

**Predominance of anaerobic bacterial community over aerobic community contribute to intensify ‘oxygen minimum zone’ in the eastern Arabian Sea**

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**Abstract**

The presence of a delicately poised mid-depth oxygen minimum zone (OMZ) makes the Arabian Sea a unique and important ecosystem. So far, various aspects responsible for its formation have been studied. However, the contributions from bacterial groups mediating its formation and maintaining its intensity are described for the first time in this study. Thus, we hypothesize that the bacterial dynamics along with organic carbon loading result in bringing about differences in the intensity of OMZ between two stations in the Eastern Arabian Sea (EAS). Water column from these 2 stations one from offshore and another from slope, in the EAS were examined for phytoplankton diversity and pertinent groups of culturable bacteria. Vertical profiles of dissolved oxygen, productivity, chlorophyll *a*, total organic carbon and different physiological groups of bacteria showed well-defined stratified patterns in tandem with physical and chemical stratifications of the water column. The phytoplankton diversity was higher at the slope station (SS) and was dominated by the heterotrophic dinoflagellates. The offshore station (OS) on the other hand, showed lower diversity dominated by diatoms ( $p < 0.05$ ). This observation could imply relatively higher autotrophy at the OS. Our results show that OMZ from these ‘oligotrophic’ regions is dominated by anaerobic bacteria. We believe that these bacteria contribute to intensify the OMZ in the EAS. Further, a higher abundance of viable anaerobic bacteria ( $TVC_{\text{anaero}}$ ) and other anaerobic groups at the SS than the OS suggests that the OMZ is relatively much more intense near the slope. Besides, total organic carbon (TOC) load is three-fold higher at the slope than at OS implying its higher accumulation and lower degradability in slope waters. Settling of this more preserved organic carbon in mid-slope sediments in contact with OMZ results in one of the highest enrichments of sedimentary TOC in the world oceans.

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## 1. Introduction

The Arabian Sea is one of the most productive regions of the world's oceans (Ryther et al., 1966) and is an intensely dynamic region driven by strong seasonal reversals in monsoon winds (Campbell et al., 1998) resulting in reversal in surface currents. During June to September (southwest summer monsoon, SWM), the main driving force is the Findlater Jet which blows from the southwest with high velocities often exceeding  $14 \text{ m s}^{-1}$  (Bauer et al., 1991) along the coasts off Somali and Arabian Peninsula causing clockwise surface currents. In contrast, from November to February (northeast winter monsoon, NEM), winds are predominantly from the northeast with mild speed of  $\sim 5 \text{ m s}^{-1}$  (Bauer et al., 1991) during which anticlockwise surface currents prevail. This reversal in regional current patterns results in seasonally opposing currents in the eastern Arabian Sea (EAS) and the western Arabian Sea (WAS). These opposing trends on either side are mainly due to the geographical configuration of the Arabian Sea which is bound by land on its three sides and is connected to the open sea only on its southern side. The summer SWM currents induce intense upwelling in the WAS resulting in extremely high surface productivity ( $>1 \text{ gC m}^{-2}\text{d}^{-1}$ ) characterizing it as one of the most productive region of the world's oceans (Qasim, 1977). During the same period, relatively weaker equator-ward coastal current prevail in EAS (Shetye et al., 1994) which results in mild upwelling and moderate surface productivity ( $>0.5 - 1 \text{ gC m}^{-2}\text{d}^{-1}$ ) all along the southwestern coast of India as a narrow coastal belt (Madhupratap et al., 2001). In contrast, during NEM, prevailing anticlockwise currents are mild and are unable to cause upwelling anywhere in the Arabian Sea. Nevertheless, during this winter period sinking of cold surface waters in the region off the Gulf of Kachchh in the northeastern Arabian Sea causes upwelling resulting in high productivity ( $> 1 \text{ gC m}^{-2}\text{d}^{-1}$ ) over a very limited area (Madhupratap et al., 1996). Thus, the productivity in the Arabian Sea is highly episodic due to variable climatological stresses resulting in extremes in primary production, from eutrophic coastal upwelling to oligotrophic open ocean conditions, in close proximity (Campbell et al., 1998). Moreover, despite relatively lower primary productivity, the presence of much thicker and more intense OMZ in the EAS compared to the WAS is an enigma. Although the OMZ is caused due to excessive biological oxygen consumption, its mid-depth position is maintained by subsurface circulation patterns. The role of microbes in the development of OMZ is well appreciated. However, the bacterial contribution in its development and intensification is poorly understood. Interestingly, the OMZ is more intense and thicker in the EAS (Wyrski, 1971) despite lower annual column productivity than WAS (Qasim, 1977). Unlike other areas influenced by upwelling, the zones of highest primary productivity and lowest oxygen concentrations are not proximate but geographically separated in the Arabian Sea (Balch et al., 1991). In order to

understand this phenomenon, we have examined different physiological groups of bacteria in two water columns sampled in November 2001 (inter-monsoon period). As, this study is the first of its kind in the Arabian Sea it was difficult to compare bacterial community in the EAS with that in WAS. However, we have compared the data from the offshore station (OS) representing the open ocean environment and the slope station (SS) representing slope environment.

This study answers the following questions: Is the preservation of organic matter higher at the SS than at the OS due to lower dissolved oxygen regimes? Consequently, is this preservation marked by the prevalence of higher abundance of anaerobic bacterial communities than aerobic leading to higher net accumulation than degradation of organic matter (OM)? The study highlights that the lower rates of degradation by dominating anaerobes due to environmental forcing leads to net accumulation or preservation of OM, notwithstanding the relative oligotrophy in EAS compared to WAS. Thus this study hypothesizes that bacterial dynamics contribute to the differences in the intensity of OMZ between two stations –the SS and OS in the Eastern Arabian Sea.

## **2. Materials and Methods**

### *2.1 Sampling*

Water samples were collected using 12 litre Go-Flow Niskin bottles in November 2001 (winter monsoon) from two stations; SS (15° 8'44.1 N, 72°46.2' E) and OS (15°02'20.6 N, 72°17'2.1 E) (Fig. 1), during the cruise SK-172 on board ORV Sagar Kanya. These stations are 120 km and 185 km away from the coast respectively. Two separate approaches were adopted for water column sampling. For shallow water (100 m depth referred in this study as euphotic waters) related studies, a CTD (Sea-Bird SBE 9plus CTD with SBE 11plus Deck Unit) was used to measure salinity, temperature and fluorescence. Water samples were collected from 1, 10, 25, 50, 75 and 100 m depths for phytoplankton related studies at both the stations. For the second approach for deeper waters, samples were collected from 5, 100, 150, 250, 500, 750 and 919 m at SS and from 5, 100, 150, 250, 500, 750, 1000, 1200, 1400 m at OS.

### *2.2 Dissolved oxygen and total organic carbon*

Dissolved oxygen was measured by Winkler method (Grasshoff et al., 1983) on board the vessel. Total organic carbon (TOC) was analyzed by Shimadzu TOC-5000 (precision, 5%) analyzer using HTCO (high temperature catalytic oxidation) by platinum catalyst. The system blank and instrument blank were determined using UV oxidized milli-Q water. The acidified samples were homogenized

and inorganic carbon was removed by purging a stream of carbon-dioxide-free air through the samples just before the analyses.

### *2.3 Primary productivity and phytoplankton biomass*

Chlorophyll *a* (Chl *a*) and phaeopigment (Phaeo) concentrations were determined fluorometrically (Turner Designs, USA) by filtering duplicate 1L water samples from each depth. GF/F filters were used and the extraction was done by adding 10 ml of 90% acetone in the dark for 24 h under refrigerated condition. Phaeopigments were measured by adding 2 drops of 0.5 N hydrochloric acid to the same sample.

For primary productivity measurements, water samples were drawn from the above mentioned depths up to 100 m at predawn and collected into four 300 ml polycarbonate bottles, three light and one dark at each depth.  $\text{NaH}^{14}\text{CO}_3$  (Board of Radiation and Isotope Technology, Mumbai with activity of 185 kBq) was added to each bottle and incubated in situ using a mooring system at respective depths for 12 h -from just before sunrise to half an hour after sunset. Incorporation of  $^{14}\text{C}$  was determined by filtering 100 ml of the sample from each bottle through GF/F filters (Whatman, USA). The filters were transferred to scintillation vials and exposed overnight to HCl (0.5 N) fumes in vial. Liquid scintillation cocktail (5 ml) was added and the radioactivity measured in Packard 2500 TR liquid scintillation counter.

To evaluate the numerical abundance and major species composition of phytoplankton, 250 ml of water samples from each depth up to 100 m were preserved with Lugol's iodine, allowed to settle for 3-4 days and concentrated to a known small volume. One ml in triplicate of this concentrate was counted on Sedgewick-Rafter plankton counting chamber using Olympus microscope. The phytoplankton cell density (PCD) was expressed as numbers  $\times 10^3$  cells  $\text{l}^{-1}$ . Generic and species identification were done according to various keys (Lebour, 1978; Constance et al., 1985; Tomas, 1996). The statistical software, PRIMER ver. 6 was used to calculate Shannon-Wiener diversity index on  $\log_e$  basis ( $H'$ ), Margalef's index ( $d$ ) and Pielou's evenness index ( $J'$ ).

### *2.4 Bacterial Abundance and Bacterial Production*

Total direct bacterial counts (TBC) estimated by the acridine orange direct count (AODC) method (Hobbie et al., 1977) were expressed as numbers per litre. Quantitative enumeration of total viable bacteria ( $\text{TVC}_t$ ) was done (Kogure et al., 1980) under aerobic ( $\text{TVC}_{\text{aero}}$ ) and anaerobic ( $\text{TVC}_{\text{anaero}}$ ) conditions. For anaerobic viability a reductant i.e.  $\text{Na}_2\text{S}$  at a final concentration of 0.125% was

added before incubating the samples. To minimize cell damage, very low vacuum (<1 kPa) and a small filtration volume (< 100 ml) was filtered.  $TVC_t$  includes  $TVC_{aero}$  and  $TVC_{anaero}$  where there could be certain amount of overlap of facultative forms which could be counted under both conditions.

Bacterial production (BP) was estimated by measuring the rate of [methyl- $^3H$ ] thymidine incorporation (Fuhrman and Azam, 1982). Thymidine incorporation rates were converted to bacterial carbon production (BCP) by using a conversion factor of  $2.0 \times 10^{18}$  cells produced mole $^{-1}$  thymidine incorporated (Kirchman et al., 1982) and a carbon conversion factor of  $2.0 \times 10^{-14}$  gC cell $^{-1}$  (Lee and Fuhrman, 1987).

### *2.5 Physiological bacterial groups*

The estimation of sulphate reducing bacteria (SRB) was according to Pfennig et al. (1981) and Loka Bharathi et al. (1991). Colony forming units (CFU) were counted after 15-20 days of incubation at room temperature (RT) ( $28 \pm 2^{\circ}C$ ) in the dark. Colonies in SRB agar shake tubes that did not reduce sulphate were counted as fermentors (FERM). Thiobacillus denitrificans-like organisms (TDLO) were isolated on modified Leiske's medium (Rodina, 1972; Loka Bharathi, 1989; Loka Bharathi et al., 1997) by the agar shake technique. Nitrifiers were estimated using Winogradsky medium (Rodina, 1972; Ram et al., 2001). The medium was substituted with ammonium sulphate at 2 mM (final concentration) for ammonia oxidizing bacteria (AOB) or sodium nitrite at 0.5 mM (final concentration) for nitrite oxidizing bacteria (NOB) and incubated at RT. The colonies were enumerated and expressed as CFU l $^{-1}$ . The DNT were enumerated using the medium described by Sugahara et al (1974).

Two-way analysis of variance (ANOVA) was performed to determine the depth wise and station wise differences among the studied variables. Post hoc test was also performed. Statistical analysis was performed using software 'Statistica'.

## **3. Results and discussion**

The euphotic zone in the study areas extends to 60-80 m depth. Hence, in the present investigation, the data upto a depth of 100 m was collected using a CTD not only to understand the variation in temperature, salinity and fluorescence but also the spatial variability in Chl *a*, primary productivity and other phytoplankton-related parameters (Figs. 2, 3 & 4). In addition to the euphotic water columns of 100 m, entire water columns at both the stations, i.e. up to 919 m at SS and up to 1400 m

at OS, were studied for dissolved oxygen (DO), total organic carbon (TOC), bacterial counts their productivity and viability (Figs. 5). Besides these bacterial parameters, physiological groups of bacteria like sulphate and nitrate reducing bacteria were also studied (Figs. 6).

### *3.1 Euphotic water columns (0 - 100 m depth)*

#### *3.1.1 Phytoplankton related parameters*

##### *3.1.1.1 Primary Productivity (PP)*

PP values ranged between 0.41- 84.47 and 0.34 - 69.13 mgC m<sup>-3</sup> d<sup>-1</sup> at OS and SS respectively with maximum values at 10 m depth (Fig. 3a). Column productivity was 2900 mgC m<sup>-2</sup> d<sup>-1</sup> at OS and 2010 mgC m<sup>-2</sup> d<sup>-1</sup> at SS. These integrated values are higher than the values of 1016 and 1348 mgC m<sup>-2</sup> d<sup>-1</sup>, reported by Balachandran et al., (2008) from stations to the north in December 2000. These values are also higher than the values of 243 and 555 mgC m<sup>-2</sup> d<sup>-1</sup> during September 2003 given by Jyothibabu et al., (2008) for the south. This comparison clearly shows that the central offshore region of west coast of India is relatively more productive than northern and southern regions. However, similar or higher column production values were recorded in the WAS, especially off Omani coast, during comparable times (Ryther et al., 1966; Ducklow 1993; Jochem et al., 1993; Savidge and Gilpin, 1999; Garrison et al., 2000). The primary productivity values obtained presently are much higher than those reported earlier. There can be substantial changes in PP within a period of few weeks (Savidge and Gilpin, 1999). Hence these high values could be attributed to the snap shot observation during a temporary algal bloom. The primary productivity values obtained during International Indian Ocean Expeditions (IIOE) (Qasim, 1977) off Arabian Peninsula varied between 500 to >1000 mgC m<sup>-2</sup> d<sup>-1</sup>. However, in the present study area, the values were between 250 to 500 mgC m<sup>-2</sup> d<sup>-1</sup> implying that EAS is less productive. Moreover, the column productivity values having good spatial coverage were also lower in EAS than WAS. These results suggested prevalence of oligotrophy in the EAS.

##### *3.1.1.2 Chlorophyll a (Chl a)*

The Chl *a* concentrations ranged from 0.09 to 0.64 mg m<sup>-3</sup> at OS and 0.09 to 0.26 mg m<sup>-3</sup> at SS in the present study (Fig. 3b). The surface values of both the stations are almost similar and match with those from SeaWiFS data. The integrated Chl *a* values were 26.8 mg m<sup>-2</sup> at OS and 15.2 mg m<sup>-2</sup> at SS. These integrated values are similar to those reported earlier from the study region (Radhakrishna et al., 1978) and are comparable to the values reported from adjacent northern region (45 and 58 mg

$\text{m}^{-2}$ , Dec. 2000, Balachandran et al., 2008; 29 and  $16.4 \text{ mg m}^{-2}$  in Sept. 2003 Jyothibabu et al., 2008) and those from the entire southern offshore region in southeast Arabian Sea ( $0.89$  to  $16.90 \text{ mg m}^{-2}$ , Bhargava et al., 1978). Incidentally, these values are also close to those from WAS during comparable season ( $20 \text{ mg m}^{-2}$ ; Savidge and Gilpin, 1999). This lack of difference in values at WAS and EAS is attributed to the temporary formation of algal blooms in EAS. According to Qasim (1978) the integrated Chl *a* concentrations obtained during International Indian Ocean Expeditions (IIOE) ranged from  $5\text{-}15 \text{ mg m}^{-2}$  up to 50 m depth and  $15\text{-}25 \text{ mg m}^{-2}$  up to 100 m depth in the study area. However, it ranged from  $15\text{-}25 \text{ mg m}^{-2}$  up to 50 m depth and  $25\text{-}35 \text{ mg m}^{-2}$  up to 100 m depth in the WAS. These findings support the prevalence of relative oligotrophy in the EAS as mentioned above based on primary productivity.

### 3.1.1.3 Phaeopigment (Phaeo)

The phaeopigment values in surface levels in the present study are  $\sim 0.2 \text{ mg m}^{-3}$ . Bhargava et al., (1978) reported similar values of phaeophytin  $0.007 - 0.32 \text{ mg m}^{-3}$  in surface waters in the entire offshore of south India including the study area. Like Chl *a*, these values also suggest that there is little variation in phaeopigments all along the EAS.

### 3.1.1.4 Primary productivity, Chlorophyll *a* and Phaeopigments

Relatively higher PP values and highest Chl *a* and Phaeo occur at a depth of 25 m at both stations (Fig. 3a, b and c). The coincidence of sharp peak of fluorescence and presence of thermocline at this depth confirms the presence of subsurface chlorophyll maxima (SCM) (Fig. 2). Occurrence of SCM around this depth, from EAS (Bhargava et al., 1978; Sawant and Madhupratap, 1996; Jyothibabu et al., 2008). and in the Arabian Sea around 35 - 70 m depth is well established (Ducklow, 1993; Jochem et al., 1993; Savidge and Gilpin, 1999; Pomeroy and Joint, 1999; Gardner et al., 1999; Barber et al., 2001). The association of thermocline with SCM and chlorophyll concentrations close to the present values ( $0.3\text{-}0.4 \text{ mg m}^{-3}$ ) has also been reported by Pomeroy and Joint, (1999) in the adjacent region of Central Arabian Sea (CAS). The association of primary productivity peak with the SCM suggests the presence of photosynthetically-active phytoplankton adapted to low light conditions at this depth (Anderson, 1969).

Maximum PP value in this study occurs at a depth of 10 m above the SCM at 25 m. A similar observation has been made by Sawant and Madhupratap (1996) in the EAS. Likewise, Ducklow (1993) also reported a broad, well-defined productivity maximum at 25-50 m, slightly above the chlorophyll maximum in CAS and WAS. The primary productivity values decreased from  $37.7 \text{ mgC}$

$\text{m}^{-3}\text{d}^{-1}$  at 25 m to a negligible value of  $2.2 \text{ mgC m}^{-3}\text{d}^{-1}$  at 50 m, indicating the lower limit of the euphotic zone. Typical CTD data from these two stations also showed the pycnocline around this depth (Fig. 2). This data confirms the presence of the mixed-layer close to this depth ( $\sim 50 \text{ m}$ ) as has been previously recognized by Gardner et al., (1999). The investigations of Jochem et al., (1993) from regions covering areas off Oman and Pakistan and CAS showed that the chlorophyll maximum is due to active phytoplankton biomass. The subsurface increase of primary production in our study proved that this subsurface population was actively growing and was not formed as a result of sedimenting biomass.

The average values of Chl *a*, phaeopigments and primary productivity at OS ( $0.275 \text{ mg m}^{-3}$ ,  $0.412 \text{ mg m}^{-3}$  and  $37.454 \text{ mgC m}^{-3} \text{ d}^{-1}$  respectively) are higher compared to SS ( $0.167 \text{ mg m}^{-3}$ ,  $0.162 \text{ mg m}^{-3}$ ,  $28.83 \text{ mgC m}^{-3} \text{ d}^{-1}$  respectively). The ratio of Chl *a* to phaeophytin is higher at OS suggesting higher degradation. This observation is consistent with the earlier reported results of Radhakrishna et al. (1978) from the study region and the area just north of ours (Balachandran et al., 2008). The column Chl *a* at OS is 76% higher than that of SS. Similarly, high concentrations have been obtained by Radhakrishna et al., (1978) for this region. Higher concentration of phaeopigments at OS ranging between  $0.11$  to  $0.84 \text{ mg m}^{-3}$  and lower concentrations varying from  $0.01$  to  $0.33 \text{ mg m}^{-3}$  at SS (Fig. 3b) indirectly suggests that decomposition of algal chlorophyll is more intense at OS than at SS. During the inter-monsoon period, the organic material at the surface layer is probably considerably reworked by microbes before descending to the euphotic zone (Ramaiah et al., 1996). Thus, higher PP and Chl *a* values at OS than at SS can be attributed to availability of conducive light conditions and abundance of recycled / remineralized nutrients. Though earlier reports (Radhakrishna et al., 1978) showed values one order lower than ours, they also showed that OS was more productive than the slope. A similar observation was made for an offshore station from southern CAS by Sawant and Madhupratap (1996) despite the absence of upwelling signature. This finding is also supported by earlier observation of Savidge and Gilpin (1999) who have shown relatively higher PP in the offshore oligotrophic waters of the NW Indian Ocean. Despite the absence of upwelling, the higher column production may be sustained at the SCM of the offshore oligotrophic station by vertical nutrient transfer especially nitrate as reported by Woodward et al. (1999) for the WAS.

The depth distribution patterns of these three parameters changed little at OS but varied substantially at SS (Fig. 3d, e and f) and showed a sudden increase beyond 25 m depth coinciding with the depth of SCM. Occurrence of phytoplankton and intact diatoms at similar or even at greater depths (Sawant and Madhupratap, 1996) and relatively higher abundance of diatoms in the EAS has also been



reported earlier (Madhupratap et al., 2001). It is likely that diffusive flux of nutrients is efficiently trapped by the SCM (Banse, 1987) below 25 m at SS thereby increasing the number of diatoms and dinoflagellates underneath. Studies on vertical distribution of dinoflagellates on the continental shelf have shown that these organisms are mostly concentrated near the bottom (Tseng et al., 2005; Huang et al., 2008). Possible reasons that could be attributed to such a phenomenon suggest that the dinoflagellates accumulate where nutrient availability is more. Yet another reason could be a weak top down control i.e. predation on these dinoflagellates by organisms of higher trophic level is minimal (Sellner et al., 2001).

#### 3.1.1.5 Phytoplankton diversity

The average density of phytoplankton cells, diatoms and dinoflagellates at SS was higher (13400, 4680, and 8720 cells l<sup>-1</sup> respectively) than that at OS (1551, 1472.5, and 78.5 cells l<sup>-1</sup> respectively) (Fig. 3, Table 1). A total of 20 phytoplankton taxa were identified, of which 16 belonged to the diatom Bacillariophyta group (Fig. 4). At the SS around 18 different taxa were recorded of which 14 belonged to the Bacillariophyta group. The percent composition of dinoflagellates varied between 19-80 % of the phytoplankton abundance with the maximum percentage at 100 m. The dinoflagellates *Peridinium* and *Prorocentrum* dominated in high numbers especially at depths > 50 m. Species diversity ranged from 1.61 to 2.45 at different depths at this SS.

Diatoms pre-dominated the offshore waters and their abundance ranged from 88-100 % of the total phytoplankton at different depths. A total of 12 taxa were encountered of which 9 belonged to the Bacillariophyta group. Bottom dwelling species such as *Navicula*, *Nitzschia* were conspicuously absent. The species diversity was less than the slope waters and ranged between 1.73 and 1.95.

This difference between species compositions at the two stations could be attributed to variations in nutrients. Perhaps the OS could be influenced by autochthonous nutrients replenished by remineralization, whereas SS could be more influenced by allochthonous nutrients of terrestrial / coastal origin. Further, the higher concentrations of phytoplankton at SS could also be due to accumulation of dead fraction of phytoplankton sinking in the water column as a part of rapid pumping of organic matter. This is corroborated by the Chl *a* profiles which clearly show that the offshore values are much higher than the SS. This result occurred despite the higher diatom abundance at the SS. Although, the diatom abundance is higher at the slope than the OS, the diatoms at the OS predominates the phytoplankton counts. Hence, we believe that the higher column productivity in the OS could be attributed not only to the clearer water but also due to predominance

of diatoms. Studies have shown that the diatom species showed lower carbon/Chl *a* ratios and higher photosynthetic rates per unit carbon than the dinoflagellates (Chan, 1980).

### 3.1.2 Bacterial parameters

The total bacterial counts (TBC) and total viable counts (TVC<sub>t</sub>) (Fig. 3g and h) both varied little at SS but increased from surface to enhanced concentrations at 25 m at OS. Earlier, such an increase in TBC was reported just above the thermocline in 20-30 m depth in the central and western Arabian Sea (Ducklow et al., 2001). As mentioned above, SCM is associated with this depth where highest Chl *a* concentrations are also observed. According to Li et al. (2004), a negative relationship exists between Chl *a* and TBC where high chlorophyll regimes prevail. However, in the present investigation, Chl *a* and TBC are positively related ( $p < 0.05$ ). This result may be due to the relatively low chlorophyll concentrations in the study area as compared to those in the WAS (Qasim, 1978). Conversely, these observations suggest that EAS is microbially dominated. In support, our data also shows high abundance of TBC and TVC<sub>t</sub> in the EAS water column, as in previous studies ( $\sim 1 \times 10^9$  cells l<sup>-1</sup> Ramaiah et al., 1996). Although similar values of TBC have been reported in the WAS and CAS ( $0.8 \times 10^9$  cells l<sup>-1</sup> Pomeroy and Joint, 1999;  $1.1 \times 10^{11}$  cells m<sup>-1</sup> Koppelman et al., 2005) those areas are chlorophyll dominated. In addition to high abundance of TBC, high bacterial production (Ramaiah et al., 1996) was observed in the EAS during the intermonsoon, the time coinciding with our sampling period. There was a significant negative relationship between PCD and TVC<sub>t</sub> at both the stations (SS  $p < 0.001$ ,  $n = 6$ ,  $r = -0.965$  and OS  $p < 0.05$ ,  $n = 6$ ,  $r = -0.792$ ), suggesting trophic decoupling of the viable bacteria with phytoplankton cell density (Nagata, 2000; Morán et al., 2002).

## 3.2. Entire water column

### 3.2.1 Dissolved oxygen (DO)

Dissolved oxygen varies similarly at both stations (Fig. 5a). The surface waters are highly oxidic up to 120 m with dissolved oxygen  $\sim 4.5$  ml l<sup>-1</sup> at the surface which decreases to 0.3 ml l<sup>-1</sup> at 120 m depth. Further, at deeper depths, oxygen levels are extremely low ( $< 0.5$  ml l<sup>-1</sup>), sometimes even below detection limits, between 120 to 1200 m and this depth zone has been termed as oxygen minimum zone (OMZ). Beyond 1200 m, the waters again become oxidic with oxygen concentrations increasing beyond 0.5 ml l<sup>-1</sup>. Several workers have reported similar distribution of dissolved oxygen in the waters of the EAS (e.g. Wyrski, 1971; Naqvi, 1987; Bange et al., 2005). Thus, the waters of the study

region clearly show vertical stratification reflecting presence of different biogeochemical provinces related to DO concentrations.

### 3.2.2 Total Organic Carbon (TOC) and Bacterial Production (BP)

On an average, TOC and BP were 71  $\mu\text{M}$  and 763  $\mu\text{g l}^{-1}\text{d}^{-1}$  respectively at OS. Investigations of Hansell and Peltzer, (1998) showed almost similar TOC concentrations off Arabian Peninsula in WAS during comparable period. At SS, TOC values were almost 1.6 times higher than OS (114  $\mu\text{M}$ ) while BP values were higher by an order of magnitude (1635  $\mu\text{g l}^{-1}\text{d}^{-1}$ ). This result suggests that the entire water column at SS is much more dominated by bacterial activity than at OS. Similar increment in BP from offshore to coast was observed in the region off Arabian Peninsula (Ducklow, 1993). At OS, TOC and BP values peaked at 150 m just beneath the upper boundary between oxic-anoxic waters and then steadily decreased with depth. As such, TOC followed a similar pattern of BP with depth at both the stations (Fig. 5b and c). However, it was significantly related to BP only at OS ( $r=0.737$ ,  $p<0.05$ ,  $n=8$ ) suggesting an excess TOC near the SS. Such fuelling of bacterial metabolism by the availability of TOC has also been observed by Ramaiah et al., (2000) in the Arabian Sea. Sharp increases in TOC and BP values just beneath the upper boundary of OMZ in the oxygen deficient waters, particularly at SS, could be attributed to nitrate-reduction (Kirchman, 2000) which may allow bacterial population to flourish. Incidentally, higher TOC and BP values occur at 250 m at SS which is not discernible at OS. This increase in SS could be attributed to the presence of 'deep nitrite maxima' (DNM, 0.5 M  $\text{NO}_2^-$ ) characterized by 'permanent' denitrification (Naqvi, 1991) at this depth. Devol et al., (2006) have defined the DNM as core of the OMZ where severe depletion of oxygen occurs. Perhaps this level is characterized by more bacterial populations taking part in denitrification. This result is supported by earlier observation wherein presence of intermediate nepheloid layer caused by bacterial populations in association with the DNM has been reported (Naqvi et al., 1993; Morrison et al., 1999; Jayakumar et al., 2009).

At both the stations TOC and BP values decreased sharply at 500 m. Below this depth, in the vicinity of the slope, these values increased substantially in tandem with a decrease in oxygen. Hence, even at deeper depths of SS more labile organic material could be available as sinking undecomposed particles. More TOC at SS than OS is indicative of low degradability of organic matter especially under relatively more anaerobic conditions. This inference is drawn despite the similarity in variations of DO with depth at both the stations (Fig. 5) which we attribute to oxygen detection limits of the Winkler protocol. Perhaps, this method may not be able to detect very low concentrations of oxygen especially when these approach values of near zero. Similarly, Koppelman et al., (2005)

also observed sharper increase in bacteria at CAS than WAS in response to the increase in intensity of the OMZ at CAS. This response was despite the close similarity in the trends of DO at both the stations. The poor relationship between TOC and BP at SS ( $r = 0.18$ ,  $p > 0.05$ ,  $n = 7$ ) suggests that TOC present could be more non-limiting than at OS ( $r = 0.77$ ,  $p < 0.05$ ,  $n = 8$ ). Consequently, the degradation of TOC compared to accumulation, at the slope is much slower than offshore. This result could be responsible for higher transport of TOC to the slope in contact with OMZ along western margin of India. This conclusion is similar to that of Paropkari et al., (1991) who proposed that TOC enrichments in mid-slope sediments are due to its better preservation and accumulation under reducing conditions developed by the interception with OMZ.

### 3.2.3 Total Bacterial Counts (TBC) and Total Viable Counts (TVC)

The TBC values at the surface at both the stations are similar (Fig. 5d). Highest TBC values of  $11.5 \times 10^8 \text{ l}^{-1}$  were noted just beneath the oxic-anoxic boundary at 150 m depth at SS. However, at OS, highest TBC values of  $21 \times 10^8 \text{ l}^{-1}$  coincided with 250 m depth. The  $\text{TVC}_t$  values of the surface (Fig. 5e) at OS ( $5.42 \times 10^7 \text{ l}^{-1}$ ) is an order higher than at SS ( $0.82 \times 10^7 \text{ l}^{-1}$ ). These observations suggest the availability of more nutrients in the surface waters or that there is less grazing pressure at OS. The same inference is drawn in the previous section, based on abundance of TOC and BP. From 100 m onwards the  $\text{TVC}_t$  values increase gradually up to 250 m at both the stations. These observations suggest that the increased TBC and  $\text{TVC}_c$  values at 250 m reflect the presence of DNM around this depth. Raghukumar et al. (2001) have also recorded an increased bacterial abundance between 250 and 500 m in the OMZ of the eastern and central Arabian Sea. Further, relatively higher values of TBC and  $\text{TVC}_t$  in the upper layers of OMZ at SS indicate more intense bacterial activity in the vicinity of the slope as compared to offshore, perhaps in response to increasing TOC and intensity in OMZ. Koppelman et al. (2005) also showed a sharp increase in bacterial populations at 250 m depth coinciding with the DNM at CAS and marginal increase at WAS. This difference also further strengthens the view that intensity of denitrification increases towards east simultaneously with the intensity of OMZ.

The variations of  $\text{TVC}_{\text{aero}}$  and  $\text{TVC}_{\text{anaero}}$  (Fig. 5f and g) show coexistence of these groups at both stations exhibiting minimal spatial disconnect between them. Coexistence of anaerobic groups with aerobic groups of bacteria has been also reported off Oman margin in the WAS between 210 m to 4727 m (Marty, 1981). Such coexistence is highly favoured in organically-rich environments even at low oxygen concentrations. In surface waters, population of  $\text{TVC}_{\text{aero}}$  ( $4.71 \times 10^7 \text{ l}^{-1}$ ) at the OS exceeds those at the SS ( $0.68 \times 10^7 \text{ l}^{-1}$ ), implying prevalence of a more oxic environment at OS. With

increase in depth, the  $TVC_{aero}$  values decrease and are more or less similar at both stations within the OMZ. However, beyond 1200 m depth, the counts of  $TVC_{aero}$  at OS increased in response to the oxic conditions.

The values of  $TVC_{anaero}$  also fluctuated in response to dissolved oxygen. The counts were lower in the oxic environment of surface waters and in the deeper layers beyond 1200 m at both stations (Fig. 5g). Its distribution was marked by a peak at 250 m at both the stations. The  $TVC_{anaero}$  population were more abundant at slope than at OS perhaps in response to the intensity of oxygen depletion. Conversely their proliferation could contribute to the intensity of OMZ. This observation augments our inference that the intensity of OMZ increases closer to the slope, particularly along the core of OMZ (Devol et al., 2006) centred around 250 m depth. The ratio of  $TVC_{aero} / TVC_{anaero}$  varied from 0.13 to 13 at OS and from 0.28 to 4.86 at SS, suggesting more reducing conditions in the latter. Based on data of two central and one eastern Arabian Sea stations, Moffet et al. (2007) showed increasing nitrite concentrations eastwards in the area closer to our study region. This result agrees with the observations made in the present study where increasing reducing conditions were observed from open ocean towards the coast.

Two-way ANOVA of the variables in this study showed significant variations between the 2 stations and also depth wise (Table 2). Post hoc test showed significantly high values of  $TVC_{anaero}$ , TOC and BP at the SS between 150 to 250 m water depth.

### 3.2.3 Physiological groups of bacteria in the N cycle

#### 3.2.3.1 Nitrifying bacteria (AOB & NOB)

In surface waters of SS nitrifying bacteria which comprise of ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB) ( $14.4$  and  $35.2 \times 10^1 \text{ l}^{-1}$ ) were 2 and 3 orders higher than that at OS ( $0.1$  and  $0.025 \times 10^1 \text{ l}^{-1}$ ) respectively (Fig. 6b and c). AOB increased to  $57.6 \times 10^1 \text{ cells l}^{-1}$  at SS and to  $91.2 \times 10^1 \text{ l}^{-1}$  at OS in the oxic zone just above the OMZ. The maximum values of NOB were  $35.2 \times 10^1 \text{ l}^{-1}$  at SS and  $8.0 \times 10^1 \text{ l}^{-1}$  at OS at the same depth of  $\sim 100$  m. Similarly, maximum nitrifiers have been reported near the bottom of the photic zone in the vicinity of the primary nitrite maximum in the upper 100 m in the OMZ off Peru (Ward et al., 1989). The simultaneous occurrence in abundance of both these physiological groups of nitrifying bacteria at this depth is because oxidation of ammonia and nitrite is often coupled (Krishnan et al., 2008). Archaea also contribute to the processes of ammonia oxidation both aerobically and anaerobically (Francis et al., 2007). The values of AOB and NOB decrease sharply at  $\sim 250$  m coinciding with DNM. Moreover, in the central

eastern Arabian Sea, nitrite oxidation is a major bacterial activity in the core of DNM (Shailaja, 2001). Despite this, the lowest concentrations of AOB and NOB observed at DNM in the present study are rather intriguing. This could be either due to low culturability of these groups or this could be due to artefact caused by our sampling interval strategy. Similar reasoning could be assigned for low populations of the AOB and NOB in the water depth beyond 250 m at OS. Nevertheless, our studies suggest that DNM may not be a product of a dynamic coupling of denitrification/nitrification but could be due to sluggish physical forcing. Activities in the OMZ perhaps reach the feedback threshold much faster, thus retarding the coupling process. The dynamic processes could be more prevalent near the oxic-anoxic boundary where higher numbers of nitrifiers and denitrifiers are present.

### 3.2.3.2 Nitrate-reducing bacteria (NRB)

NRB were more dominant at SS throughout the water column than at OS (Fig. 6a) implying slightly more intense nitrate reduction in the water column near the slope. However, at OS a peak ( $34.33 \times 10^3 \text{ l}^{-1}$ ) of nitrate-reducing bacteria is observed below the oxic-anoxic boundary  $\sim 150$  m. This peak may be attributed to the presence of more nitrates in the offshore region than the slope. In fact, nitrate is likely to be the dominant nutrient present in this region which fuels enhanced productivity despite absence of upwelling in the intermonsoon period.

### 3.2.4 Physiological groups of bacteria in the S cycle

#### 3.2.4.1 *Thiobacillus denitrificans* like organisms (TDLO)

At both stations the population of TDLO increases from surface to 100 m ( $31.2 \times 10^1 \text{ l}^{-1}$  at slope,  $7.36 \times 10^1 \text{ l}^{-1}$  at OS) (Fig. 6d). Here reduced sulphur species are likely to be brought to the surface during upwelling from OMZ to the oxic waters and the TDLO metabolize them, leading to their oxidation just above the oxic-anoxic boundary. Further, the TDLO concentration decreases and reaches the lowest concentration in the region of DNM. At deeper depths, TDLO is negligible at OS, whereas at SS, highest abundance ( $50.0 \times 10^1 \text{ l}^{-1}$ ) of TDLO, almost twice the surface value, occurs at 500 m. This abundance is perhaps due to its proximity to the adjacent organic rich slope sediments. Colourless sulfur-oxidizing bacteria have been encountered extensively in the Arabian Sea. These oligotrophs have been known to mediate nitrogen cycle reductively even under autotrophic conditions (Loka Bharathi et al., 1994). Naqvi et al., (1993) also observed intermediate nepheloid layer caused by bacteria associated with the DNM. They inferred that most of these bacteria could be denitrifiers. Koppelman et al. (2005) felt that this layer could be due to an increase in nitrifying

bacteria (both ammonium and nitrite oxidizers), which are of great importance in oxygen minimum zones. However, during the present study, this region was dominated more by SRB than by denitrifiers. SRB are also known to participate in nitrate reduction (Mitchell et al., 1986). Nevertheless, attempts have been made to identify the potential bacteria causing denitrification by molecular characterization. Based on such studies, Jayakumar et al. (2009) and Ward et al. (2009) found that denitrifying bacteria dominate the biomass of the OMZ. The denitrifying bacteria in this zone may perhaps be in a viable but non culturable state.

#### 3.2.4.2 Sulphate reducing bacteria (SRB)

SRB are present throughout the water column (Fig. 6e) at both stations. Loka Bharathi and Chandramohan (1990) reported that the SRB occur not only in the bottom sediments but also in aerobic surface waters and beach sediments. SRB populations increased from the surface waters up to the oxic-anoxic boundary, particularly at SS which could possibly be due to exchange of oxidized and reduced sulphur species in and out of OMZ. At SS, SRB also decreased at DNM like the other groups of bacteria such as NOB. Perhaps the concentration of nitrite in these waters is beyond the tolerance levels of the groups examined. Nitrite is known to inhibit the growth of bacteria. The extent of inhibition could be different for different groups of bacteria. For example the median inhibitory concentration (IC<sub>50</sub>) of free nitrous acid (FNA) was 0.10 mg l<sup>-1</sup> (=2.13 μmol) and the IC<sub>10</sub> of FNA was 0.05 mg l<sup>-1</sup> (=1.06 μmol) for *Acinetobacter* species (Weon et al., 2002). The nitrite concentrations encountered in the OMZ could range from 1- 5 μmol l<sup>-1</sup> (Moffet et al., 2007). However, at the OS the prevalence of higher number of SRB, particularly at DNM, suggests that the accumulation of nitrite at OS is less than that at SS.

#### 3.2.5 Fermentors

The fermentors increased slightly with depth in the surface oxic waters at both stations (Fig. 6f). Their abundance was maximum at 150 m depth at OS while at SS their population showed uniform distribution throughout the water column with a peak at 750 m (Fig. 6f). A high concentration of TOC at 750 m depth coincides with a secondary peak of abundance of almost all groups of bacteria especially fermentors. Following aerobic breakdown, it is the fermentors which feed the other anaerobic forms of bacteria like sulfate reducers or nitrate reducers. Incidentally, Paropkari et al., (1991, 1993) have reported maxima of TOC enrichment in the adjacent slope sediments at this depth due to severe reducing conditions. It is likely that such a condition promotes the increase in the bacterial populations at the SS at this depth.

### 3.2.6 Conceptual figure

A conceptual figure is drawn to summarize the finding in this study (Fig. 7).

Though the prevalence of OMZ and its intensification along continental margins is known, very little has been understood about the contribution from microbes which are the driving force behind this phenomenon. The conceptual model presents the variables studied in the water columns, especially the OMZ. The figure shows higher TOC at SS. Though the primary producers are higher at the SS, PP is higher at OS. This system is more dominated by bacteria especially the anaerobic forms in the slope region. Their abundance and activity is intensified by sluggish physical forcing. However, if efficiently managed there could be means for either using them to trap CO<sub>2</sub> or renewing and rejuvenating these sluggish water masses for improving local fisheries.

## 4. Summary, conclusions and future scope

The vertical stratification in the study region exerts an influence on the distribution of biological parameters in the water column. The distributions of various hydrographic and phytoplankton related parameters, have helped delineate that SCM is at 25 m, euphotic zone and MLD (mixed layer depth) at ~50 m and the DNM at 250 m.

The positive relationship between TBC and Chl *a* suggests that the study region is more bacterially dominated. Based on abundance of bacterial populations, especially the TVC<sub>aero</sub> and TVC<sub>anaero</sub>, the formation and intensification of OMZ is attributed to the higher use of oxygen by bacteria than its replenishment by either phytoplankton or physical forcings. Significantly greater abundance of TOC, BP and TVC<sub>anaero</sub> in the 'deep nitrite maxima (DNM)' especially at SS, suggests more intense OMZ at this region. Statistically significant higher values of TVC<sub>anaero</sub>, TOC and BP were noted at the SS between 150 to 250 m water depth. The NRB, AOB, NOB, TDLO, SRB, FERM all exhibit invariably higher populations in OMZ except at the upper oxic-anoxic boundary at the SS. The above findings suggest that these bacterial groups influence the OMZ which become much more intense and thicker in the EAS than WAS. Alternatively, this association of bacterial groups with OMZ reflects the biogeochemical niche where these can thrive.

A comparison between two stations in the present study shows that TOC is three-fold higher at the SS than at OS implying its low degradability in slope waters. Consequently, settling of this well preserved organic carbon in mid-slope sediments adjoining the OMZ along the western margin of India, results in one of the highest sedimentary accumulation of TOC in the world oceans. This



inference supports the earlier conclusion by Paropkari et al. (1991), where it was proposed that the TOC enrichments in mid-slope are due to its better preservation under reducing conditions. For the first time we were able to delineate the bacterial response and its role in intensification of the OMZ. Understanding the system would help us to manage these systems better for example in carbon sequestration and climate modulation. Further studies comparing WAS with EAS holistically at molecular, cellular and community levels including viruses and protozoans will further enlighten the microbial role in the formation and intensification of OMZ.

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## Tables

Table 1 Diversity indices, species richness and evenness of the slope and offshore station.

Station No.	total genera richness (d)	total individuals index (J')	Margalef species index H'(loge)	Pielou's evenness	Shannon-Wiener diversity	(S)	(N)
C-1	13	1710	1.61	0.96	2.45		
C-10	11	4328	1.19	0.67	1.61		
C-25	11	1223	1.41	0.95	2.29		
C-50	17	23799	1.59	0.58	1.65		
C-75	15	27720	1.37	0.67	1.87		
C-100	14	21648	1.30	0.64	1.69		
s7-1	7	1083	0.86	0.89	1.73		
s7-10	6	307	0.87	0.98	1.75		
s7-25	6	749	0.76	0.94	1.68		
s7-50	8	3224	0.87	0.85	1.76		
s7-75	8	1166	0.99	0.85	1.77		
s7-100	8	2777	0.88	0.94	1.95		

Table 2 Summary of two-way analyses of variance of the effect of station location and water depth on the studied variables in the slope station and offshore station.

Variables	Source of variation	df	F	p value
TOC	Station (S)	1	168.40	***
	Depth (D)	6	59.75	***
	S x D	6	15.38	***
BP	S	1	3016.05	**
	D	6	1216.15	**
	S x D	6	1421.57	**
SRB	S	1	37.91	***
	D	6	27.76	***
	S x D	6	14.70	***
TDLO	S	1	15027	**
	D	6	2028.81	**
	S x D	6	1725.26	**
NRB	S	1	6.81	*
	D	6	10.82	***
	S x D	6	2.01	ns
AOB	S	1	1040.51	**
	D	6	13278.20	**
	S x D	6	1687.82	**
NOB	S	1	6649.96	**
	D	6	1346.48	**
	S x D	6	718.23	**
FERM	S	1	28.08	***
	D	6	271.03	***
	S x D	6	199.26	***
TC	S	1	1.31	ns
	D	6	10.29	***
	S x D	6	13.21	***
TVC <sub>t</sub>	S	1	0.0285	ns
	D	6	39.03	***
	S x D	6	46.99	***
TVC <sub>aer</sub>	S	1	80.77	***
	D	6	96.89	***
	S x D	6	87.75	***
TVC <sub>anaer</sub>	S	1	31.33	***
	D	6	16.38	***
	S x D	6	7.34	***

Significance levels: \*p<0.05, \*\*p<0.001, \*\*\*p<0.001, ns: p>0.05



## Figure Captions

Fig. 1. Sampling locations in the eastern Arabian Sea off the west coast of India.

Fig. 2. Representative CTD profile showing variation of fluorescence, salinity and temperature at the slope station (SS) and offshore station (OS).

Fig. 3. Depth profiles of primary production (PP), chlorophyll *a* (Chl *a*), phaeophytin (Phae), phytoplankton cell density (PCD), diatoms, dinoflagellates, total bacterial counts (TBC) and total viable counts (TVC) in the euphotic water columns (100 m depth). Solid line represents SS and dashed line represents OS.

Fig 4. Depth wise distribution of the different phytoplankton genera along the SS (solid line) and the OS (dashed line).

Fig. 5. Depth profiles of dissolved oxygen (DO), total organic carbon (TOC), bacterial productivity (BP), total bacterial counts (TBC), total viable counts (TVC<sub>t</sub>), total viable aerobic counts (TVC<sub>aero</sub>) and total viable anaerobic counts (TVC<sub>anaero</sub>) of bacteria in the entire water columns. Solid line represents SS and dashed line represents OS.

Fig. 6. Depth profiles of culturable bacterial groups like nitrate reducing bacteria (NRB), ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), *Thiobacillus denitrificans* like organisms (TDLO), sulphate reducing bacteria (SRB) and fermentors (FERM) in the entire water columns. Solid line represents SS and dashed line represents OS.

Fig. 7. Conceptual figure depicting the variation in the parameters studied. The bar diagrams are drawn to scale of the ratios of average of parameters. For offshore station (OS) the ratios have been calculated as OS/(OS+SS) and for slope station (SS) as SS/(OS+SS). OMZ - oxygen minimum zone, DNM - deep nitrite maximum, DO- dissolved oxygen, TOC - total organic carbon, PCD - phytoplankton cell density, Chl *a* - chlorophyll *a*, Phaeo- phaeophytin, PP - primary productivity, BP- bacterial productivity, TBC- total bacterial counts, TVC<sub>t</sub> - total viable counts, TVC<sub>anaero</sub> - total viable anaerobic counts, TVC<sub>aero</sub> - total viable aerobic counts, AOB - ammonia oxidizing bacteria, FERM - fermentors, NOB - nitrite oxidizing bacteria, NRB - nitrate reducing bacteria, TDLO - *Thiobacillus denitrificans* like organisms, SRB - sulphate reducing bacteria.

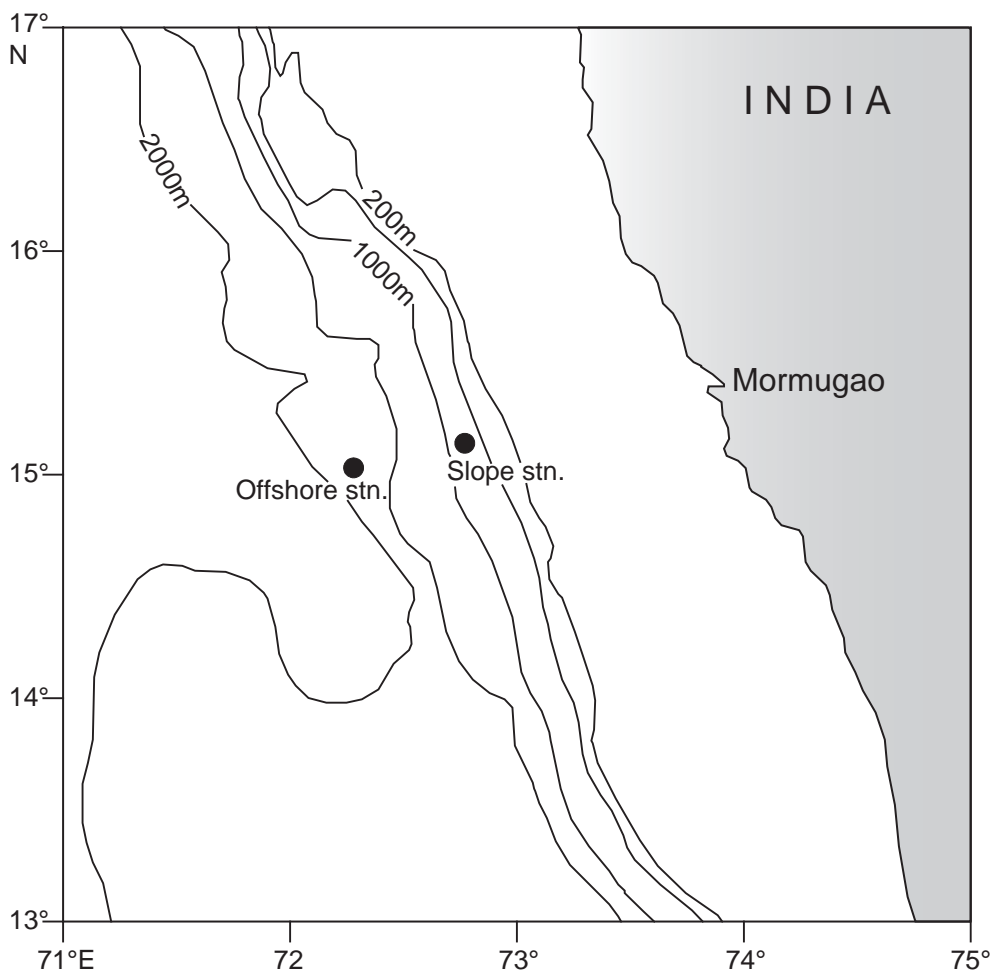


Fig.1

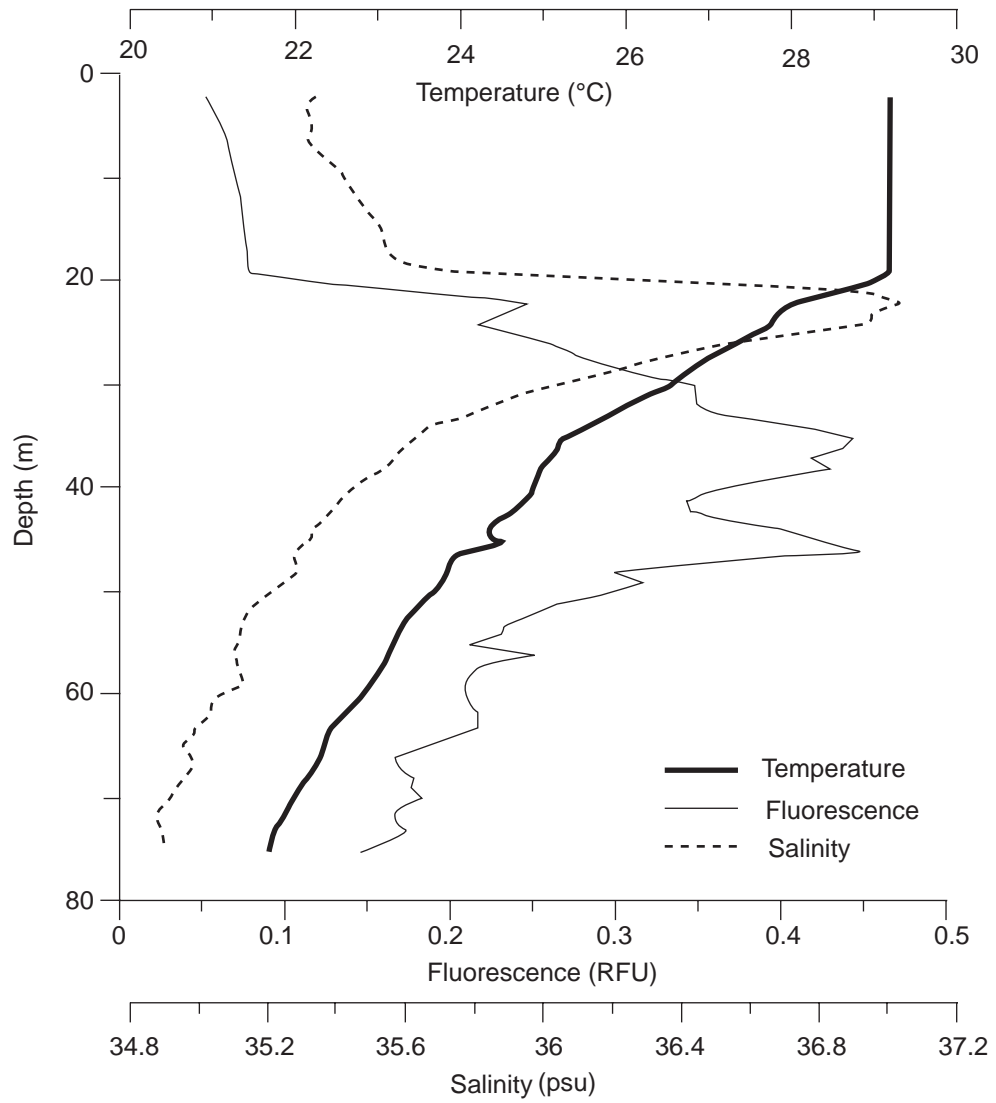


Fig. 2

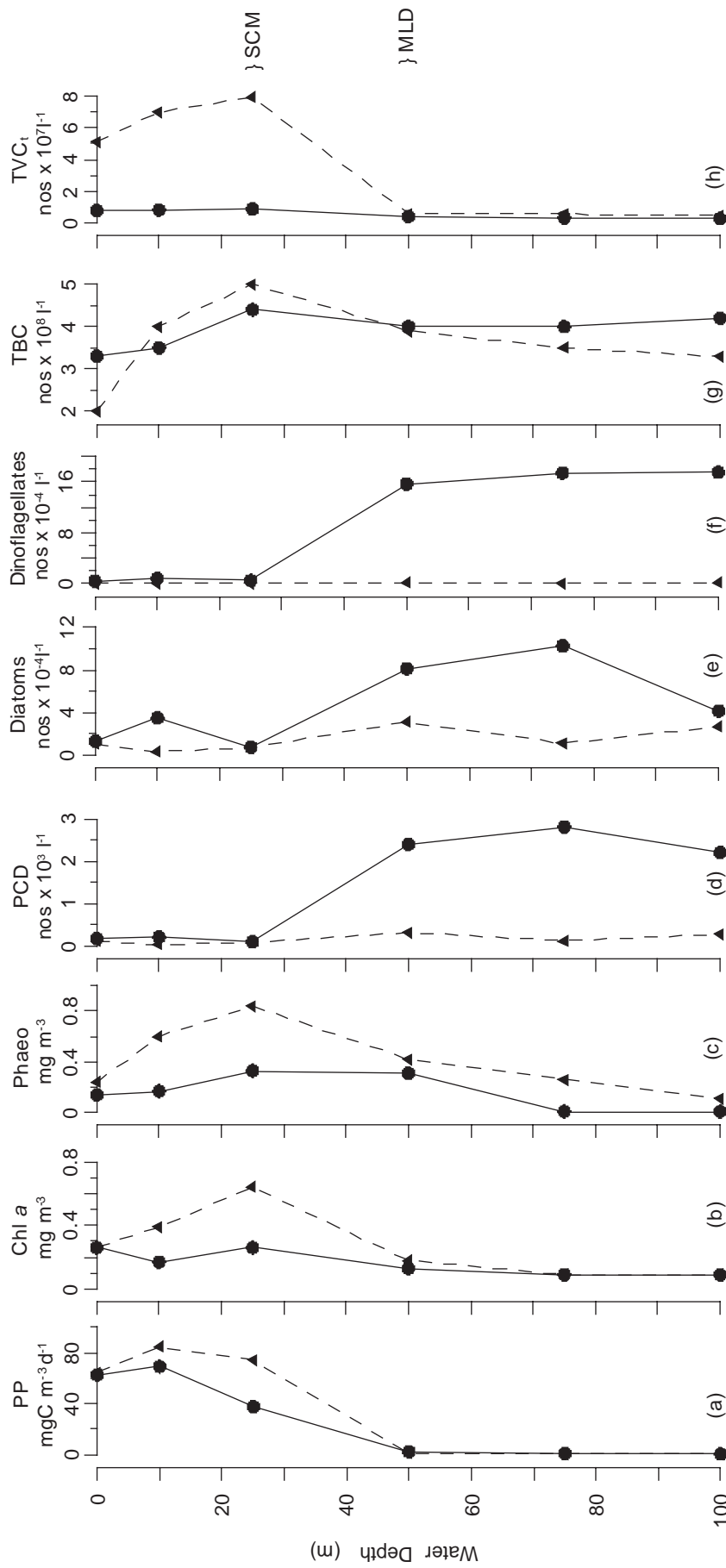


FIG. 3.

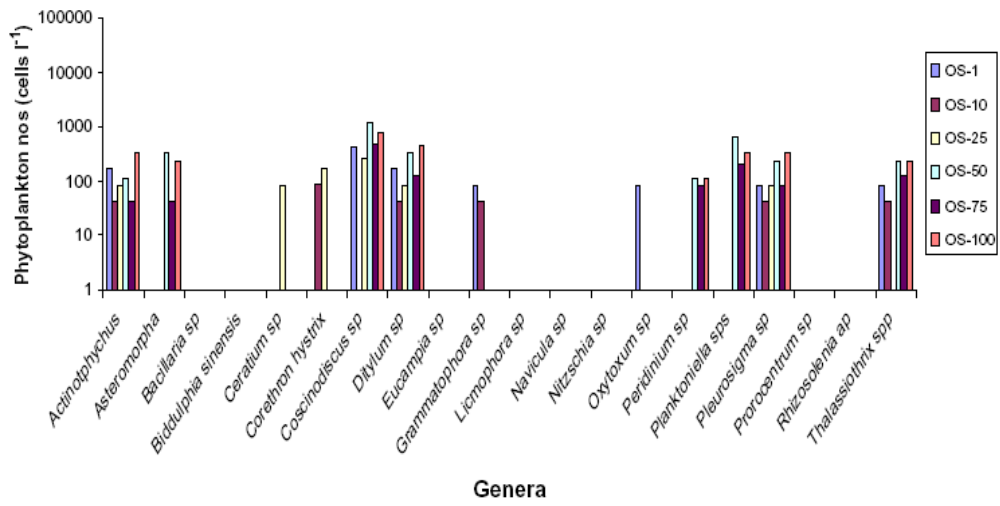
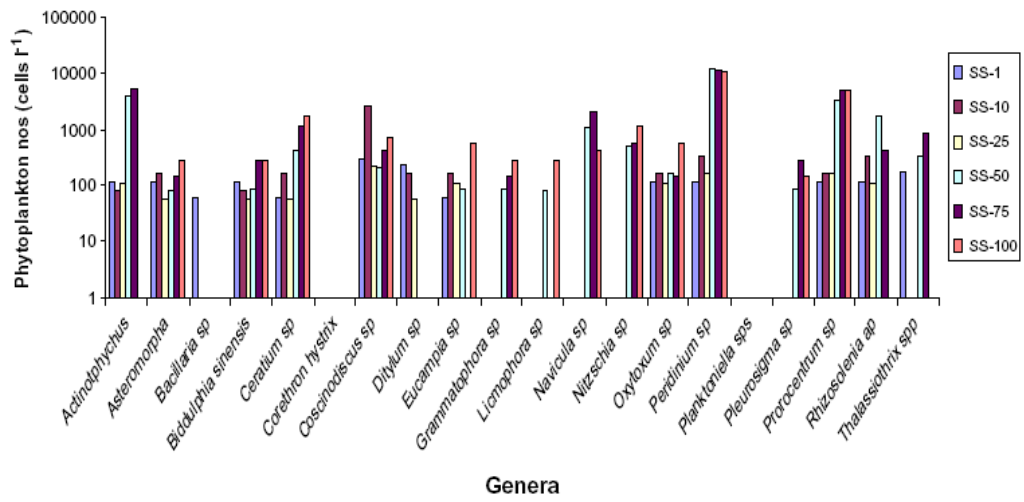


Fig 4

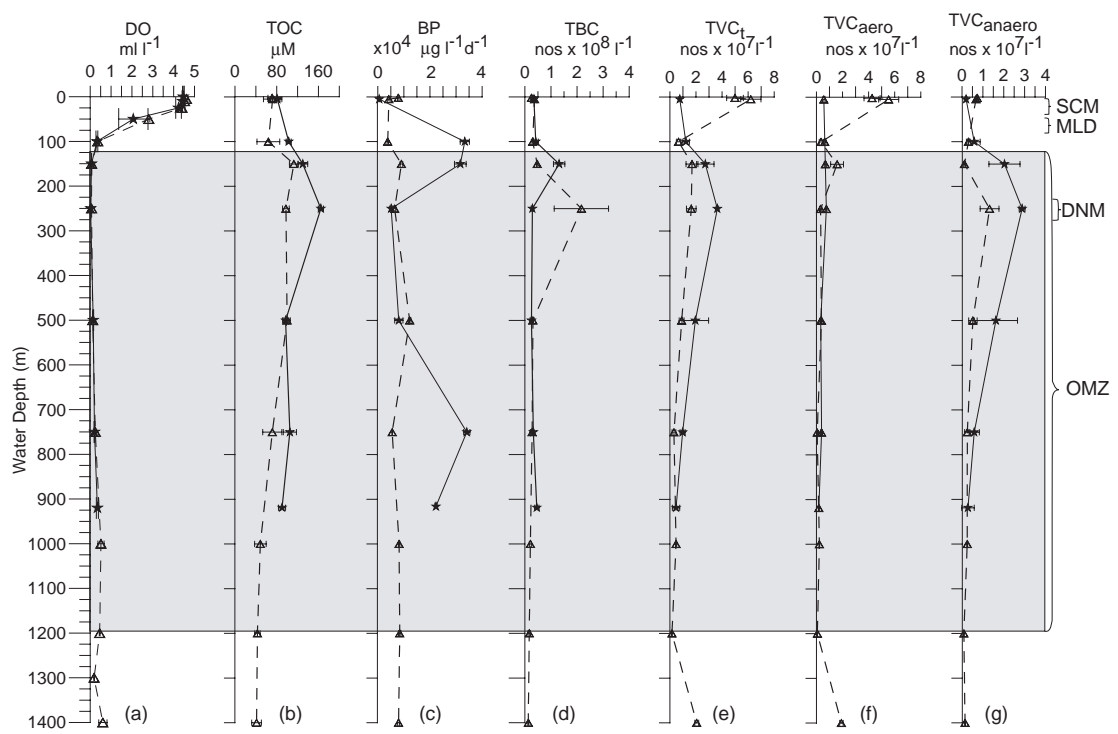
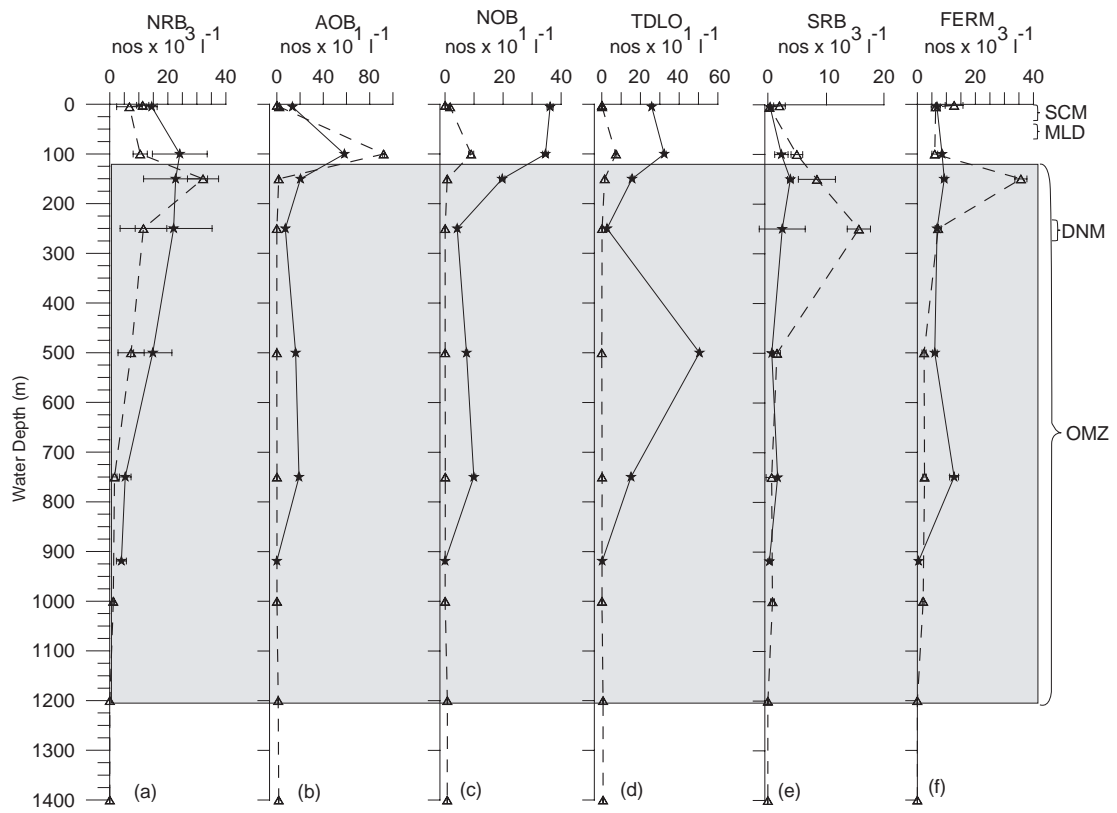
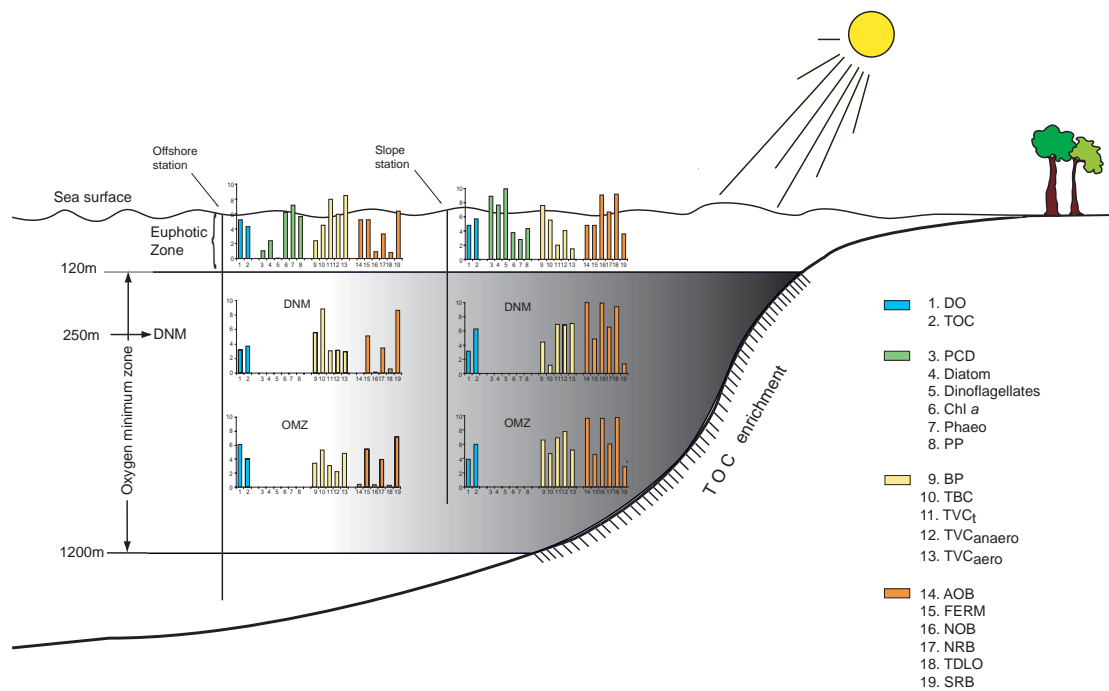


Fig.5



**Fig. 6**



**Fig. 7**