

Nitrogen fixation rates in the eastern Arabian Sea

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Abstract

The Arabian Sea experiences bloom of the diazotroph *Trichodesmium* during certain times of the year when optimal sea surface temperature and oligotrophic condition favour their growth. We measured nitrogen fixation rates in the euphotic zone during one such event in the Eastern Arabian Sea using $^{15}\text{N}_2$ tracer gas dissolution method. The measured rates varied between 0.8 and 225 $\mu\text{mol N m}^{-3}\text{d}^{-1}$ and were higher than those reported from most other oceanic regions. The highest rates (1739 $\mu\text{mol N m}^{-2}\text{d}^{-1}$; 0-10m) coincided with the growth phase of *Trichodesmium* and led to low $\delta^{15}\text{N}$ (<2‰) of particulate organic matter. At stations not experiencing *Trichodesmium* bloom nitrogen fixation rates were low (0.9 - 1.5 $\mu\text{mol N m}^{-3}\text{d}^{-1}$). Due to episodic events of diazotroph bloom, contribution of N_2 fixation to the total nitrogen pool may vary in space and time.

Keywords: Nitrogen cycle, N_2 fixation, *Trichodesmium*, Arabian Sea, Indian coast

Introduction

The availability of nitrogen (N) is essential for biological production in the ocean. However, most parts of the world oceans are depleted in bioavailable nitrogen at the surface (Montoya et al., 2004). Supply of nitrate from deep water is the major source of new nitrogen sustaining primary productivity. This flux of new nitrogen into the euphotic zone is balanced by loss through sinking particles (Dugdale and Goering 1967) and export of organic nitrogen out of the upper ocean (Eppley and Peterson, 1979; Lewis et al., 1986; Platt et al., 1992; Capone et al., 2005). Estimates of the nitrogen demand for new production, however, often exceed nitrate flux into the euphotic zone (Lewis et al., 1986) prompting speculation about other nitrogen inputs (Legendre and Gosselin 1989, Karl et al., 2002). Several studies have shown that biological nitrogen fixation plays a critical role in supporting new production (Carpenter and Romans, 1991; Gruber and Sarmiento, 1997; Karl et al., 1997; Capone et al., 1997; Montoya et al., 2002; Gruber, 2008; Großkopf et al., 2012) by providing the largest external input of nitrogen to the ocean, thereby also exerting important control on the ocean's nitrogen inventory (Falkowski et al., 1998; Karl et al., 2002 and Deutsch et al., 2007). The inventory of bioavailable (fixed) nitrogen in the ocean is largely a function of microbially mediated reduction-oxidation reactions occurring throughout the water column (White 2012) and is mostly regulated by nitrogen fixation, denitrification and anaerobic ammonium oxidation (anammox) (Galloway et al., 2004; Hamersley et al., 2011).

Recent estimates derived from both field data and model analyses for the global oceanic fixed N budget range from sources roughly balancing sinks (Gruber and Sarmiento, 1997; Gruber, 2004; Eugster and Gruber, 2012; DeVries et al., 2013) to a rather large net deficit with losses exceeding inputs by 140- 234 Tg N yr⁻¹ (Codispoti et al., 2001; Karl et al., 2002; Galloway et al., 2004; Codispoti, 2007). In order to address this imbalance, the nitrogen fixation process (the largest N source) has received a great deal of attention. It has long been believed that biological fixation of atmospheric nitrogen occurs when nitrate (NO₃⁻) concentrations are very low. However, nitrogen fixation has been shown to occur even at relatively high nitrate concentrations (Mulholland et al., 2001; Holl and Montoya, 2005). The organisms responsible for nitrogen fixation convert dissolved N₂ to ammonium. Despite the high energy requirement for this process, a few cyanobacteria (especially *Trichodesmium* spp.) are capable of reducing N₂ to NH₄⁺ (Bange et al., 2005). Further, there are also some heterotrophs which have the capability to fix dissolved nitrogen (Shiozaki et al., 2014).

The Arabian Sea is known to be an active site for N₂ fixation (Devassy et al., 1978, Capone et al. 1998). Surface bloom of filamentous diazotrophic cyanobacteria (*Trichodesmium* sp.) are well

documented and a recurring phenomenon in this region (Desaet al., 2005; Parabet al., 2006; Basuet al., 2011). The main factor that favors the growth of nitrogen fixers in the Arabian Sea is the excess phosphate in surface waters arising from denitrification (Bange et al., 2005). The ecological significance of this organism in the Arabian Sea is not very well known, although it is an important contributor to productivity of the Arabian Sea during oligotrophic conditions (Parab et al., 2012). Based on limited measurements of N_2 uptake rates (Dugdale et al., 1964; Capone et al., 1998), the estimated N_2 fixation rate for the region is 3.3 Tg N yr^{-1} (Bange et al., 2000).

Previous estimates of N_2 fixation from the eastern and the central Arabian Sea were based on volumetric conversion of trichomes and $^{15}N_2$ gas bubble addition method (Montoya et al., 1996; Gandhi et al., 2011; Parab et al., 2012) that might suffer from methodological problems. We use here the more recent $^{15}N_2$ gas dissolution technique developed by Mohr et al. (2010) that overcomes these problems.

1. Materials and methods

2.1 Study area and sampling

The study was conducted on board *R.V. Sindhu Sankalp* (Cruise SSK 46) during late north-east monsoon (NEM) (February 2013). The stations (Fig. 1), S1 to S4 (off Goa), S5 and S6 (off Mangalore) and S7 (off Cochin) were sampled based on the presence of *Trichodesmium* bloom (Fig. 2). Stations S1 to S4 are grouped as northern stations and S5 to S7 as southern stations. Water samples were collected with 10L Niskin samplers mounted onto a rosette which is fitted with a conductivity-temperature-depth profiler (CTD: Sea-Bird Electronics). Subsamples for nutrients were frozen onboard at -20°C and later analyzed in the shore laboratory for nitrate and phosphate using a Skalar auto analyzer following the methods given in Grasshoff et al. (1983).

2.2 Biological measurements

2.2.1 HPLC pigments

Water samples (0.5 to 1 L) for the analysis of phytoplankton pigments were collected in amber-colored bottles and filtered through Whatman GF/F filters (pore size $0.7 \mu\text{m}$) in dark and stored at -80°C until the analysis. The frozen filters were extracted at 0°C for 1-2 minutes in 3 ml of 100% methanol using Fisher Scientific ultrasonic dismembrator at 23 kHz. The extracts were filtered using a Teflon syringe cartridge (pore size $0.45 \mu\text{m}$) to remove cellular debris and analyzed with an HPLC (Agilent Technologies) using an Eclipse XDB C8 column. Pigments were separated following the procedure described by Roy et al. (2006) and Kurian et al. (2012) using a gradient program, and detected at 450 and 665 nm (Soret and Q-bands) by the diode array detector. Commercially available

standards from DHI Inc. (Denmark) were used for the identification and quantification of pigments including both chlorophylls and carotenoids. Identification was done based on the retention time and visible spectra matching. Phytoplankton size fractions based on the concentrations of accessory pigments were derived following Vidussi et al. (2001).

2.2.2 Primary productivity

Primary productivity was measured at three stations S1, S2 and S5. Water samples were collected in triplicates from upper euphotic depths in pre-cleaned acid-washed 250ml Nalgene polycarbonate bottles. From each depth three light bottles and one dark bottle were spiked each with 1 ml of ^{14}C -labelled sodium bicarbonate ($5\mu\text{Ci}$). The bottles were incubated for 12h in light using appropriate neutral density light cut-off screens. Samples were filtered after incubation through 25mm GF/F ($0.7\mu\text{m}$) filters and stored in vials for measurements in the shore laboratory. Filters were exposed to HCl fumes for 20 minutes in a desiccator to remove residual tracer. Samples were then placed in a vial having scintillation cocktail and activity was measured with a Wallace Scintillation counter (model no. 1409). All counts were quench corrected (UNESCO, 1994).

2.3 Nitrogen fixation

N_2 fixation rate measurements were made following the procedure described by Mohr et al. (2010) and Großkopf et al. (2012), wherein $^{15}\text{N}_2$ tracer-enriched seawater is directly added. Filtered water (Acropac filter $0.2\mu\text{m}$) degassed with argon for 15 minutes was spiked with $1\text{ml}^{15}\text{N}_2$ gas (98 atom%; Cambridge Isotope Laboratories, Massachusetts, USA) per 100 ml. The schematic representation for degassing is presented in Figure 3. Incubations were initiated by adding this water to the samples in 1.25 L polycarbonate bottles (~4% of the total incubation volume, Raaset al., 2014). All incubation bottles (triplicate) were acid washed prior to sampling and rinsed 3 times with seawater. Incubation bottles were gently shaken at least 50 times to ensure proper mixing prior to the incubation. These bottles were placed in on-deck incubator where temperature was regulated by a continuous surface seawater flow. A range of neutral density screens was used to mimic light attenuation at different depths. The solubility of N_2 in seawater was calculated according to Hamme and Emerson (2004).

All fixation experiments were terminated by filtering each sample under vacuum ($<10\text{ kPa}$) through a 25mm precombusted GF/F filter. In addition, water samples (4L) were also filtered for the measurement of the natural abundance of particulate organic nitrogen (PON) at $t=0$. Filters were frozen and stored at -20°C until the analysis. The filters were oven dried at 60°C and pelletized into tin cups and flash combusted in an elemental analyzer (EURO3000 Eurovector) coupled to a mass spectrometer (ThermoFinnigan DELTA V) to determine PON content and isotopic ratios. A working

standard, ACA (Amino-n-caproic acid) was intermittently run between samples to check for isotopic reproducibility (Maya et al., 2011; Bardhan et al., 2015).

For the calculation of N₂ fixation rate, we used the following equation (Montoya et al., 1996; Mohr et al., 2010):

$$N_2 \text{ Fixation rate} = \frac{A_{PN}^{final} - A_{PN}^{t=0}}{A_{N_2} - A_{PN}^{t=0}} \times \frac{(PN)}{\Delta t}$$

Where A = atom% ¹⁵N in the particulate nitrogen (PN) at the end or the beginning (t=0) of the incubation or in the dissolved N₂ pool (N₂).

2. Results

3.1 Hydrographic conditions

The temperature and salinity profiles are presented in Fig.4. Warm surface water (SST: 28-29.5 °C) having low salinity (~34) was present in shallow mixed layer depths (30–60m) in the south (stations S5, S6 and S7). NO₃⁻ concentration was below the detection limit (BDL) at the surface at station S3 compared to other stations (max upto 1.75 μM at S6, Fig.5a). The water column (< 100m) profiles showed a maximum concentration of ~9 μM NO₃⁻ at S1 and S5. Likewise, PO₄⁻ concentration varied between 0.03 μM (S3) and 0.57 μM (S7) with low concentration in surface waters at stations S5 and S6 (Fig. 5a).

3.2. Phytoplankton biomass and pigments

Calm weather conditions, higher temperature and low nutrient concentrations during this period were the most likely cause for the occurrence of the *Trichodesmium* bloom in the eastern Arabian Sea. The bloom was observed to occur in patches at most of the stations except at stations S3 and S4. Despite the bloom, Chl *a* concentration was low (<1 mg m⁻³) at the surface at sites sampled (Fig. 5b). Carbon uptake measurement showed higher rates (12.8 mg C m⁻³ d⁻¹) at station S4. HPLC-derived phytoplankton pigments comprised five major marker pigments [fucoxanthin (Fuco), 19'-Hexanoyloxyfucoxanthin (19HF), diadinoxanthin (diad), zeaxanthin (zea) and divinyl chlorophyll *a* (div chl *a*), corresponding to various phytoplankton functional groups at these stations (Table 1). Zeaxanthin (marker pigment of cyanobacteria) and div chl *a* (marker pigment of *Prochlorococcus*, a cyanobacterium) were the dominant pigments in the water column at all stations except S3 and S4 where fucoxanthin was dominant (134–188 ng L⁻¹ in the surface water). Nutrient deficient surface waters were generally dominated by pigments (zeaxanthin and div chl *a*)

derived from picoplankton size fraction ($69\text{-}85\text{ ng L}^{-1}$) at all the stations except at S3 and S4 (off Goa), which were dominated by microplankton fractions ($189\text{-}264\text{ ng L}^{-1}$) (Fig 6).

3.3 Nitrogen fixation and carbon uptake rates

Figure 7 shows depth profiles of the nitrogen fixation rate in the upper 10 m of the water column. N_2 fixation rates in the surface water ranged between 0.8 and $225\text{ }\mu\text{mol N m}^{-3}\text{ d}^{-1}$ with the highest rate occurring at S1 followed by S6 and S7. The rates were higher at the surface and decreased considerably with depth.

N_2 fixation was detectable even at stations (S3 and S4) not visibly affected by the bloom, but the rates measured here were lower ($0.9 - 1.5\text{ }\mu\text{mol N m}^{-3}\text{ d}^{-1}$) than those at the bloom stations. Interestingly, the rates were moderately high ($15\text{ }\mu\text{mol N m}^{-3}\text{ d}^{-1}$) in the region having thick *Trichodesmium* bloom (S5), and the highest rates (214 and $225\text{ }\mu\text{mol N m}^{-3}\text{ d}^{-1}$) were recorded at stations S1 and S7 where *Trichodesmium* bloom was less intense. Productivity measurement along the Goa transect (S1, S2 and S4) showed wide variations (from 0.07 to $12.8\text{ mg C m}^{-3}\text{ d}^{-1}$, Fig 8a). Primary production rates at S4 were an order of magnitude higher than at the other two stations ($<0.1\text{ mg C m}^{-3}\text{ d}^{-1}$).

The column integrated nitrogen fixation, carbon uptake rates and Chl *a* are shown in Figures 8a and 8b. N_2 fixation rates integrated over the top 10 m varied significantly (6 to $1739\text{ }\mu\text{mol N m}^{-2}\text{ d}^{-1}$) with the highest rate at station S7. Integrated N_2 fixation rates at the non-bloom stations were comparatively low (6.5 and $7.5\text{ }\mu\text{mol N m}^{-2}\text{ d}^{-1}$). The depth integrated carbon uptake rate in upper 20 m of the water column varied between 4 and $76.4\text{ mg C m}^{-2}\text{ d}^{-1}$, with higher value at station S4 (Fig 8b). Since the sampling depth was variable at these stations, we integrated carbon uptake rates over a depth of 20 m. The integrated Chl *a* varied between 3 and 28.8 mg m^{-2} with higher values at station S5 and S6 where the dense *Trichodesmium* bloom was observed. The $\delta^{15}\text{N}$ values of particulate organic matter varied between 0.5 and 6.1 ‰ . The higher value was recorded at station S3, whereas the lowest value (0.6 ‰) was observed at station S5 where intense bloom occurred.

3. Discussion

The Arabian Sea is one of the five major upwelling zones of the World Ocean. It also houses one of the three major oceanic oxygen minimum zones where intense loss of fixed nitrogen occurs through denitrification (Naqvi, 1987). However, unlike other areas, the most vigorous upwelling in the Arabian Sea occurs along its western boundary (Naqvi, 2008) during the south-west monsoon (SWM, from June to September). This process affects a very large area where nutrient-rich

subsurface water is brought to the surface, enhancing biological production (Banse, 1968; Sankaranarayanan et al., 1978; Naqvi and Jayakumar, 2000; Wiggert et al., 2005; Habeebrehman et al., 2008). During the NE monsoon, the convective mixing induced by cool and dry winds increases productivity in the northeastern Arabian Sea (Madhupratap et al., 1996; Weller et al., 1998; Lee et al., 2000). Thus the monsoons cause large-scale fertilization of surface waters through upwelling in summer and convective mixing in winter. The high rates of carbon fixation support the export of particulate organic matter to the deep sea thus contributing to the sustenance of oxygen minimum zone (Naqvi et al., 2009) an important feature of the biogeochemistry of the region.

Our observations showed relatively low (27.9°C) sea surface temperature (SST) in the north than those (29.5°C) in the south. The sea surface salinity (SSS) showed an opposite trend with higher values at the north (33.9-35.4). This north-south gradient in SST and SSS is a known feature of the Arabian Sea during NEM (Madhupratap et al., 1996; Prasannakumar and Narvekar, 2005; Prakash and Ramesh, 2007; Gandhi et al., 2011). The low concentrations of NO_3^- and PO_4^- in the surface water at most of the stations are associated with downwelling caused by the northward flow of the West India coastal current in winter (Naqvi et al., 2009). During the late north east monsoon and spring inter monsoon seasons, the upper water column in the Arabian Sea becomes nutrient deficient. This in conjunction with calm weather creates favourable conditions for the growth of diazotrophic cyanobacteria (*Trichodesmium*) as observed in the present study. *Trichodesmium* has an advantage over other phytoplankton to fix atmospheric nitrogen by using available PO_4^- (Chang et al., 2000; Parab et al., 2012) and bioavailable iron (Mills and Arrigo, 2010). Representatives of unicellular diazotrophic cyanobacteria have been reported to be P-limited in the Eastern Tropical North Atlantic Ocean (Turk-Kubo et al., 2012). The low PO_4^- concentration observed at station S5 in the present study is probably due to extensive uptake by *Trichodesmium*. The presence of *Trichodesmium* bloom under vertically stratified condition has been reported earlier from various areas such as the North Pacific sub tropical gyre (Karl et al., 1995). In the present study, the bloom of *Trichodesmium* was observed at five out of seven stations with varying intensity. Fresh seeding of *Trichodesmium* was observed at station S1, S2 and S7, whereas bloom was in the senescent phase at S5 and S6. Bloom was not visible at stations S3 and S4 where diatoms were dominant as confirmed by the presence of their marker pigment, fucoxanthin (Table 1).

As mentioned earlier, phytoplankton biomass (Chl a) integrated over the water column was higher at stations S5 and S6 (28.8 and 21.86 mg m^{-2} respectively) having intense bloom of *Trichodesmium*. However, integrated carbon uptake rate was higher at station S4 ($76.5 \text{ mg C m}^{-2} \text{ d}^{-1}$)

where bloom was not observed. Carbon uptake rate in the surface water at this station was more elevated ($12.8 \text{ mgCm}^{-3}\text{d}^{-1}$) than at station S1 ($0.17 \text{ mgCm}^{-3}\text{d}^{-1}$). Unfortunately, carbon uptake measurements could not be made at other stations due to logistic reasons. Parabet al. (2012) reported primary productivity upto $3.4 \text{ gCm}^{-3}\text{d}^{-1}$ subsequent to the *Trichodesmium* bloom in the Arabian Sea. High rates of primary productivity ($804\text{-}1080 \text{ mgCm}^{-2}\text{d}^{-1}$) has also been reported from other area such as the North Atlantic during *Trichodesmium* bloom (Carpenter et al., 2004). Several observations made in the Eastern Equatorial Atlantic Ocean during *Trichodesmium* bloom yielded a mean primary productivity of $247 \text{ mgCm}^{-2}\text{d}^{-1}$ (Bauerfeind, 1987).

The N_2 fixation rate varied widely, ranging between 0.8 and $225 \text{ }\mu\text{M Nm}^{-3}\text{d}^{-1}$ with the highest values at stations S1 and S7 and the lowest at the stations S3 and S4. High rates of N_2 fixation at these stations were obviously caused by the *Trichodesmium* bloom. These rates are much higher than those reported earlier from central Arabian Sea (Capone et al., 1998; Carpenter and Capone 2008; Shiozakiet al., 2014) and North Pacific Ocean (Church et al., 2009). However, Gandhi et al. (2011) and Parabet et al. (2012) reported even higher N_2 fixation rates in the Eastern Arabian Sea (Table 2). These wide variations in the estimated rates of N_2 fixation from the Arabian Sea and other oceanic regions could in part result from the methodological differences. The $^{15}\text{N}_2$ gas bubble method has long been used for measuring N_2 fixation rates. However, it has been demonstrated recently that this method underestimates nitrogen fixation as compared to the $^{15}\text{N}_2$ gas dissolution method. In the present study, nitrogen fixation was measured following the latter technique (Mohr et al., 2010). To our knowledge this is the first study from the region with the recently amended method.

N_2 fixation rates integrated over a depth of 10m varied significantly (6 to $1739 \text{ }\mu\text{molNm}^{-2}\text{d}^{-1}$). The highest rate was recorded from station S7 ($1739.5 \text{ }\mu\text{mol Nm}^{-2}\text{d}^{-1}$) followed by S6 and S1 (938 and $737 \text{ }\mu\text{molNm}^{-2}\text{d}^{-1}$ respectively) (Fig. 8a) coinciding with the seeding of *Trichodesmium* sp. Nitrogen isotopic composition ($\delta^{15}\text{N}$) of POM is often used as a proxy for N_2 fixation in the water column (Gandhi et al., 2011). Bacteria fix dissolved nitrogen with little isotopic fractionation and therefore $\delta^{15}\text{N}$ of a natural sample likely reflects that of its nitrogen source (Altabet and McCarthy, 1985; Kumar et al., 2004, 2005; Gandhi et al., 2011). The $\delta^{15}\text{N}$ values of particulate organic nitrogen (PON) in the surface waters varied between 0.6 and 6‰ (Fig. 8a) with lower values (1.4 - 2‰) corresponding to the sites with maximum N_2 fixation rates (stations S1, S6 and S7). Earlier studies documented low values of $\delta^{15}\text{N}$ in POM associated with *Trichodesmium* bloom (Liu et al. 1996). In the eastern Arabian Sea Maya et al. (2011) also found low values of $\delta^{15}\text{N}$ associated with

Trichodesmium. However, the lowest $\delta^{15}\text{N}$ value (0.6‰) was observed at station S5 where N_2 fixation rate was low, indicating that the production could have occurred earlier.

It is interesting to note that nitrogen fixation also occurs in the N-rich waters and in the absence of *Trichodesmium* bloom. However, N_2 fixation rates at the non-bloom stations were comparatively lower (6.5 and 7.5 $\mu\text{molNm}^{-2}\text{d}^{-1}$). Proliferation of diazotrophic cyanobacteria and N_2 fixation have been known for long from the nitrogen-depleted waters (Capone et al., 1997). Since the process is energetically expensive, it has been assumed that the assimilation of N in the form of NO_3^- and NH_4 is most preferred, before the N_2 fixation takes over (Karl et al., 2002). Our understanding of nitrogen fixation is constantly evolving with recent findings of nitrogen fixation or diazotrophic presence in a wide range of ecosystems including N-rich systems at high latitudes (Needoba et al., 2007), estuarine and coastal waters (Short and Zehr, 2007; Rees et al., 2009; Grosse et al., 2010; Bombaret et al., 2011; Bhavya et al., 2015), upwelling areas (Moutinet et al., 2008), eddies (Church et al., 2009) and even High Nutrient Low Chlorophyll (HNLC) waters (Bonnet et al., 2009). N_2 fixers can grow efficiently on combined N as long as it is sufficiently available and switch to a more energetically costly process of N_2 fixation when combined N forms have been exhausted to the nanomolar range (Holl and Montoya, 2005; Agawin et al., 2007). Recent data on algal cultures confirmed that micromolar levels (10 μM) of nitrate do not suppress N_2 fixation in diazotrophic cyanobacterium *Crocospaera* (Dekazemacker and Bonnet, 2011) unlike, *Trichodesmium erythraeum* where inhibition is known to occur to some extent (Holl and Montoya, 2005).

Generally filamentous cyanobacteria were considered as the only organisms contributing significantly to new production by N_2 fixation in oceans (Montoya et al., 2004). However, it is now recognized that nitrogen fixation by picoplanktonic forms (Wasmund et al., 2001; Zehr et al., 2001; Montoya et al., 2004) and marine heterotrophs may also be an important source of new N to the pelagic food web. HPLC based pigment analysis revealed more contribution of picoplankton at the bloom stations (Fig. 6). Further, nitrogen fixing diazotrophs has been reported from the Arabian Sea during non *Trichodesmium* bloom period (Bird et al., 2005) thus signifying the presence of N_2 fixation during the other period of the year. Bonnet et al. (2011) reported that 45 to 75% of N_2 fixation rates occurred in the picoplankton size fraction indicating that these smaller forms may be responsible for significant part of total N_2 fixation rates.

Iron, a micronutrient element, has been believed to potentially limit nitrogen fixation in the marine environment (Paerlet et al., 1994; Berman-Frank et al., 2001). The Saharan dust is known to stimulate N_2 fixation in the Northeastern Atlantic, increasing N:P ratios above Redfield levels

making P as the ultimate limiting nutrient for diazotrophs in this basin (Sohmet et al., 2011). Saharan dust deposition is suggested to cause *Trichodesmium* bloom as far as off the Florida coast (Leneset et al., 2001) and increase N₂ fixation rates by two-fold (Mills et al., 2004). Recent report also suggests that the diazotrophic populations may be limited by different nutrients on basin-wide spatial scales in the North Atlantic with the eastern basin more P-limited rather than Fe-limited (Turk-Kubo et al., 2012). However in the present study these nutrients could be supplied by atmospheric deposition and also by mobilization from shelf sediments (Pratihary et al., 2014).

Earlier estimates of oceanic nitrogen fixation based on canonical genera (*Trichodesmium* sp. and the diatom endosymbiont *Richelia intracellularis*) have been revised (Codispoti, 2007) and may require further revision (Davis and McGillicuddy, 2006). Recent data also suggest a substantial contribution of nitrogen fixation by smaller microbes (Montoya et al., 2004). Existing estimates of the total oceanic nitrogen fixation rate (Gruber, 2004; Codispoti et al., 2005; Deutsch et al., 2005, 2007) do not exceed 160 TgNa⁻¹, but this rate is based on observations and models focused on the photic zone and coastal sediments. Despite the widespread prevalence of the N₂ fixation genome in the oceans (Zehr et al., 1998), a comprehensive study of autotrophic and heterotrophic nitrogen fixation in the Arabian Sea is still lacking.

The observed variability of rates of N₂ fixation by us may be due to the patchiness of the diazotrophic abundance and the intensity of bloom as well as the physiology of the cells. A comparison of the rates of N₂ fixation with other oceanic regions (Table 2), shows that the Arabian Sea is an active N₂ fixation site. This is not unexpected given the excess phosphate produced by widespread denitrification in the region (Naqvi, 2008). However, the very fact that the N: P ratio in the Arabian Sea is lower than the Redfield value implies that N-loss substantially exceeds N-inputs through N₂ fixation in the region. However, while the N-loss is pretty well constrained, the same is not the case for N₂ fixation (Naqvi, 2008). More measurements following the improved procedure are required to better constrain this important term in the N-budget.

Conclusion

The present study reports first measurements of N₂ fixation rates in the eastern Arabian Sea following the improved (gas dissolution) method. The results show that the upper euphotic zone that is N-deficient but P-rich provides favourable conditions for N₂ fixation. However, this study focuses on *Trichodesmium*. In addition to generation of additional data with improved methodology, efforts must also be made to study N₂ fixation in N-replete waters and by other organisms such as heterotrophic bacteria and picocyanobacteria.

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References

- Agawin, N. S., Rabouille, S., Veldhuis, M. J., Servatius, L., Hol, S., van Overzee, H. M., Huisman, J., 2007. Competition and facilitation between unicellular nitrogen-fixing cyanobacteria and non-nitrogen-fixing phytoplankton species. *Limnol. Oceanogr.*, 52(5), 2233-2248.
- Altabet, M. A., McCarthy, J. J., 1985. Temporal and spatial variations in the natural abundance of ¹⁵N in PON from a warm-core ring. *Deep-Sea Res. Part A. Oceanographic Research Papers*, 32(7), 755-772.
- Bange, H. W., Rixen, T., Johansen, A. M., Siefert, R. L., Ramesh, R., Ittekkot, V., Hoffmann, M. R., Andreae, M. O., 2000. A revised nitrogen budget for the Arabian Sea. *Global Biogeochem Cy.*, 14(4), 1283-1297.
- Bange, H. W., Naqvi, S. W. A., Codispoti, L. A., 2005. The nitrogen cycle in the Arabian Sea. *Prog Oceanogr.*, 65(2), 145-158.
- Banase, K., 1968. Hydrography of the Arabian Sea shelf of India and Pakistan and effects on demersal fishes. In *Deep Sea Research and Oceanographic Abstracts*. 15(1), pp. 45-79. Elsevier.
- Banase, K., 1987. Seasonality of phytoplankton chlorophyll in the central and northern Arabian Sea. *Deep-Sea Res. Pt I*, 34(5), 713-723.
- Bardhan, P., Karapurkar, S. G., Shenoy, D. M., Kurian, S., Sarkar, A., Maya, M. V., Naik, H., Varik, S., Naqvi, S. W. A., 2015. Carbon and nitrogen isotopic composition of suspended particulate organic matter in Zuari Estuary, west coast of India. *J. Mar. Syst.* 141, 90-97. doi:10.1016/j.jmarsys.2014.07.009
- Basu, S., Matondkar, S. P., Furtado, I., 2011. Enumeration of bacteria from a *Trichodesmium* spp. bloom of the Eastern Arabian Sea: elucidation of their possible role in biogeochemistry. *J Appl Phycol*, 23(2), 309-319.
- Bauerfeind, E., 1987. Primary production and phytoplankton biomass in the equatorial region of the Atlantic at 22°W. *Oceanologica Acta*, 10, 131-136.
- Berman-Frank, I., Cullen, J. T., Shaked, Y., Sherrell, R. M., Falkowski, P. G., 2001. Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*. *Limnol. Oceanogr.*, 46(6), 1249-1260.
- Bhavaya, P. S., Kumar, S., Gupta, G. V. M., Sudheesh, V., Sudharma, K. V., Varrier, D. S., Dhanya, K. R., Saravanane, N., 2015. Nitrogen uptake dynamics in a tropical eutrophic estuary (Cochin, India) and adjacent coastal waters. *Estuar. Coast*, pp 1-14.

- Bird, C., Martinez Martinez, J., O'Donnell, A. G., Wyman, M., 2005. Spatial distribution and transcriptional activity of an uncultured clade of planktonic diazotrophic gamma-proteobacteria in the Arabian sea. *Appl. Environ. Microbiol.* 71, 2079–85. doi:10.1128/AEM.71.4.2079-2085.2005
- Bombar, D., Moisaner, P. H., Dippner, J. W., Foster, R. A., Voss, M., Karfeld, B., Zehr, J. P., 2011. Distribution of diazotrophic microorganisms and nifH gene expression in the Mekong River plume during intermonsoon. *Mar EcolProg Ser.*, 424, 39–52.
- Bonnet, S., Biegala, I. C., Dutrieux, P., Slemmons, L. O., Capone, D. G., 2009. Nitrogen fixation in the western equatorial Pacific: Rates, diazotrophic cyanobacterial size class distribution, and biogeochemical significance. *Global Biogeochem Cy.*, 23, GB3012, doi:10.1029/2008GB003439.
- Bonnet, S., Grosso, O., Moutin, T., 2011. Planktonic dinitrogen fixation along a longitudinal gradient across the Mediterranean Sea during the stratified period (BOUM cruise). *Biogeosciences*, 8(8), 2257-2267.
- Brandes, J. A., Devol, A. H., 2002. A global marine-fixed nitrogen isotopic budget: Implications for Holocene nitrogen cycling. *Global BiogeochemCy*, 16(4), 67-71.
- Capone, D. G., Zehr, J. P., Paerl, H. W., Bergman, B., Carpenter, E. J., 1997. *Trichodesmium*, a globally significant marine cyanobacterium. *Science*, 276(5316), 1221-1229.
- Capone, D. G., Subramaniam, A., Montoya, J. P., Voss, M., Humborg, C. J. A. M., Johansen, A. M., Seifert, A. L., Carpenter, E. J., 1998. An extensive bloom of the N₂-fixing cyanobacterium *Trichodesmiumerythraeum* in the central Arabian Sea. *Mar EcolProg Ser.*, 172, 281-292.
- Capone, D. G., Burns, J. A., Montoya, J. P., Subramaniam, A., Mahaffey, C., Gunderson, T., Michaels, A. F., Carpenter, E. J., 2005. Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. *Global Biogeochem Cy.*, 19(2).<http://dx.doi.org/10.1029/2004GB002331>
- Carpenter, E. J., 1983. Nitrogen fixation by marine Oscillatoria (*Trichodesmium*) in the world's oceans, in *Nitrogen in the Marine Environment*, edited by E. J. Carpenter and D. G. Capone, pp. 65–103, Elsevier, New York.
- Carpenter, E. J., Romans, K., 1991. Major role of the cyanobacterium *Trichodesmium* in nutrient cycling in the North Atlantic Ocean. *Science*, 254(5036), 1356-1358.
- Carpenter, E. J., Subramaniam, A., Capone, D. G., 2004. Biomass and primary productivity of the cyanobacterium *Trichodesmium* spp. in the tropical North Atlantic ocean. *Deep-Sea Res. Pt I: Oceanographic Research Papers*, 51(2), 173-203.
- Carpenter, E. J., Capone, D. G., 2008. Nitrogen fixation in the marine environment, in *Nitrogen in the Marine Environment*, edited by E. J. Carpenter and D. G. Capone, pp. 141–198., Elsevier, New York, doi:10.1016/B978-0-12-372522-6.00004-9.
- Chang, J., Chiang, K. P., Gong, G. C., 2000. Seasonal variation and cross-shelf distribution of the nitrogen-fixing cyanobacterium, *Trichodesmium*, in southern East China Sea. *Cont Shelf Res.*, 20(4), 479-492.

- Church, M. J., Mahaffey, C., Letelier, R. M., Lukas, R., Zehr, J. P., Karl, D. M., 2009. Physical forcing of nitrogen fixation and diazotroph community structure in the North Pacific subtropical gyre. *Global BiogeochemCy*, 23. GB2020.
- Codispoti, L. A., Brandes, J. A., Christensen, J. P., Devol, A. H., Naqvi, S. W. A., Paerl, H. W., Yoshinari, T, 2001. The oceanic fixed nitrogen and nitrous oxide budgets: Moving targets as we enter the anthropocene?. *Scientia Marina*, 65(S2), 85-105.
- Codispoti, L. A., Flagg, C., Kelly, V., Swift, J. H., 2005. Hydrographic conditions during the 2002 SBI process experiments. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 52, 3199–3226. doi:10.1016/j.dsr2.2005.10.007
- Codispoti, L. A., 2007. An oceanic fixed nitrogen sink exceeding 400 Tg N a⁻¹ vs the concept of homeostasis in the fixed-nitrogen inventory. *Biogeosciences*, 4(2), 233-253.
- Davis, C. S., McGillicuddy, D. J., 2006. Transatlantic abundance of the N₂-fixing colonial cyanobacterium *Trichodesmium*. *Science*, 312(5779), 1517-1520.
- Dekaezemacker, J., Bonnet, S., 2011. Sensitivity of N₂ fixation to combined nitrogen forms (NO₃⁻ and NH₄⁺) in two strains of the marine diazotroph *Crocospaerawatsonii* (Cyanobacteria). *Mar. Ecol. Prog. Ser.*, 438, 33-46.
- Desa, E. S., Suresh, T., Matondkar, S. G. P., Desa, E., Goes, J., Mascarenhas, A. A. M. Q., Parab, S.G., Shaikh, N., Fernandes, C. E. G., 2005. Detection of *Trichodesmium* bloom patches along the eastern Arabian Sea by IRS-P4/OCM ocean color sensor and by in-situ measurements. *Indian J Mar Sci*, 34(4), 374-386.
- Deutsch, C., Gruber, N., Sarmiento, J. L., Sigman, D. M., 2005. Pacific dominance of oceanic N₂ fixation diagnosed from nutrient distributions. In AGU Fall Meeting Abstracts (Vol. 1, p. 05).
- Deutsch, C., Sarmiento, J. L., Sigman, D. M., Gruber, N., Dunne, J. P., 2007. Spatial coupling of nitrogen inputs and losses in the ocean. *Nature*, 445(7124), 163-167.
- Devassy, V. P., Bhattathiri, P. M. A., Qasim, S. Z., 1978. *Trichodesmium* phenomenon. *Indian J. Mar. Sci*, 7, 168-186.
- DeVries, T., Deutsch, C., Rafter, P. A., Primeau, F., 2013. Marine denitrification rates determined from a global 3-D inverse model. *Biogeosciences*, 10(4).doi:10.5194/bg-8-827-2011.
- Dugdale, R. C., Goering, J. J., Ryther, J. H., 1964. High nitrogen fixation rates in the Sargasso Sea and the Arabian Sea. *LimnolOceanogr*, 9(4), 507-510.
- Dugdale, R. C., Goering, J. J., 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. *LimnolOceanogr*, 12(2), 196-206.
- Eppley R. W., Peterson, J., 1979. Particulate organic matter flux and planktonic new production in the deep ocean. *Nature*, 282, 677-680.
- Eugster, O., Gruber, N., 2012. A probabilistic estimate of global marine N-fixation and denitrification. *Global BiogeochemCy*, 26(4).

- Falkowski, P. G., Barber, R. T., Smetacek, V., 1998. Biogeochemical controls and feedbacks on ocean primary production. *Science*, 281(5374), 200-206.
- Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G.P., Cleveland, C. C., Green, P. A., Holland, E. A., Karl, D. M., Michaels, A. F., Porter, J. H., Townsend, A. R., Vöosmarty, C. J, 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry*, 70(2), 153-226.
- Gandhi, N., Singh, A., Prakash, S., Ramesh, R., Raman, M., Sheshshayee, M. S., Shetye, S. 2011. First direct measurements of N₂ fixation during a *Trichodesmium* bloom in the eastern Arabian Sea. *Global BiogeochemCy*, 25(4).
- Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. *Methods of Seawater Analysis*. Verlag Chemie, Weinheim (419 pp.).
- Grosse, J., Bombar, D., Doan, H. N., Nguyen, L. N., Voss, M., 2010. The Mekong River plume fuels nitrogen fixation and determines phytoplankton species distribution in the South China Sea during low and high discharge season. *LimnolOceanogr*, 55(4), 1668-1680.
- Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M. M., Lavik, G., Schmitz, R.A., Wallace, D., LaRoche, J., 2012. Doubling of marine dinitrogen-fixation rates based on direct measurements. *Nature*, 488(7411), 361-364.
- Gruber, N., Sarmiento, J. L, 1997. Global patterns of marine nitrogen fixation and denitrification. *Global BiogeochemCy*, 11(2), 235-266.
- Gruber, N, 2004. The dynamics of the marine nitrogen cycle and its influence on atmospheric CO₂ variations. In *The ocean carbon cycle and climate* (pp. 97-148). Springer Netherlands.
- Gruber, N, 2008. The marine nitrogen cycle: overview and challenges. *Nitrogen in the marine environment*, 1-50.
- Habeebrehman, H., Prabhakaran, M. P., Jacob, J., Sabu, P., Jayalakshmi, K. J., Achuthankutty, C. T., Revichandran, C., 2008. Variability in biological responses influenced by upwelling events in the Eastern Arabian Sea. *J Marine Syst*, 74(1), 545-560.
- Hamme, R.C., Emerson, S.R., 2004. The solubility of neon, nitrogen and argon in distilled water and seawater. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 51, 1517–1528. doi:10.1016/j.dsr.2004.06.009
- Hamersley, M. R., Turk, K. A., Leinweber, A., Gruber, N., Zehr, J. P., Gunderson, T., Capone, D. G., 2011. Nitrogen fixation within the water column associated with two hypoxic basins in the Southern California Bight. *AquatMicrobEcol*, 63(2), 193.
- Holl, C. M., Montoya, J. P., 2005. Interactions between nitrate uptake and nitrogen fixation in continuous cultures of the marine diazotroph *Trichodesmium* (cyanobacteria). *J Phycol*, 41(6), 1178-1183.
- Karl, D. M., Letelier, R., Hebel, D., Tupas, L., Dore, J., Christian, J., Winn, C., 1995. Ecosystem changes in the North Pacific subtropical gyre attributed to the 1991–92 El Nino. *Nature*. 373.230–234.

- Karl, D., Letelier, R., Tupas, L., Dore, J., Christian, J., Hebel, D., 1997. The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. *Nature*, 388(6642), 533-538.
- Karl, D., Michaels, A., Bergman, B., Capone, D., Carpenter, E., Letelier, R., Lipschultz, F., Paerl, H., Sigman, D., Stal, L, 2002. Dinitrogen fixation in the world's oceans pp. 47-98. Springer Netherlands.
- Kumar, S., Ramesh, R., Bhosle, N. B., Sardesai, S., Sheshshayee, M. S., 2004. Natural isotopic composition of nitrogen in suspended particulate matter in the Bay of Bengal. *Biogeosciences*, 1(1), 63-70.
- Kumar, S., Ramesh, R., Sheshshayee, M. S., Sardesai, S., Patel, P. P., 2005. Signature of terrestrial influence on nitrogen isotopic composition of suspended particulate. *Curr. Sci.*, 88(6), 770-774.
- Kurian, S., Roy, R., Repeta, D. J., Gauns, M. U., Shenoy, D. M., Suresh, T., Sarkar, A., Narvenkar, G., Johnson, C.G., Naqvi, S. W. A., 2012. Seasonal occurrence of anoxygenic photosynthesis in Tillari and Selaulim reservoirs, Western India. *Biogeosciences*, 9, 2485–2495.
- Lee, C. M., Jones, B. H., Brink, K. H., Fischer, A. S., 2000. The upper-ocean response to monsoonal forcing in the Arabian Sea: seasonal and spatial variability. *Deep-Sea Res. Pt II: Topical Studies in Oceanography*, 47(7), 1177-1226.
- Legendre, L., Gosselin, M, 1989. New production and export of organic matter to the deep ocean: consequences of some recent discoveries. *Limnol. Oceanogr.*, 34(7), 1374-1380.
- Lenes, J. M., Darrow, B. P., Cattrall, C., Heil, C. A., Callahan, M., Vargo, G. A., Byrne, R.H., Prospero, J.M., Bates, D.E., Fanning, K.A., Walsh, J. J., 2001. Iron fertilization and the *Trichodesmium* response on the West Florida shelf. *Limnol. Oceanogr.*, 46(6), 1261-1277.
- Lewis, M. R., Harrison, W. G., Oakey, N. S., Herbert, D., Platt, T., 1986. Vertical nitrate fluxes in the oligotrophic ocean. *Science*, 234(4778), 870-872.
- Madhupratap, M., Kumar, S. P., Bhattathiri, P. M. A., Kumar, M. D., Raghukumar, S., Nair, K. K. C., Ramaiah, N., 1996. Mechanism of the biological response to winter cooling in the northeastern Arabian Sea.
- Mahaffey, C., Michaels, A. F., Capone, D. G., 2005. The conundrum of marine N₂ fixation. *Am J Sci*, 305(6-8), 546-595.
- Maya, M. V., Karapurkar, S. G., Naik, H., Roy, R., Shenoy, D. M., Naqvi, S. W. A., 2011. Intra-annual variability of carbon and nitrogen stable isotopes in suspended organic matter in waters of the western continental shelf of India. *Biogeosciences*, 8, 3441–3456.
- Mills, M. M., Ridame, C., Davey, M., La Roche, J., Geider, R. J., 2004. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature*, 429(6989), 292-294.
- Mills, M. M., Arrigo, K. R., 2010. Magnitude of oceanic nitrogen fixation influenced by the nutrient uptake ratio of phytoplankton. *Nat Geosci*, 3(6), 412-416.

- Mohr, W., Grosskopf, T., Wallace, D. W. R., LaRoche, J., 2010. Methodological underestimation of oceanic nitrogen fixation rates. *PLoS ONE* 5,e12583.
- Montoya, J. P., Voss, M., Kahler, P., Capone, D. G., 1996. A Simple, High-Precision, High-Sensitivity Tracer Assay for N₂ Fixation. *Appl Environ Microb*, 62(3), 986-993.
- Montoya, J. P., Carpenter, E. J., Capone, D. G., 2002. Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic. *LimnolOceanogr.*, 47(6), 1617-1628.
- Montoya, J. P., Holl, C. M., Zehr, J. P., Hansen, A., Villareal, T. A., Capone, D. G., 2004. High rates of N₂ fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean. *Nature*, 430(7003), 1027-1032.
- Moutin, T., Karl, D. M., Duhamel, S., Rimmelin, P., Raimbault, P., Van Mooy, B. A., Claustre, H., 2008. Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean. *Biogeosciences*, 5, 95–109.
- Mulholland, M. R., Ohki, K., Capone, D. G., 2001. Nutrient controls on nitrogen uptake and metabolism by natural populations and cultures of *Trichodesmium* (Cyanobacteria). *J Phycol.*, 37,1001–1009.
- Naqvi, S. W. A., 1987. Some aspects of the oxygen-deficient conditions and denitrification in the Arabian Sea, *J. Mar. Res.*, 49, 1049– 1072.
- Naqvi, S. W. A., Jayakumar, D. A., 2000. Ocean biogeochemistry and atmospheric composition: Significance of the Arabian Sea. *Curr. Sci.*, 78(3), 289-299.
- Naqvi, S. W. A., 2008. The Indian Ocean, in *Nitrogen in the Marine Environment*, edited by D. G. Capone et al., Elsevier, New York, 631-681.
- Naqvi, S.W.A., Naik, H., Jayakumar, A., Pratihary, A. K., Narvenkar, G., Kurian, S., Agnihotri, R., Shailaja, M.S., Narvekar, P. V., 2009. Seasonal anoxia over the western Indian continental shelf. *Indian Ocean Biogeochemical Processes and Ecological Variability*, 333-345.
- Needoba, J. A., Foster, R. A., Sakamoto, C., Zehr, J. P., Johnson, K. S., 2007. Nitrogen fixation by unicellular diazotrophic cyanobacteria in the temperate oligotrophic North Pacific Ocean. *LimnolOceanogr.* 52(4), 1317-1327.
- Paerl, H. W., Prufert-Bebout, L. E., Guo, C., 1994. Iron-stimulated N₂ fixation and growth in natural and cultured populations of the planktonic marine cyanobacteria *Trichodesmium* spp. *Appl Environ Microb.* 60(3), 1044-1047.
- Parab, S. G., Matondkar, S. P., Gomes, H. D. R., Goes, J. I., 2006. Monsoon driven changes in phytoplankton populations in the eastern Arabian Sea as revealed by microscopy and HPLC pigment analysis. *Cont Shelf Res*, 26(20), 2538-2558.
- Parab, S. G., Matondkar, S. G. P., 2012. Primary productivity and nitrogen fixation by *Trichodesmium* spp. in the Arabian Sea. *J Mar Syst*, 105, 82-95.
- Platt, T., Jauhari, P., Sathyendranath, S., 1992. The importance and measurement of new production. In *Primary productivity and biogeochemical cycles in the sea* pp. 273-284. Springer US.

- Prakash, S., Ramesh, R., 2007. Is the Arabian Sea getting more productive? *Curr Sci.*, 92(5), 667-670.
- Prasannakumar, S., Narvekar, J., 2005. Seasonal variability of the mixed layer in the central Arabian Sea and its implication on nutrients and primary productivity. *Deep-Sea Res Pt II: Topical Studies in Oceanography*, 52(14), 1848-1861.
- Pratihary, A. K., Naqvi, S. W. A., Narvenkar, G., Kurian, S., Naik, H., Naik, R., Manjunatha, B. R., 2014. Benthic mineralization and nutrient exchange over the inner continental shelf of western India. *Biogeosciences*, 11(10), 2771-2791.
- Raes, E.J., Waite, A.M., McInnes, A.S., Olsen, H., Nguyen, H.M., Hardman-Mountford, N., Peter, A.T., 2014. Changes in latitude and dominant diazotrophic community alter N₂ fixation. *Mar. Ecol. Prog. Ser* 516, 85–102. doi:10.3354/meps11009
- Rees, A. P., Gilbert, J. A., Kelly-Gerreyn, B. A., 2009. Nitrogen fixation in the western English Channel (NE Atlantic ocean). *Mar EcolProgSer*, 374, 7-12.
- Roy, R., Pratihary, A., Mangesh, G., Naqvi, S. W. A., 2006. Spatial variation of phytoplankton pigments along the southwest coast of India. *Estuar Coast Shelf S*, 69(1), 189-195.
- Sankaranarayanan, V. N., Rao, D. P., Antony, M. K., 1978. Studies on some hydrographical characteristics of the estuarine and inshore waters of Goa during the south-west monsoon 1972. *Mahasagar*, 11(3-4), 125-136.
- Shiozaki, T., Ijichi, M., Kodama, T., Takeda, S., Furuya, K., 2014. Heterotrophic bacteria as major nitrogen fixers in the euphotic zone of the Indian Ocean. *Global Biogeochem. Cycles* 28, 1096–1110. doi:10.1002/2014GB004886
- Short, S. M., Zehr, J. P., 2007. Nitrogenase gene expression in the Chesapeake Bay Estuary. *Environ Microbiol*, 9(6), 1591-1596.
- Sohm, J. A., Webb, E. A., Capone, D. G., 2011. Emerging patterns of marine nitrogen fixation. *Nat Rev Microbiol*, 9(7), 499-508.
- Turk-Kubo, K. A., Achilles, K. M., Serros, T. R., Ochiai, M., Montoya, J. P., Zehr, J. P., 2012. Nitrogenase (nifH) gene expression in diazotrophic cyanobacteria in the Tropical North Atlantic in response to nutrient amendments. *Front Microbiol*, 3,386.
- UNESCO, 1994. Protocols for the Joint Global Ocean Flux Study (JGOFS). Core Measurements, IOC Manual and Guides. 29 (170 pp.).
- Vidussi, F., Claustre, H., Manca, B.B., Luchetta, A., Marty, J.C., 2001. Phytoplankton pigment distribution in relation to upper thermocline circulation in eastern Mediterranean Sea during winter. *J. Geophys. Res.* 106, 19939-19956.
- Wasmund, N., Voss, M., Lochte, K., 2001. Evidence of nitrogen fixation by non-heterocystous cyanobacteria in the Baltic Sea and re-calculation of a budget of nitrogen fixation. *Mar. Ecol. Prog. Ser.* 214, 1–14.

- Weller, R. A., Baumgartner, M. F., Josey, S. A., Fischer, A. S., Kindle, J. C., 1998. Atmospheric forcing in the Arabian Sea during 1994–1995: Observations and comparisons with climatology and models. *Deep-Sea Res Pt II: Topical Studies in Oceanography*, 45(10), 1961-1999.
- White, A. E., 2012. Oceanography: The trouble with the bubble. *Nature* 488, 290–1. doi:10.1038/nature11481
- Wiggert, J. D., Hood, R. R., Banse, K., Kindle, J. C., 2005. Monsoon-driven biogeochemical processes in the Arabian Sea. *Prog Oceanogr*, 65(2), 176-213.
- Zehr, J. P., Mellon, M. T., Zani, S., 1998. New nitrogen-fixing microorganisms detected in oligotrophic oceans by amplification of Nitrogenase (*nifH*) genes. *Appl. Environ. Microbiol.* 64, 3444–50.
- Zehr, J. P., Waterbury, J. B., Turner, P. J., Montoya, J. P., Omoregie, E., Steward, G. F., Hansen, A., Karl, D. M., 2001. Unicellular cyanobacteria fix N₂ in the subtropical North Pacific Ocean. *Nature*, 412(6847), 635-638.

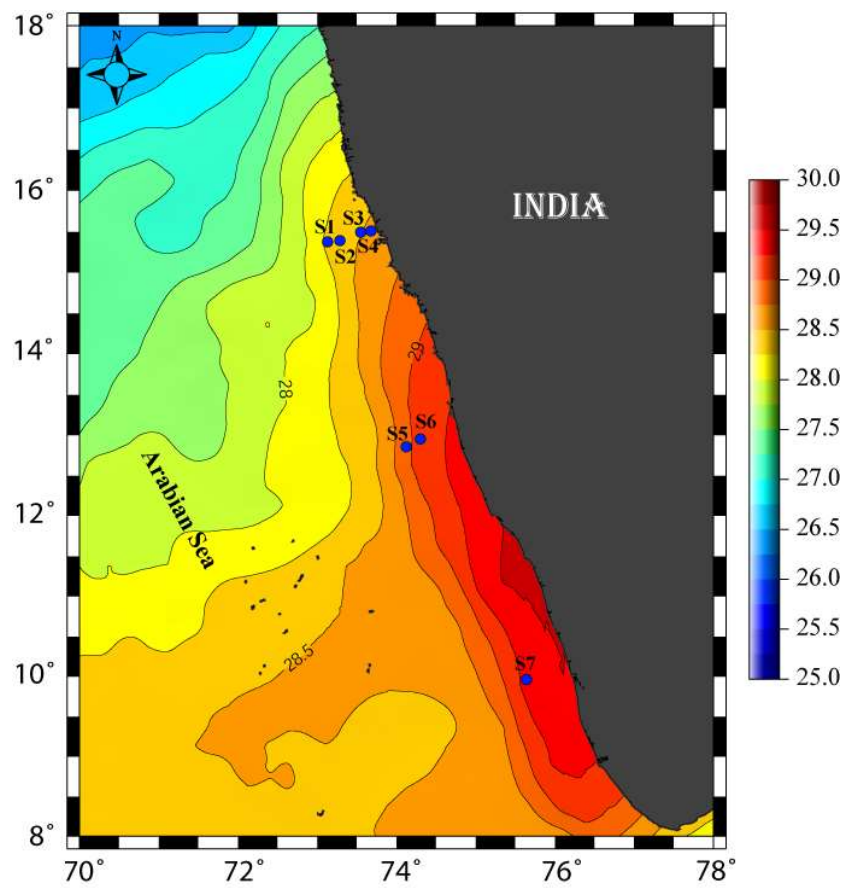


Figure 1. Satellite derived sea surface temperature (SST) (Feb. 2013) overlaid with sampling stations in the eastern Arabian Sea

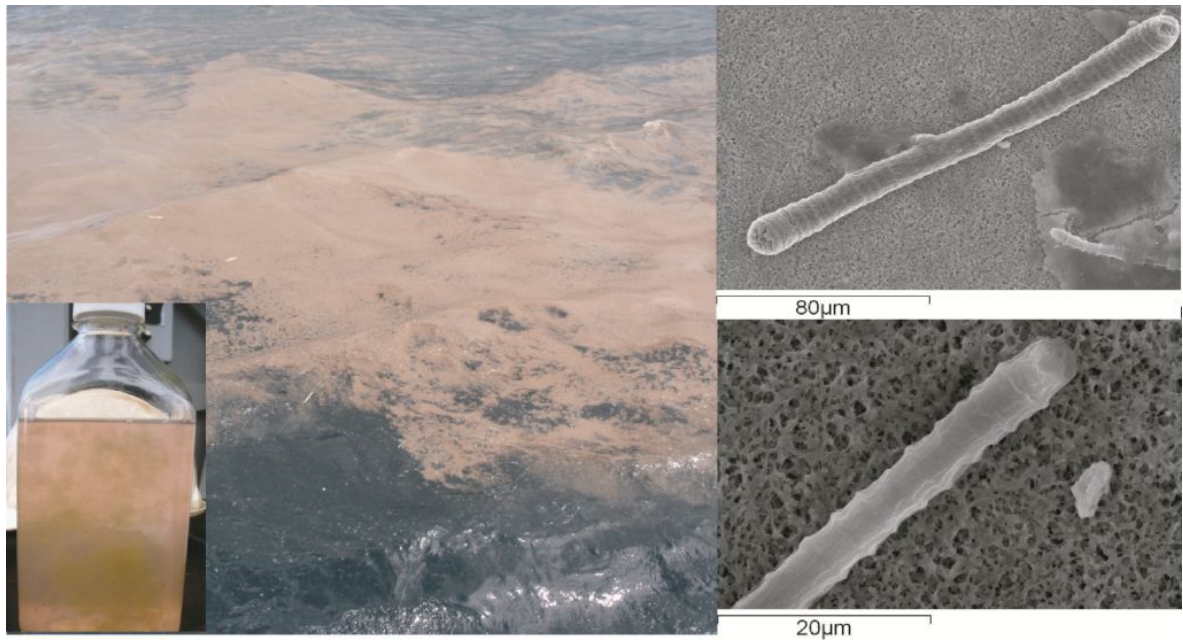


Figure 2. A view of *Trichodesmium* bloom and scanning electron micrograph of a trichome

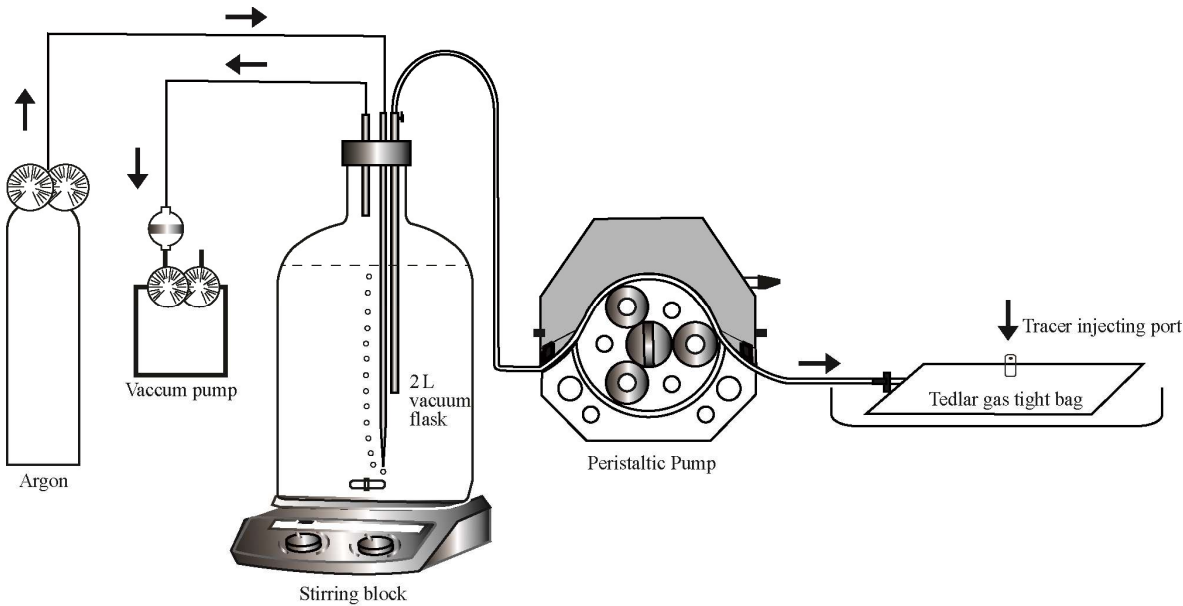


Figure 3. Schematic presentation of the experimental setup for the preparation of sea water enriched with $^{15}\text{N}_2$ gas. The apparatus include filtered sea water, purged with argon prior to vacuum degasification. The $^{15}\text{N}_2$ gas was added to the filtered sea water after it was transferred to gas tight Tedlar bag

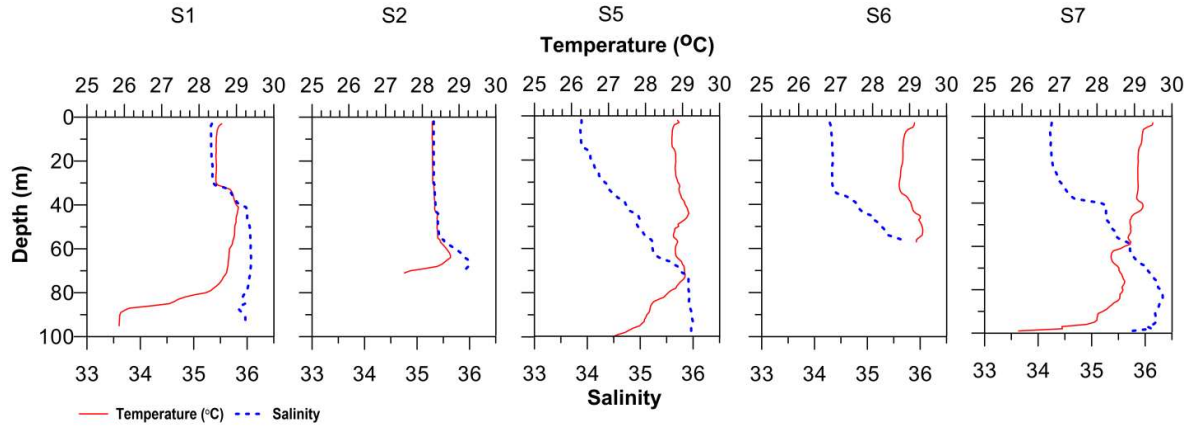


Figure 4. Vertical profiles of salinity and temperature ($^{\circ}\text{C}$) at the stations

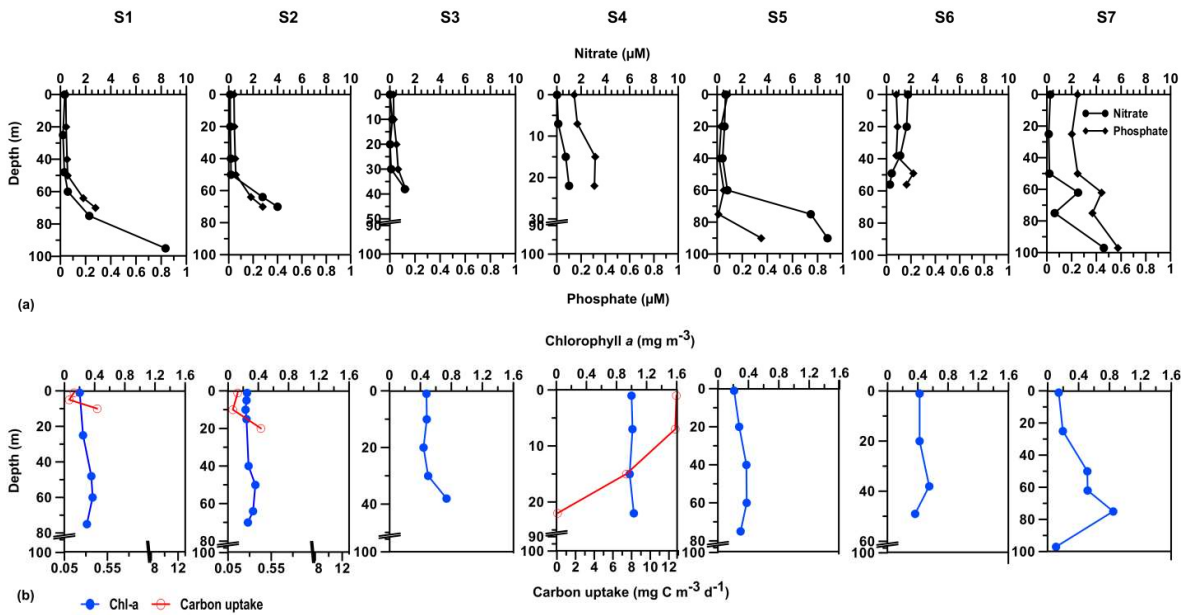


Figure 5. Vertical profiles of (a) nitrate and phosphate (μM) (b) chlorophyll a (mgm^{-3}) and carbon uptake ($\text{mg C m}^{-3} \text{d}^{-1}$), Note: Carbon uptake was measured only at S1, S2 and S4 and average values are plotted

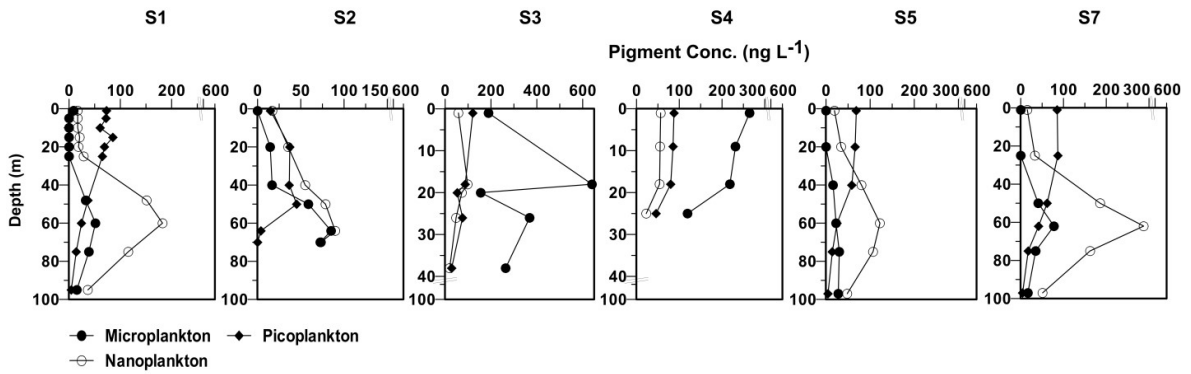


Figure 6. Vertical profiles of pigment derived fractions of microplankton, nanoplankton and picoplankton (ng L⁻¹) at the stations

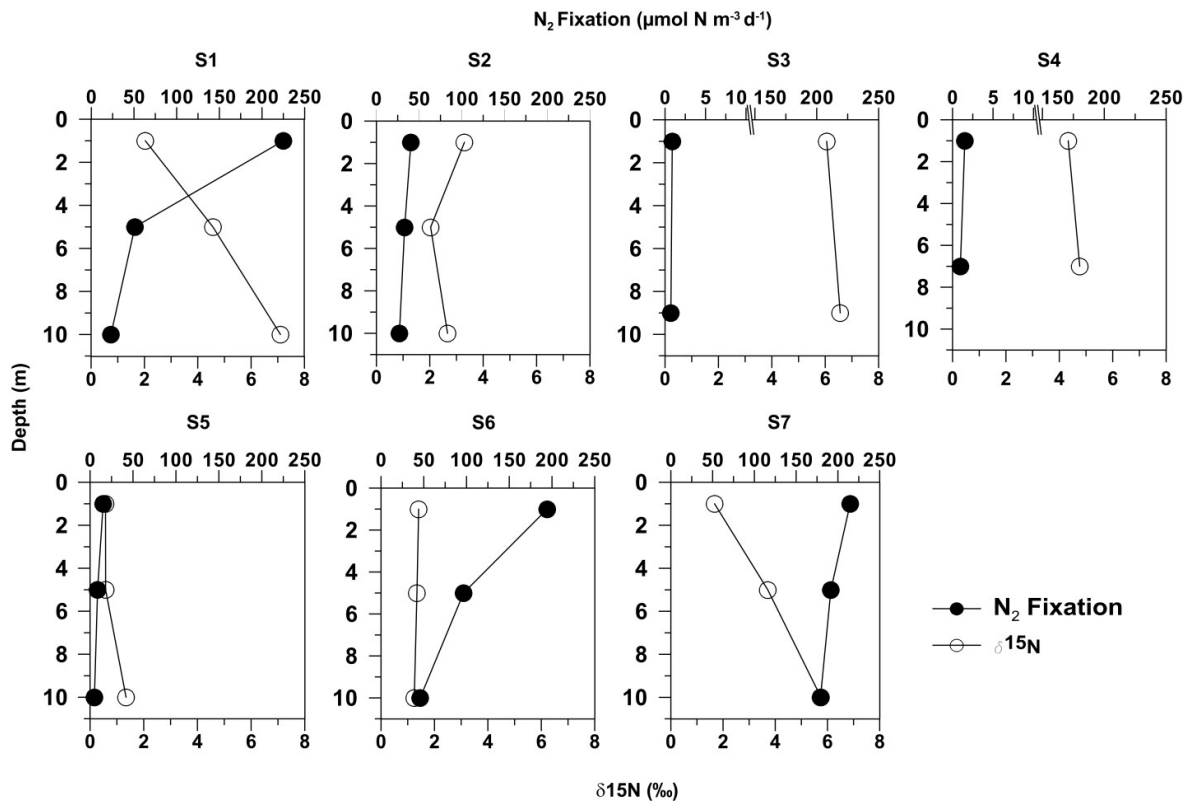


Figure 7. Vertical profiles of nitrogen fixation ($\mu\text{mol N m}^{-3} \text{d}^{-1}$) and $\delta^{15}\text{N}$ (‰) of POM at each station

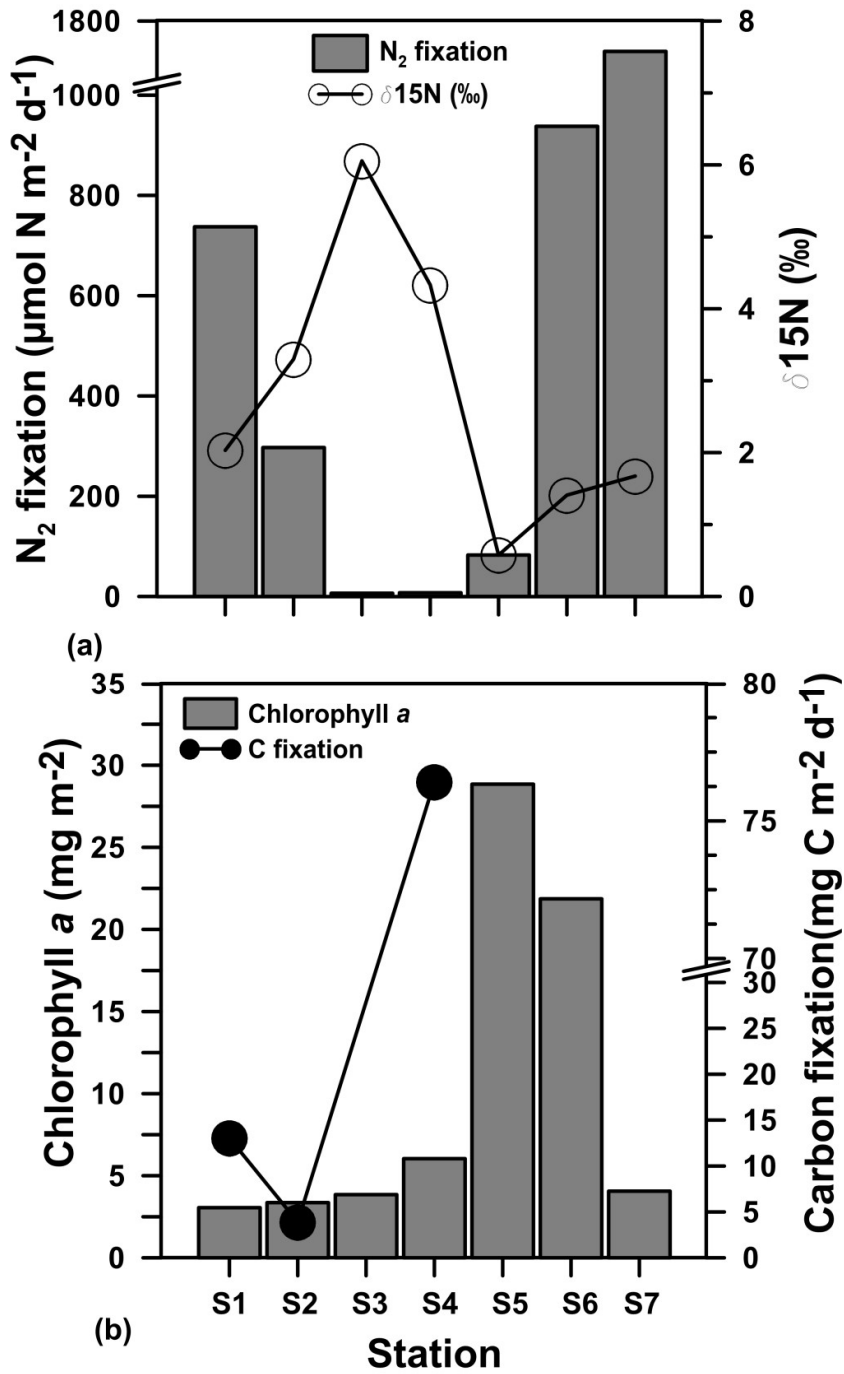


Figure 8. Depth integrated a) N_2 fixation ($\mu\text{mol N m}^{-2} \text{d}^{-1}$) and $\delta^{15}\text{N}$ (‰) of surface particulate organic matter; and b) Chlorophyll *a* (mgm^{-2}) and primary productivity ($\text{mg C m}^{-2} \text{d}^{-1}$) at various stations. Primary productivity was measured only at S1, S2 and S4 only. Please note the break in scale at the y-axis

Table 1. Dominant phytoplankton marker pigments (ng L^{-1}) at the stations

Station	Depth (m)	Fucoanthin	19 Hexanoyl oxy fucoxanthin	Diadinoxanthin	Zeaxanthin	DivinylChla
S1	1	6.5	13.3	–	85.0	67.3
	5	–	13.3	–	83.9	68.8
	10	–	13.3	3.7	70.5	48.9
	15	–	16.3	–	99.5	67.3
	20	–	14.5	3.7	80.3	64.2
	25	–	19.9	4.1	75.6	71.9
	48	23.1	103.4	7.1	42.9	96.4
	60	36.6	125.7	7.9	28.3	156.0
	75	27.4	74.3	4.9	16.3	48.9
95	10.8	21.8	–	5.1	–	
S2	1	–	13.5	–	17.4	48.9
	20	10.4	24.8	4.1	27.2	65.8
	40	12.0	38.9	4.1	17.8	61.2
	50	25.5	56.7	5.9	11.7	71.9
	64	60.3	57.2	6.3	4.5	61.2
	70	51.5	46.4	4.8	–	38.2
S3	1	134.1	38.9	25.9	89.3	–
	18	454.2	64.2	28.1	37.5	–
	20	110.2	52.9	11.1	18.9	–
	26	261.4	34.0	15.1	28.4	–
	38	187.1	13.5	11.5	5.3	–
S4	1	187.6	41.0	18.5	49.2	–
	9	164.2	39.9	16.6	48.1	–
	18	154.9	38.9	15.1	46.2	–
	25	84.7	16.2	6.6	34.1	–
S5	1	–	15.1	5.9	79.8	85.6
	20	–	27.0	7.4	76.8	108.6
	40	11.4	56.7	7.0	68.1	175.9
	60	16.1	83.6	6.6	29.1	119.3
	75	21.3	69.1	5.2	16.6	91.8
	103	19.7	28.6	–	5.3	22.9
S7	1	–	12.1	4.9	99.2	70.4
	25	–	26.0	4.1	101.0	94.8

50	20.4	125.7	10.1	71.9	192.7
62	45.7	187.4	12.0	48.7	119.3
75	24.8	104.0	6.7	20.3	64.2
97	11.8	32.6	–	5.1	18.4

Table 2. Nitrogen fixation and dominant diazotroph (s) in the euphotic zone of different Oceanic Region

Region	N₂ Fixation (μM N m⁻² d⁻¹)	Method	Dominant Diazotroph (s)	Reference
Arabian Sea <i>Trichodesmium</i> bloom	1739	N ₂ gas dissolution	<i>Trichodesmium</i> sp.	Present study
Central Arabian Sea	328±19	N ₂ gas dissolution	Unknown	Unpublished data (Ayaz et al.)
Arabian Sea	35±11.3	N ₂ gas bubble	<i>Trichodesmium</i> and 44 heterotrophic bacteria	<i>Shiozaki et al.</i> , (2014)
Arabian Sea <i>Trichodesmium</i> bloom	40	Acetylene reduction	<i>Trichodesmium</i> , outside bloom	<i>Capone et al.</i> , (1998)
Arabian Sea <i>Trichodesmium</i> bloom	129	Acetylene reduction	<i>Trichodesmium</i>	<i>Capone et al.</i> , (1998)
Arabian Sea (<i>Trichodesmium</i>)	100-34,000	N ₂ gas bubble	<i>Trichodesmium</i>	<i>Gandhi et al.</i> , (2011)
Arabian Sea (<i>Trichodesmium</i>)	24-1108	Trichome based	<i>Trichodesmium</i>	<i>Parab et al.</i> , (2012)
Equatorial and Southern Indian Ocean	10±5.47	N ₂ gas bubble	Heterotrophic bacteria	<i>Shiozaki et al.</i> , (2014)
Western North Pacific	39.2±38.3	N ₂ gas bubble	Unicellular bacteria	<i>Shiozaki et al.</i> , (2010)
Eastern North Pacific	111±66	N ₂ gas bubble	<i>Trichodesmium</i> and UCYN and diatom diazotroph association	<i>Church et al.</i> , (2009)
Western South Pacific	193±170	N ₂ gas bubble	<i>Trichodesmium</i>	<i>Shiozaki et al.</i> , (2014b)
South Pacific gyre	93.8±61.3	N ₂ gas bubble	Heterotrophic bacteria	<i>Halm et al.</i> , (2012)
North Atlantic	200	N ₂ gas bubble	<i>Trichodesmium</i> and UCYN, DDAs near the Amazon	<i>Moore et al.</i> , (2009)
South Atlantic	<20	N ₂ gas bubble	unknown	<i>Moore et al.</i> , (2009)