthic species density matrix for each station of each estuary during the three seasons. An earlier study had established unequivocally that salinity was the major determinant of macrobenthic species composition in all three estuaries (Mulik et al., 2020b). Therefore, in this investigation, to remove the salinity bias, the ecological status assessment of the three estuaries by the five benthic indices were undertaken considering three salinity zones in each estuary (euhaline, poly-mesohaline and oligohaline) based on the Venice classification system. A seasonal average approach was adopted to arrive at a final mean status for each salinity zone by averaging ecological status of non-monsoonal seasons (premonsoon and postmonsoon) of each index as suggested by Dias et al. (2018). The thresholds for the biotic indices used in the present study to determine different classes of ecological quality status are mentioned by Mulik et al. (2017).

#### (1) AMBI

AMBI is computed on the basis of classification of macrobenthic species into five ecological groups (EGs) according to their sensitivity i.e. from most sensitive species (EG I) to most opportunistic species (EG V). The index varies between 0 (best condition) and 7 (worst condition). The AMBI version 5.0 software that is freely available at <http://ambi.azti.es> (as per June 2017 species list), was used to calculate this index. Fish larvae, juveniles, decapod larvae were excluded from the calculation following guidelines of Borja and Muxika (2005).

#### (2) M-AMBI

M-AMBI values are derived by the application of discriminant analysis techniques, together with factor analysis employing the combination of AMBI values, species richness and diversity through the use of the Shannon-Wiener index. This fulfills the requirement of the use of more than a single index to evaluate the overall ecological status of an area (Zettler et al., 2007). For deriving M-AMBI values, reference conditions for three salinity zones, i.e. euhaline, poly-mesohaline and oligohaline of all the three estuaries were obtained separately (Feebarani et al., 2016) by increasing the highest species richness and diversity of all replicates by 15% (Cai et al., 2014) for each zone. The reference conditions for calculating M-AMBI were provided in Table 1.

Estuary	Euhaline			Poly-mesohaline			Oligohaline		
	AMBI	<i>H'</i>	<i>S</i>	AMBI	<i>H'</i>	<i>S</i>	AMBI	<i>H'</i>	<i>S</i>
Ulhas	1.1	4.7	40	3.0	2.5	22	2.3	2.0	6
Amba	1.8	5.1	32	1.0	4.5	30	3.9	2.7	7
Savitri	0	2.8	14	1.4	3.0	13	1.5	2.4	7

**Table 1:** Reference conditions for the three salinity zones of the three estuaries. *H'*: diversity, *S*: richness.

(3) BENTIX

BENTIX index was calculated using BENTIX Add-In v. 1.0 which is freely downloadable from [http://www.hcmr.gr/english\\_site/services/env\\_aspects/bentix.html](http://www.hcmr.gr/english_site/services/env_aspects/bentix.html). This index considers sensitive and tolerant taxa based on the reduction of five EG's (as per AMBI) to two EG's, i.e. EG I, II as EG I and EG III, IV, V as EG II. BENTIX index ranges between 0 (azoic) to 6 (pristine).

(4) BOPA

BOPA is based on the taxonomic sufficiency principle that uses relative frequencies of opportunistic polychaetes and amphipods (except for *Jassa* species). The BOPA will be null when there are no opportunistic polychaetes, indicating an area with a very little amount of organic matter. Also, when the index gives low values it is considered that the area has a 'good' ecological quality status with a small number of opportunistic species.

(5) BO2A

BO2A is an adaptation of BOPA that incorporates the abundance of annelid groups such as Clitellata along with the opportunistic polychaetes instead of only the latter group being used in BOPA estimations.

Considering the inherent natural complexity of the estuarine ecosystem (i.e. Estuarine Quality Paradox) (Elliott and Quintino, 2007), identification of a common estuarine index that can detect anthropogenic pressures requires assessment of the relative performance of indices against *a priori* established ecological status of differently modified estuaries (Borja and Dauer, 2008, Patrício et al., 2009). The ecological status of the salinity zones were classified initially based on a combination of personal observations, best professional judgement, abiotic descriptors and pressure index (Pelletier



et al., 2018) against which each of the five benthic indices were validated. Hence, it was concluded that, the euhaline zone of all three estuaries was slightly impacted ('good' status). The poly-mesohaline zone of Ulhas was heavily polluted ('poor' status) while that of Amba and Savitri were moderately stressed ('moderate' status). The oligohaline zones of Ulhas and Savitri were stressed to some extent ('moderate' status) and Amba was comparatively less disturbed ('good' status).

## **2.4 Statistical treatment**

The statistical programs, PRIMER v7 (Clarke and Gorley, 2015) was used to conduct a Principal Component Analyses (PCA) on abiotic variables to view the distribution pattern of stations of individual estuaries based on their environmental factors. Spearman's rank correlation was calculated between the different index values, the pressure index (PI) and the contamination proxies ( $\text{NO}_2^-$ -N,  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N and  $\text{C}_{\text{org}}$ ) using Statistica 7 to verify the response of each index. The  $p$ -value  $<0.05$  was selected as a threshold for significance. A shade plot, an easy visualization of the frequency matrix (Tweedley et al., 2015), was constructed to view the frequency of occurrence of the most abundant macrobenthic taxa (y-axis) across estuaries, seasons and salinity zones (x-axis). The species abundance data were square-root transformed for better visualisation. The shade plot was restricted to the taxa that contributed  $\geq 10\%$  to any sample (50 taxa).

## **3. Results**

### **3.1 Environmental characteristics**

The constituent stations of the salinity zones during three seasons of the estuaries are given in Fig. 2. In Amba, oligohaline zone during the premonsoon could not be established due to marine dominated nature of the estuary.

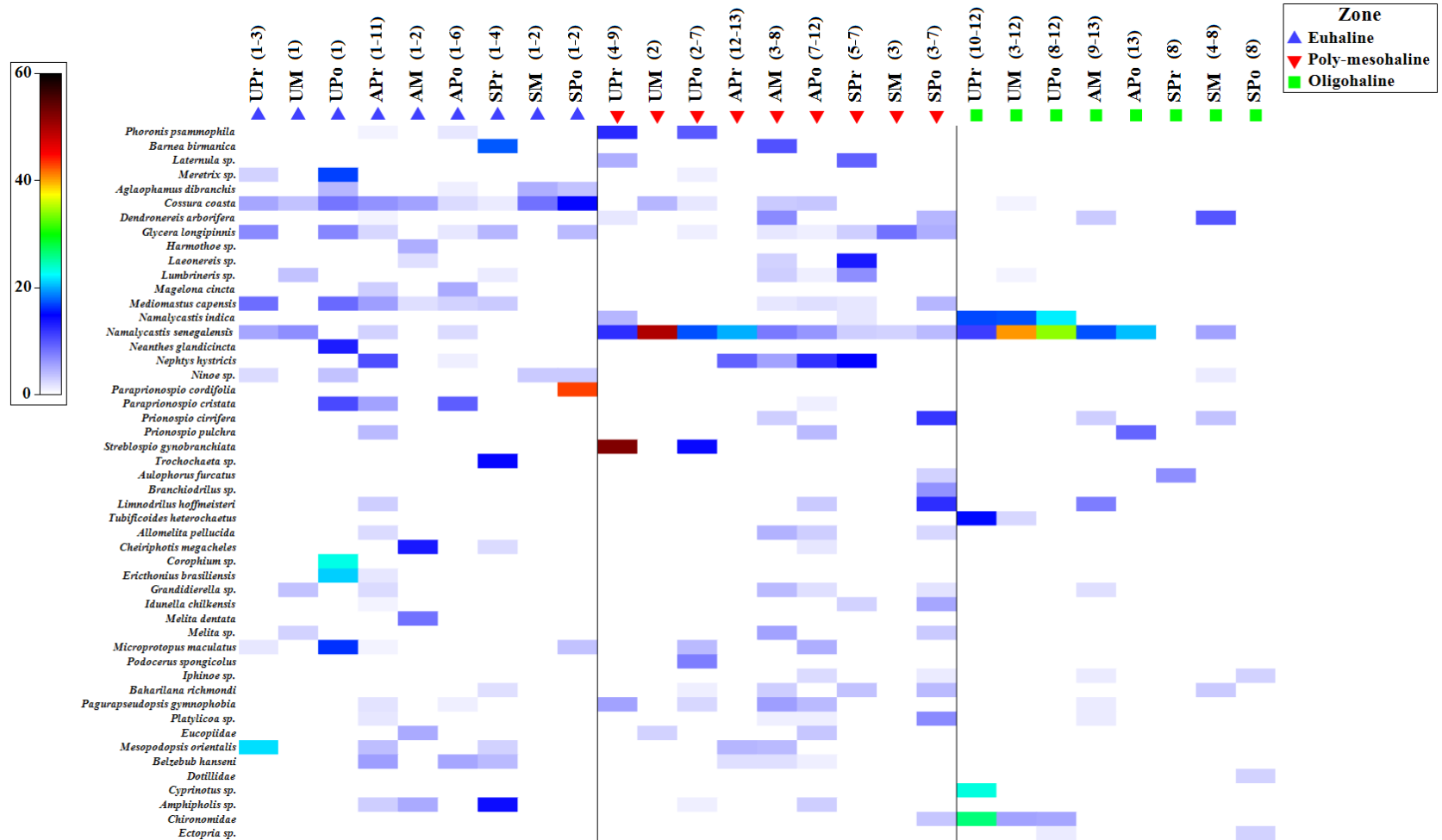


Fig. 2 Shade plot representing the square root transformed densities of the 50 most abundant macrobenthic taxa across estuaries (U=Ulhas, A=Amba, S=Savitri), seasons Pr=Premonsoon, M=Monsoon, Po=Postmonsoon) and salinity zones (Euhaline, Poly-mesohaline, Oligohaline)

Spatial and temporal variations in salinity were distinct in all three estuaries (Appendix 1). In comparison with the other two estuaries, significantly lower DO values were recorded at the poly-mesohaline zone of Ulhas during the premonsoon ( $2.9 \pm 0.5 \text{ mg l}^{-1}$ ) and postmonsoon ( $3.0 \pm 1.3 \text{ mg l}^{-1}$ ) seasons. Amba and Savitri were comparatively well oxygenated ( $> 4.0 \text{ mg l}^{-1}$ ) during the entire study period except at the oligohaline zone ( $2.9 \text{ mg l}^{-1}$ ) of Savitri during the premonsoon. High  $\text{NO}_2^-$ -N levels were observed at the poly-mesohaline zone of Ulhas during the premonsoon ( $16.9 \pm 12.8 \text{ } \mu\text{mol l}^{-1}$ ) and postmonsoon ( $16.8 \pm 6.1 \text{ } \mu\text{mol l}^{-1}$ ) whereas low concentrations were recorded at Amba and Savitri.  $\text{NO}_3^-$ -N was noticeably high throughout the Ulhas with maximum value recorded at euhaline zone during premonsoon ( $46.4 \pm 41.7 \text{ } \mu\text{mol l}^{-1}$ ) and poly-mesohaline zone during postmonsoon ( $46.4 \pm 43.8 \text{ } \mu\text{mol l}^{-1}$ ). In Amba, the elevated value of  $\text{NO}_3^-$ -N ( $22.4 \pm 10.4 \text{ } \mu\text{mol l}^{-1}$ ) was observed at the euhaline zone during premonsoon. Similarly, the highest value of  $\text{NO}_3^-$ -N ( $26.8 \pm 6.7 \text{ } \mu\text{mol l}^{-1}$ ) was observed in the poly-mesohaline zone of Savitri during premonsoon. High values of  $\text{NH}_4^+$ -N were noticed in the poly-mesohaline ( $34.0 \pm 27.3 \text{ } \mu\text{mol l}^{-1}$ ) and oligohaline ( $43.4 \pm 38.9 \text{ } \mu\text{mol l}^{-1}$ ) zones of Ulhas during postmonsoon, but were lower in the other two estuaries. The zones having higher concentrations of nutrients during non-monsoonal seasons (mostly euhaline and poly-mesohaline) indicated lower nutrient concentrations during the monsoon in zones. The sediments were dominated by the silt fraction in Ulhas and Amba during all seasons, while Savitri had mostly sandy sediments. The  $\text{C}_{\text{org}}$  content in sediments was higher in the oligohaline and poly-mesohaline zones of Ulhas and Amba during all three seasons ( $> 2.0 \%$ ). In Savitri, higher  $\text{C}_{\text{org}}$  were observed in the oligohaline zone during the non-monsoonal seasons (premonsoon;  $2.5 \%$  and postmonsoon;  $3.6 \%$ ) than monsoon ( $1.3\%$ ).

In the PCA undertaken to explain the patterns of stations based on abiotic data, the primary three principal components (PC1, PC2 and PC3) together explained 80.1%, 71.6% and 79.8% of the total variability in Ulhas, Amba and Savitri respectively (Fig. 3). PCA indicated that the poly-mesohaline stations of Ulhas were associated with high nutrients and low DO; euhaline stations with higher DO and oligohaline stations with high DO and  $\text{C}_{\text{org}}$ . The euhaline stations of Amba were marked by high  $\text{NO}_3^-$ -N and salinity whereas the oligohaline stations had higher DO and low salinity. Poly-mesohaline stations of Savitri were marked by higher nutrients and euhaline zone by higher DO.

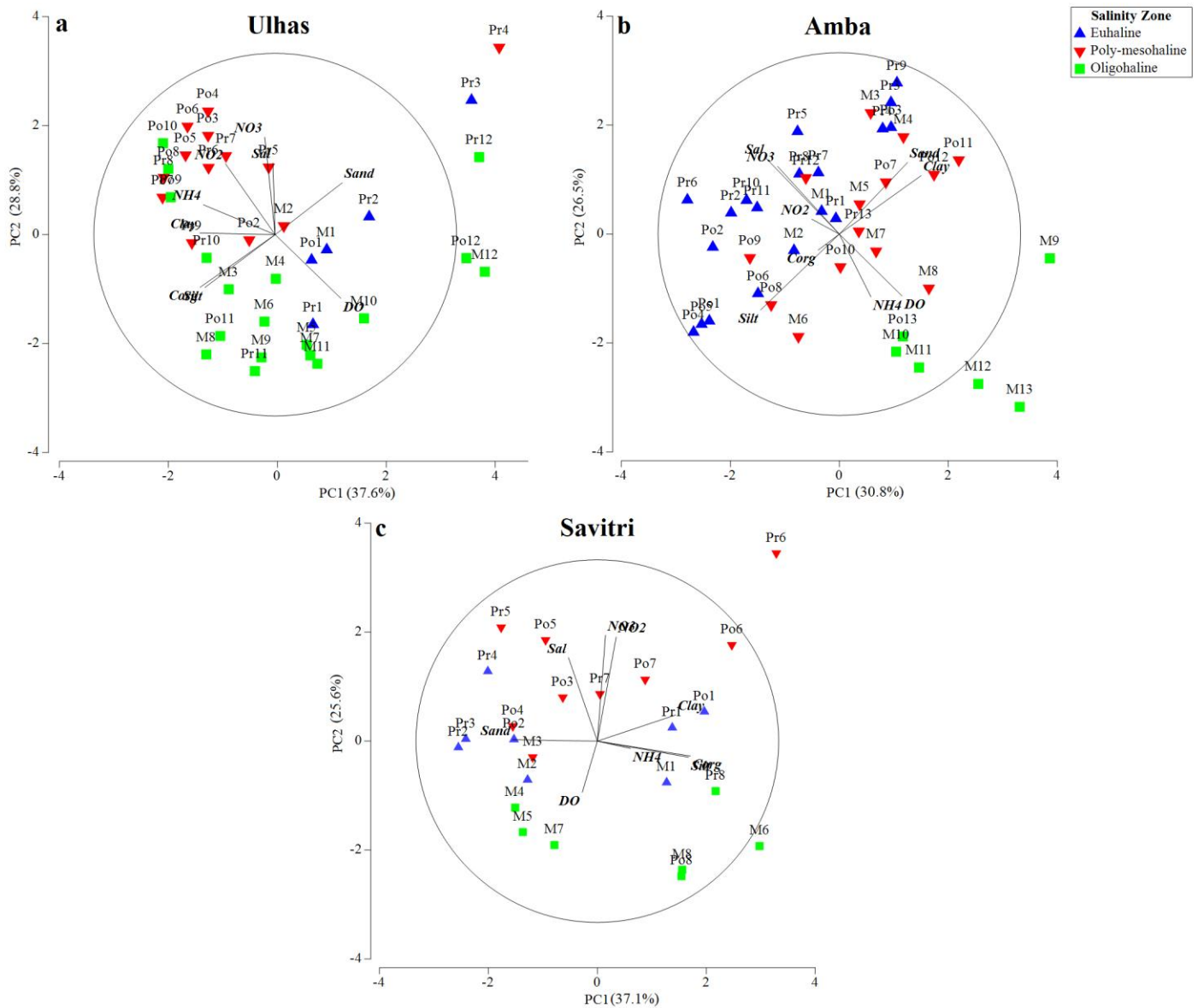


Fig. 3 Two-dimensional Principal Component Analysis (PCA) ordination of the environmental variables of the three estuaries during all seasons (Pr=Premonsoon, M=Monsoon, Po=Postmonsoon)

The Pressure Index estimated to evaluate the stress levels at each station of the three estuaries that are subjected to diverse anthropogenic pressures is presented in Table 2 and Fig. 1. All three estuaries are impacted by multipronged anthropogenic activities such as agricultural inputs, domestic waste and sewage, industrial effluents and dredging, though in varied intensities. However, as apparent in the PI, Ulhas is comparatively heavily impacted. Amba and Savitri are exposed to relatively low levels of pressures, mainly related to domestic waste and sewage and dredging.

**Table 2:** Pressures determined for each ecosystem and sampling station. From 0 to 3: 0= no pressure, 1= low pressure, 2 =moderate pressure 3= high pressure

	<b>Agricultural inputs</b>	<b>Domestic waste and sewage</b>	<b>Industrial load</b>	<b>Dredging</b>	<b>Pressure index</b>
<b>Ulhas</b>					
U1	0	1	0	0	1
U2	0	2	0	0	2
U3	0	2	0	0	2
U4	0	2	0	1	3
U5	0	2	1	0	3
U6	0	2	1	1	4
U7	1	3	2	1	7
U8	1	3	2	2	8
U9	1	3	2	2	8
U10	2	3	3	0	8
U11	2	2	0	0	4
U12	0	1	0	0	1
					<b>51</b>
<b>Amba</b>					
A1	0	1	1	0	2
A2	0	1	1	0	2
A3	0	1	2	0	3
A4	0	1	1	0	2
A5	0	1	1	0	2
A6	0	1	2	0	3
A7	0	1	1	1	3
A8	0	1	0	1	2
A9	0	1	2	2	5
A10	0	1	1	0	2
A11	0	1	1	0	2
A12	1	2	0	0	3
A13	1	2	0	0	3
					<b>34</b>
<b>Savitri</b>					
S1	0	1	0	0	1
S2	0	1	0	0	1
S3	0	1	0	0	1
S4	0	1	0	1	2
S5	0	2	1	2	5
S6	0	3	2	1	6
S7	1	2	0	0	3
S8	1	2	0	0	3
					<b>22</b>

### 3.2 Spatial and seasonal variations in macrobenthic assemblages

Overall, 189 taxa were identified from the three estuaries during the entire study period. The shade plot (Fig. 2) clearly emphasized the role of salinity in determining the species diversity of all three estuaries given their varying environmental characteristics. Thus, the euhaline and poly-mesohaline zones of all estuaries were inhabited by more species types than the oligohaline zone. The species that significantly dominated in Ulhas were *Streblospio gynobranchiata* (87.6%; poly-mesohaline premonsoon), *Mesopodopsis orientalis* (59.8%; euhaline premonsoon) and *Namalycastis senegalensis* (98%; poly-mesohaline monsoon). In Amba, the most abundant species was *N. senegalensis* (72.5%; poly-mesohaline premonsoon to 82.8%; oligohaline postmonsoon). In Savitri, *Paraprionospio cordifolia* (84.3%) dominated in the euhaline zone during the postmonsoon whereas only *Aulophorus furcatus* was observed in the oligohaline zone during premonsoon. *Glycera longipinnis* (91%) was abundant in the poly-mesohaline zone during monsoon.

### 3.3 Biotic indices

Of the 189 taxa identified, 118 taxa were assigned to their respective ecological groups as per AMBI species list. Thirty-nine taxa were assigned based on the genus, eighteen taxa were not assigned, nine taxa which were not included in the species list were assigned using expert opinion and five taxa were ignored (Appendix 2).

All Ecological Groups were present in each of the three estuaries. The constituent percentage of each Ecological Group varied between estuaries, zones and seasons (Fig. 4). Abundance of EG I and EG IV were higher in Savitri, EG II in Amba, EG III and EG V in Ulhas. In Ulhas, the sensitive groups EG I and EG II were dominant (> 60%) in the euhaline zones and lower in the other two zones, where the opportunistic groups EG IV and EG V groups were dominant. Generally, a similar trend was visible in Amba too except during the postmonsoon. EG V were mainly present at the poly-mesohaline and oligohaline zones of all estuaries. The oligohaline zone of Savitri was mostly populated by EG I and EG II except during monsoon when EG IV were dominant. Generally, more EG types were present in the euhaline zone of all the estuaries. EG types reduced during monsoon but increased again in post monsoon. The oligohaline zones of all estuaries had few EG types. The estimated values of the five indices and

the corresponding ecological status of each station and the salinity zones of the three estuaries during three seasons are presented in Appendices 3 to 5.

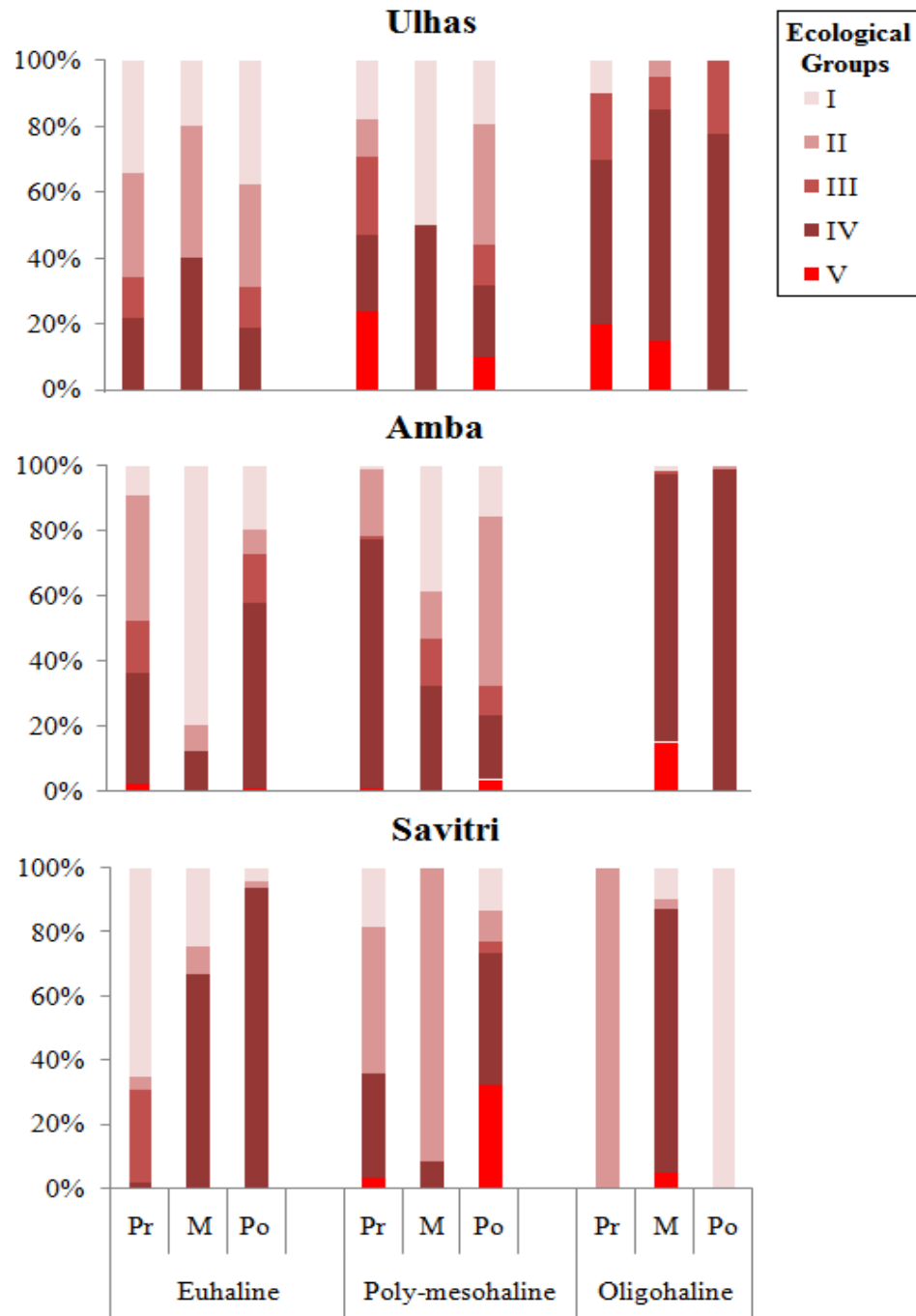


Fig. 4 Percentage of macrobenthic fauna belonging to ecological groups of AMBI (I= sensitive, II= indifferent, III =tolerant, IV=second order opportunistic, V= first order opportunistic) at the different sampling stations of the three estuaries across seasons (Pr=Premonsoon, M=Monsoon, Po=Postmonsoon) and salinity zones (Euhaline, Poly-mesohaline, Oligohaline)

### **3.3.1 AMBI**

AMBI designated the euhaline zone of Ulhas as 'good' during premonsoon, 'moderate' during monsoon and 'high' during postmonsoon. The poly-mesohaline zone was 'poor' during the premonsoon and 'moderate' during the other two seasons. The oligohaline zone was 'moderate' during all three seasons (Appendix 3). In Amba, the euhaline zone was deemed 'good' during premonsoon, 'poor' during monsoon and 'moderate' during postmonsoon. Poly-mesohaline was 'moderate' during premonsoon and monsoon and 'good' during the postmonsoon. Oligohaline was termed 'moderate' during monsoon and 'bad' during postmonsoon (Appendix 4). In Savitri, the AMBI status for euhaline and poly-mesohaline zones was 'good' which did not fluctuate temporally. Oligohaline zone was assigned 'good' status during the premonsoon, 'moderate' during the monsoon and 'poor' during the postmonsoon (Appendix 5).

### **3.3.2 M-AMBI**

M-AMBI classified the euhaline zone of Ulhas as 'moderate' during premonsoon, 'poor' during monsoon and 'high' during the postmonsoon. Poly-mesohaline zone improved from 'poor' (premonsoon and monsoon) to 'moderate' (postmonsoon) whereas the oligohaline zone was 'moderate' during premonsoon and 'poor' during the monsoon and postmonsoon. In Amba, the euhaline zone showed 'good' status during the premonsoon and 'poor' during the subsequent seasons. The poly-mesohaline zone was 'poor' during premonsoon and 'moderate' during the monsoon and postmonsoon. At the oligohaline zone, 'moderate' status was observed during the monsoon and 'poor' during the postmonsoon. In Savitri, both euhaline and poly-mesohaline zones of Savitri were considered as 'good' during the non-monsoonal months and 'poor' during monsoon. The oligohaline zone was 'poor' during all seasons.

### **3.3.3 BENTIX**

In Ulhas, a 'good' status was assigned to the euhaline zone during both the premonsoon and postmonsoon and 'moderate' during the monsoon by BENTIX. The poly-mesohaline zone improved from 'bad' (premonsoon) and 'poor' (monsoon) to 'moderate' (postmonsoon). The oligohaline zone was 'moderate' during premonsoon and 'poor' during monsoon and postmonsoon. In Amba, the euhaline zone was 'good' during premonsoon and monsoon seasons and 'moderate' during the postmonsoon. The poly-mesohaline zone showed 'good' status during premonsoon and postmonsoon and 'moderate' during the monsoon. The oligohaline zone was



'poor' throughout all seasons. In Savitri, the euhaline zone was classified as 'high' during premonsoon and 'good' during the monsoon and postmonsoon. The 'high' status observed in the poly-mesohaline zone during premonsoon and monsoon changed to 'good' during postmonsoon. In the oligohaline zone, 'high' status was observed during the non-monsoonal seasons and 'poor' during monsoon.

### **3.3.4 BOPA and BO2A**

In Ulhas, both the BOPA and BO2A designated euhaline zone as 'good' during the non-monsoonal seasons and 'moderate' during the monsoon. 'Poor' status was assigned to the poly-mesohaline zone during the non-monsoonal seasons and 'bad' status during the monsoon. In the oligohaline zone, BO2A value was expectedly higher due to the dominance of opportunistic nereids and oligochaetes that indicated 'poor' status during the non-monsoonal months and 'bad' during the monsoon. Similar ecological status was obtained by BOPA and BO2A for the three zones in Amba. Euhaline zone was 'good' during the premonsoon and monsoon and 'moderate' during the postmonsoon. The poly-mesohaline zone was classified as 'moderate' during premonsoon and monsoon and 'good' during the postmonsoon. In the oligohaline zone, 'poor' and 'bad' status were assigned during the monsoon and postmonsoon respectively. In Savitri, the euhaline zone indicated 'high' status during the premonsoon and 'moderate' during the monsoon and postmonsoon. The poly-mesohaline zone was assigned as 'good' by the BOPA index for all three seasons. BO2A gave the poly-mesohaline zone 'good' status during the premonsoon and monsoon and 'moderate' status during the postmonsoon. The oligohaline zone was classified as 'high' during non-monsoonal seasons and 'moderate' during the monsoon by BOPA. BO2A designated oligohaline zone as 'bad' during premonsoon, 'poor' during monsoon and 'high' during postmonsoon.

### **3.4 Seasonal averaged approach**

Table 3 represents the zonewise index values averaged for the non-monsoonal months, the corresponding ecological status and the comparison of the established status with the estimated status for all five applied indices in the three estuaries. Overall, the ecological status obtained by different indices for all the salinity zones of Ulhas were congruent except for BENTIX and BO2A which underestimated ('poor') the ecological status of the oligohaline zone. In Amba, AMBI correctly evaluated the status of the euhaline zone as 'good' and poly-

mesohaline as 'moderate' but underestimated the oligohaline as 'bad'. M-AMBI could accurately classify the euhaline zone as 'good' but failed to evaluate the ecological status of other zones. The remaining three indices either underestimated or overestimated the ecological status. All indices except BENTIX appropriately assigned a 'good' status to the euhaline zone of Savitri. However, all indices also classified the impacted poly-mesohaline zone as 'good' erroneously, thus failing in differentiating between two differently impacted zones. The oligohaline zone of Savitri was correctly assigned only by AMBI and BO2A as 'moderate', the rest either underestimating (M-AMBI) or overestimating (BENTIX, BOPA) the status.

Estuary	Zones	EES	AMBI	MAMBI	BENTIX	BOPA	BO2A
Ulhas	Euhaline	Good	2.0 ✓	0.68 ✓	4.1 ✓	0.0740 ✓	0.0740 ✓
	Poly-mesohaline	Poor	5.1 ✓	0.34 ✓	2.0 ✓	0.2276 ✓	0.2276 ✓
	Oligohaline	Moderate	4.5 ✓	0.41 ✓	2.2 ↓	0.1779 ✓	0.2278 ↓
Amba	Euhaline	Good	3.1 ✓	0.48 ✓	3.4 ↓	0.1408 ↓	0.1428 ↓
	Poly-mesohaline	Moderate	3.8 ✓	0.37 ↓	4.4 ↑	0.1170 ↑	0.1198 ↑
	Oligohaline	Good	6.4 ↓	0.24 ↓	2.0 ↓	0.2983 ↓	0.2983 ↓
Savitri	Euhaline	Good	2.3 ✓	0.59 ✓	4.6 ↑	0.0737 ✓	0.0737 ✓
	Poly-mesohaline	Moderate	2.5 ↑	0.61 ↑	4.3 ↑	0.0830 ↑	0.1143 ↑
	Oligohaline	Moderate	3.4 ✓	0.31 ↓	6.0 ↑	0.0000 ↑	0.1505 ✓

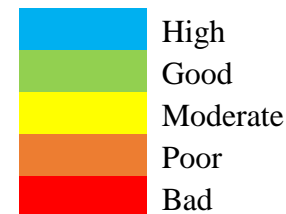


Table 3: Comparative mean status of all the benthic indices obtained by averaging non-monsoonal seasons (premonsoon and postmonsoon) according to the salinity zones of the three estuaries

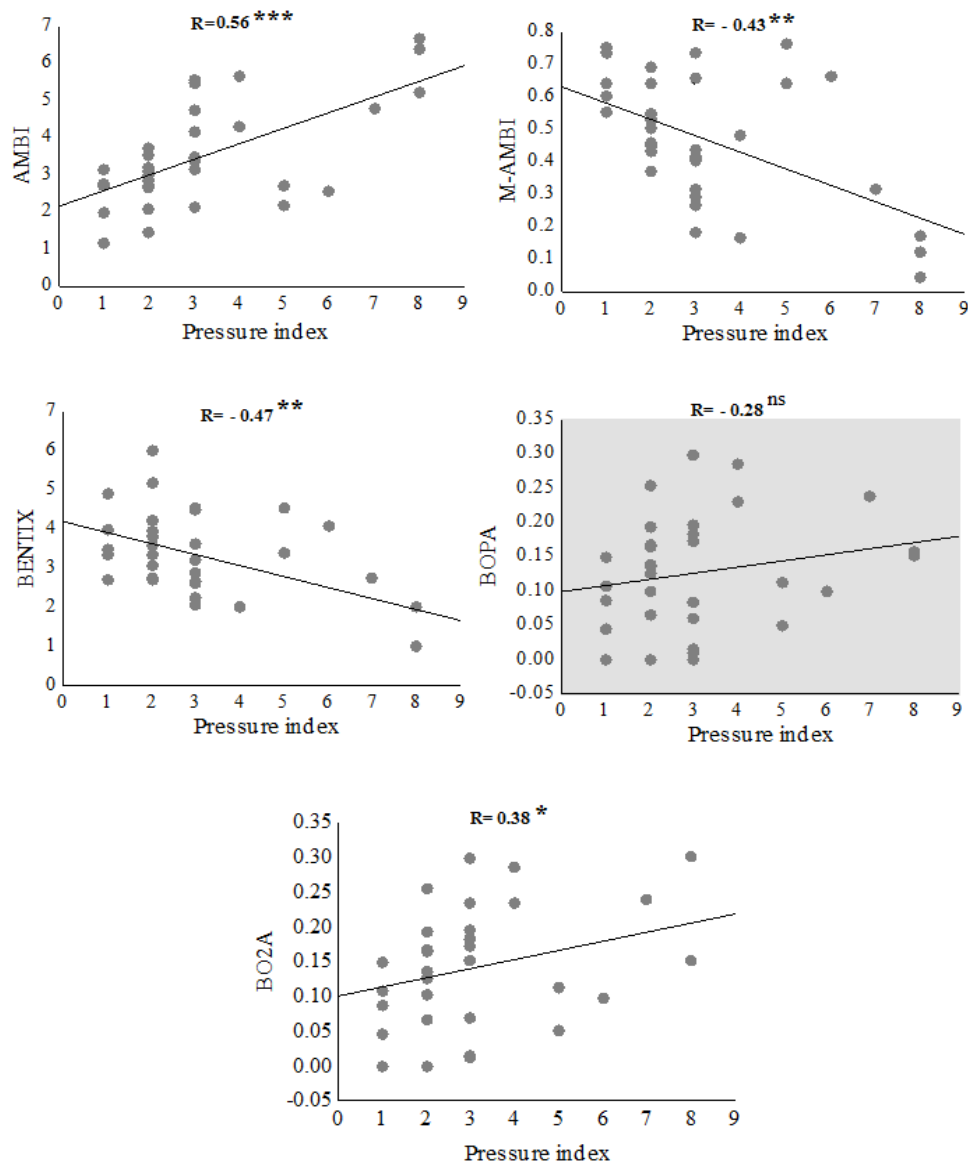
✓= agreement with *a priori* established ecological status (EES)

↑= Overestimation of ecological status

↓= Underestimation of ecological status

### 3.5 Performance of the indices vis-à-vis the PI and contamination proxies

The pressure index (PI) was significantly correlated with all the applied indices except BOPA. The best correlation was obtained between PI and AMBI, followed by BENTIX, M-AMBI, and BO2A (Fig. 5).



**Fig. 5** Spearman's rank correlation between the Pressure Index (PI) and biotic indices (AMBI, M- AMBI, BENTIX, BOPA and BO2A). R= Spearman correlation coefficient,  $***p < 0.001$   $**p < 0.01$   $*p < 0.05$ , ns= not significant

All indices, except BO2A, showed relationship with at least one contamination proxy. Hence,  $\text{NO}_2^-$ -N was negatively correlated with BENTIX and positively correlated with BOPA.  $\text{NO}_3^-$ -N had positive correlation with BOPA.  $\text{NH}_4^+$ -N was significantly correlated with AMBI, BENTIX and BOPA.  $\text{C}_{\text{org}}$  had a positive association with AMBI and negative with M-AMBI and BENTIX.

#### 4. Discussion

A major attribute of a successful ecological index is its universal applicability (Wetzel et al., 2012). Thus, five most frequently employed benthic indices were tested for their comparative efficacy in three estuaries that were exposed to varied intensities of anthropogenic stress. The macrobenthic community structure and abiotic variables differed significantly between the estuaries, zones and seasons (Mulik et al., 2020b). The significant variation in the abiotic variables between estuaries and salinity zones emphasized the in-built spatial heterogeneity of the three estuaries (Elliott and Quintino, 2007). Adding to the complexity, apparent seasonal variations were also detected, owing mainly to the prevailing monsoonal processes that are known to affect the abiotic and biotic constituents of Indian estuaries (Sivadas et al., 2011).

Distinct spatio-temporal variations in salinity were discerned in the studied estuaries as reported for other tropical estuaries in this region (Feebarani et al., 2016; Dias et al., 2018). Sudden salinity fluctuations and increased turbidity associated with the freshwater discharge during the monsoon are known to result in reduced macrobenthic abundances, taxa and proliferation of tolerant organisms in Indian estuaries leading to the underestimation by indices (Sivadas et al., 2011; Dias et al., 2018). Similar reduction in the number of taxa during the monsoon was observed in the three estuaries as reported elsewhere (Feebarani et al., 2016, Dias et al., 2018). During the monsoon, the even distribution of low saline preferring opportunistic polychaete, *N. senegalensis* throughout the estuary resulted in a uniform ecological status estimated by AMBI ('moderate') and M-AMBI ('poor') for the Ulhas. No single index could successfully identify the three zones appropriately during the monsoon. The effect of seasonality was observed on all the indices as the monsoon season resulted in underestimation of the ecological status at majority of the zones (Appendix 3 - Appendix 5) which can be attributed to the natural disturbances. Therefore, by the exclusion of the monsoon values and averaging the

status of the non-monsoonal seasons, a more reasonable and coherent assessment of each zone was derived for all estuaries following Sivadas et al. (2016) and Dias et al. (2018).

The ambiguity existing in the application of multiple indices was reaffirmed by the dissimilar categorization of the different salinity zones of the three estuaries by the five indices and four out of five indices correlating with different contamination proxies. M-AMBI was successful in identifying the correct ecological status for all salinity zones in the heavily modified Ulhas and for the euhaline zones of other estuaries. In other zones, M-AMBI mostly underestimated the ecological status. Being a multimetric index, it relies on the univariate indices such as species diversity and species richness which are influenced by sample size and habitat type leading to possible misinterpretations of the ecological status (Simboura and Argyrou, 2010). Furthermore, the need of a reference site for the computation of M-AMBI renders the application of this index more complicated. Further, the results of M-AMBI may change when new samples are added in the assessment. It has been recommended that robust results of this index are obtained by analyzing at least 50 samples (Borja et al., 2008b). M-AMBI provided an adequate response similar to AMBI in highly impacted Ulhas estuary but demonstrated weak performance in the other two estuaries that were less anthropogenically impacted. This agrees with the view of Borja and Tunberg (2011) that M-AMBI worked well in highly stressed estuaries. In India too, M-AMBI has been advocated for the assessment of highly stressed estuaries like Cochin estuary (Feebarani et al., 2016) and Kundalika estuary (Dias et al., 2018).

BENTIX correctly identified only the euhaline and poly-mesohaline zones of Ulhas and did not perform effectively in the other two estuaries. BENTIX downgraded the ecological status of the oligohaline zones of Ulhas and Amba, as its formula gives equal weightage to all the opportunistic (AMBI- EG IV and V) and tolerant taxa (AMBI- EG III) during estimation. Similar observations were made by Pitacco et al. (2018) in three Slovenian transitional water bodies where low BENTIX scores led to the underestimation of ecological status due to the discrepancy in EG assignation. BENTIX was originally developed for an oligotrophic, microtidal and euhaline environment (Eastern Mediterranean ecoregion) where the macrobenthos is generally extremely diverse with even distribution (Simboura and Reizopoulou, 2008). Savitri had a patchy distribution of poorly diverse macrobenthic communities. Therefore, the index could not perform well in Savitri where the index overestimated the ecological status of all the

salinity zones. For the same reason BENTIX probably failed in the oligohaline zones of the other two estuaries as well. Studies have highlighted the constraints of BENTIX in transitional waters such as estuaries or lagoons where higher abundance of opportunistic species exists naturally (Simboura and Zenetos, 2002; Blanchet et al., 2008; Sánchez-Moyano et al., 2017).

Although BOPA and BO2A are the simplest to apply among the indices, one of the major shortcomings of these two indices is that only polychaetes (or annelids) and amphipods (except *Jassa*) are being considered in the computation and other species are not taken into account. For instance BO2A in Ulhas failed to accurately assign the oligohaline zone as 'moderate' because it did not consider other taxa (EG I; *Cyprinotus* sp. and EG III; Chironomidae) that contributed 27.3% of the total abundance. Similarly, in Amba, both these indices either underestimated or overestimated the ecological status at all the salinity zones as sensitive species other than amphipods were not accounted for. In areas typified by sparse soft-bottom macrobenthic communities such as the Savitri estuary, stations with complete absence of amphipod and opportunistic polychaetes are frequently encountered. This results in wrong interpretation of ecological status by BOPA as demonstrated in the case of the poly-mesohaline and oligohaline zones of Savitri where the absence of amphipods and opportunistic polychaetes resulted in overestimation of ecological status. The dismal performance of these indices in these estuaries was probably due to the fact that the sensitivity to stress varied from species to species in the same taxonomic group (Andrade and Renaud, 2011). Also the natural absences of either amphipods or annelids along the salinity gradient tend to distort the BOPA/BO2A results (Wetzel et al., 2012). Indices like BOPA or BO2A are based on few groups and hence are probably not suitable for expansive geographical comparisons (Keeley et al., 2012).

Among the applied indices, AMBI could correctly classify major part of the three estuaries. However, AMBI underestimated ('bad') the oligohaline zone of Amba which was only slightly affected. This inconsistency occurred as only a single station of only postmonsoon (as no oligohaline station could be established during premonsoon) populated by just EG IV and V was included in the estimation. The sensitivity of AMBI is reduced when the number of taxa is equal or lesser than three (Borja and Muxika, 2005, Afli et al., 2008). In naturally stressed estuaries, a poor ecological status can be denoted by indices even when the site is low impacted (Sánchez-Moyano et al., 2017). AMBI also overestimated ('good') the ecological status of poly-mesohaline

zone of Savitri which was moderately modified due to presence of a larger proportion of EGI and EGII at this zone. Such aberrations also occur as dominance of some species can happen in relatively low impacted zones due to the ability of these organisms to tolerate adverse environmental conditions present in the estuaries (Patrício et al, 2009). Therefore, especially in estuaries caution is advised while interpreting the index values. In low abundance sites, Borja and Muxika (2005) have advised using AMBI along with other metrics for better devolvement of ecological status. However, in the two incorrectly identified zones, all tested indices were unsuccessful either due to low sample size and taxa (Amba oligohaline) or due to the unexpected presence of sensitive species in an area of evident stress (Savitri polyemesohaline). Despite the inconsistencies, AMBI could explain the level of stress better than other applied indices.

While testing several indices, it is imperative to substantiate the ecological significance of each benthic index by using the pressure index (Wetzel et al., 2012). The three estuaries had most of the pressures that varied in types and intensities. In all the estuaries, pressure index was lower in the euhaline zone whereas the poly-mesohaline zone with multiple stressors had higher PI. The oligohaline zones of Amba and Savitri were impacted by domestic waste and agricultural inputs whereas the same zone in Ulhas was furthermore influenced by the industrial release downstream. AMBI was most correlated with the PI amongst the indices. Therefore, it can be concluded that AMBI fulfills the primary criteria for being considered as a common estuarine benthic index for estuaries along the northwest coast of India i.e. (i) the index was most correlated with the PI (validation of the first hypothesis) (ii) AMBI could mostly discriminate the differently impacted zones of the three estuaries more effectively than other indices tested (validation of the second hypothesis).

Extensive reviews on the performance of various benthic indices in terms of their response to 15 types of pressures in 19 biogeographical zones have been undertaken wherein AMBI was rated to be most responsive to aquaculture, sewage, physical alteration, chemical pollution, organic enrichment, hydrographic modifications, harbours and multipressures (Borja et al., 2015). In the Ulhas, facing multiple stressors, AMBI performed the best. AMBI index has been successful in the ecological status assessment of estuaries of tropical or sub-tropical waters (Khan et al., 2014; Brauko et al., 2015; Sigamani et al., 2015; Sivadas et al., 2016; Mulik et al., 2017). Also, this index has performed well in various geographical areas, across the globe, in



diverse environmental settings (Borja et al., 2019). However, the suboptimal performance of AMBI in other estuaries have also been reported (Nebra et al., 2014, Sánchez-Moyano et al., 2017).

This study established that even though the different indices were based on similar principles, results inferred were disparate leading to ambiguous conclusions regarding the final ecological status. Disparities in ecological status of estuaries evaluated by the different benthic indices have been reported by others (Ruellet and Dauvin, 2007; Tataranni and Lardicci, 2010; Pitacco et al., 2018). AMBI, M-AMBI and BOPA could identify the correct ecological status of all salinity zones of Ulhas. With the exception of oligohaline zone, BENTIX and BO2A too performed well in Ulhas. However the performance of indices (except AMBI) was erratic in Amba and Savitri. Hence, it was concluded that indices worked best and provided similar ecological status in estuaries most affected by anthropic activities with a definite pollution gradient (i.e. Ulhas) than the estuaries with intermediate impact (i.e. Amba, Savitri). The indices also seemed to underperform in 'moderate' ecological conditions whereas they performed appropriately when ecological status was 'good' or 'poor' as was also observed by Quintino et al. (2006) and Puente and Diaz (2008).

In the absence of locally derived benthic indices, adoption and examination of those derived elsewhere are observed in Indian ecological status assessment investigations. While studies employing estuarine benthic indices are fast gaining credence in the Indian subcontinent, they have been mostly restricted to a single estuary (Feebarani et al., 2016; Sivadas et al., 2016; Rehitha et al., 2017; Mulik et al., 2017; Dias et al., 2018) or in case of multiple domains only the Azti indices have been tested so far (Khan et al., 2014; Sigamani et al., 2015). Validation of benthic indices by way of testing them in different geographical provinces and diverse pressures are important while making informed decisions. It is preferable to opt for one index as even indices based on the same paradigms do not interpret the information similarly (Ruellet and Dauvin 2007) as was well demonstrated in this study. Usage of multiple indices has resulted in more ambiguity and less clarity of the ecological status in many studies (Blanchet et al., 2008). It is also vital for large environmental monitoring programs to employ an ecological index that is comparable across broad-scales for deriving easy, effective and comparable conclusions. This investigation endorses the applicability of AMBI in the north-west Indian estuaries as a reliable

estuarine index as amongst all indices tested, it appeared to have functioned the best in delineating the ecological status of three different estuaries. The performance of AMBI could well be enhanced if sufficient data points are established in low saline zones of estuaries in future studies. Also similar studies need to be replicated in the other estuaries of NW India to reaffirm the pre-eminence of AMBI.

## **5. Conclusion**

An efficient estuarine benthic index should be valid across estuaries of a geographical zone and capable of providing regional evaluation of ecological health status by comparing the benthic ecological conditions between the estuaries. The results indicated that in heavily modified environments (Ulhas), indices concurred in accurately defining the slightly impacted (euhaline) and heavily impacted (poly-mesohaline) zones. Ambiguity in ecological status assignment by various indices was more acute in the estuaries that were comparatively less impacted. What stands out from our results is that among different benthic indices, AMBI could more appropriately discriminate between diversely impacted areas, despite a couple of inconsistencies that were common to all tested indices. AMBI also correlated the best with the Pressure Index indicating excellent responsiveness to stressors of all kinds. Hence, it is suggested that this index alone can be used in future studies for assessing the ecological quality status of the estuaries of NW India so as to reduce the effort and time required for the application of multiple indices and complexities arising from having to choose between multiple ecological status. This would be the practical approach for monitoring programs of developing economies beset with fund shortages and scarce baseline data.

## **Supplementary material (SM)**

Appendix 1 - Appendix 5

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