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 $^{15}$N tracer gas dissolution method. The measured rates varied between 0.8 and 225 $\mu$mol N m$^{-3}$ d$^{-1}$ and were higher than those reported from most other oceanic regions. The highest rates (1739 $\mu$mol N m$^{-2}$ d$^{-1}$; 0-10m) coincided with the growth phase of Trichodesmium and led to low $\delta^{15}$N (<2‰) of particulate organic matter. At stations not experiencing Trichodesmium bloom, nitrogen fixation rates were low (0.9 - 1.5 $\mu$mol N m$^{-3}$ 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 fixedN 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 (NO\(_3^–\)) 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 N\(_2\) to ammonium. Despite the high energy requirement for this process, a few cyanobacteria (especially *Trichodesmium* spp.) are capable of reducing N\(_2\) to NH\(_4^+\) (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 N\(_2\) fixation (Devassy et al., 1978, Capone et al., 1998). Surface bloom of filamentous diazotrophic cyanobacteria (*Trichodesmium* spp.) 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 (Bangeet 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 N₂ uptake rates (Dugdale et al., 1964; Capone et al., 1998), the estimated N₂ fixation rate for the region is 3.3 Tg N yr⁻¹ (Bange et al., 2000).

Previous estimates of N₂ fixation from the eastern and the central Arabian Sea were based on volumetric conversion of trichomes and ¹⁵N₂ 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 ¹⁵N₂ 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 10 L 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 μm) 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 μm) 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 depth in pre-cleaned acid-washed 250 ml 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 (5μCi). The bottles were incubated for 12 h in light using appropriate neutral density light cut-off screens. Samples were filtered after incubation through 25 mm GF/F (0.7μm) 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 $^{15}N_2$ tracer-enriched seawater is directly added. Filtered water (Acropac filter 0.2 μm) degassed with argon for 15 minutes was spiked with 1 ml $^{15}$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, Raeset 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 an 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 kPa) through a 25 mm 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 N\textsubscript{2} fixation rate, we used the following equation (Montoya et al., 1996; Mohr et al., 2010):

\[
N_2\text{ Fixation rate} = \frac{A_p^{t=0}}{A_N^{t=0} - A_N^{t=0}} \times \frac{(PN)}{\Delta t}
\]

Where A = atom\% ¹⁵N in the particulate nitrogen (PN) at the end or the beginning (t=0) of the incubation or in the dissolved N\textsubscript{2} pool (N\textsubscript{2}).

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 – 60 m) in the south (stations S5, S6 and S7). NO\textsubscript{3}⁻ 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 (< 100 m) profiles showed a maximum concentration of ~9 µM NO\textsubscript{3}⁻ at S1 and S5. Likewise, PO\textsubscript{4}⁻ 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\textsubscript{a} concentration was low (<1 mg m\textsuperscript{-3}) at the surface at sites sampled (Fig. 5b). Carbon uptake measurement showed higher rates (12.8 mg C m\textsuperscript{-3} d\textsuperscript{-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 chla)], corresponding to various phytoplankton functional groups at these stations (Table 1). Zeaxanthin (marker pigment of cyanobacteria) and div chla (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\textsuperscript{-1} in the surface water). Nutrient deficient surface waters were generally dominated by pigments (zeaxanthin and div chla)
derived from picoplankton size fraction (69-85 ng L\(^{-1}\)) at all the stations except at S3 and S4 (off Goa), which were dominated by microplankton fractions (189-264 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 µmol N m\(^{-3}\) 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 µmol N m\(^{-3}\) d\(^{-1}\)) than those at the bloom stations. Interestingly, the rates were moderately high (15 µmol N m\(^{-3}\) d\(^{-1}\)) in the region having thick *Trichodesmium* bloom (S5), and the highest rates (214 and 225 µmol N m\(^{-3}\) 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 mg C m\(^{-3}\) d\(^{-1}\), Fig 8a). Primary production rates at S4 were an order of magnitude higher than at the other two stations (<0.1 mg C m\(^{-3}\) d\(^{-1}\)).

The column integrated nitrogen fixation, carbon uptake rates and Chla are shown in Figures 8a and 8b. N\(_2\) fixation rates integrated over the top 10 m varied significantly (6 to 1739 µmol N m\(^{-2}\) 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 µmol N m\(^{-2}\) d\(^{-1}\)). The depth-integrated carbon uptake rate in upper 20 m of the water column varied between 4 and 76.4 mg C m\(^{-2}\) 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 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 δ\(^{15}\)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; Sankaranarayananet al., 1978; Naqvi and Jayakumar, 2000; Wiggertet al., 2005; Habeebrehmanet 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$_4^{3-}$ 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 (Chla) 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 mg C m$^{-2}$ d$^{-1}$)
where bloom was not observed. Carbon uptake rate in the surface water at this station was more elevated (12.8 mgCm$^{-3}$d$^{-1}$) than at station S1 (0.17 mgCm$^{-3}$d$^{-1}$). Unfortunately, carbon uptake measurements could not be made at other stations due to logistic reasons. Parab et al. (2012) reported primary productivity up to 3.4 gCm$^{-3}$d$^{-1}$ subsequent to the *Trichodesmium* bloom in the Arabian Sea. High rates of primary productivity (804-1080 mgCm$^{-2}$d$^{-1}$) has also been reported from other areas 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 mgCm$^{-2}$d$^{-1}$ (Bauerfeind, 1987).

The N$_2$ fixation rate varied widely, ranging between 0.8 and 225 µM Nm$^{-3}$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; Shiozaki et al., 2014) and North Pacific Ocean (Church et al., 2009). However, Gandhi et al. (2011) and Parab 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 $^{15}$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 $^{15}$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 µmolNm$^{-2}$d$^{-1}$). The highest rate was recorded from station S7 (1739.5 µmol Nm$^{-2}$d$^{-1}$) followed by S6 and S1 (938 and 737 µmolNm$^{-2}$d$^{-1}$ respectively) (Fig.8a) coinciding with the seeding of *Trichodesmium* sp. Nitrogen isotopic composition ($\delta^{15}$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 $\delta^{15}$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 $\delta^{15}$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 $\delta^{15}$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 $\delta^{15}$N associated with
Trichodesmium. However, the lowest $\delta^{15}$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 $\mu$molNm$^{-2}$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 (Needobaet al., 2007), estuarine and coastal waters (Short and Zehr, 2007; Rees et al., 2009; Grosse et al., 2010; Bombaret al., 2011; Bhavyaet al., 2015), upwelling areas (Moutinet 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 $\mu$M) of nitrate do not suppress $N_2$ fixation in diazotrophic cyanobacterium Crocosphaera (Dekaezemacker 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; Zehret al., 2001; Montoya et al., 2004) and marine heterotrophs may also be an importance 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 nonTrichodesmium 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 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 al., 2011). Saharan dust deposition is suggested to cause *Trichodesmium* bloom as far as off the Florida coast (Leneset 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; Codispotiet 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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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 $^{15}$N$_2$ gas. The apparatus include filtered sea water, purged with argon prior to vacuum degasification. The $^{15}$N$_2$ gas was added to the filtered sea water after it was transferred to gas tight Tedlar bag.

![Schematic presentation of the experimental setup for the preparation of sea water enriched with $^{15}$N$_2$ gas.](image)

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

![Vertical profiles of salinity and temperature ($^\circ$C) at the stations.](image)

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

![Vertical profiles of nitrate and phosphate (µM), chlorophyll a (mg m$^{-3}$) and carbon uptake (mg C m$^{-3}$ d$^{-1}$).](image)
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 ($\mu$mol N m$^{-3}$ d$^{-1}$) and $\delta^{15}$N (‰) of POM at each station.
**Figure 8.** Depth integrated a) N$_2$ fixation (µmol N m$^{-2}$ d$^{-1}$) and δ$^{15}$N (‰) of surface particulate organic matter; and b) Chlorophyll $a$ (mg m$^{-2}$) and primary productivity (mg C m$^{-2}$ 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

<table>
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<th>Depth (m)</th>
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<th>Diadinoxanthin</th>
<th>Zeaxanthin</th>
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