

## **Elucidation of the tidal influence on bacterial populations in a monsoon influenced estuary through simultaneous observations**

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### **Abstract**

The influence of tides on bacterial populations in a monsoon influenced tropical estuary was assessed through fine resolution sampling (1 to 3 hours) during spring and neap tides from mouth to the freshwater at four stations during pre-monsoon, monsoon and post-monsoon seasons. Higher abundance in surface water near the river mouth of total bacterial count (TBC), compared to the upstream, during pre-monsoon, was followed by an opposite scenario during the monsoon. When seasonally compared, it was during the post-monsoon season when TBC in surface water was highest, with simultaneous decrease in their count in the river bottom sediment. The total viable bacterial count (TVC) was influenced by the depth-wise stratification of salinity, which varied with tidal fluctuation, usually high and low during the neap, and spring tides, respectively. The abundance of both the autochthonous *Vibrio* spp. and allochthonous coliform bacteria was influenced by the concentrations of dissolved nutrients and suspended particulate matter (SPM). It was concluded that depending on the interplay of riverine discharge and tidal amplitude, sediment re-suspension mediated increase in SPM significantly regulates bacteria populations in the estuarine water, urging the need of systematic regular monitoring for better prediction of related hazards, including those associated with the rise in pathogenic *Vibrio* spp. in the changing climatic scenarios.

**Keywords** Estuary. Simultaneous sampling. *Vibrio cholerae*. Total coliforms. Tides. Bacterial dynamics

## Introduction

Estuaries are transition zones between marine and freshwater environments, highly productive and experience continuous tidal forcing and mixing of the water column (Correll 1999; McLusky and Elliott 2004). The large amount of freshwater influx from different sources brings high amount of pollutants, untreated sewage and microbial contaminants which can alter the estuarine biological communities. Bacteria are an important component of the microbial loop that influence food web dynamics and ecosystem functioning, are passive drifters and their population is expected to be influenced by the physical processes and anthropogenic impact.

Bacteria play an important role in the biogeochemical cycling in an ecosystem (Azam et al. 1983). However, some bacteria are pathogenic which are either allochthonous or autochthonous and responsible for causing diseases in marine organisms and human beings (Neogi et al. 2014 and references therein). *Vibrio* spp. are autochthonous to estuaries and possesses chitinolytic enzymes, can use crustacean exoskeleton made of chitin and thus play an important role in nutrient recycling (Bassler et al. 1991). Among *Vibrio*'s *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* are the most important pathogens known to cause diseases in humans, shellfish, and shrimps (Daniels and Shafaie 2000; Aguirre-Guzmán et al. 2004; Oliver and Kaper 2007). *V. cholerae* is a causative agent of epidemic cholera in humans that results in rapid dehydration and even death of infected persons (Finkelstein, 1996). More than 200 O-antigen serogroups of *V. cholerae* are identified till date, among which only two serogroups, O1 and O139, are known to cause epidemics and pandemics (Sack et al. 2003). Outbreak of cholera has been reported in various countries (Sur et al. 2000; Smirnova et al. 2004; CDC 2010; Hendriksen et al. 2011). In India cholera epidemics crop up with seasonal regularity in the Gangetic delta (Colwell 1996). Their distribution and outbreaks in estuaries is often related to elevated water temperature and salinity (Blackwell and Oliver 2008; Oberbeckmann et al. 2012).

The allochthonous bacteria, especially fecal coliforms are released directly through wastewater, raw sewage, human and animal waste to the rivers and the estuaries. *Escherichia coli* and intestinal *Enterococci* are used to check microbiological quality of water (Ouattara et al. 2011) and is crucial for understanding patterns of water quality impairment, establishing management planning, and developing appropriate mitigation strategies (Stumpf et al. 2010). Evaluating the survival and persistence of such pathogens is of concern to human health and has implications in microbial ecology of such monsoon influenced estuary.

Earlier studies have revealed that salinity changes with the tidal action are major factors in controlling the survival and viability of the bacterial populations in estuaries (Hyun et al. 1999; Devanathan et al. 2010). A study by Eldridge and Sieracki (1993) in the York River sub estuary also indicated that the bacterial abundance in the surface water was regulated by spring – neap tidal cycle. Generally tides provide various functions such as transport of organisms and nutrients in estuaries. Further the tidal currents are also important in re-suspension of sediments at various estuaries (Lindsay et al. 1996; Morris and Howarth 1998). These bed sediments are known as reservoir for fecal bacteria and enteric pathogens in aquatic systems (Alm et al. 2003). Resuspension of such bed sediments to the water column increases persistence and transport of pathogens in the estuaries and risks to public health (Desmarais et al. 2002). Other than physical processes, enhancement of nutrient load via inflows may also trigger the growth of human pathogens. Role of nutrients in influencing the bacterial population is well known (Allen et al. 2002). Since bacteria are passive, their population is expected to be influenced by physical processes. Such influence of physical processes on pathogens in this estuary is poorly studied.

This study was undertaken in the Zuari estuary, a highly productive tide dominated monsoonal estuary. A significant contamination from untreated sewage and effluent discharge has been reported in this estuary (Sawkar et al. 2003). Generally low saline estuarine areas have been recognized as region of severe physiological stress for both marine and freshwater organisms (Kinne 1971). Transition from fresh to brackish and marine conditions influence the biogeochemical process. Taking this into consideration, four sampling stations depicting the mouth of the estuary (M), lower middle estuary (LM), upper middle estuary (UM) and upstream (U) were simultaneously sampled using four trawlers anchored midstream in the study, first of its kind from the region. The sampling was repeated for three seasons in order to evaluate the temporal variations.

Previous studies have reported distribution and occurrence of different pathogens (such as *E. coli*, total coliforms, *Vibrio*'s, *Salmonella* spp., fecal indicators, and *E. coli* O157:H7) (Nagvenkar and Ramaiah et al. 2009; Rodrigues et al. 2011; Khandeparker et al. 2015) and pollution (Shirodkar et al. 2012; Kessarkar et al. 2015) in this estuary. Earlier studies carried out in this estuary so far have provided snapshot views. In this study the emphasis was on characterizing the bacterial distribution through high resolution simultaneous observations. The present study was interdisciplinary during which the stratification and currents were also measured (Sunder et al. 2015). As the bacterial population is influenced by physical processes, in this study we elucidate the differences in their population in the water column in relation to stratification in a monsoon influenced estuary.

## Materials and methods

### Study sites and sampling

Zuari estuary (15° 27.5' N, 73° 48' E) is located along the west coast of India. The river source of this estuary originates in the Sahyadri Hills from Dighi Ghat and experiences distinct climatic variations due to the impact of monsoon. The estuary remains well mixed in PreM season, while it gets stratified during SW-MoN. Both ebb and flood currents are strong in Zuari estuary and receives high amount of freshwater during SW-MoN (Qasim and Sengupta 1981). Tides in this estuary are of mixed semidiurnal type normally occurring twice a day and the tide ranges are approximately 2.5 m and 1.5 m during spring and neap tides respectively (Shetye et al. 2007). The tidal influence is more at the mouth as compared to upstream end of the estuary. These tides as well as monsoon run-off are responsible for the mixing and circulation. The main channel of Zuari estuary is about 50 km long and 5 km wide with approximate depth of 5m. But their cross section area decreases towards the riverine end (0.5 km) (Shetye et al. 1995) and receives freshwater from a number of streams and rivers. During the SW-MoN (June – September) fresh water influence is comparatively more than the PreM summer season (March to May) wherein there is less river discharge and is vertically mixed throughout the estuary till riverine region (Shetye et al. 1995).

The four sampling stations included were Dona Paula (DP; 15° 25' 16.9" N 73° 47' 36.9" E ) - bay station situated at the mouth of the estuary, Cortalim (CR; 15° 24' 32.0' N, 73° 54' 50.2' E) - lower middle estuary, Borim (BR; 15° 25' 03.6" N, 73° 47' 58.0" E) – upper middle estuary and Sanvordem (SV; 15° 16' 01.1' N, 74° 06' 36.0' E) – upstream station (Fig. 1). No observations were carried out during the SW-MoN season due to rough weather only at DP station (Mouth of the estuary). CR and BR are at the mid part of the estuary and SV is at the head of the estuary with near zero salinity. Fresh water and human activities influence the environmental characteristics of the upstream station. The water is highly stratified at lower middle estuary (CR) during the flood phase, whereas, at upper middle estuary (BR) it is well mixed. The upstream station (Sanvordem - SV), being the river end showed no such variation.

Observations were carried out every hour for TBC, every three hour for TVC and pathogens, over a 24 hour period. The study was carried out during the year 2011 for three different seasons of and details of the sampling are provided in the Table 1. Surface and near bottom water samples were collected using 5L capacity Niskin sampler at an interval of 1 and 3 hrs, while sediment samples were collected at an interval of 5 hr using van Veen grab from three different locations for 24 hr.

Water samples (surface and near bottom) were also collected for analysis of suspended particulate matter (SPM), temperature, salinity, and dissolved nutrients. After collection, samples were stored in an ice box and transported to the laboratory for further analyses.

#### Physicochemical parameters

Temperature and salinity were recorded by using portable conductivity-temperature-depth (CTD) probe (Seabird Electronics Inc., USA of model 19 plus) having an accuracy of 0.005 °C in temperature and 0.005 psu in salinity measurements and was operated from anchored boats every hour. Tide and flow were recorded using a current meter. SPM was obtained by filtering water through pre-weighed, ashed GF/F filter paper and dried in oven at 60 °C for 48 hrs. After drying, filter papers were weighed using weighing balance and expressed as mg l<sup>-1</sup> (Mettler Model-AT/20; Kumar et al. 2004). Dissolved nutrient concentrations i.e. nitrate, nitrite, phosphate, ammonia, and silicate were analyzed using autoanalyser (Skalar SAN PLUS 8505 Interface v3.331, Netherland; Khandeparker et al. 2015; Roy et al. 2015).

#### Enumeration of total bacterial count (TBC)

For total bacterial count analysis 5 ml of the samples were fixed with formaldehyde (2% final concentration) and 1 ml of this fixed sample was used in the analysis. In the case of sediment, 1 g of the sediment was weighed and diluted in 10 ml of sterile sea water and fixed as described above. For flow cytometric (FCM) analysis of sediment, samples were sonicated at 50% power in water bath sonicator (Ultrasonic cleaner, Equitron) for 1 min three times to separate the sediment particles from cells. Further, sonicated samples were centrifuged at 3000 rpm for 1 min and the supernatants were recovered (Luna et al. 2002). The clear supernatants of sediment and water samples were passed through BD cell strainer cap (BD-Biosciences, USA; Cat no: 352235) to remove larger particles. Subsequently, the samples were stained with SYBR Green I (Molecular Probes, USA) at 1: 10,000 final concentrations (Marie et al. 1999) and incubated for 15 min in dark at room temperature. After incubation, the samples were analyzed using a BD FACSAria™ II (BD-Biosciences, USA) flow cytometer equipped with a nuclear blue laser 488 nm which can differentiate green fluorescence excited by blue laser. Emitted light was collected through the filter sets 488/10 band pass (BP) for right angle light scatter (SSC) and 530/30 BP for green fluorescence. Fluorescent beads (1µm, Polysciences) were used as internal standards and for calibration of the above parameters. Gating was done against SSC versus green fluorescence (FITC). Flow cytometry data were processed using

BD FACSDIVA software (BD-Biosciences, USA). The bacterial abundance is expressed as cells ml<sup>-1</sup> for water or g<sup>-1</sup> for sediment respectively.

#### Enumeration of viable and pathogenic bacteria

The samples were diluted as required and spread plated (0.1ml) on Zobell Marine Agar 2216 (ZMA; Hi-media). ZMA has been extensively used to grow marine bacteria. Pathogenic bacteria were quantified using specific media following the manufacturer's instructions (Hi-media). Thiosulphate-Citrate-Bile Salts (TCBS) medium was used for different *Vibrio* spp. (*V. cholerae*, *V. parahaemolyticus* and *V. alginolyticus*). The presence of sucrose in TCBS allows differentiation of *Vibrio* species (Pfeffer and Oliver 2003). Mac Conkey agar was used for enumeration of total coliforms. The samples were inoculated on selective agar plates and incubated at 37°C for 24 h before colonies were enumerated. Cultivable and pathogenic bacterial abundance thus quantified is expressed as CFU ml<sup>-1</sup> for water or g<sup>-1</sup> for sediment respectively. Bacteria which were grown on TCBS and Mac Conkey agar were further confirmed as described by Khandeparker et al. (2015). In brief, bacterial colonies which were grown on selective agar were randomly picked and confirmed using appropriate biochemical tests (Himedia, India). Subsequently, the bacterial species were also confirmed using MALDI-TOF-MS Bio typing (MTB) method.

#### Data analysis

Data were log (X+1) transformed in order to meet the normality assumptions. The relationship between bacterial numbers (TVC, TBC, *V. cholerae*, *V. alginolyticus*, *V. parahaemolyticus* and Total coliforms) and the environmental parameters was investigated by means of canonical correspondence analysis (CCA), using the CANOCO version 4.5 (ter Braak and Smilauer 2002). Prior to CCA analysis, Detrended Correspondence Analysis (DCA) was performed to determine the variability within species data set. The length of the first axis gradients for all data sets were >2 standard deviation unit (SD) indicating unimodal character of dataset. Due to the unimodal characteristics of the species data set CCA analysis was performed (ter Braak and Smilauer 2002). A forward selection was performed on the set of environmental variables and statistical significance of each variable was tested using Monte Carlo permutation test under the reduced model with 999 numbers of permutations. Further, correlation analysis was performed between abiotic (temperature, salinity, dissolved nutrients and stratification factor ( $\Delta S$ ) and bacterial components. The above statistical analysis was performed using Statistica 6.0 statistical package at a significance level of  $\leq$

0.05 (Statsoft, Oklahoma, USA). The stratification factor  $\Delta S$ , is computed as a difference between the near bottom and surface salinity (Sundar et al. 2015).

## Results

### Physicochemical parameters and dissolved nutrients

The details of the results of the physico-chemical parameters and nutrient dynamics are provided in the supplementary Table. 1a-f. Briefly, at the mouth of the estuary (DP), the variation in the mean salinity was minimal ( $\sim 1$  psu) in the surface water (34.97 – 35.93 during PreM and 31.96 – 32.82 during PostM). While, near bottom water salinity remained constant during the PreM ( $35.44 \pm 0.1$ ) and the PostM seasons ( $33.38 \pm 0.20$ ). The details of salinity variations are reported in Sundar et al. (2015). Large salinity variations were observed at lower middle (CR) and upper middle (BR) estuarine stations during the PreM season which peaked during the SW-MoN; the influence was more during the high waters (Suppl. Table. 1e). The stratification factor, ( $\Delta S$ ) was high during neap tide irrespective of stations except during the SW-MoN which showed strong stratification at the lower middle estuary irrespective of tides (Sundar et al. 2015).

Suspended particulate matter (SPM) was high during the spring tide of PostM season in surface water (Suppl. table. 1f). During the PreM and the SW-MoN seasons, SPM was high at the lower middle estuarine station (CR;  $178 \pm 50$  mg L<sup>-1</sup>) followed by mouth of the estuary (DP ;  $167 \pm 52$  mg L<sup>-1</sup>), and BR ( $132 \pm 35$  mg L<sup>-1</sup>) which is the upper middle estuarine station (CR > DP > BR). However, during the PostM, it was high at the mouth of the estuary ( $229.5 \pm 61$  mg L<sup>-1</sup>). The lowest SPM was observed at the upstream station (SV;  $21 \pm 2.5$  mg L<sup>-1</sup>) during PreM season. A similar trend was also observed in the near bottom water (CR ( $265.77 \pm 72$ ) > DP ( $215.49 \pm 71$ ) > BR ( $150.63 \pm 56$ ) > SV ( $21 \pm 11$ ) during PreM. In the case of dissolved nutrients, nitrate was high during PreM ( $18.27$   $\mu\text{mol l}^{-1}$ ) and low during PostM ( $0.87$   $\mu\text{mol l}^{-1}$ ) whereas an opposite trend was observed in the case of phosphate which was higher ( $13.03$   $\mu\text{mol l}^{-1}$ ) during spring tide of PostM at the river mouth station. Dissolved nutrients in the surface water were higher during the spring tide and showed gradual decrease with the advection of neap tide. Nitrate and phosphate concentrations were higher in the upstream (SV) station and decreased towards the mouth of the estuary. The silicate concentration ranged from  $0.21 - 222.7$   $\mu\text{mol l}^{-1}$  without any remarkable site-wise variation (Suppl. Table. 1a-d).

## Total Bacterial Count (TBC)

During PreM season, TBC in surface water varied from  $1.56 \times 10^5$  -  $2.1 \times 10^6$  cells ml<sup>-1</sup> and was high at the mouth (DP;  $2.1 \pm 0.002 \times 10^6$  cells ml<sup>-1</sup>) followed by upper middle (BR;  $3.11 \pm 0.023 \times 10^5$  cells ml<sup>-1</sup>), lower middle (CR;  $1.60 \pm 0.021 \times 10^5$  cells ml<sup>-1</sup>) and upstream end (SV;  $1.56 \pm 0.025 \times 10^5$  cells ml<sup>-1</sup>) of the estuary during spring tide (DP > BR > CR > SV; Fig. 2 a, d, g, j). TBC during neap tide ranged from  $5.55 \times 10^3$  -  $2.75 \times 10^6$  cells ml<sup>-1</sup> and was low at all the stations.

In the case of SW-MoN, a reverse trend was observed wherein abundance was high at the upstream end and decreased towards the mouth (Fig. 2e, h, k). Overall, abundance ranged from  $4.04 \pm 0.10 \times 10^4$  -  $1.43 \pm 0.004 \times 10^5$  cells ml<sup>-1</sup>. Whereas, the abundance of TBC in the near bottom water remained similar as PreM and decreased from lower middle to upstream end (CR > BR > SV) of the estuary (Fig. 3e, h, k).

During PostM, the TBC was high throughout the estuary (DP - SV) irrespective of tides in both surface and near bottom waters (Fig. 2 & 3c, f, i, l). It ranged from  $1.16 \pm 0.003 \times 10^6$  -  $1.8 \pm 0.003 \times 10^6$  cells ml<sup>-1</sup> in surface and  $4.75 \pm 0.01 \times 10^5$  -  $1.35 \pm 0.006 \times 10^6$  cells ml<sup>-1</sup> in near bottom water.

In the case of the sediment, TBC was high and almost similar during PreM and PostM seasons at the mouth and upper middle estuary (Table. 1). On the other hand, lower middle estuary (CR) showed high TBC during the PreM followed by SW-MoN and least in the PostM season (Table 1). Sediment samples could not be collected at the upstream station.

Overall, during the PreM season, the TBC was high towards the mouth and low at the upstream end and vice-versa was observed in the SW-MoN. During PostM, TBC was high irrespective of the stations and the tides in the surface water. TBC in near bottom water ranged from  $9.9 \times 10^3$  -  $4.12 \times 10^6$  cells ml<sup>-1</sup> and was high throughout the estuary during PreM and PoM seasons. The bacterial numbers were low during SW-MoN season.

The CCA axis 1 and 2 (eigenvalues of 0.822 and 0.223 respectively) explained 90.07% of the cumulative variance of the relation between the species (TBC, TVC, TC, and pathogens) and environmental variables (Salinity, SPM, and nutrients; Fig. 4a). Abundance of TBC was separated along axis 1 and showed a positive relation with nitrate during spring tide in surface water ( $r = 0.45$ ,  $p \leq 0.05$ ). This relation was prominent during the PostM season (Fig. 4b).



## Total Viable Count (TVC)

During PreM and PostM season, TVC was high at the mouth ( $2.82 \pm 0.2 \times 10^5$  and  $8.96 \pm 0.003 \times 10^5$  CFU ml<sup>-1</sup>) followed by lower middle ( $1.34 \pm 0.06 \times 10^4$  and  $8.37 \pm 1.53 \times 10^4$  CFU ml<sup>-1</sup>), upper middle ( $2.84 \pm 0.6 \times 10^3$  and  $2.10 \pm 1.7 \times 10^4$  CFU ml<sup>-1</sup>), and upstream end ( $1.27 \pm 0.3 \times 10^3$  and  $3.10 \pm 0.4 \times 10^2$  CFU ml<sup>-1</sup>) of the estuary irrespective of the tides. In the case of sediment, the trend remained same and abundance steadily decreased from mouth to upstream end during PreM (DP > CR > BR > SV; Table. 1). In the present study at the mouth and lower middle estuary, average TVC in near bottom water and sediment was 0.6 % - 0.08% and 3.12 % - 5.67% respectively during PreM and vice – versa was observed during PostM spring tide (1.33 and 1.80% in near bottom water; 0.10 and 1.51% in sediments). Moreover, spring tide showed 1 – 2 fold high abundance than neap tide irrespective of the stations except at upper middle estuary (BR) where the trend was reversed (Table. 1).

In the case of SW-MoN also TVC decreased from lower middle to upstream end of the estuary (CR>BR>SV; Suppl. Table. 2). However, not much variation in TVC numbers were observed between the tides as seen in other seasons (Suppl. Table. 2).

In the CCA analysis, TVC was located close to the center of the axis and didn't show a significant relation with any of the environmental variables (Fig 4 & 5). However, correlation analysis showed that  $\Delta S$  had inverse relationship with TVC abundance ( $r = -0.73$ ,  $p \leq 0.02$ ). In addition, SPM also showed a significant positive relation with TVC and this relation was more evident during PostM ( $r = 0.672$ ;  $p \leq 0.001$ ).

## Pathogenic bacteria

Mean abundance of total coliforms (TC) in the surface water decreased from the mouth to the upstream end of the estuary (DP>CR>BR>SV) irrespective of seasons during the spring tide (Fig. 6a, b). The abundance of TC was more at BR>SV>DP>CR during the neap tide. Similar trend was also observed for near bottom water (Fig. 6c, d). Overall, the abundance was high during the PostM season. TC abundance in sediment was also higher during postM when compared to others. On an average 0.14 – 0.24 % of abundance increased from mouth to upstream end of the estuary (Table. 1).

The variation in the *Vibrio* spp. along the estuarine stretch was influenced by tides and seasons (Fig. 6e-p). In the case of *V. alginolyticus*, abundance was high at the mouth (DP;  $5.11 \pm 0.7 \times 10^2$  CFU ml<sup>-1</sup>), followed by lower middle (CR;  $2.78 \pm 0.5 \times 10^2$  CFU ml<sup>-1</sup>), upper middle (BR;  $1.02 \pm 0.3 \times$

$10^2$  CFU ml<sup>-1</sup>) and upstream end (SV;  $2.07 \pm 2.3$  CFU ml<sup>-1</sup>) of the estuary during spring tide. The trend reversed during neap tide in both surface and near bottom waters (Fig. 6f, h). Similarly, *V. parahaemolyticus* and *V. cholerae* abundance also decreased from mouth to upstream end (DP > CR > BR > SV) of the estuary during spring tide (Fig. i, k, m, o). No such variation was observed during neap tide.

The CCA and correlation analysis indicated that the TC abundance was related to silicate ( $r = 0.515$ ;  $p \leq 0.05$ ), salinity ( $r = -0.655$ ;  $p \leq 0.01$ ) and SPM ( $r = -0.570$ ;  $p \leq 0.05$ ). The CCA biplot indicated that the association between silicate and TC was not direct and rather related to the monsoonal influences such as freshwater influx and run off (Fig. 4 & 5b). For example, SW-MoN (irrespective of the stations) in the biplots separated along the axis 1 and closely associated with the increase of silicate and TC abundance (Fig. 4 & 5b) in surface and near bottom waters.

The abundance of *Vibrio* spp., showed significant positive relationship with the salinity ( $r = 0.636$ ;  $p \leq 0.05$ ) and SPM ( $r = 0.757$ ;  $p \leq 0.01$ ). The CCA biplots also revealed that PreM and PostM seasons were clearly separated from SW-MoN (Fig. 4 & 5b, d) indicating a possible influence of monsoon on *Vibrio* spp. abundance. Other than salinity and SPM, *V. cholerae* also showed a significant positive relation with nitrite during neap tide in near bottom water (Fig. 5c). This relation was prominent at lower middle estuary (CR) during PreM where the mean abundance of *V. cholerae* was high (Fig. 6p) and could be related to high nitrite ( $39.76 \mu\text{M l}^{-1}$ ).

## **Discussion:**

The observations carried out simultaneously at fine resolution to elucidate spatial and temporal variations in the tropical Zuari estuary points out the bacterial population to be influenced by the physical processes.

### **Bacterial response to diel - tidal changes**

The mouth of the estuary was supported by high total and viable bacterial abundance (TBC and TVC). The tidal range between spring – neap phase is about 1 m at the mouth and it causes significant variability in  $\Delta S$  (Sundar et al. 2015).  $\Delta S$  were low during high waters at the mouth (0.5) indicating that the water mass was well-mixed which possibly resulted in high bacterial abundance. A concurrent study carried out on the biofilm formation in Zuari estuary at the same location also showed high bacterial abundance in the biofilms developed at the mouth region during PostM (Khandeparker et al. 2015 Personal observation). At the mouth, SPM was also high in the water

column during PostM season. Tide mediated mixing of the water column induces significant changes in physico-chemical variables and suspended load, this variation has indirect effect on bacterial abundance. Average percentage of TVC in sediments with respect to TBC was also low (0.10 %) as compared to the water (1.33 %) which indicates the possible re-suspension of bacterial inoculum in the overlaying water column. One to three fold variations in TVC ( $\sim 10^1 - 10^3$ ) was evident between the tidal cycle (spring and neap tide) with high values during the spring phase. High bacterial abundance and SPM in the water column can be attributed to resuspension events of bed sediments due to tidal currents. Tidal turbulence can inoculate detritus, bacteria, and pigments from the sediment into the water column (Poremba et al. 1999). A study by Anand et al. (2014a) on sub hourly changes in biogeochemical properties in the surface water at the bay station of this estuary suggested that enhanced turbulence due to rising tide also enables the release of substances from the bottom sediments resulting in the increase in nutrients. Other than tidal turbulence, freshwater input also facilitates the sedimentary release and contaminants in the Zuari estuary (Shirodkar et al. 2012; Anand et al. 2014b). Additionally, south west monsoon carries bulk amount of fine grained material to this estuary and alters the composition of SPM (Shetye et al. 1995; Kessarkar et al. 2009). Thus, it seems that other than  $\Delta S$ , SPM also has a significant influence on the bacterial abundance. During SW-MoN, influence of salinity at lower middle estuary was also evident where TBC and TVC decreased. During monsoon, high stratification observed during high waters at the lower middle estuary, weakens marginally at the peak of the high waters, during both spring and neap phases. Because of this, the salinity of bottom layer remains constant for about 2-3 hrs while the surface salinity continues to increase up to the peak of high water, resulting in the weakening of stratification (Sunder et al. 2015). This could be a responsible factor for the decrease in TBC and TVC.

#### Bacterial response to nutrient dynamics

In the present study, nutrients such as nitrite, nitrate, phosphate and silicate showed seasonal and tidal variation and were low during PostM (mainly nitrate and nitrite). These nutrients showed a significant negative correlation with TBC and can be attributed to their active utilization. The most common forms of inorganic nitrogen in aquatic ecosystems are ammonium, nitrite, and nitrate which originate from surface and groundwater runoff,  $N_2$  fixation by certain prokaryotes, and biological degradation of organic matter (Camargo and Alonso 2006). Three major factors control N and P limitation in an aquatic ecosystem: (i) nitrogen to phosphorus ratio in nutrients (ii) loss of N due to denitrification; and (iii) extent of nitrogen availability in an ecosystem through nitrogen fixation (Ward et al. 2009). The main limiting nutrient in terrestrial and marine ecosystem is nitrogen (Elser

et al. 2007). Similarly, a study by Anand et al. (2014b) showed notable intra – seasonal variation in nitrate and nitrite during PostM season in this estuary. Earlier study on daily variation in total and pathogenic bacterial abundance at the mouth of this estuary during 2009 – 2011 pointed out surge of nitrate during premonsoon. This correlated well with the increased abundance of total bacterial numbers and phosphate (Khandeparker et al. 2015). These observations were carried out at fixed time (between 10:00 and 11:00 h) and did not bring out the variations related to the tidal cycle. The removal of nitrate could be linked with increase in total bacterial numbers and enhancement of phosphate.

#### Factors influencing the occurrence and distribution of coliforms in Zuari estuary

Estuaries are reservoir for disease– causing microorganisms due to interference of human waste. In the present study, allochthonous coliforms ranged from  $< 10^1 - 10^5$  CFU ml<sup>-1</sup> in surface and near bottom waters. These values are consistent with the previous studies (Shirodkar et al. 2012; Khandeparker et al. 2015) and other estuaries (Blackwell and Oliver 2008; Neogi et al. 2012). Total coliforms (TC) are among the major groups of indicators of fecal bacteria stated in European standards regarding microbiological quality of surface waters. We observed that mean abundance of TC was higher at the mouth of the estuary where TBC was high. Li et al. (2014) reported that TC distribution was mainly influenced by the total bacterial abundance at Jiahe River estuary. In the present study, occurrence and abundance of TC varied between the spring – neap phase. For example, during spring the abundance was high towards the mouth. Whereas, it was high at the upper middle and upstream end of the estuary during the neap tide. Sediment resuspension and high anthropogenic impact during high waters could be one of the possible reasons for high TC abundance at the mouth during spring tide. Sediment also showed low TC abundance during high waters indicating possible resuspension. During flood tides, re-suspension of sediments tends to be maximal, and the sediment is rapidly transported landside and deposition of pathogenic estuarine bacteria is a risk to public health (Lee et al. 2006; Mallin et al. 2007). In addition, previous studies have pointed out that the load of sediment (SPM) in seawater can lead to an increase in the survival of fecal coliforms mainly *E. coli* (Gerba and Mcleod 1976; Solo-Gabriele et al. 2000). Earlier studies have pointed out that release of particle – attached pathogens from bed sediments to the water column via re-suspension process play a vital role in the survival and transport of pathogens in the estuaries (Smith et al. 1978; Desmarais et al. 2002). A study by Shirodkar et al. (2012) also reported high TBC, TC and *Vibrio* spp. in this estuary and concluded that these pathogens could have been transported via waste discharge during high waters from Mormugao sewage treatment plant (STP)

which is located at the sides of the estuarine mouth. The tidal influence and different nutrient load also regulate and enhance abundance of pathogens (Lafferty and Holt 2003; Johnson and Carpenter 2008). While, high abundance of TC at upstream station sediment could be due to its geographical location, as it is located at the entrance of channel near the junction between bay and upstream region of Zuari river and influenced by tidal currents, waves, movement of fishing trawlers, ferry boat, shallow in depth (~1 m) and sediment is silty-clayey in nature. A recent observation on the sediment texture revealed that upstream station was rich in silt and clay (54.6%) content as compared to mouth (19.3%) (Desai and Atchuthan; personal observation). A study by Perkins et al. (2014) reported a significant correlation between high abundance of pathogenic bacteria and sediment containing high amount of silt, clay and organic matter at the Conwy estuary, UK. Thus, the sediment texture could be one of the possible reasons for high abundance of TC in the sediment towards the upstream station. In the present study, TC abundance was influenced by high silicate irrespective of tides in both surface and near bottom waters during SW-MoN. There could be two possible sources for high silicate in this estuary especially during SW-MoN. Silicate is added in this estuary during SW-MoN at the upstream regions via land run-off, and naturally occurring physical and chemical weathering (Shirodkar et al. 2012; Anand et al. 2014a). Another possible reason could be the silicate contributed by disintegrating freshwater phytoplankton which is transported to saline water (Morris et al. 1978). During SW-MoN, the fluctuation in salinity is maximal due to high river discharge in this estuary which can impact the phytoplankton community. Studies have also shown that pore water from marine sediments could also be a significant source for silicate especially during non-monsoon (Fanning and Pilson 1973; Balls et al. 1994). A study by Sarma et al. (2009) at tropical monsoon driven Godavari estuary also pointed out negative correlation between chl *a* and river discharge. But this was attributed to river – borne suspension during monsoon and clear water during non-monsoon.

Factors influencing the occurrence and distribution of *Vibrio* spp. in Zuari estuary

*V. cholerae* (VC) ranged from 0 –  $2.79 \times 10^4$  CFU ml<sup>-1</sup> and were high in marine water. Similarly, other two *Vibrio* spp., i.e., *V. alginolyticus* (VA) and *V. parahaemolyticus* (VP) were also high in marine water and decreased towards the freshwater zone during spring tide. However, during neap tide, *Vibrio* spp. were high towards the freshwater end. This trend was more evident in VA population. These observation points out survival of *Vibrio* spp. in wide salinity range. *Vibrio* spp. are members of marine *Gamma proteobacteria* and they can survive in freshwater as well as marine systems (Hagstrom et al. 2000; Mookerjee et al. 2014). Recent study by Khandeparker et al. (2015) although observed that the abundance of *Vibrio* spp. was high towards the mouth of Zuari estuary

indicating that their growth is favored by saline conditions, tidal influence was not evaluated. A study carried out by Neogi et al. (2012) along the tropical Karnaphuli estuary which is located at the southern part of the Bangladesh too showed high *Vibrio* spp. abundance in saline water ( $\sim 10^2 - 10^4$  CFU ml<sup>-1</sup>) which declined towards the freshwater end ( $< 10^1$  CFU ml<sup>-1</sup>). Also, the abundance was high during PostM compared to PreM, a trend which is similar to this estuary. Changes in abundance of *Vibrio* spp. have been linked to changing environmental conditions (Lipp et al. 2001; Louis et al. 2003). In an estuarine system, factors such as temperature, vertical mixing, tidal flushing, precipitation and nutrient load can change the environment and further alter microbial community structure, including *Vibrio* spp. (Hsieh et al. 2008). Sudden change or alteration in environment factors may also trigger the virulence genes in pathogens and risk to nearby human populations.

The present study showed that viable *Vibrio* spp. were positively influenced by high SPM during PostM along the estuary. The increase was  $\sim 10^1 - 10^3$  folds higher when compared to PreM and SW-MoN. The influence of SPM was more at the upper middle and upstream stations. The upper middle estuarine study area is fringed with thick mangroves which receives high amount of terrestrial derived organic matter in this estuary. In addition, it also receives a significant quantity of pathogen inoculum through many small streams and rivers located at the upstream. A study at Karnaphuli estuary showed high cultivable *Vibrio* abundance to be associated with high suspended load (Lara et al. 2009). Suspended matter is often found densely packed by pathogens. Association of pathogens with sediment particles provide shelter from UV radiation, predation, environmental stress and thus enhance their survival in aquatic systems (Davies et al. 2000; Craig et al. 2004). Thus, the changes in the SPM can influence the microorganisms (especially pathogens) associated with them. Members of *Vibrio* spp. are known to form biofilms on plankton and suspended matter for their persistence (Islam et al. 2007). The interaction between microorganisms (pathogens) and particulate matter is most prevalent in estuarine environments due to the formation of suspended sediment (Flocs) at the marine – freshwater interface (Malham et al. 2014). Generally, *Vibrio* spp. are autochthonous organisms in estuaries and are associated with zooplankton, shellfish (Novotny et al. 2004) and phytoplankton (Rehnstam-Holm et al. 2010), consisting of chitin as the main structural polymer. *Vibrio* spp. are known as active consumers of chitin in various systems. Moreover, C: N ratio analysis of SPM derived from Sundarbans mangrove system showed significant contribution of chitin ( $\sim 1 - 2$  mg l<sup>-1</sup>) to the total SPM pool (Lara et al. 2011).

In the present the study, VC abundance was strongly related with salinity, and nitrite. A study by Batabyal et al. (2014) at Ganges estuary pointed out that the influence of salinity variation with tidal

cycles and sediment re-suspension from tidal flats can regulate the *Vibrio* spp. abundance in the aquatic systems. Nutrients can directly impact algae and zooplankton which may further affect the associated *V. cholerae* populations (Huq et al. 1996; Mourino-Perez et al. 2003). Pathogens such as *V. cholerae* and *V. parahaemolyticus* preferentially attach to planktonic copepods and are important pathogens for fish (*V. alginolyticus*) and humans (*V. cholerae* and *V. parahaemolyticus*) and are closely linked with diatoms (Rehnstam-Holm et al. 2010). The present study also noted that VC abundance correlated with high nitrite in near bottom water and could be due to the reduction of nitrate. Similar relation was also reported by Lara et al. (2011) at eastern Sunderban mangrove forest, Bangladesh after a storm event and concluded that it may be due to the increase of nitrate reducing *Vibrios*. The present study indicates a clear spatio-temporal variation within closely related species, controlled by nutrient load and tidal cycles in this estuary. Further, sediment resuspension could be a significant source for pathogen inoculum (both allochthonous and autochthonous) in this estuary and thus has relevance to estuarine pathogen ecology.

The use of culture dependent techniques in monitoring bacterial populations are reported to have some limitations like over estimation of a single taxa and non-detection of metabolically active but non-cultivable forms (Laiz et al. 2003; Dickson et al. 2014). The use of such tools to characterize the bacterial populations in this estuary would provide new insights on pathogens and their physiological changes and will be a step ahead.

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## Legends to figures

**Fig. 1.** Map showing the sampling locations along the Zuari estuary. Dona Paula (DP) - mouth of the estuary, Cortalim (CR) - lower middle estuary, Borim (BR) – upper middle estuary and Sanvordem (SV) – upstream station.

**Fig. 2.** Seasonal variation in bacterial abundance with respect to time, tide and salinity in surface water at Dona Paula (DP) - mouth of the estuary, Cortalim (CR) - lower middle estuary, Borim (BR) – upper middle estuary and Sanvordem (SV) – upstream station in Zuari estuary. Maximum bubble size corresponds to  $2.84 \times 10^6$  cells  $\text{ml}^{-1}$  and minimum bubble size corresponds to  $5.5 \times 10^3$  cells  $\text{ml}^{-1}$ .

**Fig. 3.** Seasonal variation in bacterial abundance with respect to time, tide and salinity in near bottom water at Dona Paula (DP) - mouth of the estuary, Cortalim (CR) - lower middle estuary, Borim (BR) – upper middle estuary and Sanvordem (SV) – upstream station in Zuari estuary. Maximum bubble size corresponds to  $4.12 \times 10^6$  cells  $\text{ml}^{-1}$  and minimum bubble size corresponds to  $4.14 \times 10^3$  cells  $\text{ml}^{-1}$ .

**Fig. 4.** Canonical Correspondence Analysis (CCA) for bacteria in water column together with associated environmental parameters during PreM, SW-MoN, and PostM spring tide. (a) Surface water species biplot. (b) Surface water stations biplot where different stations are indicated by symbols. (c) Near bottom species biplot. (d) Near bottom stations biplot where different stations are indicated by symbols. (a 1) Eigen values for CCA axes of Surface water analysis. (c1) Eigen values for CCA axes of near bottom water analysis. (a 2 and c 2) Lambda ( $\lambda$  I) is the eigenvalue explained by the environment variables for surface and near bottom waters respectively.  $P \leq 0.05$  are highlighted in bold.

**Fig. 5.** Canonical Correspondence Analysis (CCA) for bacteria in water column together with associated environmental parameters during PreM, SW-MoN, and PostM neap tide. (a) Surface water species biplot. (b) Surface water stations biplot where different stations are indicated by symbols. (c) Near bottom species biplot. (d) Near bottom stations biplot where different stations are indicated by symbols. (a 1) Eigen values for CCA axes of Surface water analysis. (c1) Eigen values for CCA axes of near bottom water analysis. (a 2 and c 2) Lambda ( $\lambda$  I) is the eigenvalue explained by the environment variables for surface and near bottom waters respectively.  $P \leq 0.05$  are highlighted in bold.

**Fig. 6.** Seasonal and tidal variation in total coliforms and *Vibrio* spp. abundance at Dona Paula (DP) - mouth of the estuary, Cortalim (CR) - lower middle estuary, Borim (BR) – upper middle estuary and Sanvordem (SV) – upstream station in Zuari estuary.

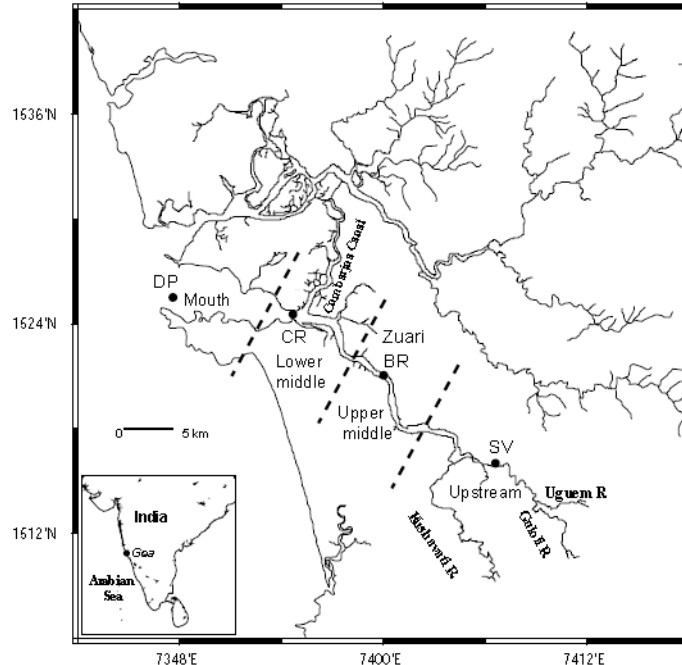
## Legends to tables

**Table 1:** Details of sampling seasons, tide, time and tidal height at the start and end of sampling.

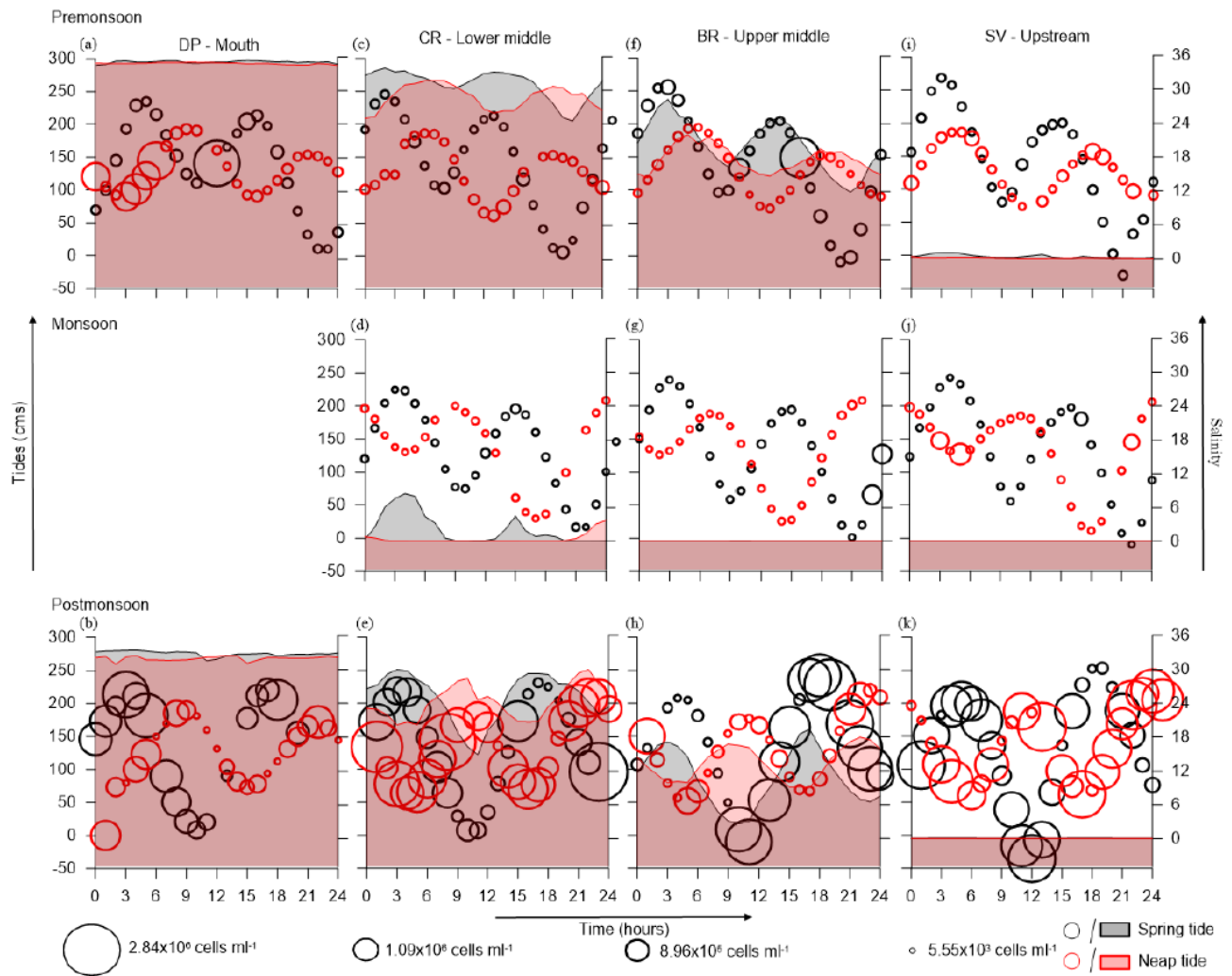
**Table 2:** Average abundance of TBC and percentage abundance of viable and pathogenic bacteria (with respect to % abundance of TBC) in the sediments at the Dona Paula, Cortalim and Borim during spring and neap tide.

**Supplementary Table 1(a-f):** Seasonal and tidal variation in dissolved nutrients (nitrite, nitrate, ammonia, silicate and phosphate) in surface and near bottom waters along the Zuari estuary. (a) Dona Paula (DP) - mouth of the estuary; (b) Cortalim (CR) - lower middle estuary; (c) Borim (BR) – upper middle estuary and (d) Sanvordem (SV) – upstream station; (e) Mean salinity variation from mouth to upstream end of Zuari estuary during spring and neap tide; (f) Mean suspended particulate matter (SPM) variation from mouth to upstream end of Zuari estuary during spring and neap tide.

**Supplementary Table. 2:** Seasonal and tidal variation in total viable counts (TVC) in surface and near bottom water at Dona Paula (DP) - mouth of the estuary, Cortalim (CR) - lower middle estuary, Borim (BR) – upper middle estuary and Sanvordem (SV) – upstream station in Zuari estuary.



**Fig. 1**



**Fig. 2**



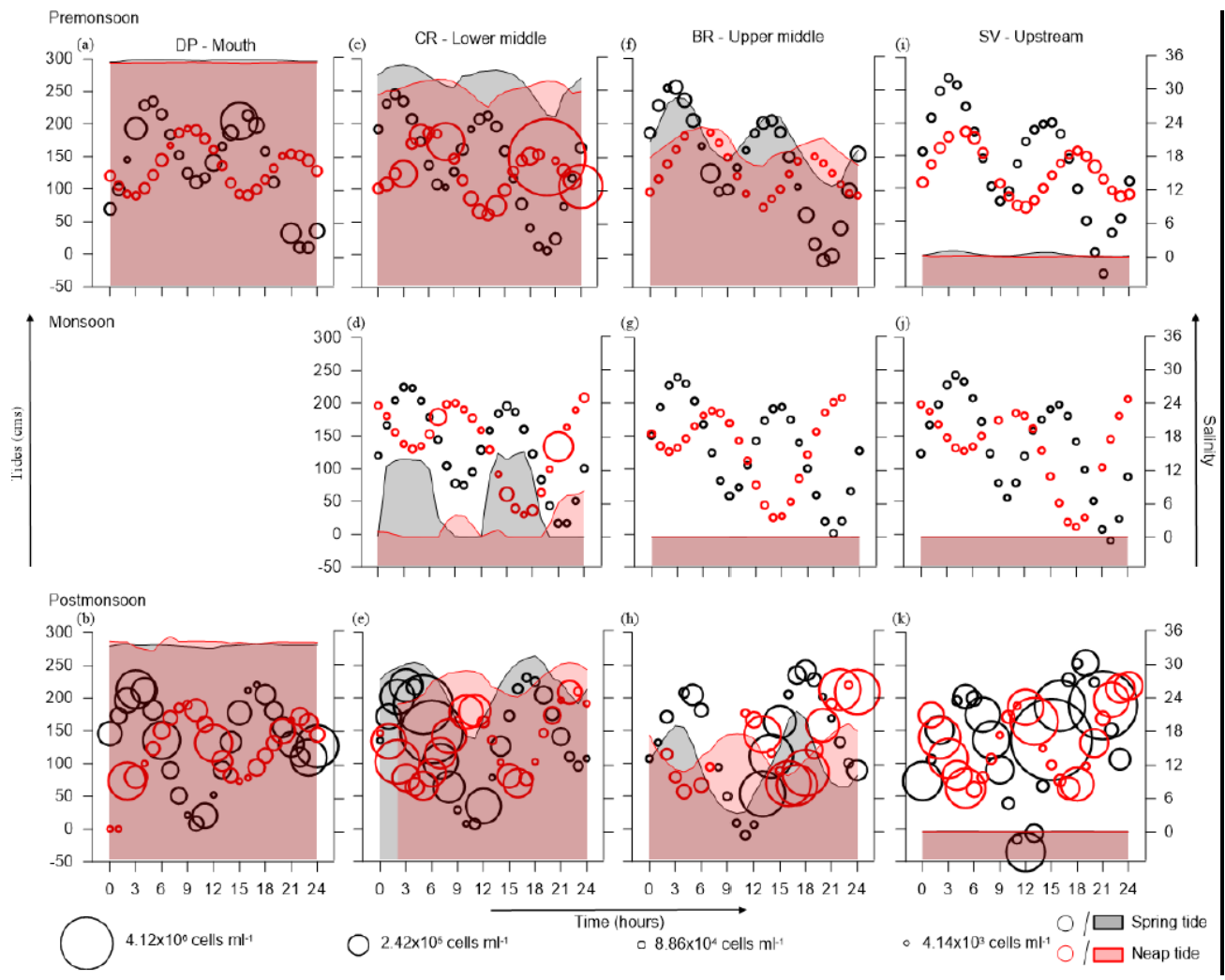


Fig. 3

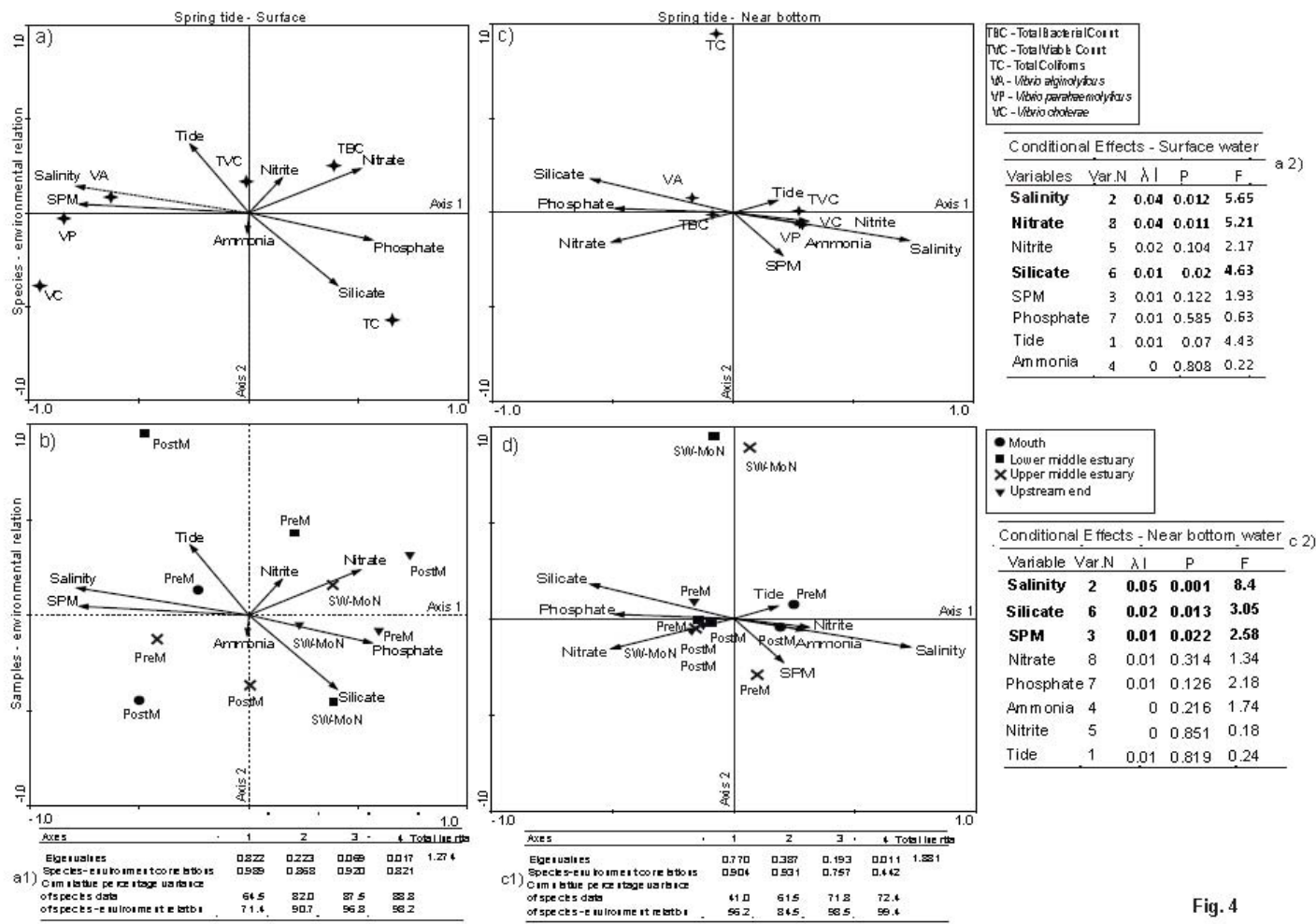


Fig. 4

Fig. 4

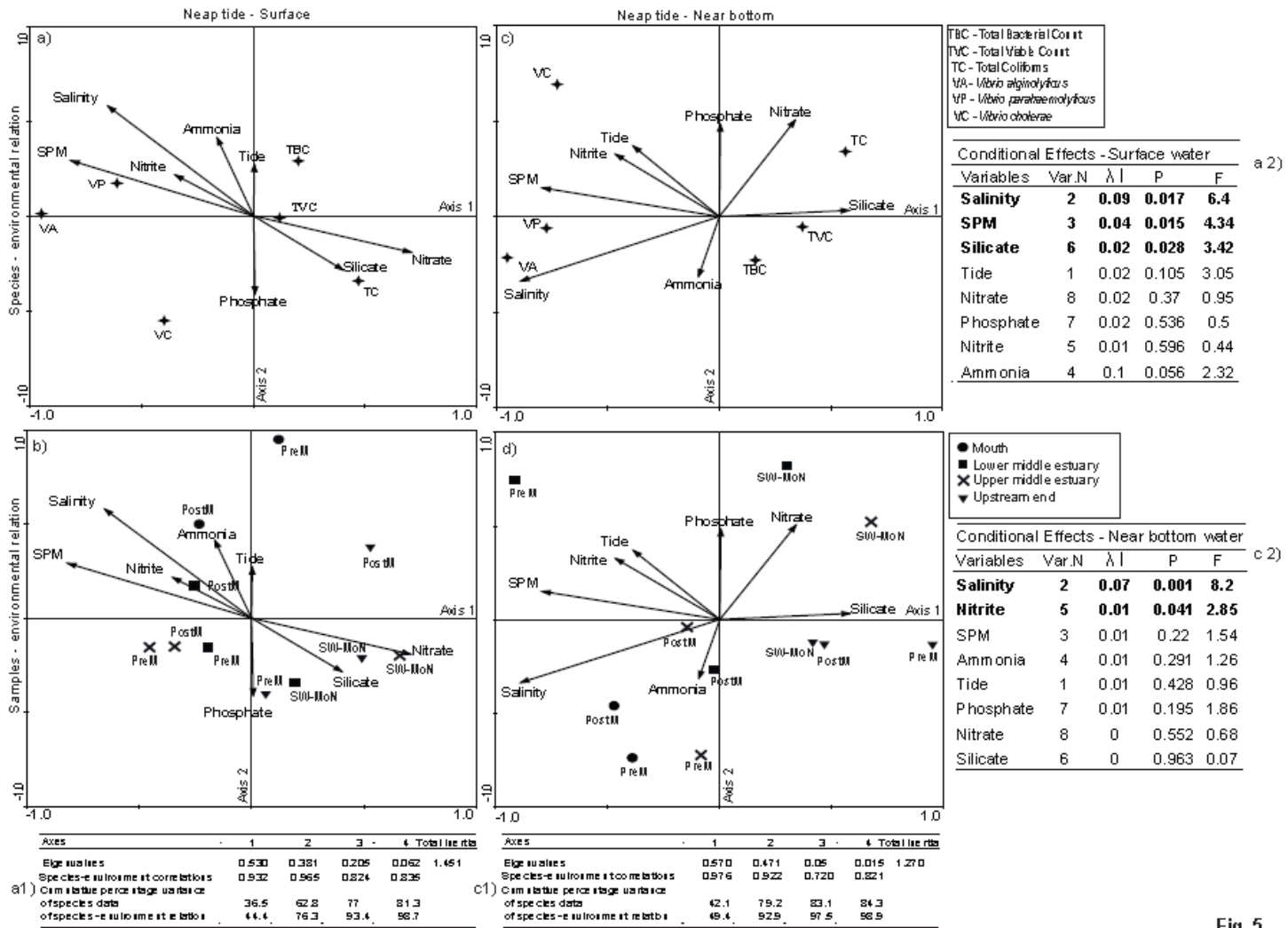
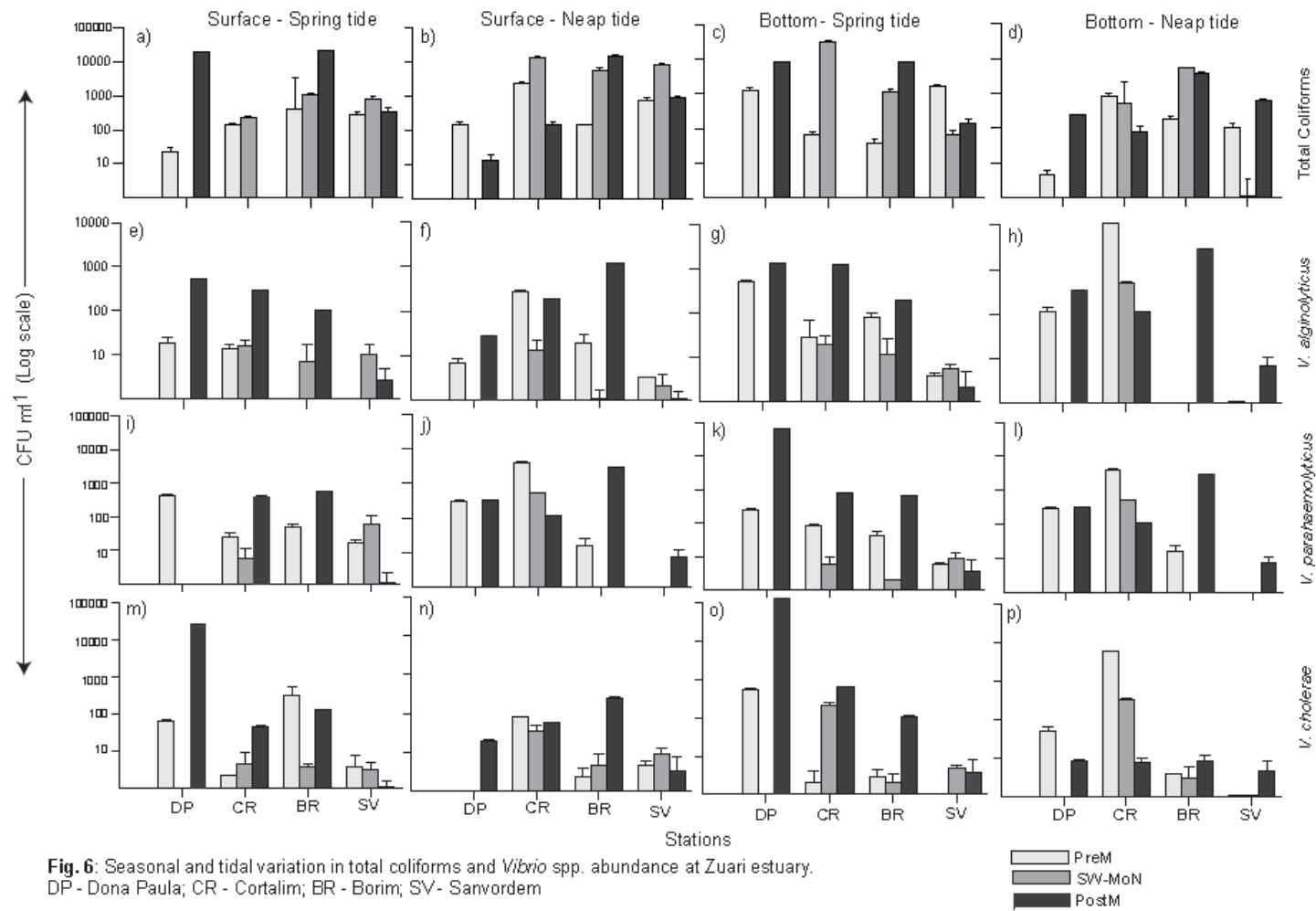


Fig. 5



**Fig. 6**

**Table 1: Details of sampling seasons, tide, date and tidal height at the start and end time of sampling.**

Seasons	Tide	Date	Time (Start time - end time)	Tidal height (At start - end time)
PreM	Spring	19 <sup>th</sup> -20 <sup>th</sup> May 2011	11:00 AM-11:00 PM	1.91 m- 2.06 m
	Neap	27 <sup>th</sup> -28 <sup>th</sup> April 2011	14:00 PM - 14:00 PM	1.08 m-1.01 m
SW-MoN	Spring	1 <sup>st</sup> -2 <sup>nd</sup> August 2011	9:00 AM-9:00 AM	1.20 m-1.01 m
	Neap	9 <sup>th</sup> -10 <sup>th</sup> August 2011	11:00 AM-11:00 AM	1.56 m-2.08 m
PostM	Spring	29 <sup>th</sup> - 30 <sup>th</sup> October 2011	10:00 AM-10:00 AM	1.72 m-1.32 m
	Neap	23 <sup>rd</sup> -24 <sup>th</sup> October 2011	11:00 AM-11:00 AM	1.3 m -1.55 m

PreM – Premonsoon; SW-MoN – Southwest monsoon; PostM - Post monsoon

**Table 2: Average abundance of TBC and percentage abundance of viable and pathogenic bacteria (with respect to % abundance of TBC) in the sediments at the Dona Paula, Cortalim and Borim during spring and neap tide.**

			TBC (Avg. $\pm$ Std. Deviation)	TVC (% Sed)	TVC ( Surf)	TVC (Bott)	VC (%)	VA (%)	VP (%)	TC (%)
Dona Paula (DP)	PreM	Spring	$4.17 \times 10^7 \pm 1.82 \times 10^7$	3.12	0.6	0.6	1.96	0	0.04	0.08
		Neap	$2.43 \times 10^7 \pm 4.52 \times 10^7$	0.10	0.001	0.001	0	0	0	0
Dona Paula (DP)	SW-MoN	Not sampled								
	PostM	Spring	$5.91 \times 10^7 \pm 1.84 \times 10^7$	0.10	1.5	1.33	0.01	0	0	0
Neap		ND	ND	-	-	ND	ND	ND	ND	
Cortalim (CR)	PreM	Spring	$3.79 \times 10^7 \pm 3.58 \times 10^7$	5.67	0.04	0.08	0.11	0.05	0.03	0.09
		Neap	$1.51 \times 10^9 \pm 3.21 \times 10^9$	0.03	0.002	0.0001	0	0	0	0
	SW-MoN	Spring	$6.75 \times 10^7 \pm 1.4 \times 10^7$	0.13	0.02	0.3	0.05	0	0.02	0.10
		Neap	ND	ND	-	-	ND	ND	ND	ND
	PostM	Spring	$5.58 \times 10^6$	1.51	1.51	1.80	0.01	0.01	0.03	0.23
		Neap	ND	ND	-	-	ND	ND	ND	ND
Borim (BR)	PreM	Spring	$2.77 \times 10^6 \pm 1.13 \times 10^6$	2.48	0.1	0.10	0.02	0.03	0.06	0.03
		Neap	$1.99 \times 10^7 \pm 7.39 \times 10^6$	5	0.12	0.002	0	0.03	0.02	0.10
	SW-MoN	Spring	$6.18 \times 10^5 \pm 1.16 \times 10^5$	2.26	0.83	0.82	0	0	0	0.08
		Neap	$1.19 \times 10^7 \pm 4.38 \times 10^6$	2.88	0.032	0.036	0.04	0.28	0.64	0.59
	PostM	Spring	$3.87 \times 10^7 \pm 3.28 \times 10^7$	0.37	0.054	0.085	0	0	0.01	0.27
		Neap	ND	ND	-	-	ND	ND	ND	ND

Note- TBC: Total Bacterial Count (cells  $\text{gm}^{-1}$ ); TVC: Total Viable Count (CFU  $\text{gm}^{-1}$ ); VC: *Vibrio cholerae* (CFU  $\text{gm}^{-1}$ ); VA: *V. alginolyticus* (CFU  $\text{gm}^{-1}$ ); VP: *V. parahaemolyticus* (CFU  $\text{gm}^{-1}$ ); TC: Total Coliforms (CFU  $\text{gm}^{-1}$ ); ND: Not Done.

Supplementary Table 1

a) Dona Paula (DP), Mouth of the estuary

Nutrients ( $\mu\text{M l}^{-1}$ )	Premonsoon				Southwest Monsoon				Post monsoon			
	Spring		Neap		Spring		Neap		Spring		Neap	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
<b>Surface Water</b>												
Silicate	0.54	26.22	2.96	8.74	×	×	×	×	11.4	27.82	9.17	36.88
Phosphate	0.16	1.44	●	0.55	×	×	×	×	1.11	13.03	0.20	4.03
Nitrite+Nitrate	2.32	18.27	0.07	11.24	×	×	×	×	●	0.82	0.71	1.04
Ammonia	26.74	95.55	3.80	52.88	×	×	×	×	4.75	17.28	1.09	31.16
Nitrite	0.49	6.06	0.14	2.31	×	×	×	×	0.08	0.75	●	1.27
<b>Near bottom Water</b>												
Silicate	5.86	54.88	1.86	30.04	×	×	×	×	1.79	2.74	10.39	21.10
Phosphate	●	3.77	●	3.59	×	×	×	×	-0.13	1.38	0.27	0.60
Nitrite+Nitrate	2.72	13.20	0.44	7.76	×	×	×	×	0.23	1.38	●	0.21
Ammonia	15.12	66.59	3.37	18.36	×	×	×	×	5.88	12.82	0.74	9.24
Nitrite	0.64	1.47	0.37	1.13	×	×	×	×	0.60	4.49	●	0.66

× - Not sampled; ● - Below detection level

b) Cortalim (CR), Lower middle estuary

● - Below detection level

Nutrients ( $\mu\text{M l}^{-1}$ )	Premonsoon				Southwest Monsoon				Post monsoon			
	Spring		Neap		Spring		Neap		Spring		Neap	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
<b>Surface Water</b>												
Silicate	●	67.30	11.08	31.93	2.34	193.38	28.27	146.85	10.04	42.52	11.60	125.48
Phosphate	0.95	20.18	0.20	1.61	1.30	26.57	1.16	4.99	0.38	20.18	0.52	3.16
Nitrite+Nitrate	●	14.45	●	5.50	6.51	17.11	7.12	14.72	●	8.79	1.35	9.01
Ammonia	●	112.52	●	36.42	●	27.33	22.65	36.67	●	12.17	●	28.22
Nitrite	●	2.80	●	43.81	●	7.88	●	0.94	●	0.12	●	0.68
<b>Near bottom Water</b>												
Silicate	0.21	58.38	●	25.36	5.49	102.99	40.11	94.73	8.02	36.78	13.56	144.37
Phosphate	1.30	8.54	●	0.57	0.80	5.27	1.35	4.42	0.41	3.52	0.68	4.24
Nitrite+Nitrate	●	13.52	●	0.74	0.38	8.25	7.89	16.42	3.10	13.16	0.14	8.51
Ammonia	●	49.55	●	1.29	●	3.00	24.87	44.43	●	19.61	●	30.51
Nitrite	●	3.77	●	39.76	●	3.16	0.50	4.58	●	0.29	●	2.19

c) Borim (BR), Upper middle estuary

Nutrients ( $\mu\text{M l}^{-1}$ )	Premonsoon				Southwest Monsoon				Post monsoon			
	Spring		Neap		Spring		Neap		Spring		Neap	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
<b>Surface Water</b>												
Silicate	25.79	133.33	9.20	35.09	19.46	82.30	0.62	102.93	26.20	82.18	19.59	44.29
Phosphate	0.08	7.08	0.20	0.83	0.80	4.69	0.04	2.21	0.25	4.42	0.17	1.96
Nitrite+Nitrate	0.49	12.15	0.02	5.24	0.76	4.31	2.12	38.59	0.87	1.74	●	2.87
Ammonia	●	51.92	●	21.82	●	18.49	●	11.10	●	17.84	●	26.51
Nitrite	●	5.5	●	3.88	●	0.47	●	●	●	1.41	●	0.09
<b>Near bottom Water</b>												
Silicate	25.23	126.58	3.64	30.47	27.20	84.58	3.19	95.34	32.18	74.72	18.55	47.12
Phosphate	●	3.19	0.15	0.97	1.06	4.35	0.26	4.12	0.20	0.69	0.12	1.63
Nitrite+Nitrate	1.74	10.51	0.53	12.36	0.09	4.76	2.27	37.71	1.01	1.56	●	4.95
Ammonia	●	225.29	●	30.01	●	19.70	●	11.44	●	18.30	1.47	26.73
Nitrite	●	6.53	●	0.88	●	2.24	●	●	●	0.56	●	0.07

● - Below detection level

d) Sanvordem (SV), Upstream

Nutrients ( $\mu\text{M l}^{-1}$ )	Premonsoon				Southwest Monsoon				Post monsoon			
	Spring		Neap		Spring		Neap		Spring		Neap	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
<b>Surface Water</b>												
Silicate	5.21	69.96	●	68.94	28.69	131.38	79.12	222.87	1.87	26.04	3.66	131.28
Phosphate	0.02	4.23	●	0.38	1.02	3.21	2.39	6.21	1.62	12.33	●	0.90
Nitrite+Nitrate	●	13.31	0.16	1.10	2.93	8.92	8.28	21.25	36.97	52.34	2.29	12.37
Ammonia	●	12.05	●	12.91	35.24	121.56	●	1.27	●	●	4.53	22.39
Nitrite	●	0.83	●	0.27	-0.15	2.23	●	●	●	1.76	0	1.76
<b>Near bottom Water</b>												
Silicate	2.91	32.96	●	59.04	53.67	115.86	73.70	219.74	2.35	31.05	80.52	135.41
Phosphate	0.04	2.95	●	0.92	1.80	3.08	3.72	6.02	0.89	10.54	0.07	1.47
Nitrite+Nitrate	●	5.33	0.11	0.84	3.95	8.65	4.96	13.52	●	48.68	1.04	7.89
Ammonia	●	11.51	●	17.12	27.12	144.68	●	●	●	●	8.42	19.47
Nitrite	●	0.46	●	0.22	-0.15	2.49	●	9.10	●	0.57	●	0.57

● - Below detection level

e) Mean salinity variation from mouth to upstream end of Zuari estuary during spring and neap tide

Salinity (PSU)	Premonsoon		Southwest Monsoon		Post monsoon	
	Spring	Neap	Spring	Neap	Spring	Neap
<b>Surface water</b>						
Mouth	35.43±0.14	34.97±0.20	×	×	32.82±0.44	31.96±0.36
Lower middle	31.75±2.54	30.37±1.26	2.19±2.74	0.06±0.01	25.17±4.27	21.47±8.56
Upper middle	20.92±4.17	19.83±2.20	0.05±0.01	0.60±1.21	10.94±5.05	13.43±2.91
Upstream End	0.44±0.34	0.13±0.07	0.03±0.00	0.03±0.00	0.05±0.02	0.04±0.01
<b>Near bottom water</b>						
Mouth	35.44±0.1	35.00±0.00	×	×	33.38±0.20	33.88±0.51
Lower middle	31.79±2.5	30.32±1.26	6.98±6.5	2.41±3.23	26.31±4.31	24.78±7.80
Upper middle	20.95±4.61	19.73±2.21	0.05±0.01	0.06±0.01	12.04±5.59	14.61±2.91
Upstream End	0.45±0.39	0.13±0.07	0.03±0.00	0.03±0.00	0.05±0.02	0.04±0.01

× – Not sampled

f) Mean suspended particulate matter (SPM) variation from mouth to upstream end of Zuari estuary during spring and neap tide

SPM (mg l <sup>-1</sup> )	Premonsoon		Southwest Monsoon		Post monsoon	
	Spring	Neap	Spring	Neap	Spring	Neap
<b>Surface water</b>						
Mouth	167.95 ±52	64.85±28	×	×	229.5±61	130±51
Lower middle	178.53±50	67±24	31.53±5	31.80±8	147.1±27	122±28
Upper middle	132±35	51±12	15.5±2	12.01±2.7	91.4±17	63.57±20
Upstream End	21±2.5	19.4±5	9.6±5	9.5±2.1	57.5±6.2	26.7±6.9
<b>Near bottom water</b>						
Mouth	215.49±71	55.27±23	×	×	207±35	65±13
Lower middle	265.77±72	64.86±30	44.3±5.6	94.6±23	176.3±71	77±12
Upper middle	150.63±56	58.45±23	7.6±4	12.02±6	109±24	70±12
Upstream End	21.63±11	22.5±9	8.6±4	9.6±4	55±4	25±6.3

× – Not sampled



**Suppl. Table. 2: Seasonal and tidal variation of total viable counts (TVC) in surface and near bottom water at all four stations viz., DP, CR, BR and SV in Zuari estuary.**

	Premonsoon (CFU ml <sup>-1</sup> )		Southwest Monsoon (CFU ml <sup>-1</sup> )		Post monsoon (CFU ml <sup>-1</sup> )	
	Spring tide	Neap tide	Spring tide	Neap tide	Spring tide	Neap tide
<b>Surface water</b>						
DP	$2.82 \times 10^5 \pm 2.2 \times 10^4$	$3.77 \times 10^2 \pm 7.34 \times 10^1$	NC	NC	$8.96 \times 10^5 \pm 2.97 \times 10^2$	$2.09 \times 10^3 \pm 2.04 \times 10^2$
CR	$1.34 \times 10^4 \pm 6.29 \times 10^2$	$3.11 \times 10^3 \pm 6.5 \times 10^1$	$1.32 \times 10^4 \pm 8.6 \times 10^2$	$2.01 \times 10^4 \pm 1.5 \times 10^3$	$8.37 \times 10^4 \pm 1.53 \times 10^4$	$2.43 \times 10^4 \pm 3.8 \times 10^3$
BR	$2.84 \times 10^3 \pm 5.53 \times 10^2$	$2.27 \times 10^4 \pm 5.8 \times 10^3$	$5.14 \times 10^3 \pm 5.2 \times 10^2$	$3.97 \times 10^3 \pm 4.0 \times 10^2$	$2.10 \times 10^4 \pm 1.7 \times 10^4$	$3.03 \times 10^5 \pm 3.4 \times 10^4$
SV	$1.27 \times 10^3 \pm 2.90 \times 10^2$	$4.20 \times 10^2 \pm 1.5 \times 10^2$	$1.34 \times 10^3 \pm 2.3 \times 10^2$	$1.27 \times 10^4 \pm 4.7 \times 10^2$	$3.01 \times 10^2 \pm 4.4 \times 10^1$	$1.52 \times 10^3 \pm 2.3 \times 10^2$
<b>Near bottom</b>						
DP	$2.85 \times 10^5 \pm 6.8 \times 10^4$	$3.77 \times 10^2 \pm 1.18 \times 10^2$	NC	NC	$7.95 \times 10^5 \pm 1.8 \times 10^4$	$2.58 \times 10^3 \pm 1.5 \times 10^2$
CR	$3.00 \times 10^4 \pm 4.6 \times 10^3$	$3.97 \times 10^3 \pm 1.3 \times 10^2$	$2.66 \times 10^4 \pm 9.1 \times 10^2$	$8.44 \times 10^4 \pm 2.1 \times 10^3$	$9.94 \times 10^4 \pm 2.0 \times 10^4$	$9.23 \times 10^2 \pm 4.3 \times 10^1$
BR	$2.90 \times 10^3 \pm 1.8 \times 10^2$	$4.20 \times 10^2 \pm 1.3 \times 10^1$	$5.16 \times 10^3 \pm 4.0 \times 10^2$	$4.22 \times 10^3 \pm 3.9 \times 10^2$	$3.34 \times 10^4 \pm 3.4 \times 10^3$	$5.19 \times 10^4 \pm 4.2 \times 10^3$
SV	$1.83 \times 10^3 \pm 7.8 \times 10^1$	$5.16 \times 10^3 \pm 3.3 \times 10^2$	$1.94 \times 10^3 \pm 2.6 \times 10^2$	$5.04 \times 10^4 \pm 7.1 \times 10^2$	$2.38 \times 10^2 \pm 4.2 \times 10^1$	$6.80 \times 10^3 \pm 6.4 \times 10^2$

NC – Not collected