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# Community structure of benthic amphipods in four estuaries of northwest India

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## Abstract

This is the first comprehensive study of the community structure and spatial-temporal distribution of amphipods in estuarine sediments of northwest India. Specifically, amphipods from Ulhas, Amba, Kundalika and Savitri estuaries were investigated seasonally to study species composition, distribution, diversity, and functional traits of Amphipods and the influence of hydrographic parameters on community structure. There were fifty-seven taxa, thirty-four genera belonging to nineteen families with Aoridae and Ampeliscidae being the most species families. The seasonal variations in abundances were influenced by the population dynamics of the dominant species. *Cheiriphotis trifucata, Grandidierella japonica* and *Podocerus spongicolus* were reported for the first time in Indian waters. Most of the amphipod taxa belonged to the functional group that was represented by detritivory and domiculous behaviour (DEDO). Salinity, suspended solids, dissolved oxygen, pH, sediment texture and  $C_{org}$  were the suite of variables that best explained the estuarine amphipod community structure. The irregular distribution trends of the amphipods in all estuaries were probably due to the low densities of this group.

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## **1. Introduction**

The dynamics of benthic assemblages of coastal ecosystems has been the focus of environmental impact assessment studies due to the increasing number of human interferences (Feebarani et al., 2016; Yang et al., 2016). Among the benthic communities, amphipods constitute one of the dominant groups that inhabit marine rocky and soft bottom habitats (de-la-Ossa-Carretero et al., 2012; Guerra-García et al., 2014). Their high turnover rates, limited mobility, direct development and high sensitivity to diverse pollutants suggest that amphipods indeed play key functional role in the ecology of these habitats (Guerra-García et al., 2014). They also represent diverse feeding and life strategies and play a vital role in energy flow through the food web (Bellan-Santini, 1980; de-la-Ossa-Carretero et al., 2012; Navarro-Barranco et al., 2017). Consequently, amphipods along with other small crustacean taxa, are responsible for providing much of the environmental sensitivity displayed by many of the commonly used benthic biotic indices such as AMBI, BOPA and BO2A (Borja et al., 2000; Dauvin et al., 2016).

Studies have highlighted the importance of benthic amphipods in various environments and have suggested that their distribution depends on both physical (e.g., sediment composition and hydrodynamics) and biological factors (e.g., food, competition and predation) (de-la-Ossa-Carretero et al., 2012, 2016; Navarro-Barranco et al., 2017). Additionally, human activities such as industry, urban sewage discharge, farming, fishing, and agriculture also alter the distribution of amphipod species at a rapid rate (Conradi et al., 1997; Gesteira and Dauvin, 2000; Carvalho et al., 2012). Thus, estuarine amphipod community dynamics may be influenced by an amalgamation of environmental and anthropogenic factors. The ecology of amphipods is generally better explained in terms of the functional traits (e.g. feeding and lifestyle). These traits are assumed to be of great significance in influencing habitat preferences, sensitivity and distribution patterns of these crustaceans (de-la-Ossa-Carretero et al., 2012). Overall, a total of 9,838 (Amphilochidea 4,140, Colomastigidea 55, Psuedingolfiellidea 4, Senticaudata 5,639) benthic (including interstitial) amphipod species (Horton et al., 2018) have been reported in many parts of the world. However, information on diversity and distribution of benthic amphipods and factors contributing to their distribution are scarce from Indian waters (Surya Rao, 1972; Nair et al., 1983; Guerra-Garcia et al., 2010; Mondal et al., 2010; Raja et al., 2013).

The 720 km long coastline of Maharashtra state, located in northwest India, is subject to rapid industrialization and urbanization resulting in the indiscriminate release of domestic and industrial effluents directly into adjacent water bodies (estuaries, creeks and bays). There are 45 estuaries along the Maharashtra coast, most of them being heavily stressed due to multiple anthropogenic

interventions (NIO, 2009; Gokhale et al., 2011). The arbitrary release of liquid and solid wastes into the coastal waters alter the water and sediment quality parameters which subsequently affect the habitat, existence and food availability of biota (Zingde and Govindan, 2000; Athalye et al., 2001). While a plethora of data are available on the hydro-sedimentological characteristics and plankton diversity of these estuaries (Paulinose et al., 2004; Quadros et al., 2004; Menon and Mahajan, 2013; Pande and Nayak, 2013a), little is known of their macrobenthic species diversity patterns (Mathew and Govindan, 1995; Athalye et al., 2003; Mulik et al., 2017). Among the limited information on macrobenthos that is available, the Polychaeta, being the dominant macrobenthic fauna, have been most studied (Mathew and Govindhan, 1995; Sukumaran and Devi, 2009). On the contrary, benthic amphipods have received scant attention probably due to lack of taxonomic expertise. Thus, the present study investigated the spatial-temporal distribution and community structure of soft-sediment benthic amphipods in four differently modified estuaries of the Maharashtra coast. The specific aims were to: 1. characterize the community structure (i.e., species composition, richness, diversity, and abundance), and functional traits both spatially (i.e., euhaline, poly-mesohaline and oligohaline) and seasonally (i.e., premonsoon, monsoon and postmonsoon), and 2. determine the important environmental parameters that influenced community structure.

#### 2. Material and methods

#### 2.1 Study area

The study was conducted in four major estuaries (Ulhas, Amba, Kundalika and Savitri) of Thane and Raigad districts, Maharashtra (Fig. 1). All four rivers originate in the western slopes of Sahyadrian ranges and drain into the Arabian Sea. The general climate of the study region is typical as that of northwest coast of India with three well defined seasons such as premonsoon (February to May), monsoon (June to September) and postmonsoon (October to January). The region receives a considerable amount of rain during monsoon and has hot weather during premonsoon and high humidity throughout the year. During monsoon, the rainfall results in more freshwater input into these estuaries, causing an intense salinity drop. In postmonsoon the salinity is at its peak.

#### Ulhas estuary

The Ulhas has a catchment area of about 4,637 km<sup>2</sup>. Following a westerly course of about 100 km, it joins the Arabian Sea at Vasai creek (19° 16' N and 72° 45'E). Average annual rainfall is about 2,943 mm. There are several industries situated along the banks of the river discharging their effluents and loading the water body heavily with pollutants which pose a threat to aquatic organisms

(Athalye et al., 2003). The estuary also receives copious amounts of domestic wastes and sewage leading to periodic hypoxia in the upper estuary (Mulik et al., 2017).

## Amba estuary

The Amba has a catchment area of about 420 km<sup>2</sup> and meanders along for over 140 km before joining the Arabian Sea at Revas village (18° 50' N and 72° 54' E). This is a shallow estuary with an average depth of 3 m. Average annual rainfall is about 2,900 mm. Large infrastructural facilities of industries are established along the estuary and a petrochemical complex releases its treated effluents (6,500 m<sup>3</sup> d<sup>-1</sup>) in the mid estuary (Naidu et al., 2016).

## Kundalika estuary

The Kundalika has a catchment area of about 294 km<sup>2</sup> and meets the Arabian Sea at Revdanda (18° 32' N and 72° 52' E). This estuary is shallow with a depth ranging from 1m at the mouth to 8m at the upstream. An average annual rainfall of 3,750 mm is reported for this area (Pande and Nayak, 2013b). The mid estuary is subject to dredging activities and acts as an ideal sink for suspended sediment matter. A chemical industrial cluster releases their effluents (10,000-15,000 m<sup>3</sup> d<sup>-1</sup>) directly into the middle estuary with minimal treatment (NIO, 2009). In addition to the industrial waste, the estuary also receives domestic waste (around 1.4 MLD; Million Litres per Day) (NIO, 2018).

#### Savitri estuary

The Savitri has a catchment area of about 2,262 km<sup>2</sup> and opens into the Arabian Sea at Bankot (17° 59' N and 73° 02'E). Annual average rain fall is about 3,560 mm (Gharde and Kothari, 2016). An industrial discharge outfall is present in the upstream zone. The estuary also receives 7.5 MLD of domestic sewage (NIO, 2009).

## 2.2 Sampling design and laboratory methods

Seasonal sampling (premonsoon, monsoon and postmonsoon) was conducted in Ulhas (7 stations), Amba (9 stations), Savitri (6 stations) during 2013 and Kundalika (7 stations) during 2014 (Fig. 1). Station K2 of the Kundalika estuary could not be sampled during monsoon season due to harsh weather conditions. The extent of each salinity zone varied seasonally depending on the freshwater input and tidal influence. Thus, the euhaline zone in Amba during premonsoon extended up to the upper estuary signifying the predominant marine influence on the estuary as compared to the other three estuaries. Some zones were not established in the studied estuaries depending on the seasons. The salinity zone of each station during all three seasons corresponding to different classes

of the Venice salinity classification system (euhaline, poly-mesohaline and oligohaline) is presented in Table 1.

Amphipods were collected in quadruplicate using a van Veen grab (0.04 m<sup>2</sup>) at each station. The sediments were then sieved through 0.5 mm mesh and the retained material was preserved in 5% buffered formalin solution containing Rose Bengal stain. Amphipod specimens were sorted from the other macrofaunal organisms and preserved in 70% alcohol for subsequent identification. They were identified to the lowest possible taxonomic resolution (i.e., genus or species) with the help of available literature (Stebbing, 1906; Barnard, 1979; Lincoln, 1979; Lyla et al., 1998; Chapman, 2007) and counted. Completely damaged individuals and juveniles were omitted from the analyses.

Sediment samples (top 2cm) were also collected in duplicate for analysis of grain size and organic carbon ( $C_{org}$ ). Grain size analysis was performed using sieving and pipette method (Buchanan 1984). Organic carbon ( $C_{org}$ ) was analyzed by a TOC analyser (Shimadzu SSM-5000A series). At each station, bottom water samples were collected in duplicate by using Niskin samplers (0.5 m above the bottom) for analyzing temperature (°C), pH, salinity, suspended solids (SS; mg l<sup>-1</sup>) and dissolved oxygen (DO; mg l<sup>-1</sup>). A digital thermometer was used to record the temperature and pH was measured by using a Cyber Scan (Model pH 510) pH meter. Samples for analysis of DO were fixed immediately and analysed by Winkler's method (Grasshoff et al., 1999). Salinity was measured by Mohr titration method and SS by filtration method following Grasshoff et al., (1999).

### 2.3 Data analysis

The average abundance of each taxon from the four replicate grabs at each station were expressed as number of individuals per m<sup>2</sup> (ind m<sup>-2</sup>) and used for univariate and multivariate analyses conducted in PRIMER v6 software (Clarke and Gorley, 2006). Several community metrics were calculated including: total number of individuals (N: average abundance), species richness (S: total number of taxa, d: Margalef index and Hurlbert index: ES 50), evenness (J': Pielou's index), species diversity (H': Shannon-Weiner index log<sub>2</sub>). Multivariate analyses included non-metric multidimensional scaling (nMDS) based on similarity matrix derived from fourth square root-transformed species abundance data using Bray-Curtis similarity index. k-Dominance plots were drawn to compare patterns of species abundances during the three seasons. Functional groups were assigned based on feeding behavior (detritivorous, detritivorous-herbivorous, detritivorous-carnivorous) and lifestyle (domicolous: species that build tubes, fossorial: species that burrow using their periopods and interstitial: species that live in the interstices between grains of sand) characteristics. Information on feeding and lifestyle of each individual species were

mined from the available literature (Appendix A). If the feeding and lifestyle information of particular taxa was not available it was assumed that the amphipods feed or behave in the same manner as congeneric species or confamiliar genus (Ramón et al., 2014). Amphipod taxa were also classified to ecological groups as a function of their sensitivity to contamination, following the criteria of AZTI Marine Biotic Index (AMBI) (Borja et al., 2000), based on the November 2014 species list available in the AMBI v.5.0 software which can be downloaded from the webpage (http://www.azti.es.). AMBI is the most applied index and classifies the amphipods into three groups: 1. Sensitive (EG-I), 2. Indifferent (EG-II) and 3. Tolerant species (EG-III) (Navarro-Barranco et al., 2014). The percentages of amphipod taxa belonging to each ecological group was calculated for each estuary and season. Spearman's rank correlation coefficients were used to visualize significant associations between amphipod abundance data, univariate diversity indices, functional groups and environmental parameters for each estuary using STATISTICA 7. The value of  $\alpha$  (0.05) was modified with the Holm-Bonferroni correction for multiple comparisons. The amphipod species abundance data was linked to abiotic variables using the BIO-ENV procedure to decipher the best suite of abiotic variables that were responsible for framing amphipod community structure.

### 3. Results

#### 3.1 Hydro-sedimentological characteristics

Spatial and seasonal variations in bottom water parameters are presented in Fig 2. The bottom water temperatures (WT) in all estuaries were expectedly higher during the premonsoon season. Lower salinity values during the monsoon indicated a significant freshwater influx in all the estuaries. The DO levels were lower in Ulhas as compared to the other estuaries with values as low as 2.1 mg l<sup>-1</sup> and 2.4 mg l<sup>-1</sup> in the upper estuary during postmonsoon and premonsoon seasons, respectively. The pH and SS in bottom waters of all estuaries displayed a decreasing trend towards oligohaline zone (Fig. 2). The granulometric analyses revealed that the sediments were predominantly silty in Ulhas, Amba and Kundalika estuaries (Fig. 3). The sediments were spatially heterogeneous with sand being the primary component in most of the stations of Savitri. The C<sub>org</sub> values were high in Ulhas, Amba, Kundalika (>1.7 %), but were comparatively lower in Savitri ( $\leq$ 1.0 %) throughout the study period.

## 3.2 Amphipod diversity

In total 4,844 individuals consisting of 57 taxa, 34 genera and 19 families were identified (Appendix B). Most of the amphipod taxa belonged to Aoridae (9 no.) and Ampeliscidae (8 no.) families. Maximum amphipod taxa were observed in the Ulhas estuary (30 no.) followed by Amba

(26 no.), Savitri (21 no.) and Kundalika (19 no.). In all estuaries, amphipod species numbers were highest during postmonsoon and lowest during the monsoon (Table 2). However, the dominant species composition of each estuary varied considerably (Table 2). In the current study, *Cheiriphotis trifurcata, Podocerus spongicolus* and a non-native amphipod *Grandidierella japonica* were reported for the first time from Indian waters.

Amphipods were not present at all stations and all seasons indicating a patchy distribution. Shannon diversity values were nil or poor (H'<1) in many stations of all estuaries (Appendix C). The highest H' values were observed in Ulhas (2.6), Amba (2.8), Kundalika (3.1) and Savitri (1.9) during the postmonsoon. The lowest H' and d values were noticed during monsoon season in all four estuaries. Evenness values (J') could not be computed for many stations, especially during monsoon due to the presence of only one species or complete absence of amphipods (Appendix C). The trend followed by Hurlbert index (ES50) was very similar to the H'. The results of the nMDS analyses for all four estuaries (Fig.4) did not indicate any clear spatio-temporal patterns in amphipod distribution. In the k-Dominance plots, the S-shaped curves pertaining to postmonsoon indicated that the more balanced amphipod assemblages with highest species diversity were present during that period in all estuaries (Fig. 5). Similarly, the sharp monsoon curves signified lower amphipod species diversity. The dominance curves for premonsoon season also suggested unfavourable conditions for amphipod species diversity in all estuaries except Amba.

## 3.3 Amphipod distribution

Among the four estuaries investigated, maximum amphipod abundance was noticed at Ulhas (1,933 ind m<sup>-2</sup>) during postmonsoon while Kundalika recorded the lowest amphipod abundances (15 ind m<sup>-2</sup>) during the monsoon. It was observed that the amphipods were present in higher numbers during the postmonsoon in all estuaries except for Amba, where the highest numbers were present during the monsoon (Fig. 6). Amphipods were more ubiquitous in the euhaline zone of Ulhas and Kundalika and in the poly-mesohaline zone of Amba and Savitri.

Though some taxa were common to two or more estuaries, many of the amphipod taxa were estuary specific (Appendix B). Also, it was observed that some amphipod taxa were specific to a certain estuarine zone or habitat. For instance, the semi-terrestrial species, *Platorchestia platensis* was present exclusively at the oligohaline zone of Ulhas where the sediments were periodically exposed during the spring low tide. The interstitial species *Idunella picta* and *Idunella* sp. and fossorial species *Urothoe pulchella* and *Urothoe* sp. were associated with the sand dominated sediments of Savitri estuary. *Dyopedos* sp. which is categorised as a EG-III group (based on its

sensitivity according to AMBI assignment) was present only during the monsoon season of Kundalika, that too in low numbers.

#### 3.4 Amphipod functional traits

In this study, seven functional groups were identified according to their feeding and lifestyle characteristics (Fig. 7). DEDO (DEtritivorous-DOmicolous) group was the most commonly occurring in all studied estuaries. DEIN (DEtritivorous-INterstitial) was the second dominant functional group, followed by CAOMIN (CArnivorous-OMnivorous-INterstitial). Considering all the seasons and zones, a maximum of five functional groups were noticed in Amba. In the euhaline zone, no noticeable trend was observed among seasons in all four estuaries whereas in the polymesohaline zone more diverse functional groups were present during the postmonsoon season. The oligohaline zone of Ulhas, Amba and Kundalika, which was sparsely populated by amphipods and had only one functional group. Savitri had two functional groups i.e. CAOMIN and DEDO in the oligohaline zone. Some of the functional groups were estuary specific. Thus, DECADO (DEtritivorous-CArnivorous-DOmicolous) and DECAIN (DEtritivorous-CArnivorous- INterstitial) in Amba; DEFO (DEtritivorous-FOssorial) in Savitri estuary were restricted to those particular estuaries. It was very interesting to observe that the amphipod taxa belonging to DEIN, DECAIN, DECADO and DEFO functional groups were present only in the euhaline and poly-mesohaline zones. All 57 amphipod taxa were assigned to Ecological Groups (EGs) according to AZTI's classification based on their sensitivities. After assignment, 33 amphipod species (57.9%) were grouped in EG-I, 9 species (15.8%) in EG-II and EG-III each. Six species (10.5%) were not assigned to any ecological group (Table 3).

## 3.5 Abiotic and biotic interaction

The Spearman's Rank correlations between univariate indices, amphipod taxa, functional groups and environmental parameters are given in Table 4. In Ulhas, amphipod diversity indices (S and N) were positively correlated with salinity. In Amba, SS was significantly correlated with diversity indices. pH had a positive correlation with *d*, *J* and *H'* in Kundalika. No significant correlations were observed between the amphipod diversity and the abiotic parameters in Savitri. The dominant DEDO functional group was positively correlated with salinity in Ulhas and with salinity and pH in Kundalika. The CAOMIN functional group showed a positive correlation with SS in Amba. While, in Savitri the CAOMIN group was negatively correlated with SS. The results of BIO-ENV analyses (Table 5) revealed that pH and salinity ( $\rho$ =0.24) in Ulhas, pH, SS, DO, sand and C<sub>org</sub>

( $\rho$ =0.28) in Amba, sand and clay ( $\rho$ =0.20) in Kundalika and pH, SS, DO and sand ( $\rho$ =0.10) in Savitri were the combination of abiotic variables that influenced the benthic amphipod assemblages.

### 4. Discussion

The present study indicated that the amphipod assemblages varied between the different salinity zones, seasons and also between estuaries. This could be possibly due to the fact that all four estuaries had varied environmental settings and different anthropogenic pressures across zones and seasons. As expected for most tropical estuaries, the salinity gradient displayed clear and marked spatial and seasonal trends in the present study (Gaonkar et al., 2013; Feebarani et al., 2016). The southwest monsoon resulted in copious fresh water discharge which lowered the salinities of the estuaries significantly. As compared to the other estuaries, a distinct situation occurred in Amba, where high salinity values were recorded during all seasons because of the sea water dominated nature of the estuary (Zingde, 1994). Low DO in the upstream region of Ulhas was due to the weak tidal impact and poor flushing which led to the accumulation of organic matter derived from urban sources (Mulik et al., 2017). The low pH observed in Savitri during monsoon season was probably due to intense fresh water discharge, when the whole estuary becomes freshwater dominated. This is in concurrence with the study conducted by Sarma et al., (2011) in Godavari estuary, where low pH values were reported during a high discharge period. In most Indian estuaries, generally the monsoon runoff leads to high suspended matter. Conversely, our results detected a reverse trend where lower SS values were observed during monsoon in Ulhas, Amba and Savitri estuaries. This may be due to the substantial flushing of the estuary owing to increased fresh water discharge (Mishra et al., 2007).

Increased sewage load, solid waste dumping, construction of jetties and dredging were mainly responsible for the silty nature of sediments in Ulhas, Amba and Kundalika (Athalye et al., 2003; Pande and Nayak, 2013a). It is well established that the type of sediment correlates with the amount of  $C_{org}$  in the sediments (Pande and Nayak, 2013b; Dias and Nayak, 2016). Thus, it is reasonable that the low  $C_{org}$  values in Savitri estuary were associated with sandy sediments, while silty sediments favored high  $C_{org}$  in other three estuaries. Besides, the high  $C_{org}$  could also be attributed to the anthropogenic input of organic waste as evidenced by Pande and Nayak, (2013b) in Kundalika and Mulik et al., (2017) in Ulhas.

The current study reported 57 amphipod taxa from the estuarine habitat of the Maharashtra coast. This was comparatively higher than the 29 species previously reported by Mondal et al., (2010) from the Vellar and Uppanar estuaries of the East coast during the premonsoon and postmonsoon. However, this study included samples from four estuaries and three seasons. It has

been reported that the seasonal patterns of peracaridean assemblages were mainly determined by the density of dominant species (Cunha et al., 1999; Valério-Berardo and Flynn, 2002). Likewise, we found that the variations in the seasonal pattern of community structure in all estuaries were evidently influenced by the population dynamics of dominant species. Many studies have concluded that the seasonal fluctuations in amphipod abundances may be dictated by the conspicuous breeding peaks of some dominant species (Moreira et al., 2008). Brown and McLachlan, (1990) have also suggested that the spatial and temporal distribution of amphipod species could depend upon the seasonal and local availability of food. This could possibly be one of the reasons for the patchy distribution of amphipods in the estuaries.

The Shannon–Wiener diversity index (H') values for amphipod species (H'=0-3.1) (Appendix C) was lower when compared with the results of Mondal et al., (2010) (H'=0-4.2) which can be attributed to the uneven distribution of amphipod species abundances in this study (Appendix B). A study conducted by Mulik et al., (2017) observed complete absence of macrobenthic organisms in the mesohaline zone of the Ulhas due to indiscriminate effluent discharge. Thus, anthropic stressors may have resulted in lower J' values in Ulhas as compared to Amba, Kundalika and Savitri, wherever the presence of amphipod swere recorded, (Appendix C). The absence of distinct grouping patterns of amphipod abundances displayed by the MDS plot was due to the null values and low amphipod abundances registered at many of the stations. Similarly, indistinct patterns in amphipod community structure were also observed by de-la-Ossa-Carretero et al., (2015) along the coast of Alicante, Mediterranean region. Dominance plots suggested balanced species assemblages during the postmonsoon season in all the studied estuaries which was also found by other studies (Nair et al., 1983; Devi, 1994; Aravind et al., 2007; Geetha and Nandan, 2014; Musale et al., 2015). According to Nair et al., (1983), the improvement of the amphipod populations during postmonsoon was mainly associated with the intermediate levels of salinity during that season.

Although many amphipod species are generally classified as sensitive to polluted sediments, various investigators have observed the dominance of detritivorous and domicolous (DEDO) amphipod species in polluted waters including harbours and brine discharge areas (de-la-Ossa-Carretero et al., 2012, 2015, 2016). As a consequence, the proliferation of amphipod taxa belonging to DEDO group was most likely due to the stressed nature of these estuaries considering the prevailing anthropogenic activities like sewage disposal, release of industrial effluents and dredging. The feeding and lifestyle types are major limiting factors shaping the distribution of amphipod species in response to their environmental conditions (Simpson and King, 2005; King et al., 2006; de-la-Ossa-Carretero et al., 2012). The availability of food usually changes with season and locality

resulting in spatiotemporal differences in amphipod functional group distributions (Brown and McLachlan, 1990) which was observed in the present study. The minor occurrence of other functional types like DEIN, DECAIN, DECADO CAMOIN and DEFO might be due to the limited availability of other food categories in the studied estuaries. According to Levin et al., (2001), hydrologically unstable areas such as the estuaries provide essential ecological functions, including decomposition, nutrient cycling and nutrient production, as well as regulation of fluxes of nutrients. As the hydro-sedimentological characteristics varied among and within the four estuaries, it resulted in the uneven amphipod distribution with diversified feeding and lifestyle. A wide variety of animals move into, out of, or across transitional zones and they have different life history patterns and use these habitats in different ways (Kneib, 2000; Rodrigues and Vanin, 2012).

The discrepancies in the assignment of amphipod species into sensitive or tolerant groups for AMBI and BENTIX indices are still a debate (de-la-Ossa-Carretero et al., 2012). In the present study, the sensitive (EG-I) and indifferent (EG-II) taxa were present in higher percentages (73.7%) while the tolerant group (EG-III) was comparatively lower (15.8%). It was observed that the EG-III taxa were mostly present in the highly impacted Ulhas as compared to the other three estuaries. The EG assignments of amphipods seems to be reasonable in the context of Ulhas and Kundalika estuaries where the AMBI indices have been applied effectively (Mulik et al, 2017, Dias et al., 2018). Indices incorporating amphipod data such as AMBI, BOPA and BO2A have been successfully employed for evaluating the Ecological Quality Status (EcoQS) of transitional water bodies (Dauvin and Ruellet, 2009 and de-la-Ossa-Carretero and Dauvin, 2010). However, considering the uneven distribution and very low abundances of amphipods, it is assumed that the EcoQS evaluation based on amphipod composition alone may not be reasonable for the studied estuaries. It may be more prudent to examine entire benthic assemblages to arrive at a logical conclusion. As previously reported by Mulik et al., 2017 and Dias et al., 2018, ecological quality status assessment studies that account for amphipod datasets (like BO2A and BOPA) can give misleading results when amphipods are present in low numbers.

BIO-ENV and Spearman's rank correlation analyses revealed that water temperature, salinity, pH, SS, DO, sediment texture and C<sub>org</sub> were the group of abiotic parameters that best explained the amphipod community structure. Many physico-chemical factors are currently evoked to explain the spatial and temporal patterns of amphipod community structure in various marine ecosystems (Nair et al., 1983; Cunha et al., 1999; Carvalho et al., 2012; Navarro-Barranco et al., 2017). Nair et al. (1983) observed that salinity was the major driving factor responsible for controlling the abundance of gammarid amphipods in the Cochin estuary. Cunha et al. (1999) reported that the patterns of

amphipod density and species diversity were related not only to water temperature, depth, sediment composition, local hydrodynamics and food availability, but also to the biology of the species. Mondal et al., 2010, concluded that the combinations of several abiotic parameters along with availability of food were the responsible factors for the recruitments, dispersion and abundances of amphipods from the two estuarine regions of southeast coast of India. The results obtained in the present study were congruent with these findings, since it was observed that a combination of abiotic factors was responsible for amphipod community patterns. However, the combination of abiotic factors varied among the four estuaries.

This study also reported the presence of non-indigenous euryhaline species, *Grandidierella japonica* at Savitri estuary (S5) and Amba estuary (A8). This species is native of brackish waters of Japan, but has been reported from several regions of the world. *G. japonica* became invasive through the oyster trade and shipping (Carlton and Eldredge, 2009; Jourde et al., 2013; Lavesque et al., 2014). The introduction of *G. japonica* into Indian waters may be due to the cargo transportation activities carried out at Mumbai, the major port nearest to the study area. This study presents the first record of this species in Indian waters, though it might have existed previously with no records. Also, this species may have not been identified previously because of the possible misidentification of *Grandidierella* genus that can be confounded with another Aorid genus *Microdeutopus*, as both share similar morphological characters (Jourde et al., 2013). *G. japonica* in terms of abundance was low (6-69 ind.m<sup>-2</sup>) in our study and distributed across the varying salinity zones (15-34) of the estuaries, while Marchini et al., (2016) had stated that these species were restricted to low saline zones in the Mediterranean.

The Amphipoda of the northwest coast of India have not been well investigated thus far. Therefore, not surprisingly, three new Indian records of amphipod species were made through this study. However, it appears that with the limited available information (Barnard, 1935; Surya Rao, 1972; Nair and Anger, 1979; Nair et al., 1983; Raja et al., 2013) much of the diversity, ecology and biology of Indian marine amphipod species remain to be explored. Further, monitoring studies such as the present study highlight the presence of non-indigenous species that may have far leading implications on native biota (Munari et al., 2016). Additional studies are further required to strengthen the biodiversity and ecology data of benthic amphipods of the Indian coast.

#### **Supplementary material**

Appendix A

Appendix B

Appendix C

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**Table 1:** Categorization of the sampled stations in different salinity zones of Ulhas, Amba,

 Kundalika and Savitri during the three seasons

Seasons	Euhaline (>30)	Poly-mesohaline (5-30)	Oligohaline (<5)				
Ulhas							
Premonsoon	U1-U3	U4-U6	U7				
Monsoon	U1	U2	U3-U7				
Postmonsoon	U1	U2-U6	U7				
	A	Amba					
Premonsoon	A1-A9	-	-				
Monsoon	A1, A2	A3-A7	A8-A9				
Postmonsoon	A1-A5	A6-A9	-				
Kundalika							
Premonsoon	K1-K4	K5, K6	K7				
Monsoon	K1,K3	K4-K6	K7				
Postmonsoon	K1-K3	K4-K6	K7				
Savitri							
Premonsoon	S1-S4	S5, S6	-				
Monsoon	S1, S2	<b>S</b> 3	S4-S6				
Postmonsoon	S1, S2	S3-S6 -					

\*U - Ulhas estuary, A - Amba estuary, K - Kundalika estuary and S - Savitri estuary

**Table 2**: Species density, species number and dominant amphipod taxa in Ulhas, Amba, Kundalika and Savitri estuaries across seasons

Season	Ulhas	Amba	Kundalika	Savitri
Premonsoon				
Density range	6-75 ind $m^{-2}$	6-69 ind $m^{-2}$	6-25 ind $m^{-2}$	6-38 ind $m^{-2}$
Total density	138 ind $m^{-2}$	300 ind $m^{-2}$	56 ind $m^{-2}$	107 ind $m^{-2}$
Species number	5	12	4	5
Dominant taxa	Dexamine spinosa (54.3%)	Grandidierella japonica	Photis longicaudata (44.6%)	Cheiriphotis megacheles
	Ericthonius sp. (27.5%)	(23.0%)	Cheiriphotis megacheles	(35.5%)
	Ampelisca sp. (9.4%)	Allomelita pellucida (16.7%)	(33.9%)	Ampelisca scabripes (29.9%)
		<i>Grandidierella</i> sp. (16.3%)	Ampelisca abdita (10.7%)	Idunella chilkensis (17.8%)
		<i>Caprella</i> sp. (12.7%)	<i>Caprella</i> sp. (10.7%)	Ampelisca sp. (11.2%)
Monsoon				
Density range	6-13 ind $m^{-2}$	6-356 ind $m^{-2}$	6-9 ind $m^{-2}$	6-31 ind $m^{-2}$
Total density	32 ind $m^{-2}$	1014 ind $m^{-2}$	15 ind $m^{-2}$	88 ind $m^{-2}$
Species number	3	9	2	5
Dominant taxa	<i>Ampelisca</i> sp. (40.6%)	Cheiriphotis megacheles	<i>Dyopedos</i> sp. (60.0%)	Idunella picta (35.2%)
	<i>Grandidierella</i> sp. (40.6%)	(35.1%)	<i>Corophium</i> sp. (40.0%)	<i>Gammaropsis</i> sp. (28.4%)
	<i>Melita</i> sp. (18.8%)	<i>Melita</i> sp. (16.7%)		<i>Lembos</i> sp. (14.8%)
		Melita dentata (12.9%)		<i>Photis</i> sp. (14.8%)
		Allomelita pellucida (12.4%)		
		<i>Grandidierella</i> sp. (11.7%)		
Postmonsoon				
Density range	$6-531 \text{ ind } \text{m}^{-2}$	$6-132 \text{ ind } \text{m}^{-2}$	6-38 ind m <sup>-2</sup>	$6-69 \text{ ind } \text{m}^{-2}$
Total density	1933 ind m <sup>-2</sup>	397 ind m <sup>-2</sup>	$228 \text{ ind m}^2$	539 ind m <sup>-2</sup>
Species number	26	14	14	
Dominant taxa	<i>Corophium</i> sp. (27.5%)	Microprotopus maculatus	Ampelisca sp. (16.7%)	Idunella chilkensis (24.5%)
	Ericthonius brasiliensis (22.3%)	(33.2%)	Ampelisca spinifer (13.6%)	<i>Urothoe</i> sp. (12.8%)
	Microprotopus maculatus	Amphilochus sp. (12.6%)	Gammarus sp. (11.0%)	Cheiriphotis trifurcata (11./%)
	(18.1%)	Allomelita pellucida (11.1%)	Isaeidae (11.0%)	Gammaropsis sp. (10.4%)
Total (ind m <sup>-2</sup> )	2103 ind m <sup>-2</sup>	1711 ind $m^{-2}$	299 ind $m^{-2}$	734 ind $m^{-2}$

Table 3: Checklist of amphipod species in studied estuaries along with the comparison with other Indian estuaries and classificati	on
into their functional and ecological groups	

Species	Ulhas	Amba	Kundalika	Savitri	Vellar & Uppanar*	Vellar**	FG	EG
Family Ampeliscidae								
Ampelisca abdita Mills, 1964	+		+				DEDO	III
Ampelisca aequicornis Bruzelius, 1859	+	+		+			DEDO	Ι
Ampelisca diadema (Costa, 1853)	+	+	+				DEDO	II
Ampelisca scabripes Walker, 1904				+	+	+	DEDO	Ι
Ampelisca sp.	+		+	+			DEDO	Ι
Ampelisca spinifer Reid, 1951	+		+				DEDO	Ι
Ampelisca spinipes Boeck, 1861		+					DEDO	Ι
Ampelisca typica (Spence Bate, 1856)		+					DEDO	Ι
Family Amphilochidae								
Amphilochus sp.		+					DECAIN	II
Gitanopsis gouriae Asari, 1998		+				+	DECADO	NA
Gitanopsis sp.		+					DECADO	NA
Family Aoridae								
Autonoe longipes (Liljeborg, 1852)			+				DEDO	Ι
Grandidierella bonnieroides Stephensen, 1947		+			+	+	DEDO	Ι
Grandidierella japonica Stephensen, 1938		+		+			DEDO	III
Grandidierella megnae (Giles, 1888)		+			+	+	DEDO	Ι
<i>Grandidierella</i> sp.	+	+		+			DEDO	Ι
Lembos sp.	+	+	+	+			DEDO	Ι
Lembos websteri Spence Bate, 1857		+					DEDO	Ι
Microdeutopus sp.		+		+			DEDO	Ι
Paramicrodeutopus schmitti (Shoemaker, 1942)			+				DEDO	II
Family Caprellidae								
<i>Caprella</i> sp.	+	+	+	+			DEIN	II
Family Corophiidae								
Americorophium sp.	+						DEDO	III
Cheiriphotis megacheles (Giles, 1885)		+	+	+		+	DEDO	Ι

Table 3 (continued)

					Vellar &			
Species	Ulhas	Amba	Kundalika	Savitri	Uppanar*	Vellar**	FG	EG
Cheiriphotis trifurcata Wongkamhaeng,		I		1			DEDO	т
Azman & Puttapreecha, 2012		Ŧ		Ŧ			DEDU	1
Corophiidae	+						DEDO	NA
Corophium sp.	+		+				DEDO	III
Family Dexaminidae								
Dexamine spinosa (Montagu, 1813)	+						DEIN	III
Family Dulichidae								
Dulichia sp.	+						DEHEIN	III
Dyopedos sp.			+				DEHEIN	III
Family Gammaridae								
Gammarus sp.	+	+	+				CAOMIN	Ι
Family Isaeidae								
Isaeidae			+				DEDO	NA
Family Ischyroceridae								
<i>Cerapus</i> sp.	+						DEDO	Ι
Ericthonius brasiliensis (Dana, 1853)	+	+	+		+	+	DEDO	Ι
Ericthonius sp.	+						DEDO	Ι
Notopoma crassicornis (Spence Bate, 1857)	+					+	DEDO	Ι
Family Liljeborgioidae								
Idunella chilkensis (Chilton, 1921)		+		+	+	+	CAOMIN	Ι
Idunella picta (Norman, 1889)				+			CAOMIN	Ι
<i>Idunella</i> sp.				+			CAOMIN	Ι
Family Melitidae								
Allomelita pellucida (Sars, 1882)		+		+			DEIN	III
Eriopisa sp.		+					DEIN	Ι
Melita dentate (Krøyer, 1842)		+					DEIN	Ι
Melita sp.	+	+		+			DEIN	Ι
Family Microprotopidae								
Microprotopus maculates Norman, 1867	+	+	+	+		+	DEDO	Ι

# Table 3 (continued)

Species	Ulhas	Amba	Kundalika	Savitri	Vellar & Uppanar*	Vellar**	FG	EG
Microprotopus sp.	+		+				DEDO	Ι
Family Photidae								
Gammaropsis sp.				+			DEDO	Ι
Photis longicaudata (Spence Bate &	I		1				DEDO	т
Westwood, 1862)	+		+	+			DEDU	1
Photis sp.	+			+			DEDO	Ι
Family Podoceridae								
Podoceridae	+	+	+				DEIN	NA
Podocerus brasiliensis (Dana, 1853)	+				+	+	DEIN	III
Podocerus spongicolus Alderman, 1936	+						DEIN	II
Family Pontogeneiidae								
Pontogeneia sp.			+				CAOMIN	II
Family Stenothoidae								
Stenothoe gallensis Walker, 1904	+						CAOMIN	II
Stenothoe marina (Spence Bate, 1856)	+						CAOMIN	II
Stenothoe sp.	+						CAOMIN	II
Family Talitridae								
Platorchestia platensis (Krøyer, 1845)	+					+	DEHEIN	NA
Family Urothoidae								
Urothoe pulchella (Costa, 1853)				+		+	DEFO	Ι
Urothoe sp.				+	+		DEFO	Ι

\*Mondal et al., 2010; \*\* Thilagavathi et al., 2013.

+ Presence, FG- Functional group; EG- Ecological group; DEDO-DEtritivorous-DOmicolous; DECADO-DEtritivorous-CArnivorous-DOmicolous; DEFO-DEtritivorous-FOssorial; DEIN- DEtritivorous-INterstitial; DEHEIN- DEtritivorous-HErbivorous- INterstitial; DECAIN- DEtritivorous-CArnivorous- INterstitial; CAOMIN- CArnivorous-OMnivorous- INterstitial.

**Table 4:** Spearman correlations between amphipod species abundance (ind  $m^{-2}$ ), community metrics (S- total number of taxa, d-Margalef index, J'- Pielou's index, H' (log 2) - Shannon-Weiner index) and N- total number of individuals, dominant functional groups and environmental variables (only significant correlations are given)

		Salinity	pН	SS (mg l <sup>-1</sup> )	C org (%)
T IIIh a a	S	0.51*			
(n=21)	Ν	0.50*			
	DEDO	0.66**			
	S			0.47*	
Amba ( <i>n</i> =27)	Ν			0.42*	
	d			0.62*	-0.06*
	H' (log <sub>2</sub> )			0.51**	
	CAOMIN			0.49**	
	d'		0.51*		
Kundalika	J'		0.53*		
( <i>n</i> =20)	H' (log <sub>2</sub> )		0.53*		
	DEDO	0.59**	0.61**		
Savitri	I. picta			-0.51*	
( <i>n</i> =18)	CAOMIN			-0.55*	

 $SS-Suspended \ Solids, \ C_{org}-Organic \ Carbon, \ DEDO-DEtritivorous-DOmicolous, \ CAOMIN-CArnivorous-OMnivorous-INterstitial$ 

**\*\*** *p*<0.01

\* *p*<0.05

Estuary	Best variable combinations	Spearman correlation (ρ)
Ulhas	pH, Salinity	0.24
Amba	pH, SS, DO, Sand, Corg	0.28
Kundalika	Sand, Clay	0.20
Savitri	pH, SS, DO, Sand	0.10

 Table 5: Summary of the BIO-ENV test results

 $DO-Dissolved\ Oxygen,\ SS-Suspended\ Solids,\ C\ _{org}-Organic\ Carbon$ 



Fig 1. Map showing the study area with sampling locations



**Fig 2**. Spatial-temporal variations of hydrographic parameters in the study area (some of the y-axes do not start at zero)



Fig 3. Spatial-temporal variations of sedimentological parameters in the study area



**Fig 4.** Non-metric multidimensional scaling (nMDS) ordination of amphipod species abundance from the four estuaries (numbers indicates stations)



Fig 5. k- Dominance curves of four estuaries for premonsoon, monsoon and postmonsoon seasons



**Fig 6.** Bubble plots showing the seasonal abundance patterns of benthic amphipods (average of four replicates at each station) for the four estuaries



Fig 7. Seasonal variations of amphipod functional groups (%) at each salinity zone for the four estuaries