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Environmental influences on the species diversity, biomass and population density of soft bottom macrofauna in the estuarine system of Goa, west coast of India

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A total of 58 species were recorded belonging to polychaetes, molluscs, crustaceans and other minor groups in order of species abundance. eighteen species are new to the local fauna that were not reported earlier. The maximum mean species diversity index (Shannon-Wiener), total biomass (wet) and total population density recorded were 2.3 (Z1), 6.7 g/m^2 (M1) and 703 no./m^2 (M2) respectively. Significantly higher species diversity was observed at high salinity, fine sand and high sedimentary biochemical parameters of total organic carbon (TOC), total organic nitrogen (TON) and carbon of biopolymeric fraction (C-BPF) sites. Medium grain size sites supported significant high biomass whereas population density showed no significant difference among the sites. The best multiple linear regression model revealed that all the 13 parameters studied were significant influencing parameters on species diversity, biomass and population density with exception of temperature. Among these salinity and TON were the main significant parameters. The combination of significant influencing environmental parameters, % variation and Mallows' C*p* values varied from sites to biotic parameters. This explained 32-72% of the total variance. The regression model derived from this data helps in detection of these biotic parameters and detection of pollution-induced effects.

[**Keywords**: Soft bottom macrofauna, species diversity, biomass, population density, linear regression model, Mandovi estury, Zuari estuary, Goa]
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Introduction

One of the aims of benthic ecologist is to understand the ecological process, which is achieved by examining the interrelationship between environmental parameters and benthic community structure[1-4], anthropogenic impacts [5], and modelling of the ecosystem[6]. This is usually accomplished by inferential analysis of empirical data by multivariate techniques and by conducting manipulative experiments[1-4]. Estuaries form an ideal ecosystem to examine such interactions between the abiotic and biotic factors due to their wide range of these parameters[1-4]. Several studies have examined relative influence of environmental parameters on species diversity, biomass and population density of soft bottom macrofauna in temperate estuaries[1-6] more than the tropical estuarine systems[7-10]. However, magnitude of variation will change at a particular geographical area[4]. There have been some studies on the Goa estuaries soft bottom macrobenthos[9,10] . These studies were restricted to a few abiotic and biotic parameters and seldom multivariate techniques have been used. The estuaries of Goa are used for fishing, aquaculture, ore transport, harbour development, waste disposal, adjacent land for human settlement and water recreation[8,10,11]. Ore refuge run off during monsoon and its impact on marine biota is one of pollution issues in these estuaries[10]. Spatio-temporal variations in abiotic and biotic parameters in this estuarine system are affected by tropical southwest monsoon, riverine and tidal flows contributing to the ecological complexity of the ecosystem[8,10,11] . Hence, a study was conducted (1997-98) at four sites in Mandovi-Zuari twin tropical estuarine system of Goa, central west coast of India, to test the hypothesis that environmental parameters (abiotic parameters) significantly affect the soft bottom macro-fauna such as species diversity (Shannon-Wiener), total wet biomass and total population density (biotic parameters). Best linear multiple regression models and ANOVA tests were used for one year (1997-98) spatio-temporal data at 4 sites in these estuaries. The regression model derived from such studies helps in detection of these biotic parameters and detection of pollution-induced effects by knowing the environmental parameters.

Materials and Methods

The Mandovi and Zuari estuaries of Goa interconnected by Cumbarjua canal and fringed with

mangroves are located on central west coast of India (Fig. 1). The site M1 is at the proximity of Arabian Sea in Mandovi estuary (depth 3.5 m). The salinity varies between 9.1-32.5 psu. The substratum is sandy and the sediment is composed of 81.2% sand, 7.6% silt and 11.1% clay. Site M2 is situated 3 km further upstream from M1 (depth 3 m) and the salinity varies between 1.1-33 psu. The substratum is sandy and sediment is composed of 94.2% sand, 3.5% silt and 2.1% clay. Site Z1 lies upstream from the mouth of Zuari estuary (depth 3.5 m) and the salinity varies between 20.5-33 psu. The substratum is silty sand and sediment is composed of 62.3% sand, 19.2% silt and 18.4% clay. Site Z2 is about 3 kms upstream from Z1 (depth 3.5 m). The salinity varies between 3-33 psu. The substratum is sand-silt-clay and sediment is composed of 35.6% sand, 27.9% silt and 36.5% clay. The average annual rainfall of Goa is about 3000 mm of which nearly 80% occurs during the southwest monsoon period (June-September), while relatively stable conditions prevail during postmonsoon (October-January) and premonsoon (February-May) period.

Four sites (Fig. 1) were sampled monthly from October 1997 to September 1998. Triplicate samples were obtained at each site with a van Veen grab (0.04 m^2, 15-20 cm penetration). Soft bottom macrofauna were sieved (0.5mm), preserved, assorted as reported earlier[12,13]. Macrofauna were identified and food and feeding habits of benthic fauna were ascertained[14]. Population density was converted into no./m^2 and biomass was expressed as wet weight g/m^2 after removing the hard parts. Sediment texture were analysed according to Buchanan[15] . Total organic carbon (TOC) and total nitrogen (TON) of sediment were determined using dried sediment sub-samples (5-10 mg) using NCS 2500 elemental analyser (Italy) after acidification with 1 *N* HCl. Proteins[16], carbohydrates[17], lipids[18] and carbon of biopolymeric fraction[19] (C-BPF) were also estimated. Bottom water was also collected using a Niskin bottle from the same locations for chlorophyll *a*[20] (Chl-*a*) and dissolved oxygen[21] (DO) estimation. Bottom water salinity and *p*H were measured using a Guildline Autosal (Model 8400 A, USA) and *p*H meter Model LI 612 (Elico Pvt Ltd, India) . All the estimates were made in triplicate.

Shannon-Wiener species diversity index (H′-log2 base) was calculated according to Pielou[22] for months data of different sites. ANOVA tests were carried out for 13 environmental and 3 biotic parameters to test any significant differences among the sites. Further,

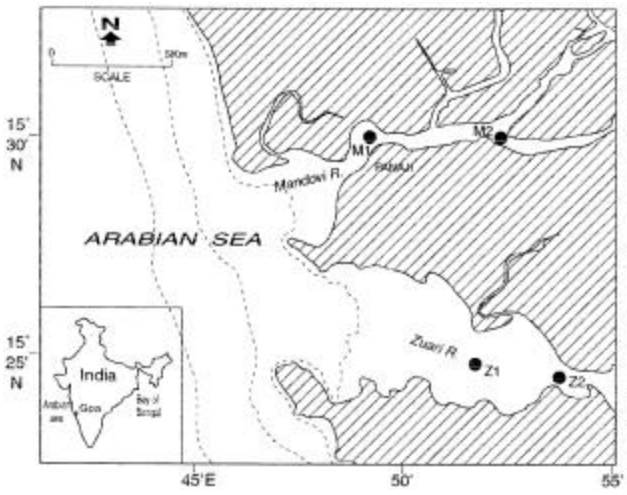

Fig. 1—Map of study sites (solid circle)

one-way Tukey`s HSD multiple comparison was used when significance was detected ($P < 0.05$). The best multiple linear regression models[23] were used to assess the relative significant influencing environmental parameters on species diversity, total wet biomass and total population density and to construct the predictive models. The regression explaining the greatest amount of variation (R^2) with all the significant parameter coefficients (r) were presented as the best fit based on adjusted R^2 and minimum Mallows` C_p[24] (low value of C_p indicates more accuracy of the regression model). The total data of four sites were first analysed to give the regression model. However, this did not give any significant regressions and variation was very low. Moreover, ANOVA tests revealed significant differences in environmental parameters among the sites. Hence, it was decided to perform these analyses on the data set of each site. All the raw data were normalized by log10 transformation before any statistical analyses. Minitab-Version 8.3 was used for these analyses[25].

Results

Environmental parameters

Table 1 shows annual mean of 13 environmental parameters studied at all the sites. All the environmental parameters varied significantly among the sites, except *p*H and Chl-*a* (ANOVA, $P < 0.0001$, Tukey's test Table 1). Temperature measurements ranged from 27.2-30.4 °C, DO 3.9-4.4 ml/l, *p*H 7.3-8.2 and Chl-*a* 0.9-1.7 µg/g . Salinity varied between 12.7 (M2) to 28.8 (Z1) and was significantly higher at sites Z1 in Zuari estuary (ANOVA

P < 0.0001, Tukey's test, Table 1). Mean grain size varied from 0.03 (Z2) to 0.3 (M2) and was significantly finer at sites in Zuari than in Mandovi estuary (ANOVA, P < 0.0001, Tukey's test Table 1). Sediment sorting varied from 0.3 (Z2) to 0.7 mm (M2) and showed well sorted to poorly sorted sediments. The muddy regions in Zuari estuary varied from silty sand to sand-silt-clay whereas sandy region in Mandovi varied from medium to fine sand. Sedimentary biochemical parameters such as TOC, TON, proteins, carbohydrates, lipids and C-BPF fraction showed wide range of variations (Table 1). TOC varied from 2587 (M1) to 17198 (Z2), TON 334.1 (M2) to 2015.5 (Z2), protein 8.4 (M1) to 18.3 (Z2) , carbohydrate 309.4 (M1) to 867.1 (Z2), lipids 75 (Z2) to 140 (M1) and C-BPF 232.4 (M1) to 422.4 (Z1) µg/g. Relatively, these values were significantly higher at sites in Zuari than in Mandovi estuary (ANOVA, P < 0.0001, Tukey's test, Table 1).

Benthic community structure

A total of 144 grab samples (0.04 m^2) were sorted equivalent to an area of 5.8 m^2. This yielded 2344 individuals belonging to polychaetes, molluscans, crustaceans and other minor groups in order of species abundance. Polychaetes formed the dominant taxa followed by molluscs and crustaceans. A total of 58 species were recorded of which 18 species were new to the local fauna that were not reported earlier. These included 24 species of polychaetes, 13 species of bivalves, 1 species of gastropod, 12 species of crustaceans, 1 species of fish and 7 other species that included Sipuncula, Nemertea, Anthozoa, Echiura, Nematoda and Coelenterata. The benthic fauna largely composed of the deposit feeding polychaetes *Prionospio pinnata* Ehlers and *Clymene annandalei* Southern at sites in Zuari estuary and the filter feeding bivalve *Meretrix casta* (Chemnitz) and the carnivorous polychaete *Nereis capensis* Wiley at sites in Mandovi estuary. The pattern of species succession and site wise species density has been described in detail elsewhere[26].

The annual mean (with standard deviation) of benthic species diversity index (Shannon-Wiener), total wet biomass and total population density at different sites is given in Table 1. The species diversity values varied between 1.5 (M2)-2.2 (M1), total wet biomass 1.7 (M2)-6.7 g/m^2 (M1) and total population density 610 (Z1)-687nos/m^2 (M2). Significant high species diversity was observed at site M1 due to high salinity and fine sand content and at site Z1 due to high sedimentary TOC, TON, C-BPF. Species diversity and biomass varied significantly among the sites However, population density showed

Table 1—Annual mean ($\bar{X}$) and standard deviation (±SD) of 13 envionmental and 3 biotic parameters at different sites. Asterisk indicates variables significant level for ANOVA tests among the sites. The superscripts refer to Tukey's HSD test, where values with same letter in each row are not different at the 0.05 significant level. ****P = < 0.0001, Ns- Not significant. N=36.

	Sites			
	M1	M2	Z1	Z2
Variables	$\bar{X}$ ±SD	$\bar{X}$ =SD	$\bar{X}$ ±SD	$\bar{X}$ ±SD
Temp.****	27.2±1.0 [a]	29.0±1.1 [b]	29.8±1.1 [b]	30.4±1.1 [b]
Sal.****	21.9±1.6 [b]	12.7±3.1 [a]	28.8±1.1 [c]	20.1±1.9 [b]
DO_2****	4.3±1.1 [b]	5.0±1.5 [c]	3.9±1.1 [a]	4.4±1.1 [b]
*p*H(Ns)	7.0±1.1	6.8±1.1	7.3±1.1	7.0±1.1
Chl-*a*(Ns)	1.0±2.8	0.9±7.0	1.7±1.8	1.6±2.5
M.gr.****	0.2±0.6 [b]	0.3±0.8 [a]	0.1±0.7 [c]	0.03±0.9 [d]
Sd.s.****	0.5±0.9 [b]	0.7±0.9 [a]	0.5±0.8 [b]	0.3±0.9 [c]
TOC****	2587.0±3.5 [a]	2759.3±2.2 [a]	11697.7±2.5 [b]	17198.0±1.7 [c]
TON****	337.7±2.1 [a]	334.1±1.8 [a]	1174.0±1.8 [b]	2015.5±2.2 [c]
Pr.****	8.4±2.3 [a]	8.5±1.5 [a]	15.3±1.5 [c]	18.3±1.3 [c]
Carb.****	309.4±2.9 [a]	355.9±1.8 [a]	615.0±2.0 [b]	867.1±1.5 [c]
Lip.****	140.0±1.1 [b]	117.2±1.4 [b]	88.4±1.9 [a]	75.0±2.1 [a]
C-BPF****	232.4±1.6 [a]	236.1±1.4 [a]	305.2±1.7 [b]	422.4±1.3 [c]
H'****	2.2±0.6 [c]	1.5±0.7 [a]	2.3±0.7 [c]	1.9±0.4 [b]
B****	6.7±0.4 [b]	1.74±0.2 [a]	1.8±0.1 [a]	2.7±0.1 [a]
P(Ns)	687.1±1.6	703.3±3.5	610.1±0.9	615.0±0.1

Temp. –Temperature (°C), Sal.-Salinity (psu), DO-Dissolved oxygen (ml/l), Chl-*a*- Chlorophyll-*a* (µg/g), M.gr.- Mean grainsize (mm), Sd.s.- sediment sorting (mm), TOC-Total organic carbon (µg/g), TON -Total organic nitrogen (µg/g), Pr.- Protein (µg/g), Carb.- Carbohydrates (µg/g), Lip.- Lipids (µg/g), C-BPF-Carbon of biopolymeric fraction (µg/g).

no significant differences (ANOVA, P < 0.0001, Tukey's test, Table 1). Relatively, significant high biomass was also observed at site M1 which had fine grain size.

The best multiple linear regression model

All the 13 environmental variables significantly (P < 0.05-0.0001) influenced the species diversity index, biomass and population density at all the sites for the significant (P < .001-0.0001) best multiple linear regression model except for temperature (Table 2). This explained 32-72% of the total variation (Table 2). No significant best regression fit could be obtained at site Z2 for species diversity. Water properties like salinity and dissolved oxygen were the significant (P < 0.05-0.0001) influencing parameters in terms of ability to explain biotic parameters at all the sites (Table 2). Similarly, sediment properties like mean grain size, sediment sorting and sedimentary biochemical variables were the significant (P < 0.05-0.0001) influencing environmental parameters to biotic parameters at all the sites except for species diversity at site Z2 (Table 2). Salinity and TON were the significant dominant abiotic parameters in influencing the biotic parameters in view of their higher frequency of occurrence in combination of two or three variables (Table 2). Although we observed significant regression fit (P < 0.001, P < 0.0001, Table 2), their combination of significant influencing parameters, % variation (R^2) and Mallows' *Cp* values varied from sites to biotic parameters.

The % variation (R^2) of best multiple regression model (Table 2) varied between 37-39% for species diversity, 41-70% for biomass and 32-72% for population density at all the sites. Similarly *Cp* values varied between –3.2-4.0, 2.0-29.8 and 5.1-71.9 for species diversity, biomass and population density respectively (Table 2). The predictive best multiple linear model derived from abiotic parameters also changed from sites to biotic parameters (Table 3)

Discussion

We have examined 13 environmental parameters and their relative influence on the species diversity, biomass and population density of soft bottom fauna. Salinity was a significant parameter to explaining the variance in especially species diversity at site M1 (P < 0.001,Table 2) and biomass at all the sites (P < 0.0001, Table 2). Wide fluctuation in salinity was mainly brought about by southwest monsoon, land runoff, riverine and tidal flow[11]. These large variations in salinity in an estuary increases physiological stress, which can result in the reduction in the number of species and restrict the species distribution depending on the species salinity tolerance thresholds[1-4]. For example, some euryhaline species like *Thalassema* sp. (echiuran worm) was

Table 2—Best multiple linear regression (BMR) model for species diversity (H'), total wet biomass (B), and total population density (P) of 13 environmental variables at different sites. Asterisk indicates variables and BMR significant level. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, Ns-no significance. N=36.

	Sites											
	M1			M2			Z1			Z2		
variables	H′	B	P	H′	B	P	H′	B	P	H′	B	P
Temp.												
Sal.	***	****			****			****			***	
DO	***						***		****			**
*p*H							**					
Chl-*a*												**
M.gr.			***	****								
Sd.S.								***	****		****	
TOC		****		*		***						
TON		****	***		**	****			****			***
Pr.	*								****			
Carb.						***	***					
Lip.				***								
C-BPF.			****		****						****	
BMR	****	****	****	****	****	****	****	****	****	Ns	****	***
Ad.R^2	0.39	0.7	0.59	0.37	0.41	0.41	0.39	0.61	0.72		0.51	0.32
C*p*	-3.2	3.2	13.9	-3.2	2	11.8	0.4	5.7	5.1		29.8	71.9

Temp. –Temperature (°C), Sal. -Salinity (psu), DO -Dissolved oxygen (ml/l), Chl-*a*-Chlorophyll-*a* (µg/g),, M.gr.- Mean grainsize mm, Sd.s.- Sediment sorting (mm), TOC-Total organic carbon (µg/g), TON -Total organic nitrogen (µg/g), Pr.- Protein (µg/g), Carb.- Carbohydrates (µg/g), Lip.- Lipids (µg/g), C-BPF.- Carbon of biopolymeric fraction (µg/g).

Table 3—Best significant linear multiple regression model for predicting the community structure from the environmental parameters at different sites. Ns-No significant regression, N=36.

M1	H′ = 0.27-4.46 DO+ 5.14 Sal. + 0.49 Pr.
	B = 0.28 +2.06 Sal. + 2.65 TOC-4.14 TON
	P = -1.94 + 0.78 M.gr.-1.80 TON + 4.11 C-BPF
M2	H =-8.27 + 1.30 M.gr. + 0.57 TOC + 2.47 Lip.
	B = 3.54-0.40 Sal. + 0.34 TON – 1.18 C-BPF
	P = 1.44 + 3.58 TOC-5.98 TON + 2.54 Carb.
Z1	H′ = 13.5 –5.69 DO – 5.93 *p*H – 0.95 Carb.
	B =-2.95 + 2.95 Sal. – 0.17 Sd.s.
	P = 9.98 – 12.3 DO + 1.31 Sd.s. + 2.64 TON – 6.07 Pr.
Z2	H′ = Ns
	B = 1.06 + 0.41 Sal. – 0.69 TOC + 0.99 C-BPF
	P = 13.0 – 0.75 Chl- *a* – 7.14 *p*H – 0.35 M.gr.

Temp. –Temperature (°C), Sal. -Salinity (psu), DO -Dissolved oxygen (ml/l), Chl-*a*-Chlorophyll-*a* (µg/g),, M.gr.-Mean grainsize (mm), Sd.s.-Sediment sorting (mm), TOC-Total organic carbon (µg/g), TON -Total organic nitrogen (µg/g), Pr.- Protein (µg/g), Carb.- Carbohydrates (µg/g), Lip.- Lipids (µg/g), C-BPF.- Carbon of biopolymeric fraction (µg/g).

only found in high salinity site (M1), whereas oligohaline species like *M. casta* (bivalve) was only observed in low salinity sites (M2) in Mandovi estuary . Such a distribution and the species succession has been described elsewhere[26]. This indicates that salinity acts as a physiological barrier for oligo- and euryhaline species due to the functional physiology of the species[1-4]. This parameter is more critical in the early larval stages of benthos[1-4]. Hence, species diversity reduced in low salinity sites and increased at high salinity sites (Table 1) supporting earlier observations[1-4]. We also observed relatively significant ($P < 0.0001$) high biomass in high salinity site (M1) (Table 1).

Dissolved oxygen variations which are dependant on the mixing process 11 was a significant parameter in explaining especially species diversity (M1 and Z1, $P < 0.001$) and population density (Z2, $P < 0.0001$, Table 2) and suggests oxygen threshold[27]. Typically, macrofauna exhibit reduced diversity and extreme dominance in oxygen minimum zone[27]. However, we did not observe such phenomenon. Oxygen was not a significant influencing parameter with respect to biomass (Table 2).

Some portion of sedimentary biochemical parameters (C-BPF) which forms the food for benthic organisms[28] was seen as a significant ($P < 0.05$-0.0001) parameter explaining the variation in species diversity (M1, M2 and Z1) biomass (M1, M2 and Z2) and population density at all the sites (Table 2). This is mainly attributed to the food and feeding relationship[28]. Bottom water Chl-*a* which forms food for the benthic organisms[29] largely depends on overlying water column productivity[29]. This parameter was significant ($P < 0.05$) in explaining the population density only at site Z1 (Table 2). The same was true for *p*H at site Z1, with respect to species diversity, which is dependent on water quality[11] .

Mean grain size and sediment sorting distribution which is a function of hydrodynamic regime[30] played a significant ($P < 0.001$, $P < 0.0001$) role in explaining species diversity, biomass and population density at all the sites (Table 2). Relatively finer sediments supported significant ($P < 0.0001$) high species diversity at sites M1 and Z1 (Table 1) corroborating earlier findings and indicating wider niches[31]. Well sorted sediments usually contain a small range of grain sizes and interstitial spaces. Such areas may provide fewer niches than poorly sorted sediment areas and which contain a less diverse fauna as noticed at site M2 (Table 1), thus supporting the earlier findings [31]. Absence of significant regression fit at some site for biotic parameters (Table 2) partly reflects the influence of unmeasured environmental parameters, such as hydrodynamic and sediment transport process, that affect the distribution of both sediments and fauna[30]. The other possible reason is that it may be linked with other unmeasured environmental parameters and biotic factors in the absence of a significant relationship.

Multivariate statistical methods can extract information from data sets containing larger amounts of variance, considering simultaneously the interrelationships of several influential variables[32-33]. The major ecological assumption for the development of this model was that the benthic community structure fluctuates greatly in relation to the environmental conditions *in situ* rather than mortality, inter-specific competition or predation[32,33]. Further, the influence of abiotic factors acting on the benthic community prior to sampling or the duration there of were not considered[32]. It is clear from an examination of the R^2 values that the predictor variable subsets selected, did not account for all the variance in the dependant variable. Several reasons may be attributed to this[23,32,33]. Firstly, the important variables with a strong influence were omitted due to multicollinearity and the procedure used to fit the linear regression model assumes that predictive variables are measured without errors[33]. Therefore, any error in benthic community structure would certainly introduce variance in the model that cannot be accounted for

any independent variable[32,33]. The accuracy did not improve by incorporating additional variables. Fitting hypothetical models to such a data-set is a compromise unless the variables and relationships are correctly chosen[23,32,33].

All the 13 environmental parameters except temperature were shown to be significant influencing parameters on species diversity, biomass and population density that explained 32-72% of the total variance. Among these, salinity and TON were the main significant factors. Unexplained variations and absence of significant regression fit suggests the influence of unmeasured environmental parameters and biotic factors. Hence, *in situ* manipulative experiments are needed to understand the biotic factors in this area. The model shown here is a pure representation of the available data set and interpretations are limited to the study site and period. The regression model derived from this data (Table 3) helps in detection of these biotic parameters and detection of pollution-induced effects by knowing the environmental parameters.

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