# Comparative efficacy of benthic biotic indices in assessing the Ecological Quality Status (EcoQS) of the stressed Ulhas estuary, India.

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## Abstract

Ecostatus of Ulhas estuary, one of the most polluted estuaries along the industrialized and urbanized northwest coast of India, was evaluated by six widely accepted benthic indices viz.  $H'(\log_2)$ , AMBI, M-AMBI, BENTIX, BOPA and BO2A to test their efficiency in a tropical setting. The mesohaline zone, which presented eutrophic conditions, was classified as 'bad' by all indices due to the azoic status. Despite significant correlations obtained between indices, there were discrepancies in the accurate level of EcoQS assigned to each station. AMBI was observed to be most efficient in indicating a clear spatial variability from a 'poor' to 'bad' ecological quality status in the middle and upstream zones to an improved status in the downstream region. Limitations of all indices are discussed in light of their suitability for assessing the estuarine environmental condition. The present results could provide a fillip to environmental improvement initiatives currently being undertaken in the estuary. Corresponding author. Tel: +91 22 2635 9605; Fax: +91 22 2636 4627

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#### **Introduction**

The consequences of rapid deterioration of estuarine ecosystems, due to urbanization and industrialization world over, have been debated and well established by a multitude of studies (McLusky and Elliot, 2004; Borja and Dauer, 2008; Merrifield et al., 2011). This has turned the focus on watershed management aimed at recovery of degraded estuarine ecosystems. Formulation of effective management policies for estuaries requires cost effective but precise evaluation of current ecological status. Lately, categorization of estuarine zones based on the ecological quality has been undertaken by employing suitable macrobenthic biotic indices (Chainho et al., 2007; Teixeira et al., 2008; Valença and Santos, 2012), that can encapsulate complex scientific data into simple information to facilitate easy interpretation by stakeholders and managers alike (Chainho et al., 2007). Among the various biological components, macrobenthos are preferred as effective indicators in determining estuarine environmental status as they have varied species composition with differential tolerances to stress (Pinto et al., 2009; Wildsmith et al., 2011) and can integrate conditions over a period of time (Dauvin et al., 2010), due to their sedentary lifestyle and longevity (Reiss and Kröncke, 2005). Several biotic indices have been formulated based on the relative sensitivity of benthic macrofauna species to stressors, macrofaunal parameters (species richness, abundance and biomass), trophic groups or even combination of some of these parameters (Pearson and Rosenberg, 1978; Word, 1978; Borja et al., 2000; Gómez-Gesteira and Dauvin, 2000; Simboura and Zenetos, 2002; Muxika et al., 2007). In naturally perturbed estuarine waters, the distinction between natural and anthropogenic disruptions requires to be examined carefully (Elliot and Quintino, 2007; Ponti et al., 2008). Therefore, it becomes even more critical to evaluate the performance of biotic indices in an estuarine environment giving due consideration to its inherent natural variability. It is pertinent to note that all widely used biotic indices were mostly developed and tested in a temperate milieu under the aegis of the European Union Water Framework Directive (WFD) (Pinto et al., 2009). Conversely, the lack of sufficient information on the taxonomical and functional characteristics of macrobenthos have hampered the applicability of benthic indices in evaluating the state of the environment (Bigot et al., 2008) in tropical regions. Studies testing the utility of biotic indices in determining the estuarine ecological status in the Indian subcontinent are rare (Khan et al., 2014; Sigamani et al., 2015; Feebarani et al., 2016; Sivadas et al., 2016). Hence, it is only appropriate to test the effectiveness of some of the widely used benthic indices in the ecological evaluation of estuaries of this biogeographical zone.

The northwest coast of India is one of the most industrialized and therefore has some of the highly polluted estuaries along its long coastline (NIO, 2009). A prime example is the profoundly deteriorated Ulhas estuary, which is in the proximity of the urbanized and industrialized Thane-Mumbai metropolis (Athalye et al., 2003). The increasing levels of pollutants being dumped into the estuary have become a major concern for regulatory authorities who have been tasked with formulating remedial and effective measures towards restoring the estuarine ecology. While considerable data on the hydrological parameters and some biological components are available (Quadros and Athalye, 2002; Athalye et al., 2003; Borkar et al., 2007; Ram et al., 2009; Menon and Mahajan, 2013), information on the subtidal macrobenthos of the estuary is meager (Mathew and Govindan, 1995). The principle aim of the present study was to test the applicability of different macrobenthic biotic indices for evaluating the ecological quality status of the Ulhas estuary which has hitherto not been attempted. From the plethora of biotic indices available, six indices viz. H'(log<sub>2</sub>) (Shannon and Weaver, 1949), AZTI's Marine Biotic Index (AMBI; Borja et al., 2000), multivariate-AMBI (M-AMBI; Muxika et al., 2007), BENTIX (Simbora and Zenetos, 2002), Benthic Opportunistic Polychaete Amphipoda index (BOPA; Dauvin and Ruellet, 2007) and Benthic Opportunistic Oligochaete Amphipoda index (BO2A; Dauvin and Ruellet, 2009) were selected based on their robustness, ease of application and sensitivity (Medeiros et al., 2012; Borja et al., 2015).

# 2. Materials and methods

#### 2.1 Study area

Ulhas river is one of the westbound flowing rivers originating from the western slopes of the Sahyadrian range in the Maharashtra state, India, nearly 400 m above the mean sea level. Following the westerly course of about 100 km, it meets the Arabian Sea at Vasai (19º 16' N and 72º 45'E). The premonsoon season in this area falls between March and May followed by the southwest monsoon from June to September. October to January forms the postmonsoon season. The estuary has vast mudflats and widespread mangroves along its intertidal zones. It is mainly a monsoon-fed river wherein the fresh water flow drops down during the dry (nonmonsoon) period (Rathod and Patil, 2009). The estuary is strongly influenced by tides. The tidal excursion which is 5-15 km in the lower estuary decreases to 2-5 km in the inner estuary during the dry season. The estuarine flushing time varies between 73 and 211 tidal cycles during the dry season leading to the possibility of accumulation of pollutants in the inner estuary (Ram et al., 2009). The estuary is heavily impacted by myriad anthropogenic activities and is susceptible to pollution from various sources such as untreated domestic sewage, runoff from terrestrial and agricultural fields, dredging activities and industrial effluents.

## 2.2 Field sampling and laboratory evaluations

A perusal of available studies on Ulhas estuary indicated that unfavorable ecological conditions like hypoxia and eutrophication peaked during the premonsoon season when there is little or no fresh water discharge (Ram et al., 2003; Ram et al., 2009). Therefore, it is presumed that response of macrobenthos to adverse conditions would be more pronounced during this period. Sivadas et al. (2016) have suggested that observations during the critical season (premonsoon along the Indian west coast) were most suitable to test the efficiency of the selected biotic indices in the tropics. Hence, sampling was carried out at 12 subtidal stations (Fig.1), located across different salinity regimes, during the premonsoon month of May 2013. The nomenclature, geographical co-ordinates, depths and type of stressors are presented in Table 1. Four sediment samples were collected at each station using a van Veen grab of 0.04  $m<sup>2</sup>$  bite area for the study of macrobenthos. Immediately after sieving the sediment samples through 0.5 mm mesh sieve, retained organisms were fixed in 5% formalin mixed with Rose Bengal. Macrobenthic organisms were then sorted up to the lowest possible taxonomic level (mostly species), enumerated and expressed as ind.m-2. Species level identification was done as per the available identification keys.

Sediment samples were also collected in duplicate for analysis of texture using combined sieving and pipette method (Buchanan, 1984) and organic carbon  $(C_{org})$  by titration method (Walkey and Black, 1934). At each station, bottom water samples were collected in duplicate during flood and ebb tides using Niskin samplers. Samples were preserved in ice immediately after the collection and were analyzed for various physicochemical parameters like temperature, pH, suspended solids (SS), salinity, dissolved oxygen (DO), phosphate-phosphorus (PO<sub>4</sub><sup>3</sup>-P), nitrite-nitrogen (NO<sub>2</sub> -N), nitratenitrogen (NO<sub>3</sub>-N) and ammonia-nitrogen (NH<sub>4</sub><sup>+</sup>-N) in the laboratory. The temperature was recorded using a digital thermometer and pH measurement was carried out using a Cyber Scan (Model pH 510) pH meter. SS was analysed by filtration method, DO by Winklers method, salinity by Mohr titration method and nutrients by photometric methods following Grasshoff et al. (1999).

## 2.3 Data analysis

To interpret the results systematically and statistically, the biological and chemical datasets were processed with the aid of appropriate statistical software. The statistical program, PRIMER v6 (Clarke and Gorley, 2006) was employed to perform univariate and multivariate analyses of average macrobenthic species abundance data. Average macrobenthic abundance (N), total number of species (S), Margalef's richness index (d), Pielou's evenness index (J') and Shannon's index (H' log<sub>2</sub>). The Bray–Curtis similarity coefficient was used to build a similarity matrix from the fourth-root

transformed macrobenthic species abundance data recorded for each station. Subsequently, this matrix was processed to non-metric multidimensional scaling (nMDS) ordination to analyze the spatial distribution of benthic assemblages along the estuarine salinity gradient. One-way ANOSIM was performed to test the significant statistical differences in macrobenthic assemblages between the different salinity zones. SIMPER (Similarity of percentage) analysis was implemented to identify the major species that contributed significantly to the discrimination between the groups formed by MDS. Standardised Principal Component Analyses were performed on abiotic data to determine distribution pattern of stations in terms of their environmental variables. Spearman's Rank correlation coefficient (r) was estimated between univariate indices, biotic indices, major macrobenthic taxa and environmental parameters using Statistica 7. The correlation results at  $p \leq$ 0.05 were considered significant. Macrofaunal abundance data was linked to abiotic variables using the BIOENV procedure. The results specify the best suite of environmental variables that were responsible for framing macrofaunal community structure.

#### 2.4 Stress level assessment using biotic indices

In the present study, six indices were employed for each station to assess the ecological quality status (EcoQS) of the environment based on macrobenthic taxocene characteristics viz. Shannon-Wiener diversity index, AMBI, M-AMBI, BENTIX, BOPA and BO2A. Shannon's index  $(H' \log_2;$  Shannon and Weaver, 1949) was calculated using the PRIMER software. AMBI indices were calculated using the AMBI index software (Borja et al., 2000); version 5.0; which is based on the classification of soft-bottom macrobenthic species into five ecological groups i.e. from most sensitive species (ecological group 1) to most opportunistic species (ecological group 5). Fish larvae were excluded from the calculation of benthic indices as they are not considered to be part of soft bottom macrobenthic fauna (Borja and Muxika, 2005). The list of identified species along with the assigned Ecological Groups (as per November 2014 species list at http://ambi.azti.es) are given in Appendix 1. Of the 39 identified taxa, 25 were assigned to their respective ecological groups and remaining 14 which were not included in the species list were assigned by using expert opinion. A modified version of AMBI, M-AMBI (Muxika et al., 2007), which is a combination of Shannon diversity, species richness and AMBI, was also derived using AMBI software. Reference conditions are a prerequisite for deriving M-AMBI values. As the salinity gradient can influence species richness and diversity, reference conditions were derived separately for each salinity zone (Feebarani et al., 2016). Given that all stations were impacted by one or more anthropogenic activities (Table 1), their species diversity and richness values could not be adapted to set the reference conditions. Hence, the reference conditions for evaluating M-AMBI index was derived by increasing the highest species

diversity and richness values of all stations in each salinity zone by 15% following Cai et al. (2014) and adapting the azoic conditions (diversity and richness equal to 0 and AMBI equal to 6) for the bad status.

BENTIX (Zenetos et al., 2004) was calculated using BENTIX Add-In v. 1.0 that is entirely based on the relative individual proportions of species sorted into two groups in accordance with their tolerance to stress. BOPA index (Dauvin and Ruellet, 2007) uses relative frequencies of opportunistic polychaetes and amphipods (except for Jassa species ) and BO2A index (Dauvin and Ruellet, 2009) which is an adaptation of BOPA index uses Annelida (including Polychaeta and Clitellata) instead of Polychaeta used in BOPA. Five EcoQS classes; 'High', 'Good', 'Moderate', 'Poor' and 'Bad' were pooled into two groups, "acceptable" and "not acceptable" to determine the level of agreement between all indices. Acceptable status was corresponded to "High" or "Good" EQS and scored as 1, whereas not acceptable status to "Moderate", "Poor" or "Bad" EQS and scored as 0. The scores given to each of the indices used were summed for each station (range: 0-5). This sum of scores was used for measuring the level of agreements (full or partial) and disagreement among the aforementioned six indices (Blanchet et al., 2008). Finally, Spearman's rank correlation coefficients ( $p = 0.05$ ) was performed in order to assess the relationship among all six indices. Thresholds adopted for all Ecological Quality Status Groups (EcoQS) for the investigated biotic indices are given in Table 2.

### 3. Results

#### 3.1 Estuarine zones and environmental variables

The multi-dimensional scale (MDS) plot using macrobenthic species abundance identified four distinct groups of stations (Fig. 2) in Ulhas estuary corresponding to different salinity zones of the Venice system namely euhaline  $(a)$ , polyhaline  $(b)$ , mesohaline  $(c)$  and oligohaline  $(d)$  (One-way ANOSIM Global R=0.928; p<0.001). The euhaline Group a comprised of stations U1 to U3 while Group b consisted of stations U4 to U7 which comprised the polyhaline zone. Group c was constituted by stations U8 and U9 (mesohaline zone) while upstream stations (U10 to U12) formed Group d which was the oligohaline zone.

The spatial variability in the environmental attributes of the estuary is depicted in Fig. 3 and Fig. 4. The depth of the water column decreased from the euhaline lower estuary to the oligohaline upper zone (Table 1). Spatial variations in water temperature were minor. A decreasing trend in the water pH and suspended solids was evident from the estuarine mouth to the oligohaline inner estuary. Dissolved oxygen values were considerably lower at polyhaline  $(2.9\pm0.7 \text{ mg } 1^{-1})$  and mesohaline

 $(2.9\pm0 \text{ mg l}^{-1})$  zones. In these two zones, NO<sub>2</sub>-N were observed to be higher ( $\geq$ 5.7 µmol l<sup>-1</sup>) than the rest of the estuary. NO<sub>3</sub>-N was high throughout the estuary with a maximum value of 84.5 µmol  $1^{-1}$ at station U3. Elevated NH<sub>4</sub><sup>+</sup>-N values at U8 (29.6 µmol  $l^{-1}$ ) and U9 (19.4 µmol  $l^{-1}$ ) indicated stressed conditions in the mesohaline zone. In general, the oligohaline stations were well oxygenated with lower nutrient levels. Ulhas estuary was mostly dominated by silty substrata except for stations U3, U4 and U12 which were sandy. The increase in sediment organic carbon values was evidenced from U6 (2.5%) to U11 (4.9%) as these stations were subject to assorted anthropogenic activities (Fig. 4).

PCA analysis (Fig. 5) of abiotic parameters demonstrated that the primary two principle components (PC1 and PC2) together explained 67.3% of the total variability. First principle component axis (PC1) showed positive correlation with sand ( $r = 0.41$ ) and negative correlation with  $C_{org}$  ( $r = -0.42$ ), clay ( $r = -0.40$ ) and silt ( $r = -0.36$ ),. The second axis (PC2) was correlated positively with NO<sub>2</sub>-N ( $r = -0.40$ )  $= 0.58$ ), NH<sub>4</sub><sup>+</sup>-N (r = 0.37) and PO<sub>4</sub><sup>3</sup>-P (r = 0.39) and negatively with DO (r = -0.49). The first axis segregated the stations based on sediment texture and  $C_{org}$  whereas the second axis grouped the stations as per DO and nutrients.

### 3.2 Diversity, distribution and community analysis of macrobenthos

Altogether 26,688 ind  $m<sup>2</sup>$  belonging to thirty-nine macrobenthic taxa were analysed in the present study (Appendix 1). Macrobenthic abundance was highest at station U7 (10,894 ind  $m^{-2}$ ) and lowest at station U4 (69 ind  $m<sup>2</sup>$ ) (Fig. 6). Stations U8 and U9, which were the nearest stations to an industrial discharge point and recipient of untreated domestic sewage, were observed to be azoic due to a complete absence of macrobenthos in all the samples. Polychaeta (73%) was the most dominant macrobenthic group followed by Chironomidae (7.7%), Ostracoda (6.1%) and Mysida (5.1%). The contribution of Nematoda, Sipuncula, Hirudinea, Cumacea and Isopoda to the total macrofauna was insignificant  $( $0.5\%$ )$  and patchiness in their distribution was also evident. The dominant macrobenthic taxon was the opportunistic polychaete, Streblospio gynobranchiata (Rice and Levin, 1998) (av 61.7%), the distribution of which was restricted only to the polyhaline zone (i.e., U4 to U7) of the estuary. The oligohaline zone was inhabited mainly by Chironomidae, Cyprinotus sp., Tubificoides heterochaetus (Michaelsen, 1926), Namalycastis indica (Southern, 1921) and N. *ouanaryensis* (Gravier, 1901). The major discriminating species between euhaline (group  $a$ ) and polyhaline (group  $b$ ) zones (av dissimilarity=98%), as inferred by SIMPER, was S. gynobranchiata which was present only in the latter zone. N. *indica*, was the major contributor towards the dissimilarity between groups a (euhaline) and d (oligohaline) (av dissimilarity=98.9%). The average dissimilarity between group  $b$  (polyhaline) and  $d$  (oligohaline) was contributed mainly by  $S$ . gynobranchiata and N. indica. (av dissimilarity=93.2%).

Spatial variability of diversity indices calculated for the study area is represented in Fig. 6. The Margalef's richness index (d) was highest at station U3 ( $d=2.2$ ) due to the presence of seventeen taxa and lowest at the defaunated stations U8 and U9  $(d=0)$ . Pielou's evenness values indicated dominance of the mysid, *Mesopodopsis orientalis* (W. Tattersall, 1908) at station U3 ( $J=0.35$ ), S. gynobranchiata at stations U6 ( $J=0.36$ ) and U7 ( $J=0.01$ ) and Tubificoides heterochaetus at station U10  $(J=0.18)$ . Zonewise, species richness was highest in the euhaline zone while the polyhaline and oligohaline stations presented low values  $(d\leq 0.8)$ .

## 3.3 Assessment of Ecological Quality Status

Along expected lines, the applied indices (with the exception of  $H$ ) indicated a decrease in ecological quality moving from the euhaline to the mesohaline zone and an improvement of ecostatus in the oligohaline zone. Shannon-Wiener species diversity index ranged from 0 (stations U8 and U9) to 2.5 (station U2) (Fig. 6). Zonewise, lowest diversity values were observed at the mesohaline zone followed by oligohaline and polyhaline zones. The EcoQS derived by the Shannon-Wiener diversity index  $(H')$  as per the thresholds mentioned in Table 2 indicated that the euhaline and polyhaline zones fell predominantly in 'poor' category (stations U1,U3,U4,U5 and U12) while most stations in the mesohaline and oligohaline zones and two polyhaline stations qualified under the 'bad' status (stations U6 to U11). The only station that was tagged with 'moderate' status was station U2 in the euhaline zone.

All 39 species of different sensitivities were assigned to five Ecological Groups (EGs) by AZTI's classification. After assignment, 13 macrobenthic taxa (33.3%) were grouped in EG I, 7 taxa (18%) in EG II, 9 taxa (23 %) in EG III, 7 taxa (18 %) in EG IV, and 3 taxa (7.7 %) in EG V (Appendix 1). The opportunistic species, S. gynobranchiata was present in large numbers and dominated the macrobenthic assemblages ( $> 10,000$  ind m<sup>-2</sup>) in the stressed polyhaline zone and hence was assigned to EG V based on expert judgement, local ecological characteristics (Teixeira et al., 2010; Valença and Santos, 2012; Sivadas et al., 2016) and available literature (Çinar et al., 2015). The derived AMBI values ranged from 2.3 to 7.0 reflecting 'good' (slightly disturbed) to 'bad' (extremely disturbed) status of the estuary. Following the defined thresholds (Table 2), euhaline stations (U1, U2 and U3) were classified as 'good'; polyhaline zone included both 'moderate' (stations U4 and U5) and 'poor' (stations U6 and U7) status. Towards upstream, status improved from 'poor' (station U10) to 'moderate' (station U11) and finally 'good' (station U12). M-AMBI values ranged from 0.0 to 0.91 and the ecological status varied between "bad" and "high". In terms of the ecological status of estuarine zones, M-AMBI classified euhaline as 'good' to 'high', polyhaline and oligohaline as 'bad to high' and mesohaline as 'bad' status. The BENTIX index (min-max 0.0-5.48) showed 'poor'

status at six stations (stations U4-U7, U10 and U11) showing heavily disturbed ecological quality in major stretch of the estuary while stations U1, U2 and U12 were categorized under 'moderate' status. BOPA and BO2A (min-max 0.0414-0.3010) indicated that the euhaline zone fell in 'moderate to 'good' status while polyhaline stations were classified as 'bad' to 'good'. The BO2A and BOPA values were similar at all stations except at U10 and U11 of the oligohaline zone. At these stations, the BO2A values were expectedly higher due to the dominance of opportunistic oligochaetes that resulted in change of ecological quality status from 'high' and 'poor' to 'bad' status respectively. Mesohaline zone (stations U8 and U9) was classified as 'bad' by all four indices  $(H, AMBI, M-$ AMBI and BENTIX) (Table 3). This was expected, as both these stations were without benthic fauna. BOPA and BO2A values could not be derived for these stations due to their azoic condition, but were given 'bad' status as per Dauvin and Ruellet (2007).

The ecological status of the individual station by the six different benthic indices was not always concordant and is represented in Table 3. M-AMBI indicated equal division (50%) of the estuary into "acceptable" and "not acceptable" quality status. AMBI and BOPA classified 66.7% of stations as "not acceptable" and 33.3% being in "acceptable" EcoQS, though in dissimilar zones. BO2A index documented 25% and 75% of the stations as "acceptable" and "not acceptable" respectively. More severe categorization was done by BENTIX where 91.7% of stations were noted as "not acceptable" with 8.3% as "acceptable". Shannon-Wiener's index  $(H')$  identified the entire estuary as "not acceptable" with 50%, 41.7% and 8.3% falling under 'bad', 'poor' and 'moderate' status, respectively. The similarity percentage among the six indices was extremely low. The ecological status of only 16.7% of the study area was in agreement though not necessarily in the same zone (Fig. 7). It was also observed that H' did not categorize any station as 'high' or 'good'. AMBI and BO2A did not assign 'high' status while the 'poor' and 'good' categories were not indicated by M-AMBI and BENTIX respectively. Only BOPA categorized stations into all five ecological classes.

Following the sum of scores method stated by Blanchet et al. (2008) (Table 4), full agreement on "not acceptable" status among the six indices was observed in 41.6% of stations (U6, U7, U8, U9 and U11). Partial agreement (i.e. 5 indices out of 6 agreed on 'acceptable' or 'not acceptable') occurred in 25% of stations (U3, U5, and U10). The different indices disagreed on the status of 33.3% of stations (U1, U2, U4, and U12). Spearman's rank correlation calculated between all six derived indices indicated statistically significant correlations with each other (Table 5). M-AMBI, BENTIX and H' were positively correlated with each other while AMBI was negatively correlated with these three indices. BOPA correlated positively with BO2A but did not display any significant correlations with remaining five indices.

#### 3.4 Interaction between abiotic and biotic measures

The Spearman's Rank correlations between biotic and abiotic variables are given in Table 6. The univariate indices  $(H', J'$  and  $d)$  were positively correlated with pH and DO and negatively with  $NH_4^+$ -N, clay and  $C_{org}$ . Margalef's index (d) was positively influenced by salinity. The polychaete, S. gynobranchiata correlated negatively with DO and positively with nutrients ( $PO<sub>4</sub><sup>3</sup>$ -P and NO<sub>2</sub>-N). Chironomidae were inversely proportional to water temperature and salinity. AMBI and BO2A indices were negatively influenced by pH and DO and positively correlated with  $NH_4^+$ -N, clay and Corg. Conversely, M-AMBI and BENTIX indices were positively related with pH and DO and negatively correlated with NH<sub>4</sub><sup>+</sup>-N, clay and C<sub>org</sub>. BIOENV analysis among environmental variables and macrobenthic abundance (Table 7) revealed the combined influence of four parameters viz. salinity,  $NO_2$ <sup>-</sup>N,  $NH_4$ <sup>+</sup>-N and SS ( $\rho$ =0.47) on the distribution pattern of macrobenthos.

### 4. Discussion

 Different sections of the Ulhas estuary were impacted to varied extent by diverse stressors as mentioned in Table 1. PCA analyses indicated that stations U5-U10 (middle estuary) had silty sediments which were associated with high organic carbon and nutrients. The low DO, high NH<sub>4</sub><sup>+</sup>-N and C<sub>org</sub> values at the defaunated mesohaline stations (U8 and U9) were most likely due to the weak tidal impact and poor flushing (Ram et al., 2009) at this zone leading to the accumulation of organic matter. The water and sediment at this estuarine stretch were observed to be dark and murky with a putrid stench (personal observ.). During low tide, this estuarine zone received untreated domestic sewage originating from the urbanized zones located upstream. Similar observations were reported by Doğan et al. (2005) and Ergen et al. (2006) in the polluted inner part of the Izmir Bay, Turkey during the summer. The opportunistic polychaete, S. gynobranchiata contributed to 88 % of macrobenthic abundances in the polyhaline zone of the estuary indicating polluted environment (Ergen et al., 2006, Çinar et al., 2012). Elevated C<sub>org</sub> accompanied by low DO were also observed at station U10 in the oligohaline zone due to the anthropogenic input of organic waste creating stressed conditions and impoverished macrobenthic assemblages at this location. Conversely, upper oligohaline stations U11 and U12 were well oxygenated due to freshwater inputs from the riverine zone. The dominance of the oligochaete, T. heterochaetus (97%) at U10 indicated that this station was relatively impacted as compared to the other two stations (U11 and U12) in the oligohaline zone as oligochaetes are usually considered to be tolerant to anthropogenic pressure (Gillett et al., 2007).

The PCA (Fig. 5) and BIOENV (Table 7) analyses demonstrated that a suite of environmental variables (salinity, DO, nutrients, sediment texture and  $C_{org}$ ) influenced the spatial distribution of macrobenthic fauna in the Ulhas estuary. Along the estuarine gradient, salinity is of paramount importance as the distribution of estuarine organisms is known to vary by the amendments in the salinity regime (Telesh and Khlebovich, 2010, Mariano and Barros, 2015). For instance, in the Ulhas estuary, S. gynobranchiata was restricted to the polyhaline zone while tubificid species were found only at the oligohaline section. While a major part of Ulhas estuary was reported to be silt dominated, a notable enhancement in the silt fraction in the last decade was observed by Athalye et al. (2003) who attributed this to the increased sewage load and solid waste dumping. Our present observations appear to reinforce this conclusion as the present silt values (av. 64 %) were higher than the values reported earlier (61.8%; Athalye et al., 2003) . The main measures of biotic integrity like species richness, diversity and evenness had stronger association with anthropogenic stressors like DO, NH<sub>4</sub><sup>+</sup>-N, C<sub>org</sub> than natural variation like salinity (Table 6).

Attributes of an efficient environmental metric include the ease of measurement and conveying information to the decision makers along with a sensitivity that can measure the environmental status and response to management initiatives (Borja and Dauer, 2008). In the present study, H', AMBI, M-AMBI, BENTIX, BOPA and BO2A were compared to evaluate their efficacy in establishing the ecological status of the highly stressed Ulhas estuary. Based on the expert judgement of the anthropogenic activities listed in Table 1, chemical measurements (Fig. 3) and macrobenthic statistics (Fig. 6), it was inferred that stations U1- U3 and U12 were less impacted, stations U4, U5 and U11 were moderately impacted, stations U6, U7 and U10 were heavily stressed and stations U8 and U9 were severely disturbed. There was no location in the entire estuarine stretch that was free from human interferences.

In general, the  $H'$  index suggested higher levels of stress than the other five indices. The discrepancies were particularly more pronounced as compared to AMBI and M-AMBI (Table 3).

 $H'$  downgraded the overall ecological status of the estuary and categorised the entire stretch as mostly 'poor' or 'bad' despite the appreciable presence of sensitive species at stations U3 (M. orientalis), U5 (Phoronis psammophila), U6 (P. psammophila) and U12 (Cyprinotus sp.). One possible reason for this could be the natural occurrence of sensitive species in comparatively high densities that affected the index value as suggested by Spagnolo et al. (2014) who indicated that H' may not respond adequately to environmental stress.  $H'$  was less efficient as an index in this study, since it only considers the number of species and its relative abundances and not the sensitivity of the macrobenthic species (Labrune et al., 2006). Low species diversity with high values of organic carbon were observed from stations U6 to U11 indicating 'bad' status at these stations. However, low diversity and high abundance of species are considered a paradigm for estuaries, inhabited by

those adapted to erratic environmental status and distributed as per their specific tolerance level (Elliott and Whitfield, 2011). Estuaries naturally harbour many opportunistic species adapted to the natural and anthropogenic stress and can underestimate the status yielded by biotic indices (Elliot and Quintino, 2007). Thus, the Shannon diversity index was unable to discriminate between moderately and severely impacted stations. Previous studies have also indicated the weakness of H' as a biotic index as this measure was sensitive to sampling effort (Hewitt et al., 2005) and also prone to classifying unimpacted areas as 'Bad', 'Poor' or ' Moderate' than AMBI (Labrune et al., 2006).

BENTIX indicated zero value at the polluted mesohaline stations (U8 and U9) which were near an outfall, signifying a response to the organic pollution in that area. These results were in agreement with those observed in Saronikos Gulf where the lowest index values were attained in stations neighboring to the Psittalia outfall (Simboura et al., 2005). Nevertheless, BENTIX overestimated station U3 as 'high' though the area was subject to minor effluent discharges as detailed in Table 1. While BENTIX was efficient in differentiating the 'bad' from the 'poor' stations in the polyhaline and mesohaline zones, it categorized all stations with minor impacts as 'moderate'. It is almost very critical for any index to evaluate the margin between 'good' and 'moderate' and to bring out an appropriate outcome (Çağlar and Albayrak, 2012). The poor discriminating power displayed by BENTIX in the euhaline and oligohaline zones is probably due to the assignment of species into only two broad ecological groups (Muniz et al., 2012, Brauko et al., 2016) as compared to AMBI and M-AMBI.

BOPA and BO2A had either underestimated or overestimated the ecostatus of majority of the stations (U1,U2,U4-U7,U10,U11) but correctly designated stations U3, U8, U9 and U12 (Table 3). BO2A index, developed for upstream (freshwater) zones of transitional waters (i.e., up to the upper limit of tidal range) performed more appropriately in the oligohaline zone than BOPA index. This could be due to the fact that it was designed specifically for such anthropogenically stressed systems with variable salinities (Dauvin and Ruellet, 2009). Although BOPA and BO2A have the advantage of minimizing taxonomical effort, they have one drawback i.e., only polychaetes (or annelids) and amphipods (except *Jassa*) are being considered in the calculation and other species which might have significant relative frequency are not sufficiently taken into account. It could be difficult to infer the results in a disturbed ecosystem where sensitive groups other than amphipods are present in appreciable numbers (for ex.  $M$ . orientalis at station U3). BOPA was not sensitive enough to identify the disturbances at the station in the vicinity of a discharge point (U10) which had high organic carbon content but was given 'high' status. This occurred as BOPA did not consider the presence of oligochaetes (T. heterochaetus) which comprised 97 % of macrobenthic abundances at U10. BO2A,

on the other hand, included this opportunistic group in its estimation and categorised U10 as 'bad'. However, BO2A failed to distinguish between the azoic stations (U8 and U9) and stations with some presence of macrobenthos (U6, U7, U10 and U11) thereby categorizing all as 'bad'.

AMBI and M-AMBI have been highly endorsed as suitable bio-indicator indices for estuaries and coastal waters by many researchers (Simboura and Reizopoulou, 2007; Li et al., 2013; Brauko et al., 2016). AMBI was observed to be appropriate in assessing the quality status of different zones of the present estuarine system and was successful in identifying the most degraded sites having high  $C_{org}$ content and low dissolved oxygen concentration. Current observations were in agreement with Borja et al. (2008) from the Chesapeake Bay and Valença and Santos (2012) from tropical estuaries of Brazil, where elevated values of AMBI coupled with high C<sub>org</sub> and low dissolved oxygen concentration represented an unhealthy environment. AMBI could also successfully distinguish the defaunated mesohaline stations ('bad') from the polyhaline stations ('moderate' to 'poor') which had better macrobenthic representation. This was in agreement with Pinto et al. (2009), who could differentiate the quality status between different salinity zones of the Mondego estuary using the P-BAT index which integrates AMBI values. The success of AMBI can be attributed to the fact that this index considers the differential sensitivities of various macrobenthic components. Also, according to Simboura (2004), AMBI was more efficient than BENTIX in estuarine sites with low species richness and high abundance, as in this case, due to better discrimination (five ecological groups as opposed to two of BENTIX ) of constituent species.

M-AMBI agreed with AMBI in 5 stations, overestimated five stations and underestimated the remaining two stations. M-AMBI indicated three stations viz. U2, U5 and U12 as 'high' despite these locations being exposed to anthropogenic elements, albeit to various degrees. Similarly, M-AMBI denoted stations U7 and U10 as 'Bad' which equated these stations with the azoic stations, U8 and U9. The magnitude of M-AMBI values relies on species diversity and species richness as it is an integrative multi-metric index. However, the diversity measures are influenced by sample size, habitat type, and natural dominance of species often leading to misinterpretations (Simboura and Argyrou, 2010). Also, the computation of M-AMBI is complicated by the need of a 'good' reference site which is often theoretically derived in most anthropogenically impacted study areas such as the Ulhas estuary, thus leading to overestimation of ecological status (Hutton et al., 2015). Therefore, it has been suggested that M-AMBI be tested with other indices (Bigot et al., 2008).

The inherent ability to distinguish between acceptable ('high' and 'good') and the unacceptable ('moderate', 'bad' and 'poor') EcoQS is crucial to the selection of a suitable biotic index (Bigot et al., 2008). Therefore, despite the significant correlations between the various indices as demonstrated in Table 5, there was little agreement between the EcoQS derived by all indices. This indicated that while all indices, except M-AMBI, agreed on the general trend that major part of the estuary had 'unacceptable' EcoQS (66.7-100%), they disagreed on the status of individual stations. Results of BENTIX index concurred with those obtained by AMBI, M-AMBI and  $H$ <sup>2</sup> at mesohaline zone showing a good agreement between the four indices for this region. A strong negative correlation between BENTIX and AMBI (Ponti et al., 2008; Spagnolo et al., 2014) was not surprising, as these two are based on the relative frequencies of species of varying sensitivity to disturbances. Positive correlation obtained between BENTIX and  $H'$  might be due to the numerical dominance of opportunistic species as Shannon's diversity index considers evenness while BENTIX takes tolerant groups (for ex. S. gynobranchiata) into account. As all the indicators are theoretically based on the benthic community succession model of Pearson and Rosenberg (1978), they are interrelated and have the power to understand the different aspects of the qualitative alterations induced by the anthropogenic disturbance of benthic habitats (Dimitriou et al., 2012). No correlation of BOPA with any of environmental variable indicated its low performance in the study area.

While the results of this study established the utility of AMBI in evaluating the environmental status of the Ulhas estuary, it is important to emphasize that its applicability required reassignment of species to appropriate ecological groups based on expert judgement. The reclassification of the opportunistic species, S. gynobranchiata to EG V improved the sensitivity of AMBI in the present study as was also endorsed by Valença and Santos ( 2012) while evaluating the ecostatus of some tropical estuaries in Brazil. While the appropriate assignment of the dominant benthic component is crucial for the proper application of AMBI, ecological assignments made available by European databases may not work in a tropical milieu and may differ depending on the geographical and environmental setting. Therefore, a number of studies (Albayrak et al.,2006) have recommended refining the EG based on expert judgment and knowledge of study area to prevent misclassification of locations and improve the utility of AZTI indices.

Several studies have reported that their indices have not correlated with reliable indicators of contamination (Brauko et al., 2015). However, in the present study, all indices except BOPA have shown a significant relationship with parameters suggesting organic pollution namely DO, NH<sub>4</sub><sup>+</sup>-N and  $C_{org}$  while there was no correlation with salinity. Thus, all indices were able to demonstrate the presence of acceptable stations in the euhaline as well as oligohaline zones. Hence, it is inferred that five of the six indices have responded appropriately to the stressors present in the ecosystem to various extent (Valença and Santos, 2012) despite the obvious salinity variations in the estuary. Estuarine gradients, especially the salinity regime, may influence resident benthic community

structure and thereby impact values derived through the indices (Teixeira, et al., 2008). Therefore, as emphasized by other studies (Chainho et al., 2008; Feebarani et al., 2016), separate reference conditions were established for different salinity zones in this study.

The application of multiple indices to measure ecological quality have been advocated by previous studies (Krõncke and Reiss, 2010). In the current study, there were inconsistencies in the ecological quality assessment produced by the various benthic indices as highlighted by others too (Labrune et al., 2006; Blanchet et al., 2008; Borja et al., 2008; Grémare et al., 2009). This disparity arises because different indices use diverse methods for species classification. Another reason for the low similarity percentage among the six studied indices might be the disagreement in the threshold values of each index that needs to be adjusted for different types of environments to obtain similar results for a given station (Zettler et al., 2007; Blanchet et al., 2008; Simboura and Reizopoulou, 2008; Munari and Mistri, 2010). However on the other hand, as per Labrune et al. (2006), a single biotic index was more efficient in converting voluminous environmental data into easily derived decision of the ecological quality of a given area.

#### 5. Conclusion

The present study is the first endeavor to test the utility of benthic indices in deriving the ecological quality status of estuaries on the industrialized and urbanized northwest coast of India. To summarize, the present results found that AMBI proved to be most efficient among the tested indices in segregating various zones of the estuary into appropriate EcoQS. Based on the EcoQS, management interventions could be devised to improve the present ecological quality. The current study could also be a model to replicate similar investigations to other estuaries impacted by diverse anthropic stressors along the northwest Indian coast. However, further studies may be required to understand the implications of seasonal variations on the efficacy and reliability of the chosen index. While AMBI is reported to be impervious to seasonality (Muxika et al., 2007), it will of interest to evaluate the effect of seasonality on the performance of indices for estuarine quality assessment along the northwestern Indian coast.

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Station	Study sites	Geographic	Depth	Sources of disturbance	
		coordinates	(m)		
U <sub>1</sub>	Bassein	19°19'8.71"N	11	Minimum anthropic action	
		72°41'8.75"E			
U <sub>2</sub>	Vasai jetty	19°19'42.95"N	5.3	<b>DSAFA</b>	
		72°49'13.10"E			
U <sub>3</sub>	Bhayandar	19°17'15.20"N	6.5	Mangrove area, MEFDA	
		72°54'31.10"E			
U4	Gaimukh	19°16'21.50"N	14	MEFDA, Dredging	
		72°59'33.20"E			
U <sub>5</sub>	Kasheli	19°13'27.70"N	10	MEFDA and industrial effluents	
		73° 0'31.60"E			
U <sub>6</sub>	Mumbra	19°12'2.30"N	12	Confluence with Thane creek, SWD	
		73° 1'34.00"E			
U7	Dombivali	19°12'24.84"N	9	<b>DSAIE</b>	
		73° 3'54.76"E			
U <sub>8</sub>	Kori gaon	19°15'27.71"N	8.5	Dredging, DSAIE	
		73° 6'28.40"E			
U <sub>9</sub>	Kalyan	19°16'8.04"N	8.5	<b>DSAIE</b>	
		73° 8'59.25"E			
U10	Ambivali	19°16'27.49"N	2.8	<b>DSAIE</b>	
		73°10'19.71"E			
U11	Pise	19°18'52.50"N	0.5	Domestic effluents	
		73°11'1.40"E			
U12	Titwala	19°18'26.50"N	0.4	Domestic effluents	
		73°11'35.60"E			

Table 1: Description of study sites and summary of their main disturbance sources (Athalye et al., 2003)

(DSAFA: Domestic Sewage And Fishing Activities., MEFDA: Minor Effluents From Domestic Areas., SWD: Solid Waste Dumping., DSAIE: Domestic Sewage And Industrial Effluents)



Table 2: Thresholds utilized for each index. References for each index are indicated by superscript numbers (see footnotes)

 $^{\circ}$ Labrune, 2006  $^{\rm b}$ Borja et al., 2000  $\mathrm{^{c}B}$ orja et al., 2007 <sup>d</sup>Simboura and Argyrou, 2010 <sup>e</sup>De-la-Ossa-Carretero and Dauvin, 2010

Station	<b>AMBI</b>	M-AMBI	<b>BENTIX</b>	H'	BO <sub>2</sub> A	<b>BOPA</b>	
U1	Good	Good	Moderate	Poor	Moderate	Moderate	
	(2.83)	(0.58)	(2.47)	(1.5)	(0.18452)	(0.18452)	
U <sub>2</sub>	Good	High	Moderate	Moderate	Moderate	Moderate	
	(3.11)	(0.79)	(3.44)	(2.5)	(0.13106)	(0.13106)	
U <sub>3</sub>	Good	Good	High	Poor	Good	Good	
	(2.5)	(0.73)	(5.48)	(1.44)	(0.04141)	(0.04141)	
U <sub>4</sub>	Moderate	Good	Poor	Poor	Good	Good	
	(4.0)	(0.56)	(2.36)	(1.24)	(0.10474)	(0.10474)	
U <sub>5</sub>	Moderate	High	Poor	Poor	Poor	Poor	
	(3.5)	(0.91)	(2.3)	(2.01)	(0.2406)	(0.2406)	
U <sub>6</sub>	Poor	Moderate	Poor	Bad	Bad	Bad	
	(5.3)	(0.43)	(2.0)	(0.83)	(0.27104)	(0.27104)	
U7	Poor	Bad	Poor	Bad	Bad	Bad	
	(6.0)	(0.09)	(2.0)	(0.01)	(0.30103)	(0.30103)	
U <sub>8</sub>	Bad	Bad	Bad	Bad	Bad*	Bad*	
	(7.0)	$\boldsymbol{0}$	$\boldsymbol{0}$	$\boldsymbol{0}$			
U <sub>9</sub>	Bad	Bad	Bad	Bad	Bad*	Bad*	
	(7.0)	$\boldsymbol{0}$	$\boldsymbol{0}$	$\boldsymbol{0}$			
U10	Poor	Bad	Poor	Bad	Bad	High	
	(5.96)	(0.16)	(2.0)	(0.18)	(0.30103)	(0.01158)	
U11	Moderate	Moderate	Poor	Bad	Bad	Poor	
	(4.3)	(0.46)	(2.0)	(0.89)	(0.26041)	(0.25181)	
U12	Good	High	Moderate	Poor	Good	Good	
	(2.29)	(0.9)	(3.42)	(1.75)	(0.08215)	(0.08067)	

Table 3: Ecological quality status derived from six biotic indices with their values in parentheses for the study stations.

\* Azoic zones are considered 'Bad' as per Dauvin and Ruellet, 2007

 Table 4: Level used for the measurement of agreement/disagreement between the six indices for each station. Overall, full agreement was measured as [a]+[g], partial agreement was measured as  $[b]+[f]$  and disagreement as  $[c]+[d]+[e]$ (Blanchet et al., 2008)







Table 6: Spearman's Rank correlation between biotic and abiotic variables (\*\*\*p<0.001; \*\*p<0.01;\*p<0.05)

(WT: Water Temperature, DO: Dissolved Oxygen,  $PO_4^{3-}$ - P: Phosphate-Phosphorus, NO<sub>2</sub> -N: Nitrite-Nitrogen, NH<sub>4</sub><sup>+</sup>-N: Ammonia-Nitrogen )



No. of Variables correlation	Variables	Spearman $(\rho)$	
	Salinity <sup>a</sup>	0.366	
2	Salinity, $NH_4^+$ - $N^b$	0.391	
$\overline{3}$	Salinity, $NH_4^+$ -N, NO <sub>2</sub> - N <sup>c</sup>	0.451	
$\overline{\mathbf{4}}$	Salinity, $NH_4^+$ -N, NO <sub>2</sub> <sup>-</sup> -N, SS <sup>d</sup>	0.478	
5	Salinity, $NO2 - N$ , $NH4+ - N$ , SS, $PO43 - P$	0.458	

 Table 7: Results of BIO-ENV analyses. Variables marked in bold indicates overall optimum Spearman correlation

<sup>a</sup> Salinity<br><sup>b</sup> Ammonia-nitrogen<br><sup>c</sup> Nitrite-nitrogen

<sup>d</sup> Suspended Solids



Fig. 1



Fig. 2



Fig. 3



Fig. 4











Fig. 7

Figure caption:

Fig.1. Map of the study area (Ulhas estuary, northwest of India coast), including the position of sampling stations

Fig.2. Multidimensional scaling (nMDS) plot of macrobenthic abundance data at all sampling stations of the Ulhas estuary. Shaded region indicates the azoic zone

Fig.3. Spatial distribution of water variables in the Ulhas estuary. Legend: (a) water temperature, (b) pH, (c) dissolved oxygen, (d) salinity, (e) suspended solids, (f) Phosphate-Phosphorous (PO<sub>4</sub><sup>3-</sup>-P), (g) Nitrite-Nitrogen (NO<sub>2</sub>-N), (h) Nitrate-Nitrogen (NO<sub>3</sub>-N) and (i) Ammonia-Nitrogen (NH<sub>4</sub><sup>+</sup>-N).

Fig.4. Spatial distribution of sediment variables in the Ulhas estuary. Legend: (a) sand %, (b) silt %, (c) clay % and (d)  $C_{org}$ %

Fig.5.Two-dimensional Principal Component Analysis (PCA) ordination of environmental variables of Ulhas estuary

Fig.6.Spatial distribution of (a) Average macrobenthic abundance; N, (b) Margalef's richness index; d, (c) Pielou's evenness index; J' and (d) Shannon-Weiner's index;  $H'log<sub>2</sub>$  in the Ulhas estuary

Fig.7.Ecological quality status of Ulhas estuary using different biotic indices (AMBI, M-AMBI, BENTIX, H' log<sub>2</sub>, BO2A and BOPA)



Appendix 1: List of macrobenthic species with their ecological groups recorded in the stations of the Ulhas estuary. Value in parenthesis is the EG used in the current study.  $\overline{\phantom{0}}$ 

Appendix 1: Contd..

<b>Faunal groups</b>	<b>Ecological Groups (EGs)</b>	<b>Stations</b>
<b>OLIGOCHAETA</b>		
Tubificoides heterochaetus (Michaelsen, 1926)	V	U10
Tubificoides amplivasatus (Erséus, 1975)	$\overline{\mathsf{V}}$	U11
<b>HIRUDINEA</b>	IV	U12
<b>AMPHIPODA</b>		
<i>Ampelisca</i> sp.	I	U <sub>2</sub>
Dexamine spinosa (Montagu, 1813)	III	U <sub>2</sub>
Dulichia sp.	Ш	U2
Microprotopus maculatus (Norman, 1867)	I	U3, U5
Ericthonius sp.	I	U <sub>3</sub>
<b>CUMACEA</b>		
<i>Iphinoe</i> sp.	I	U <sub>2</sub>
<b>ISOPODA</b>		
Cirolana aldabrensis (Schotte & Kensley, 2005)	$\mathbf{I}$	U <sub>3</sub>
Excirolana sp.	I	U <sub>3</sub>
<b>OSTRACODA</b>		
Cyprinotus sp.	I	U12
<b>TANAIDACEA</b>		
<i>Pagurapseudopsis gymnophobia</i> (Barnard, 1935)	III	U5, U6
<b>MYSIDA</b>		
<i>Mesopodopsis orientalis</i> (W. Tattersall, 1908)	$\mathbf{I}$	U <sub>3</sub>
<b>INSECTA</b>		
Chironomidae	III	U11, U12