

Anoxia over the western continental shelf of India: Bacterial indications of intrinsic nitrification feeding denitrification.

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Running title: *Coastal anoxia - Intrinsic nitrification feeds denitrification*

Abstract:

Studies on the Arabian Sea coastal anoxia have been of immense interest, but despite its ecological significance there is sparse understanding of the microbes involved. Hence, observations were carried out off Goa ($15^{\circ}30^1\text{N}$, $72^{\circ}40^1\text{E}$ to $15^{\circ}30^1\text{N}$, $72^{\circ}59^1\text{E}$) to understand the processes that mediate the changes in various inorganic nitrogen species in the water column during anoxia. Water column chemistry showed a clear distinct oxic environment in the month of April and anoxic condition in October. Our study based on microbial signatures indicated that oxygen deficit appeared as a well-defined nucleus almost 40 km away from the coast during the oxic period (April) and spreads thereafter to the entire water column synchronizing with the water chemistry. Striking results of net changes in inorganic nitrogen species in nitrification blocked and unblocked experimental systems show that denitrification is the predominant process in the water column consuming available nitrate ($\sim 0.5 \mu\text{M}$) to near zero levels within ~ 72 hours of incubation. These observations have been supported by concomitant increase in nitrite concentration ($\sim 4 \mu\text{M}$). Similar studies on denitrification-blocked incubations, demonstrate the potential of nitrification to feed denitrification. Nitrification could contribute almost $4.5 \mu\text{M}$ to the total nitrate pool. It was found that the relation between ammonium and total dissolved inorganic nitrogen (DIN) pool ($r = 0.98$, $p < 0.001$, $n = 122$) was significant compared to the latter with nitrite and nitrate. The occurrence of high ammonium under low phosphate conditions corroborates our observations that ammonium does not appear to be locked under low oxygen regimes. It is suggested that ammonium actively produced by detrital breakdown (ammonification) is efficiently consumed through nitrification process. The three processes in concert viz. ammonification, nitrification and denitrification appear to operate in more temporal and spatial proximity than hitherto appreciated in these systems and this gives additional cues on the absence of measurable nitrate at surface waters, which was earlier attributed only to efficient algal uptake. Hence we hypothesize that the alarming nitrous oxide input into the atmosphere could be due to high productivity driven tighter nitrification-denitrification coupling, rather than denitrification driven by extraneous nitrate.

Key words: Denitrifiers, Nitrifiers, Coastal anoxia, Arabian Sea

INTRODUCTION

Progressive and cyclic developing of oxygen deficient waters over the continental shelf of the western India has received much attention (Naqvi et al. 2000). West India Coastal Current (WICC) flows equatorward along the west coast of India during June-September in response to alongshore wind stress and consequent upwelling can be seen along the coast (Shetye et al. 1990). The biological activity over the continental shelf is enhanced during the summer due to intense coastal upwelling and Ekman pumping (Brock et al. 1991). The upwelled water over the shelf covers an area of 180,000 km² (Naqvi et al. 2000) and is extremely oxygen depleted (Banse 1968). Naqvi et al. (2000) reported that this coastal water body could be one of the largest low-oxygen zones, which begins its annual development in June, reaching peak intensity by September-October and dissipating by December. Further, they measured the highest accumulations of hydrogen sulphide (H₂S) and nitrous oxide (N₂O) in open coastal waters and attributed to the addition of anthropogenic nitrate and its subsequent denitrification, which is favored by hypoxic conditions. It is now well understood that coastal anoxia is more widespread geographically with periodic cyclicality. Hence, the cues that trigger such processes could be linked to large scale oceanic processes than hitherto understood. A 5-10 m thick cap of warm, relatively less saline waters over the upwelled water clearly indicate the magnitude of terrestrial runoff accompanied by precipitation (Banse 1959). However, despite its origin from land runoff with sufficient nutrient load, low nitrate values observed in this warm-water cap is attributed to efficient algal uptake (Naqvi et. al 2000). If this were to happen, then the denitrification process would be constrained by the availability of nitrate. The observed high N₂O accumulation (Naqvi et al. 2000) is indicative of availability of nitrate despite algal uptake. Consequently, there could be a more significant pathway dynamically linked to denitrification contributing significantly to NO₃⁻ and N₂O flux. The low O₂ conditions are also known to enhance the yield of N₂O during nitrification, and so the possibility of contribution coming through this pathway cannot be excluded (Goreau et al. 1980). Shailaja (2001) has shown that both denitrification and nitrification occur in several closely spaced layers in the Arabian Sea and the

contribution of nitrification to nitrite enrichment in the perennial oxygen minimum zone is significant. Despite the growing interest in mechanisms involved in the development and regression of coastal anoxia and emission of potential green house gases into the atmosphere, the role of microbial processes in driving this ecosystem has received little attention.

Ammonia-oxidizing nitrifiers and sulphide-oxidizing denitrifiers (Example: *Thiobacillus denitrificans* like organisms; TDLO) are physiologically diverse bacterial groups, which respond in tandem to environmental chemistry. Recording the spatial and temporal distribution of these groups and their synchronization with the water column chemistry gives us a better understanding of the dominant microbial processes and their potential contribution to gaseous inputs to the atmosphere. Hence we hypothesize, that ammonification fed nitrification under hypoxic conditions could be more important than previously thought of in these systems. The alarming nitrous oxide input into the atmosphere could be due to a tighter nitrification-denitrification coupling, rather than denitrification driven by extraneous nitrate alone.

MATERIALS AND METHODS

Study area and sampling:

A cross shelf transect off Goa (15°30'N, 72°40'E to 15°30'N, 72°59'E) was selected to study the spatial and temporal variation of nitrifiers and denitrifiers with respect to the water chemistry for two contrasting periods viz. April (oxic) and October (anoxic) during the cruises SaSu-40 (April, 2003), 49 (October, 2003) and 103A (October, 2005) onboard C.R.V. Sagar-Sukti. Though the cruises conducted in April and October 2003 are the best representative of the contrasting conditions, analysis of additional data from other cruises almost corresponding to the period were undertaken [SaSu-26 (September 2002), 29 (December, 2002)]. A similar transect off Mangalore (13°00'N, 74°38'E to 13°00'N, 74°03'E) during cruises SaSu 26, 30 (December, 2002), and 49 have also been

included in this analysis. Such extended analyses were undertaken to get a better perspective of the accumulation/oxidation of dissolved ammonium and inorganic phosphate in the water column.

Portable CTD (conductivity, temperature, depth) was used to obtain temperature and salinity profiles at sampling locations. Seawater was collected using Niskin water samplers attached to the winch wire and triggered with a messenger. Seawater sub-samples for microbiological analyses were collected into sterile containers immediately after retrieval of the bottle. Sub-samples for nitrate and phosphate measurements were frozen for onshore analysis, whereas ammonium and nitrite were measured onboard.

Water column chemistry:

Dissolved oxygen (DO) and nutrients (ammonium, nitrite, nitrate and phosphate) in the seawater samples were measured by colorimetric and titrimetric methods respectively (Grasshoff 1983).

Abundance of nitrifiers and sulphide oxidizing denitrifiers:

Nitrifiers and Sulphide-oxidizing denitrifiers (*Thiobacillus denitrificans* like organisms, TDLO) were enumerated as Colony Forming Units (CFU) on different selective media. Nitrifiers were enumerated by spread plating onto solidified Winogradsky's medium (Rodina 1972). After inoculation, the plates were incubated for 30-45 days at $15 \pm 2^\circ\text{C}$. The TDLO were enumerated by an agar shake method on modified Leiske's medium (Loka Bharathi 1989; Loka Bharathi et al. 1988). The culture tubes were incubated for ~30 days at $28 \pm 2^\circ\text{C}$.

Nitrification and denitrification studies:

Seawater for incubation experiments were collected at definite intervals (surface, 8, 13 and 18 m) from a 25m deep station off Goa on R.V. Sagar Sukti #103A in the month of October (2005). Sub-

samples in triplicates were then transferred into sterile, plugged, 250 ml, glass conical flasks, which were incubated in the dark onboard under ambient temperature. The temperature in the incubation flasks varied between 26-29°C. Three sets of incubation experiments were carried out viz., – (1) Nitrification was inhibited by adding Allyl thio urea to a final concentration of 86 µM (Ginestet et al. 1998), (2) Denitrification was inhibited by adding Chloramphenicol to a final concentration of 300 mg L⁻¹ (Brooks et al. 1992), and (3) Sub-samples of third set were monitored without adding any inhibitors (controls). Aliquots were analyzed at 24 hrs intervals up to 144 hrs (6 days) for nitrate, nitrite and ammonium.

Contours, plots and statistical analysis:

Bacterial abundance contour map was generated using Surfer 8.0 (Golden Software, Golden, CO). Gridding used a kriging algorithm with a linear variogram and no anisotropy, as employed in standard topographic applications. Scatter plots and line graphs were constructed using Grapher 4.0 (Golden Software, Golden, CO, USA). Statistical analyses were done using Analysis Tool Pack in Microsoft Excel.

RESULTS

Surface temperatures roughly followed the solar heating pattern with primary and secondary heating during the March-April and September-October. The salinity profile for October showed the influence of riverine input as evidenced by the development of a less saline, warm water cap. Variations in temperature and salinity during October (Figs.1a and 1b respectively) showed an upwelling signature, possibly during the waning phase of the WICC. The WICC flows northward during January-April, which results in downwelling along the coast. The temperature/salinity profiles for April (Figs.-2a and 2b respectively) also showed downwelling signatures, though weak. The SST was cooler during October than in April, possibly due to the upwelling and the imminent winter. However, the signatures were weak during the transition periods i.e. April and October.

Changes in water column chemistry (Figs.1 c-e and 2 c-e):

During October, the shelf was characterized by low-oxygenated bottom waters ($<0.5 \text{ mL}^{-1}$) of ~40m overlying the shelf. Nitrate values increased gradually to reach $>4 \mu\text{M}$ at around 90m depth. It was observed that the thickness of nitrate-poor surface waters had decreased considerably. It was also interesting to note that nitrite and nitrate values in the month of oxygen deficit (October) showed a similar distribution pattern as that of the oxic month (April) but with enhanced concentrations. Patchiness in nitrite distribution seen during April was lost but replaced by a core of elevated nitrite at around 50 km from the coast (Fig.1e). In April, the concentration of nitrate and nitrite was very low in the top 20 m of the oxic water and it gradually increased to $>1.4\mu\text{M}$ at around 90m water depth. The distribution of nitrite was patchy and elevated concentrations were noticed at around 40 km from the coast (Fig.2e).

Bacterial abundance (Table 1):

During April, the abundance of nitrifiers were in the order of $10^{7-8} \text{ CFU L}^{-1}$ and patchy. The TDLO numbers showed that there is a core of elevated abundance almost 40 km away from the coast. By October nitrifier counts fell sharply to even reach non-detectable levels. In October, the distribution of TDLO was uniform and generally increased away from the coast. There was not much difference in the abundance of TDLO ($10^{4-5} \text{ CFU L}^{-1}$) in both the contrasting periods.

Nitrification and denitrification:

In October (2005), the temperature and salinity (Fig.3a) ranged from 26.3-28.6°C and 35-35.5 psu respectively throughout the water column. The levels of nitrite, nitrate and ammonium which served to define the initial conditions during the experimental incubations is illustrated in Fig.3b. The concentration of nitrite increased with depth while nitrate remained almost constant. Ammonium

values showed two peaks viz subsurface and bottom indicating that there is considerable amount of spatial heterogeneity in ammonium levels. The results of the incubation experiments are presented in Figs. 4-6.

Denitrification blocked system (Fig 4 a-e):

At the surface (Fig.4a) the levels of nitrite did not vary much over an incubation period of 144 hours when denitrification was inhibited by chloramphenicol, but nitrate increased to almost 2 μM in 96 hours and plateaued at the end of incubation (144 hours). The ammonium levels increased at a slow rate over the incubation period to reach final values of around 5.5 μM . At 3m (Fig.4b), the values of ammonium fluctuated with a decrease followed by an increase with very little variation in nitrite but with constant increase in nitrate, which reached 2.6 μM in 144 hours. At 8m (Fig.4c), the variation in nitrite values fairly resembled the surface and 3m incubations except for a sharp decrease in the first 24 hours. At 13m (Fig.4d), nitrite and nitrate values evolved as in the 8m water depth though absolute values differed. The irregularities observed in the rise and fall of ammonium values were more pronounced at this depth. At bottom i.e., 18 m (Fig. 4e), it could be observed that both nitrite and ammonium values vary in phase at higher frequency, while nitrate values continuously increase over time, which was to all the sampled depths.

Nitrification blocked system (Fig. 5a-e):

At the entire sample depths nitrate fell below the levels of detection within 48-72 h. At the surface (Fig.5a), when nitrification was inhibited by the addition of allyl thiourea, the levels of nitrite initially increased and followed an elliptical curve to reach values below the limits of detection. The nitrate values decreased to non-detectable levels within 48 hours of incubation due to nitrate reduction/denitrification, while change in concentrations of ammonium was sigmoidal though less pronounced. The trend in the variation of all the three nitrogen species at 3m (Fig.3b) water depth

were similar to that observed at the surface waters. The concentrations in nitrite and ammonium varied inversely. Nitrite level increased at the beginning of incubation and then fell to below detection. The peak of nitrite is broader at 8m depth (Fig.5c) and decreased with increasing depth. Changes in ammonium at 8m resembled that of the surface; while at 13 (Fig.5d) and 18m (Fig.5e) it increased rapidly after an initial stationary phase and then flattened out. At 13m the peak of nitrite accumulation was observed at 48 hours while at 18m it was observed at 24 hours. It could also be observed that the fall in level of nitrite at 13m is more pronounced between 96-120 hours where as the depletion of nitrite was more gentle at 18m.

Monitoring the changes in 'N' species in the controls (Fig. 6a-e):

At the surface (Fig.6a) and 3m (Fig.6b), when neither of the inhibitors were added, the levels of nitrite initially increased and then followed an elliptical curve to reach values below the limits of detection, while ammonium levels increased at a very slow rate. At all the depths nitrate values fell to non-detectable levels within 72 – 120 hrs. The pattern of changes in ammonium and nitrite at 8m (Fig.6c), 13m (Fig.6d) and 18m (Fig.6e) were very similar to that of the experiment where nitrification was inhibited by allyl thiourea (Figs 5c-e).

DISCUSSION

The presence of a 5-10 m thick cap of warm, less saline water over the upwelled water clearly indicates the magnitude of terrestrial runoff accompanied by precipitation (Banse 1959). Low nitrate values observed in this warm water cap were solely attributed to efficient algal uptake (Naqvi et al. 2000). A more significant pathway dynamically linked to denitrification could contribute significantly to NO_3^- and N_2O flux and mediate N_2O production. It was observed that the relationship between ammonium and total dissolved inorganic nitrogen (DIN) pool ($r = 0.98$, $p < 0.001$, $n = 122$; data obtained from various cruises as described in legend to Fig.7) was stronger than the relationship between DIN to nitrite and nitrate. It is therefore inferred that ammonification is a predominant

process contributing to DIN. The oxygen-poor waters are conducive for reduction of iron and the subsequent phosphate release from ferric phosphate (Caraco et al. 1989; 1991). Enhanced dissolved inorganic phosphate in the water column is used as an indicator for the degree of anoxia in the region. The reduced compounds such as NH_3 , H_2S , and CH_4 tend to accumulate under anaerobic conditions. Hence low oxygen regimes result in the accumulation of phosphate and ammonia. However, our study showed that under low oxygen regimes with high phosphate concentrations, though ammonium is a significant fraction of DIN, accumulation of ammonium was low. This strengthens our observation that ammonium does not appear to be locked always under low oxygen regimes. Besides, the higher diffusion coefficient of ammonium as compared to other reduced compounds like sulphide (Li & Sandra, 1974) facilitates its mobility to oxic shallower layers. This could probably improve the spatial coupling between the substrate (ammonium) and the utilizer (nitrifier), which could get decoupled due to the development of acute anoxia. Unlimited substrate availability by degradation of organic matter could also eliminate the dependence of culturable bacterial abundance on the distribution of ammonia.

Studies generally reveal that 99% or more of the bacterial diversity remains uncultured and unexplored. However, estimates on data compiled for culturability indicate that in seawater and unpolluted estuarine water, percentage culturability can reach up to 0.1 and 3% of direct microscopic counts respectively (Fuhrman & Campbell, 1998). Table 1 presents contrasting distribution of TDLO and nitrifiers. While nitrifiers are mostly detectable only during the oxic period (April), TDLO could be retrieved during both the seasons (April and October) as TDLO is more metabolically versatile to respire either oxygen or nitrate. During April (2003), the culturable fraction of nitrifier population (10^7 CFU/ L^{-1}) decreased towards the open sea. It was also observed that the nitrifier counts vary with water depth at around 40 km from the coast. A zone of high nitrite, high TDLO and low nitrifiers was observed in the same area. Increase of nitrate concentration below 20m depth indicates that nitrification is more pronounced in the deeper depths than in the surface. These observations provide a glimpse of the distribution pattern of bacteria well before the onset of chemical signatures

of anoxia. In the post monsoon season (October, 2003) the nitrifier population declined to non detectable levels from $\sim 10^8$ CFU L⁻¹. During this period, very low nitrifier counts in conjunction with a more heterogeneous and regular abundance pattern of TDLO showed that the water column is at the peak of anaerobic conditions. The depth of nitrate-poor surface layers has decreased considerably but showed inputs of nitrate by nitrification from subsurface levels. Though the nitrifier counts have dropped by almost two orders, the elevated nitrate observed could be attributed to higher specific activity of nitrifying bacteria or suboxic nitrification, perhaps at the expense of iron and manganese. The nitrite and nitrate values in the anoxic period showed a similar distribution pattern as that of the oxic period, but the enhanced magnitude due to net nitrate production being more than nitrate reduction suggests that the nucleus of microbial activity spreads during the period of anoxia to cover the entire water column, changing the chemistry. Hence it is proposed that the microbial indicators of oxygen deficit appear as a well-defined nucleus almost 40 km from the coast even during the oxic period (April).

Net changes of inorganic nitrogen species in nitrification blocked and unblocked experimental systems during the period of anoxia (October) show that denitrification is the predominant process in the water column consuming available nitrate (~ 0.5 μ M) to near zero levels within 48 – 96 hours of incubation. Similar experiments conducted by Naqvi et al. (2000) showed that majority of the available nitrate (21.8 μ M) is converted to nitrite in the first 48 hours of incubation. Initial levels of nitrite varied with depth and could range between 0.44-3.87 μ M. The levels of nitrite encountered in the incubation experiments were comparatively higher (~ 7 -65 times) than that reported by Naqvi et al. (2000). When denitrification was blocked, the absence of a well-defined nitrite peak coupled to a steady increase in nitrate clearly indicates the efficiency with which ammonium is oxidized to nitrate. It was found that nitrification could contribute almost 4.5 μ M to the total nitrate pool, suggesting that ammonium is actively produced by detrital breakdown, but efficiently consumed through the nitrification process. Moreover, the highest rate of ammonia

oxidation (0-144 hours) was found to be at the surface ($2.34 \text{ ng at } \text{NH}_4^+\text{-N l}^{-1} \text{ h}^{-1}$) and the lowest rate of $0.72 \text{ ng at } \text{NH}_4^+\text{-N l}^{-1} \text{ h}^{-1}$ was observed at 13 m depth. But contrary to the observed oxidation maxima, nitrite and nitrate production maxima of 5.85 and $2.34 \text{ ng at } \text{NO}_3^-\text{-N l}^{-1} \text{ h}^{-1}$ were observed at 18m depth. Since the incubation systems were isolated and denitrification was blocked it could be assumed that a major fraction of nitrate was produced by the oxidation of ammonium with an intermediate production of nitrite. The production of nitrate occurred at a much higher rate than ammonium oxidation. This highlights the predominance of ammonification and its contribution to denitrification via nitrification. In addition, the highest rate of nitrate reduction in nitrification blocked systems (0-144 hours) was found to be at 13 m depth ($0.05 \text{ ng at } \text{NO}_3^-\text{-N l}^{-1} \text{ h}^{-1}$) and the lowest rate of $0.03 \text{ ng at } \text{NO}_3^-\text{-N l}^{-1} \text{ h}^{-1}$ at the surface. High ammonium oxidation and low nitrate reduction in surface waters suggest that the physiologically forced synthetic pathway as electron sink is much more dominant in these systems than hitherto accepted. Oxidation of NH_4^+ to NO_2^- in the euphotic zone has been observed in many regions (McCarthy et al. 1984; Ward et al. 1989; Dore & Karl, 1996). Studies by Sambrotto (2001) have also shown that ammonium regeneration rates and concentrations are higher along the coastal waters of Oman during the southwest monsoon season. The unaccounted (~20%) portion of total ammonium sink in the study was attributed to nitrification. Moreover, Mantoura et al. (1993) have attributed elevated N_2O production to nitrification from the Omani coastal region. Hence, the sequential processes viz. ammonification, nitrification and denitrification operate in more spatial and temporal proximity than hitherto believed suggesting their significant role in the dynamics of nitrogen species. Further, the study suggests the potential role of anaerobic/suboxic nitrification that could intrinsically feed the denitrification process.

CONCLUSION

The microbial indicators of ensuing oxygen deficit appear as a well-defined nucleus almost 40 km from the coast even during the oxygenated period and spreads during the period of anoxia to cover the entire zone governing the water column chemistry. Nitrification operates at the base of this

suboxic system, very efficiently feeding denitrification. The alarming nitrous oxide input into the atmosphere could be relatively more due to a tighter intrinsic coupling of nitrification-denitrification, rather than denitrification driven by extraneous nitrate alone.

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Figure legends:

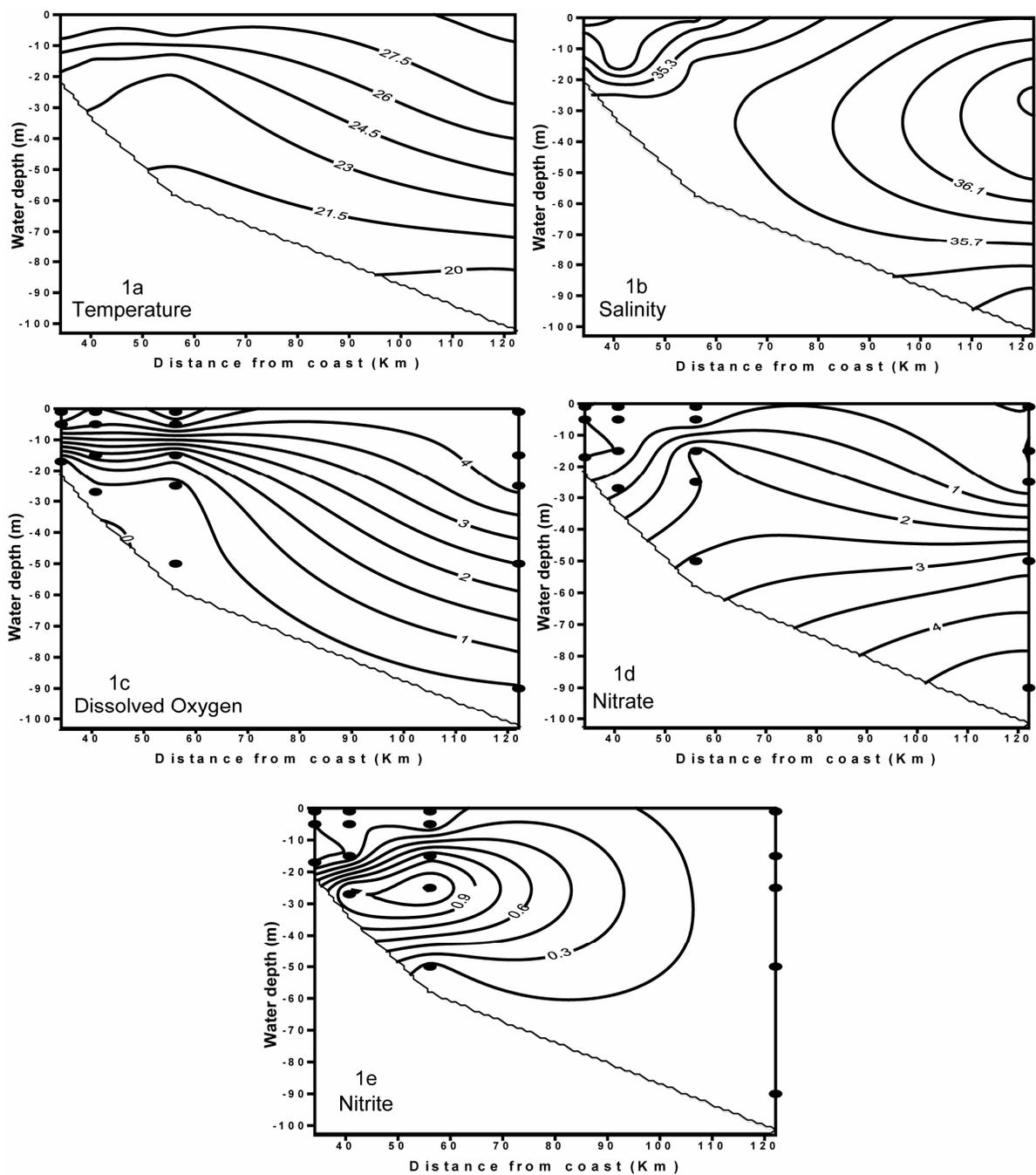
Fig.1. Hydrography along the transect Off Goa transect in October a- temperature ($^{\circ}\text{C}$); b - salinity (psu); c - oxygen (mL^{-1}); d -nitrate (μM); e- nitrite (μM). Station # 1 was sampled at 1, 5 and 17m (bottom depth, 19m); Station # 2 was sampled at 1,5,15 and 25m (bottom depth, 35m), Station # 3 was sampled at 1,15,30 and 60m (bottom depth 79m); Station # 4 was sampled at 1,15,30,60 and 90m (bottom depth, 100m).

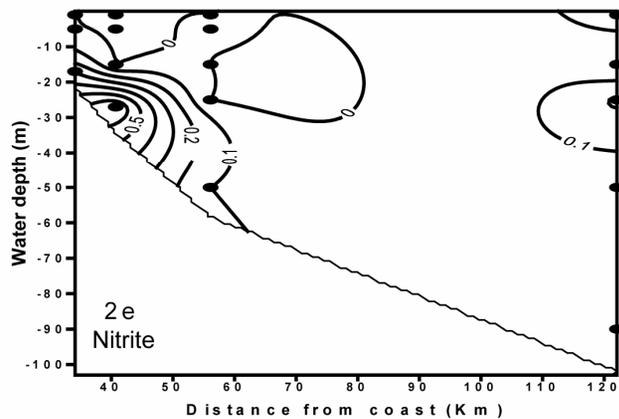
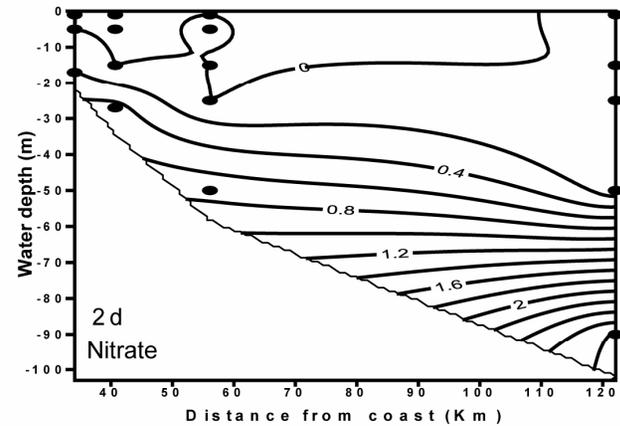
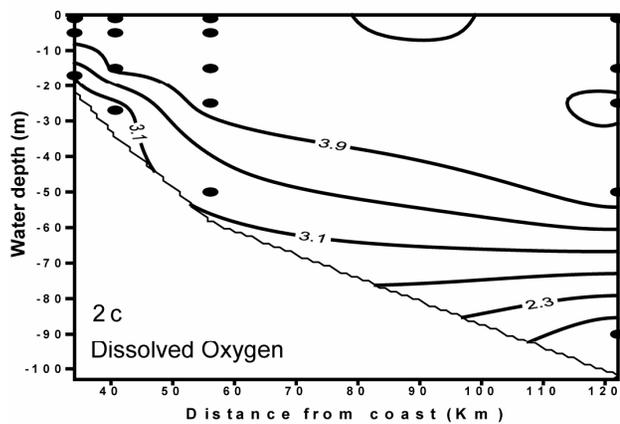
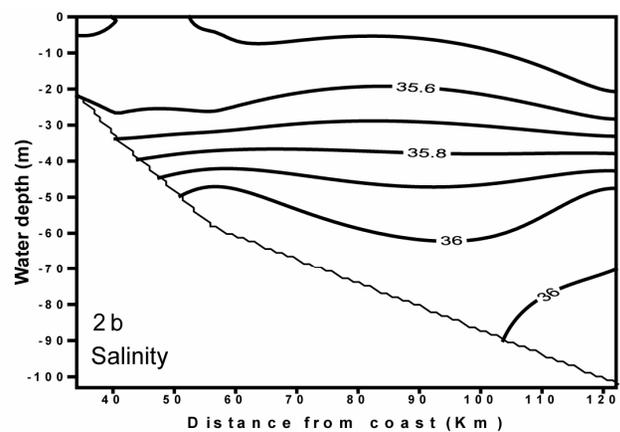
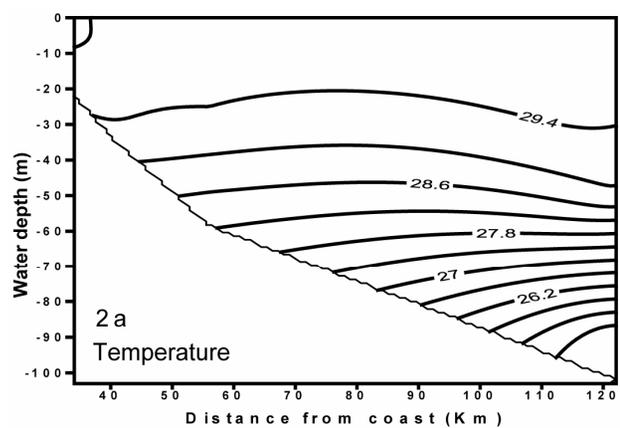
Fig.2. Hydrography along the transect Off Goa transect in April. temperature ($^{\circ}\text{C}$); b - salinity (psu); c - oxygen (mL^{-1}); d -nitrate (μM); e- nitrite (μM). Details of sampling depths same as Fig 1.

Fig.3a-b. 3a represents T-S profiles for station #2 during the cruise SaSu 103A (October, 2005). 3b represents the zero hour levels of nitrite, nitrate and ammonium at station #2 at the start of the experiment.

Figs 4a-e represents net changes in 'N' species from denitrification blocked water sample retrieved from - surface, 3,8,13 and 18m respectively. Figs 5a-e and 6a-e show, nitrification blocked and control sample respectively from similar depths. 'x'-axis represent the hours of incubation. (\square Nitrite \bullet Ammonium+ Nitrate).

Fig.7. Plot of dissolved inorganic phosphate (DIP) versus ammonium under different oxygen levels for the southwest monsoon cycle. Data from cruises covering the off Goa transect - SaSu-26 (September 2002), 29 (December, 2002), 49 (October 2003), 103A (December 2005) and a similar transect off Mangalore - SaSu 26, 30 (December, 2002), and 49 during oxic and anoxic seasons have also been included in this analysis.





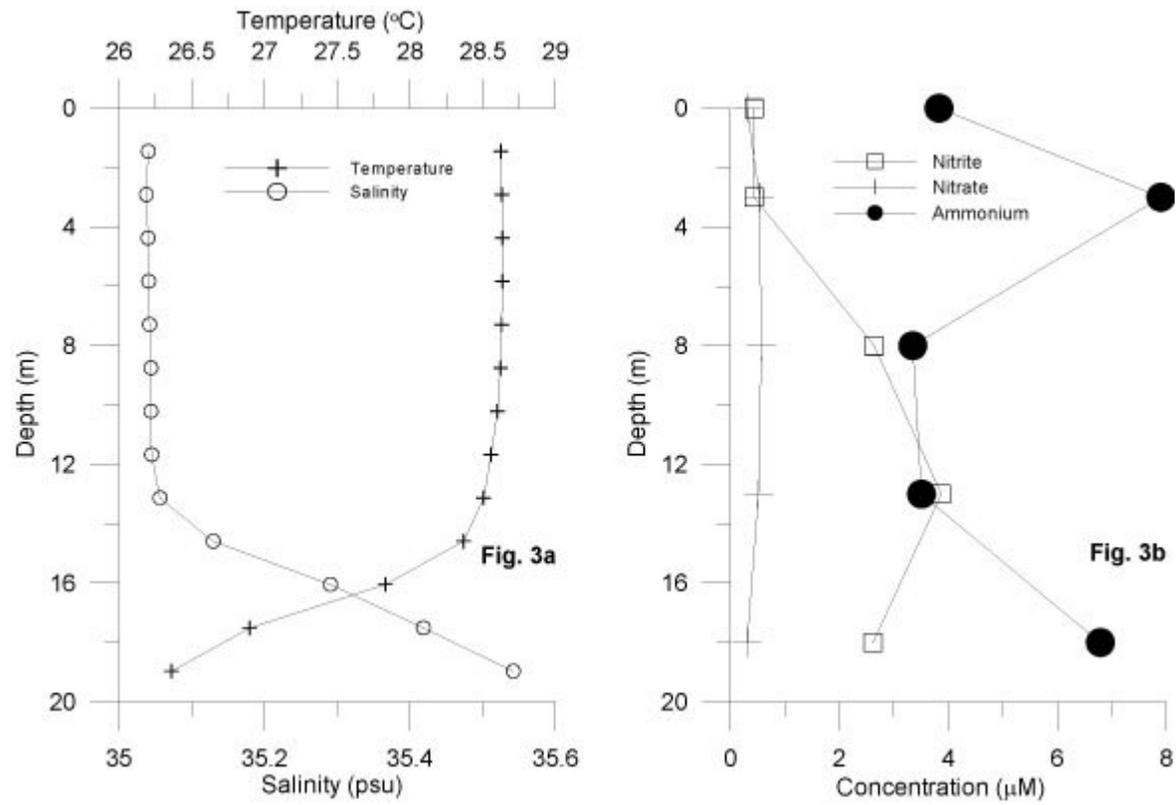


Fig.3 a and b

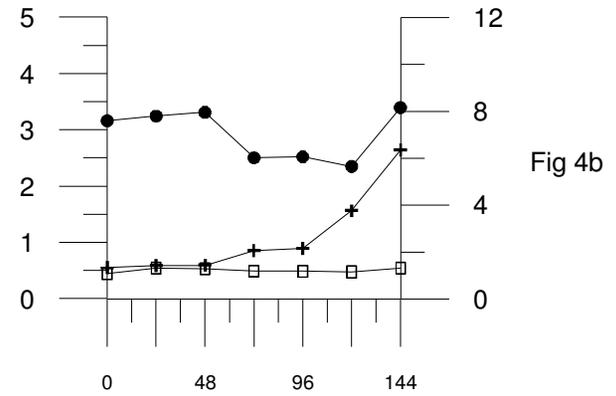
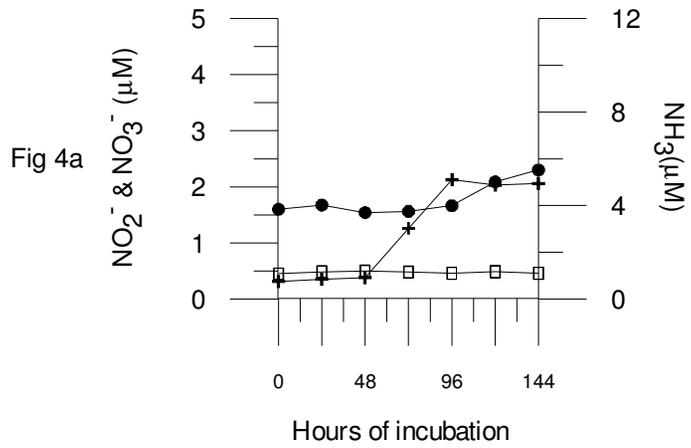


Fig 4c

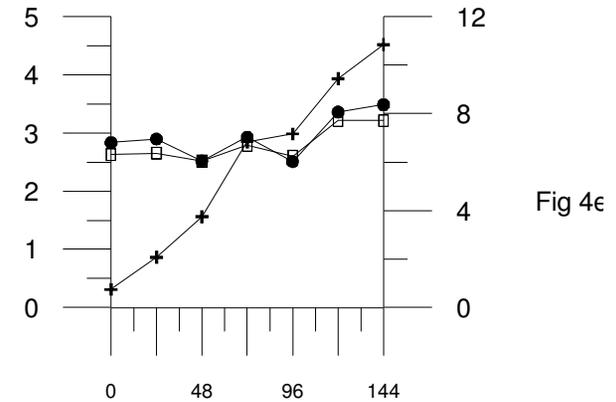
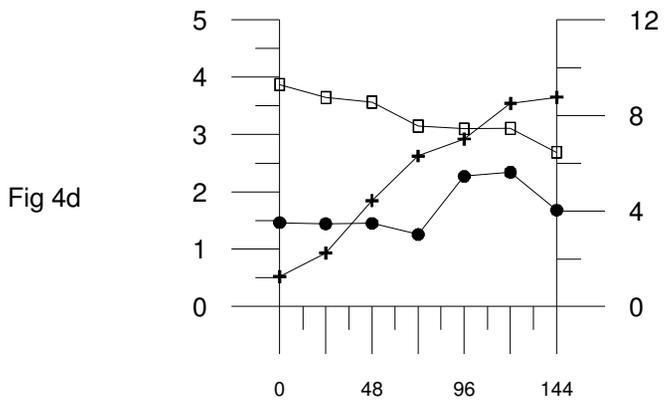
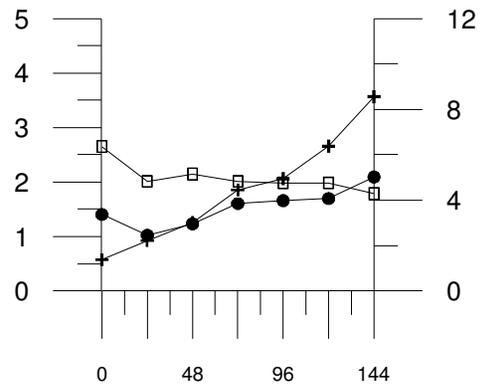


Fig 5a

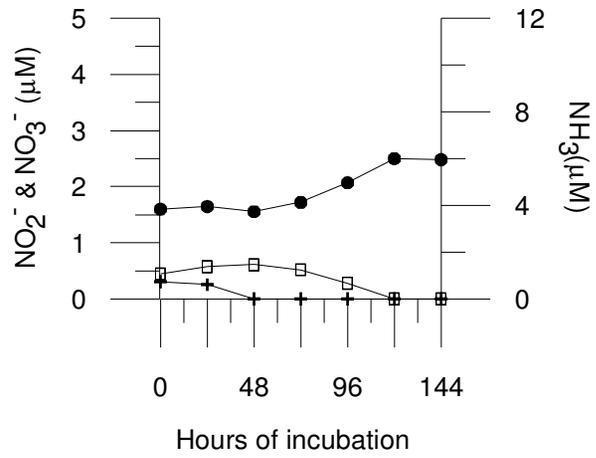


Fig 5b

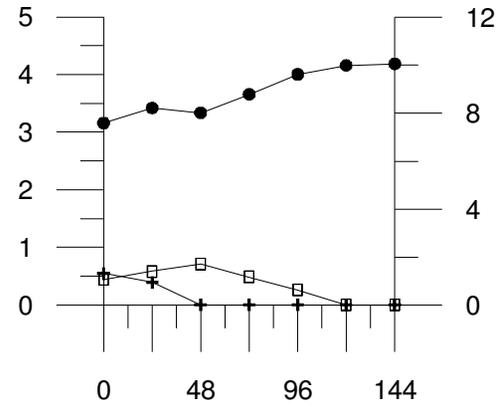


Fig 5c

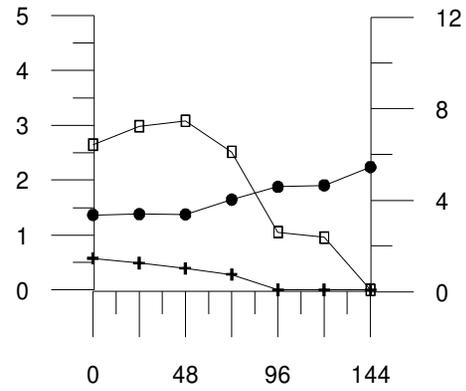


Fig 5d

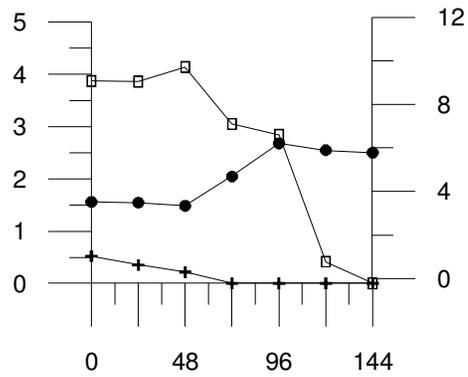


Fig 5e

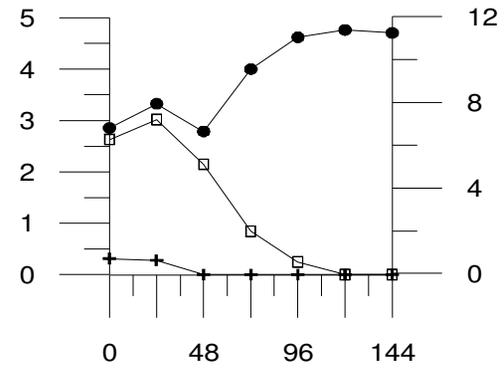


Fig 6a

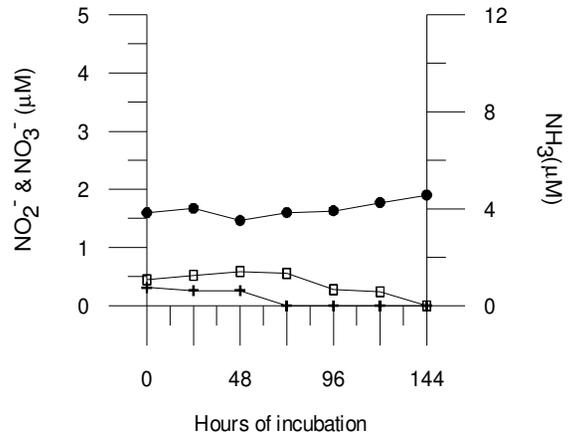


Fig 6t

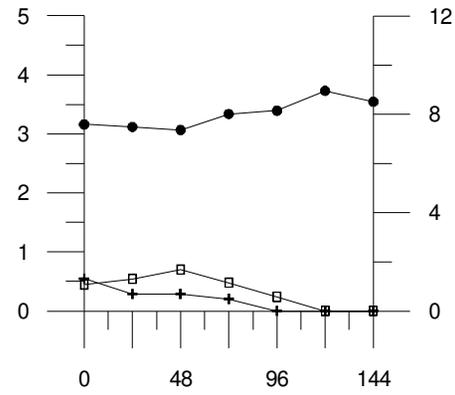


Fig 6c

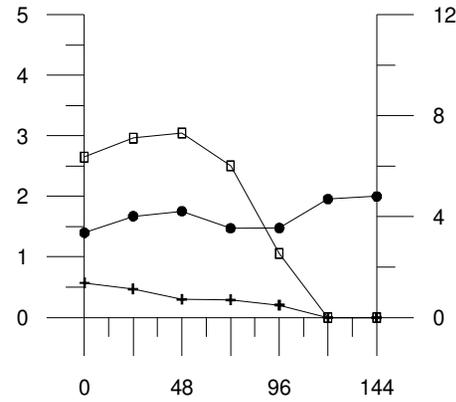


Fig 6d

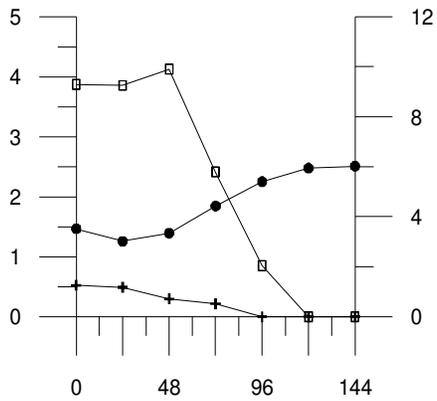
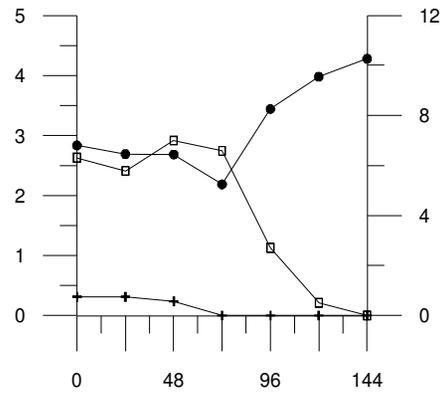


Fig 6e



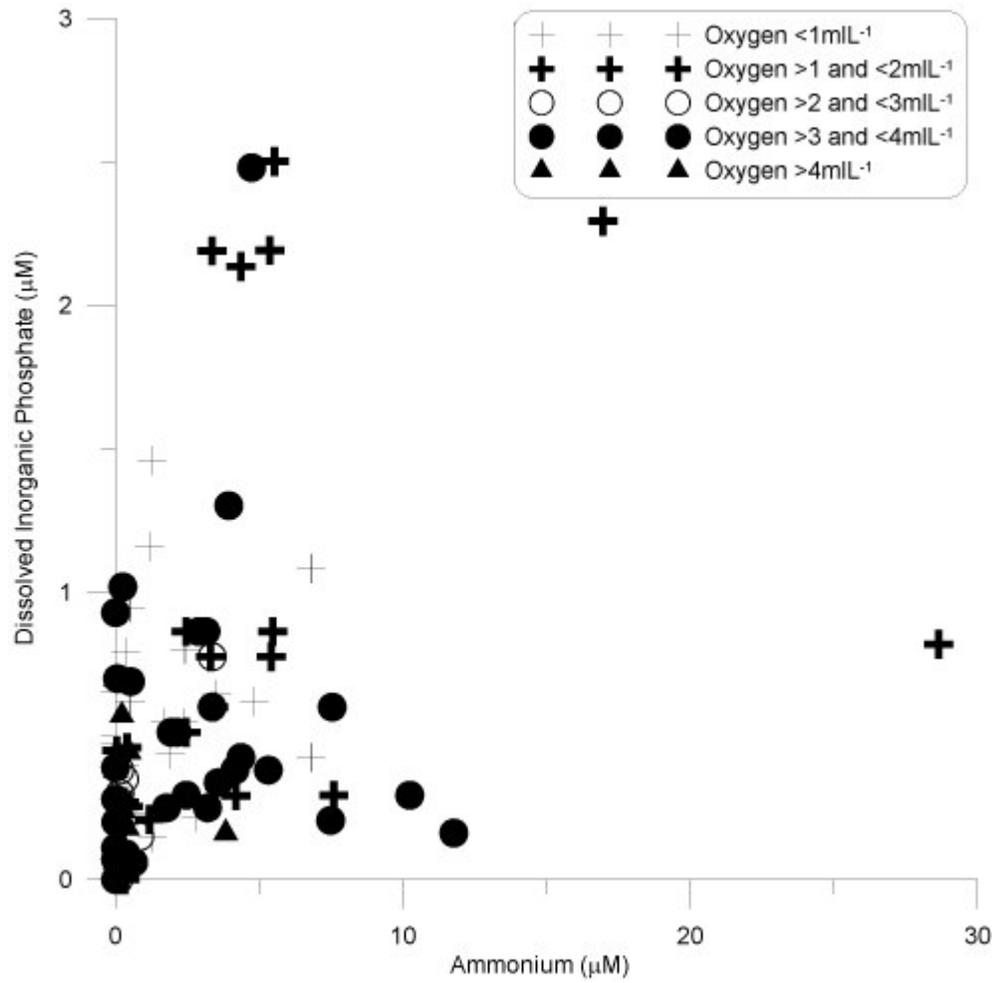


Fig.7.

Table 1. Bacterial groups along the transect Off Goa in April and October 2003

Depth (m)	April								October							
	Nitrifiers ($\times 10^8$ CFU L ⁻¹)				TDLO ($\times 10^5$ CFU L ⁻¹)				Nitrifiers ($\times 10^7$ CFU L ⁻¹)				TDLO ($\times 10^5$ CFU L ⁻¹)			
	#1	#2	#3	#4	#1	#2	#3	#4	#1	#2	#3	#4	#1	#2	#3	#4
1	2.3	1.2	2.6	0.7	0.1	0.1	0.3	0.3	ND	ND	ND	2.2	0.8	0.5	0.6	1.4
5	4.1	2.3	3.1	0.8	0.2	0.2	0.6	0.3	ND	ND	ND	ND	0.5	0.7	0.6	1.2
15	3.1	1.0	1.2	1.3	2.2	2.2	0.1	0.08	ND	ND	ND	ND	0.07	0.5	0.3	2.4
25	-	1.0	3.7	1.6	-	0.3	0.6	0.3	ND	ND	ND	ND	-	0.5	0.3	1.5
50	-	-	1.5	0.7	-		0.2	-	ND	ND	ND	ND	-	-	0.6	1.0

- station number, CFU – Colony Forming Units and ND – not detected