UNIVERSIDAD DE LAS PALMAS
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Diazotrophic activity of *Trichodesmium* and unicellular cyanobacteria in the subtropical Northeast Atlantic

Actividad diazotrófica de *Trichodesmium* y cianobacterias unicelulares en el Atlántico Nordeste subtropical

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Memoria de investigación presentada por Mar Benavides Gorostegui para optar al grado de suficiencia investigadora por la Universidad de Las Palmas de Gran Canaria.

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“Assumptions about systems in steady state have given way to the recognition that nothing is constant except change”

Michaels *et al.*, 2001.

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Abstract

Fractionated (> and <10 μm) diazotrophic activity and *Trichodesmium* abundance were studied in a macroscale cruise enclosing the boundary current system of the NE Atlantic and during two mesoscale experiments in the upwelling systems of Cape Silleiro (NW Iberia) and Cape Ghir (NW Africa). The abundance and N_2 fixation rates of *Trichodesmium* were low (<0.5 filaments/l and <0.2 μmol N m^{-2} d^{-1}) and only accumulated at frontal sites, such as the Azores Front or the edge of Cape Ghir's filament. Mean N_2 fixation rates in the macroscale phase were 0.19 nmol N l^{-1} d^{-1}, comparable to those obtained off Cape Silleiro (0.09 nmol N l^{-1} d^{-1}) and off Cape Ghir (0.1 nmol N l^{-1} d^{-1}). Unicellular cyanobacteria provided ~60% and ~80% of total N_2 fixation in the macroscale and mesoscale cruise phases, respectively. The predominance of <10 μm diazotrophic activity suggests a wider distribution and activity of these organisms than previously thought.

Keywords: N_2 fixation, *Trichodesmium*, diazotrophy, cyanobacteria, upwelling filaments

1. Introduction

Diazotrophs fix atmospheric nitrogen (N_2) into ammonium (NH_4^+) which can be ultimately transformed into substrates available for planktonic autotrophic communities. Most of the open oceanic areas are oligotrophic, being nitrogen (N) the main limiting factor for primary production (Dugdale, 1967). Diazotrophic organisms have proven to provide new N in warm and nutrient impoverished oceanic areas, permitting primary production in long periods of stratified water column (Michaels et al., 1994). Ever since N_2 fixation in marine planktonic cyanobacteria was discovered (Dugdale et al., 1964) and the first depictions of an excess of N in the ocean were attributed to diazotrophy (Michaels et al., 1996; Gruber and Sarmiento, 1997), many studies have arisen and pointed towards a greater contribution of N_2 fixation to new production than it was previously thought. Hence, discussion on the potential role of this process as a response to marine N cycle disequilibria is continuously growing. Considered to be the principal diazotroph in the ocean, the filamentous non-heterocystous cyanobacterium *Trichodesmium* has been target of intensive research, especially in the last two decades. Nonetheless, recent discoveries have broadened our conception of oceanic diazotrophy and given way to a possible significant contribution of small unicellular diazotrophs not restricted to tropical or severely oligotrophic areas (Zehr et al., 2001; Falcón et al., 2004; Moisander et al., 2010).

The N and carbon cycles are inextricably connected and the influence of one on another could be even more critical in the future than it has been until present. Among the consequences of climate change, ocean stratification, expansion of Oxygen Minimum Zones (OMZs) (Stramma et al., 2008; Paulmier and Ruiz-Pino, 2009) and increase of desert dust deposition, all would lead to an enhancement of N_2 fixation which constitutes an entrance of new N to the oceans (Michaels et al., 2001), eventually leading to an increasing withdrawal of atmospheric carbon dioxide (Tyrrell, 1999; Karl et al., 2002). N is removed in the OMZs and sediments through denitrification, but the measured rates of this process exceed the N inputs to the surface oceans available in the literature so far (e.g. Falkowski, 1997; Codispoti, 2007). Notwithstanding, some modelling efforts have suggested that there is not such an imbalance and that the loss of nitrogen in OMZs and sediments is in good agreement with N_2 fixation in nearby areas (e.g. Gruber, 2004; Deustch et

al., 2007). N_2 fixation rates available to date through geochemical and direct methods have resulted in quite distant numbers. Geochemical approaches may be overestimating diazotrophy due to isotopic fractionation (Altabet et al., 1988), preclusion of the Dissolved Organic Nitrogen (DON) influence (Mahaffey et al., 2005) or interferences with significant inputs of atmospheric fixed N (Zamora et al., 2010). Biologically-derived rates could be overestimated when regional data are extrapolated to larger areas (e.g. extrapolation of *Trichodesmium* N_2 fixation in the Caribbean Sea resulting in biased rates over the North Atlantic), or underestimated if small unicellular forms, endosymbiont diazotrophs and DON release are not taken into account (Bronk et al., 1994; Zehr et al., 2001; Montoya et al., 2007). Although new approaches are narrowing the differences between both approaches (Hansell et al., 2004), still, the steady state of the N cycle is doubtful and its unbalances are bound to have a direct impact on global carbon cycling.

Both the availability of iron through aeolian dust inputs and the growing evidence of an excess N signal (through N* and $\delta^{15}N$ parameters) have pointed towards a significant contribution of N_2 fixation to new and export production in the North Atlantic (e.g. Gruber and Sarmiento, 1997; Mahaffey et al., 2003). Particularly, the NE Atlantic region would be ideal due to its closeness to the Sahara desert. Nevertheless, diazotrophy may be restricted in this area due to the upwelling of cold nutrient-rich waters in its eastern boundary. The importance of N_2 fixation has been highlighted in several geochemical studies conducted in this region (Mahaffey et al., 2003; Álvarez and Álvarez-Salgado, 2007; Bourbonnais et al., 2009), whereas direct evidences based on biological methods have been majorly conducted on the west side of the basin, i.e. Caribbean and Sargasso seas (e.g. Orcutt et al., 2001; Falcón et al., 2004; Capone et al., 2005). The few West-East cross-basin studies available have focused on tropical areas (Voss et al., 2004; Montoya et al., 2007; Staal et al., 2007). Montoya et al. (2007) gathered a set of data from cruises conducted between 1996 and 2001, mostly over the tropical North Atlantic. They concluded that whilst *Trichodesmium* presented higher rates of N_2 fixation in the western part of the basin, its prevalence diminished towards the East, where unicellular diazotrophs became more important. Interestingly, Voss et al. (2004) found an opposite trend over the 10ºN parallel. This discrepancy might be explained by the fact that these authors used CTD-rosette systems to collect bulk seawater, a method which potentially undersamples *Trichodesmium* (Chang, 2000).

Therefore, the higher rates found over the western side of the Atlantic would agree with the conclusions drawn by Montoya et al. (2007). However, these authors stated that by the combination of all the data, N_2 fixation rates resulted in non statistically differences across the West-East transect. As an explanation, they suggested that rates are constant over the Atlantic, while protagonists change from one to the other side of the basin responding to better adaptation to environmental constraints such as temperature, nutrient availability and aeolian dust inputs.

Given that most studies regarding N_2 fixation have been restricted to the tropics, that the results obtained from geochemical and direct estimates are substantially different and that the extension of diazotroph communities in the ocean seems to be wider than previously thought, the interpretation of subtropical NE Atlantic diazotrophic activity remains unsatisfactory. More direct method studies including the whole range of diazotrophic organisms are needed in order to reassess the true contribution of diazotrophy and to better constrain N budgets. Here, we evaluate size-fractionated and *Trichodesmium* diazotrophic activity in the oligotrophic waters of the subtropical NE Atlantic. We also give, to our knowledge, the first estimations of diazotrophy in two active upwelling regions: Cape Silleiro (NW Iberia) and Cape Ghir (NW Africa).

2. Materials and methods

2.1. Sampling and hydrographic measurements

During the summer of 2009 the subtropical NE Atlantic was sampled within the framework of the project 'Shelf–ocean exchanges in the Canaries–Iberian Large Marine Ecosystem' (CAIBEX), onboard B/O Sarmiento de Gamboa. The cruise was divided in 3 legs: 2 mesoscale experiments, one off Cape Silleiro (6^{th}-24^{th} July, Galicia), the other off Cape Ghir (16^{th} August-5^{th} September, Morocco) and a macroscale phase (named CAIBOX, 25^{th} July-14^{th} August) covering the whole region of study (figure 1).

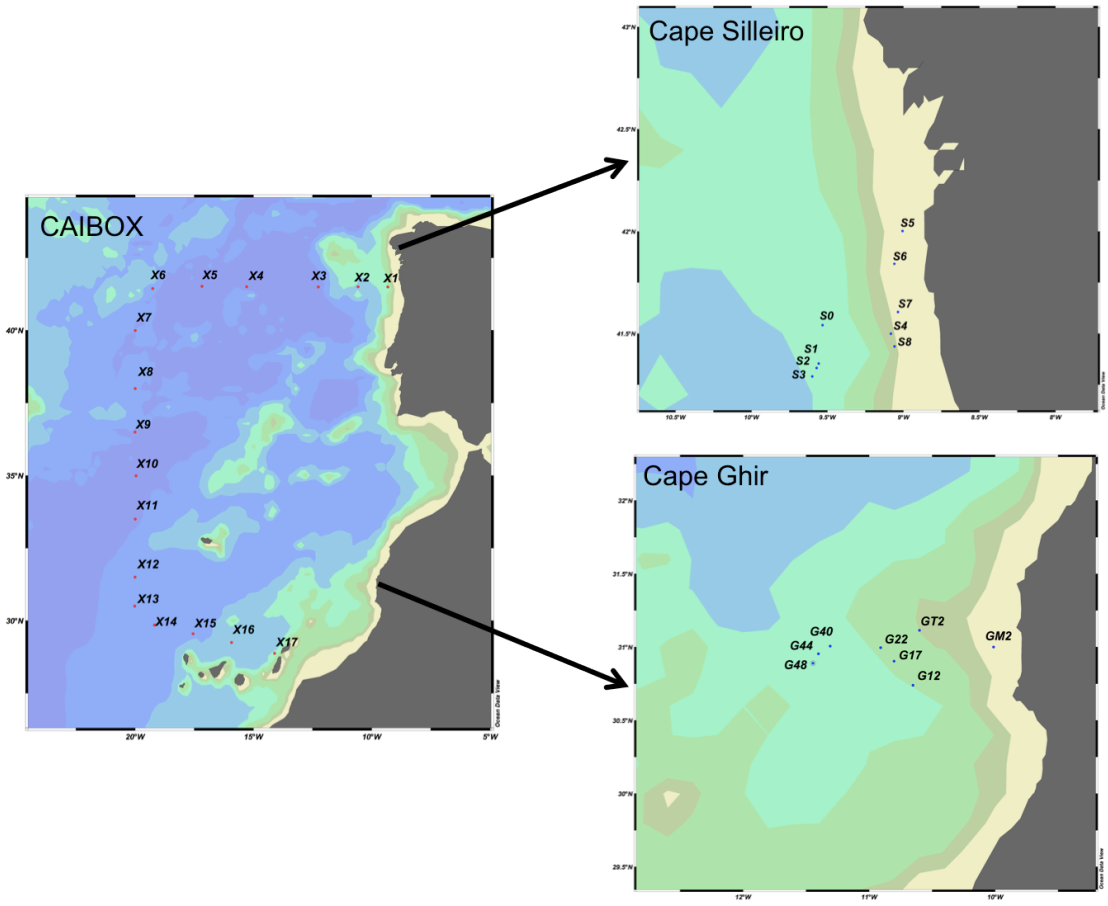

Figure 1: Study areas maps and location of sampled stations.

Cape Silleiro and Cape Ghir are sites where upwelling filaments typically develop (Barton et al., 1998; Torres et al., 2003). With the aim of tracking the biological evolution of these structures, Lagrangian experiments were carried out. For these, drifting buoy experiments were performed during the mesoscale legs of the cruise. The buoys were deployed in the morning and allowed to drift for 24 h after which they were recovered, sampled and deployed again to drift for another day. N_2 fixation assays were conducted at the same positions were buoys were deployed/recovered during the Lagrangian experiments. The filament off Cape Silleiro did not develop at the time of the cruise so the stations sampled in this first leg rather correspond to active upwelling (stations S05 to S08) and open ocean (stations S01 to S03) situations. In the other hand, a marked upwelling filament fully developed during the Cape Ghir cruise leg (figure 2) and the stations sampled represent its evolution path along 6 days. Finally, the 17 stations

sampled during the CAIBOX cruise cover the NE Atlantic upwelling region, starting off Cape Silleiro, sailing southwards along 20ºW and ending near the strait between the islands of Lanzarote and Fuerteventura (Canary Islands archipelago).

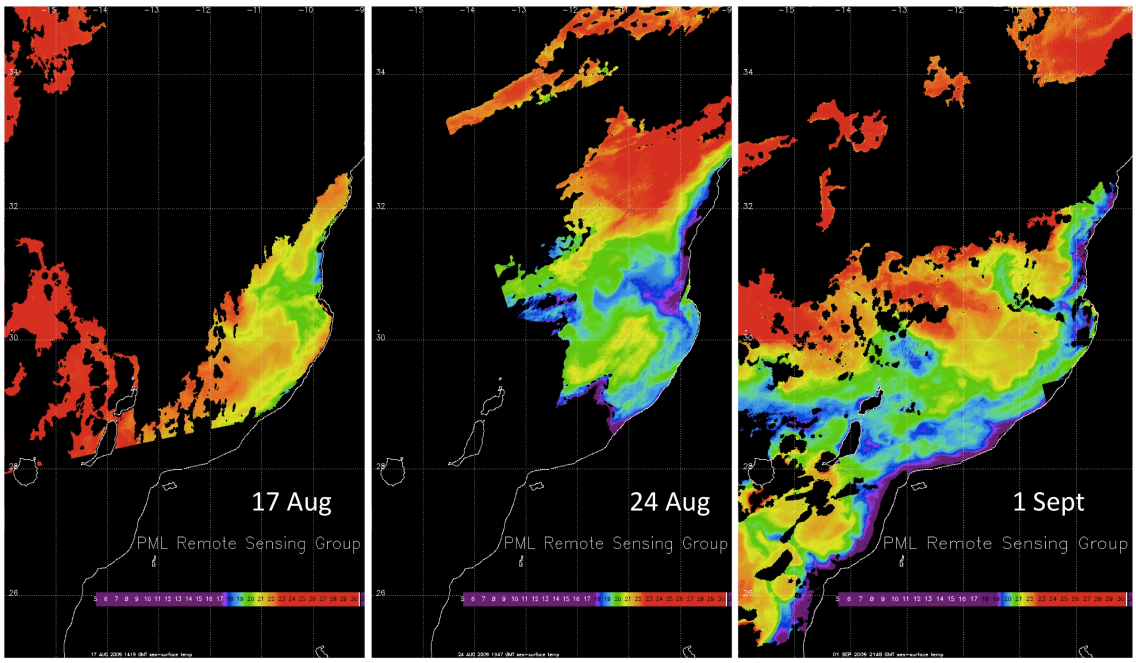

Figure 2: Cape Ghir upwelling filament evolution as depicted by Sea Surface Temperature (SST) detected by Advanced Very High Resolution Radiometer (AVHRR). Courtesy of the Plymouth Marine Laboratory Sensing Group.

2.2. N_2 fixation experiments and plankton net casts

Diazotrophic activites were measured by the Acetylene Reduction Assay (ARA) (Capone, 1993). Water from the surface (~5 m) was collected using 12 l Niskin bottles mounted on a General Oceanics rosette sampler between 9 and 11 am (UTC). On-deck incubations were performed with flow-through surface seawater. The incident light in the incubators was kept to its 80% with attenuating panels. For the ARA method, 6 replicate samples of 2 l were filtered through 25 mm Whatman GF/F filters. The filters were placed in 10 ml headspace crimp vials, moisturised with ~0.5 ml of filtered (GF/F) seawater and sealed with a rubber stopper and an aluminium seal cap using a seal crimper. After sealing, 2 ml of acetylene were injected to each of the samples using gas-tight Hamilton syringes. Of the 6 replicates, 3 were incubated in the light and 3 in the dark for

a 3 h period. Vertical hauls were performed at each station with a 55 μm plankton net. The net was deployed 2-3 times down to the DCM and recovered at a mean speed of 20 m/min. The samples were preserved with 10% buffered formaldehyde and stored in the dark at ambient temperature until they were examined for the abundance of *Trichodesmium* in the laboratory by means of an inverted microscope. A fraction of the biological material collected with the net was filtered (10-30 ml) and equally processed for the ARA, ideally corresponding to N_2 fixation rates by *Trichodesmium* or diatom endosymbiont cyanobacteria.

After the incubation, 10 ml of headspace were removed from the sealed vials and transferred to pre-vacuumed Hungate tubes which were finally sealed with thermofusible glue and stored in the dark at ambient temperature until analysis. The acetylene and ethylene content of samples was measured with an HP 5890 Gas Chromatograph equipped with a flame-ionization detector (FID). The number of light and dark hours derived from station geographical position and sampling time were considered to calculate N_2 fixation diel rates. Acetylene reduction rates were converted to N_2 fixation rates using a factor of 4:1 and equations in Stal (1988).

3. Results

3.1. Distribution and abundance of *Trichodesmium*

The abundance of *Trichodesmium* was quite low across the whole area of study and mainly made up of free trichomes with fewer colonies, either puffs or tufts (figure 3). The predominance of free trichomes over colonies could be caused by the roughness of net tows (Davis and McGillicuddy, 2006) or, more unlikely, due to disaggregation inside the sample containers between the time of collection and the time the organisms were counted. Counts were performed within 3 months after collection, which is a short period for the degradation of samples.

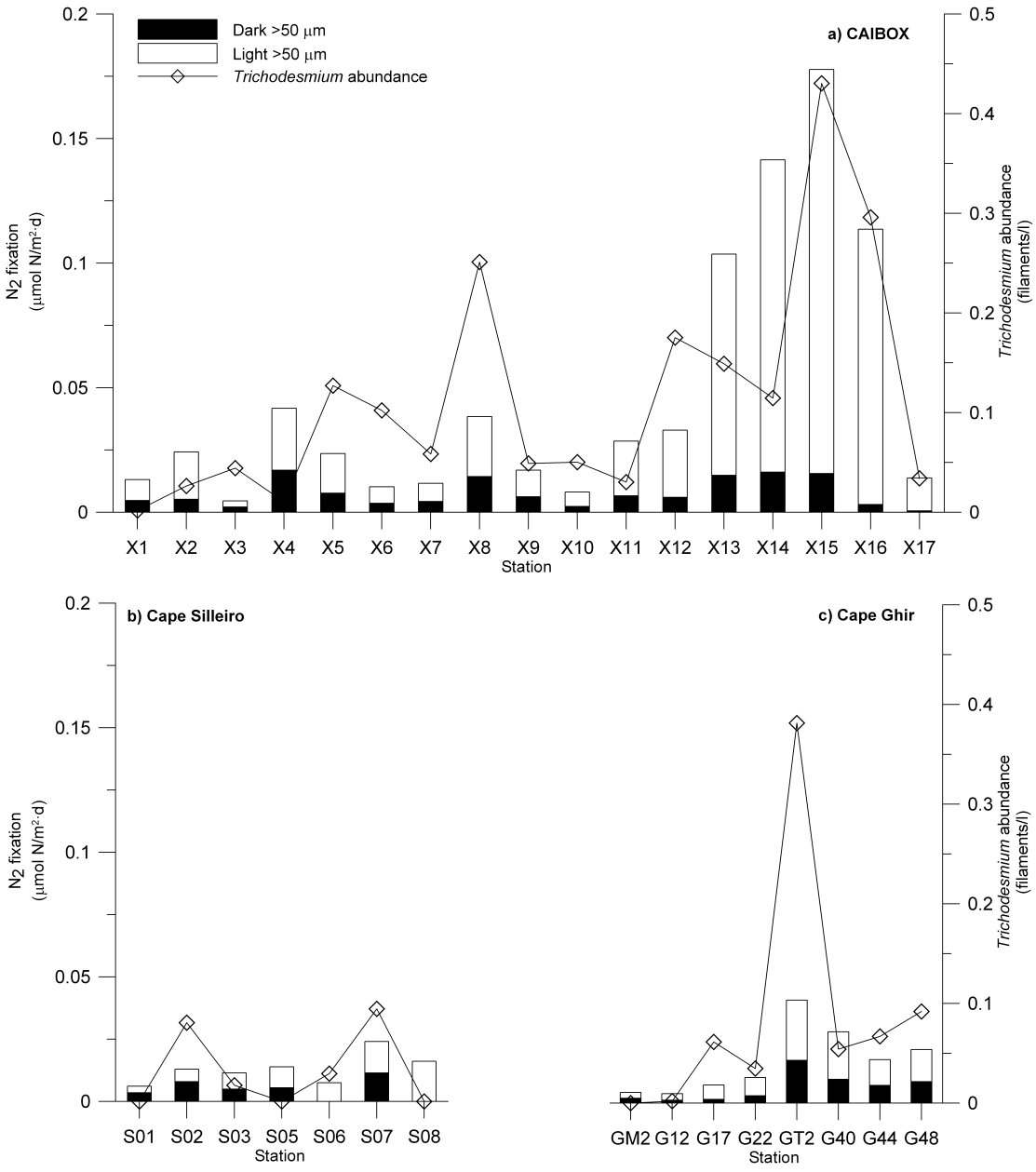

Figure 3: *Trichodesmium* abundance and N_2 fixation rates during (a) CAIBOX, (b) Cape Silleiro and (c) Cape Ghir cruise legs.

The abundance of *Trichodesmium* in the CAIBOX increased from the Galician coast to the South, ranging from 0 to a maximum of 0.43 filaments/l at station X15. A peak of 0.25 filaments/l was found at station X8, coinciding with the Azores Front (AF), which was approximately situated at 37º 30' N during our cruise. The distribution of *Trichodesmium* in Cape Silleiro was rather patchy if we consider the upwelling status at the different stations sampled. Free trichome abundance

was similar off Cape Ghir. Only station GT2 presented a maximum of 0.38 trichome/l, which corresponded to the frontal area at the edge of the filament and thus it is plausible that free trichomes accumulated there.

3.2. Fractionated N_2 fixation activity

N_2 fixation rates increased gradually from 0.09 nmol/l·d in the Galician coast to a maximum of 0.36 nmol/l·d in the AF. A nitrogenase activity drawdown was observed at station X6 (where only bulk rates are available, figure 4a). The AF clearly marked a frontier between lower N_2 fixation rates in the northern part of the CAIBOX and average higher rates in its southern part, which decreased abruptly at stations X16 and X17 with rates of 0.06 and 0.05 nmol/l·d, respectively. Although the contribution of different fractions incubated in the light or in the dark at each station remained fairly constant along the CAIBOX transect, <10 μm diazotrophic activity was the greatest. The highest rates found corresponded to incubations in the light. The activity of this fraction appeared to be greater at sites with marked temperature gradients, such as the coast-ocean transition off Galicia, the AF and the last stations North of the Canary Islands, closer to the African coast. The contribution of dark-incubated >10 μm diazotrophs to total N_2 fixation varied greatly throughout the transect, but light-incubated showed considerably steady values, specially towards the southern part of the CAIBOX.

Both the Cape Silleiro and Cape Ghir cruise legs represent upwelling conditions. Nevertheless, due to the absence of an upwelling filament during the cruise off the Galician coast, stations sampled off Cape Silleiro represent upwelling and off-upwelling conditions, while the stations off Cape Ghir are located over the track of an upwelling filament, plus 2 more stations, near the coast (GM2) and at the edge of the filament (GT2). Off Cape Silleiro, N_2 fixation in the dark dominated over light incubations in the non-upwelling stations (S01-S03; note that fractionated rates are not available for these stations). The rates of dark N_2 fixation remained in the same range in the upwelling-affected stations with a major contribution of >10 μm diazotrophs, but in these last stations a high activity was found in the light incubations. Diazotrophs >10 μm were responsible for these rates, but the picture changed completely in the last station S08, where >10 μm diazotrophy was undetectable and substituted by a <10 μm N_2 fixation rate as high as 0.2

nmol/l·d. The contribution of dark >10 μm diazotrophs was undetectable along all the upwelling-affected stations of Cape Silleiro, providing only a small percentage at station S08.

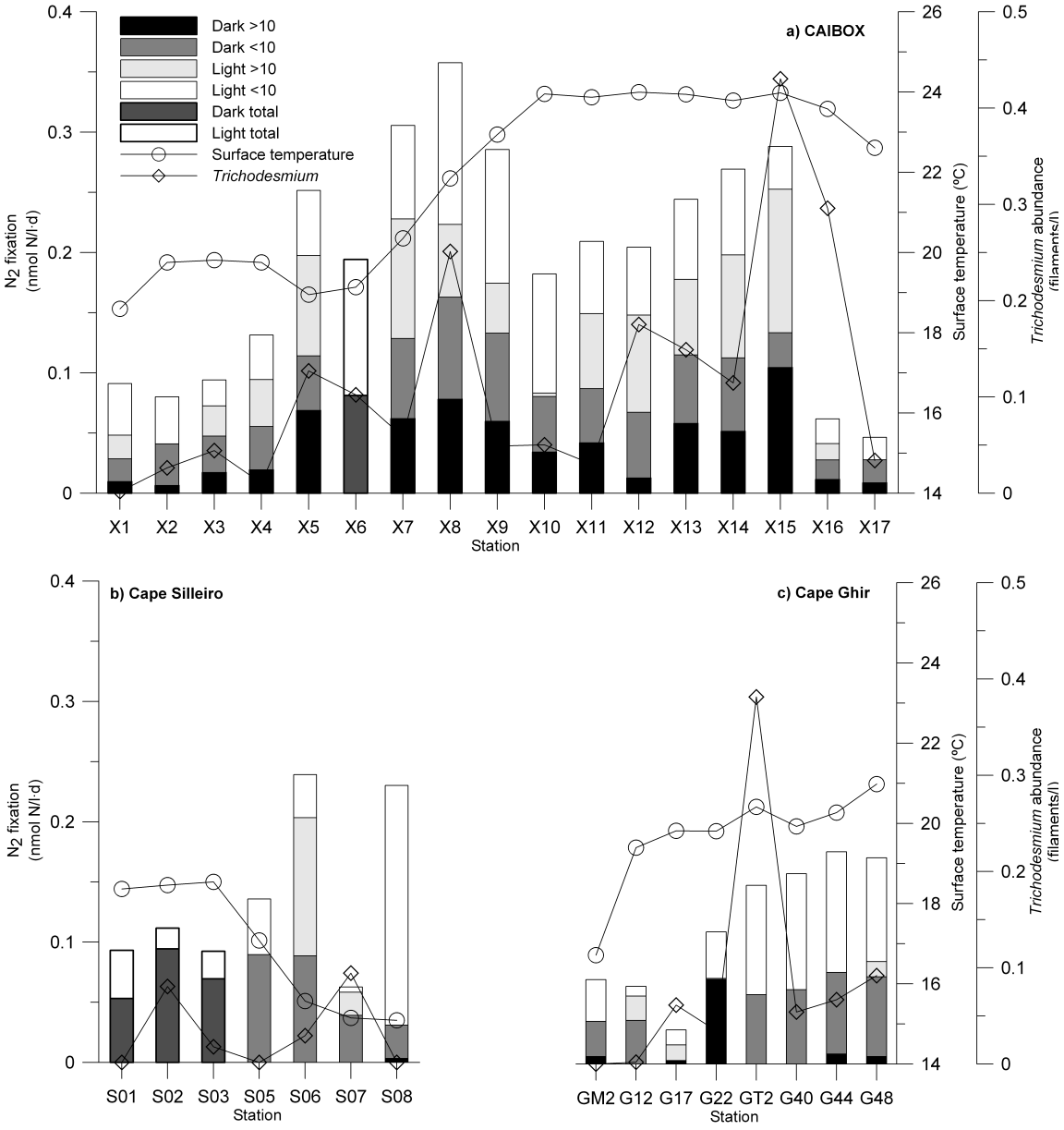

Figure 4: Fractionated light and dark N_2 fixation rates, and surface temperature during (a) CAIBOX, (b) Cape Silleiro and (c) Cape Ghir cruise legs.

As abovementioned, the situation found in Cape Ghir was quite different to that of Cape Silleiro, not only hydrographically, but also with regards to diazotrophic activity. Dark-incubated activity

was greater off Cape Silleiro, but light-activity increased towards the upwelling-affected area. On average, total rates were lower in Cape Ghir than in Cape Silleiro. Diazotrophic <10 μm in-the-light activity dominated in Cape Ghir, but both light and dark activity increased along the coastal-offshore path of the filament, following a rise in temperature. The activity of >10 μm was minimal or undetectable during this cruise. Comparing the first drifter experiment (stations G12 to G22) to the second (stations G40 to G48), a general rising of diazotrophic activity was observed, only truncated by a drop at station G17.

N_2 fixation rates from net samples (>50 μm) are presented as areal rates (i.e. μmol/m^2·d, figure 3) given that net tows necessarily integrate the water column above the DCM. The rates associated with this fraction where fairly low along the whole study. Significant rates where only found in the southern part of the CAIBOX, from station X13 to X16 (0.1-0.18 μmol/m^2·d). *Trichodesmium* abundance correlated well with light-incubated >50 μm N_2 fixation during the CAIBOX and Cape Ghir cruise legs (r^2 = 0.47 and 0.63, respectively, both $p<0.05$), but not in Cape Silleiro (r^2 = 0.28, $p>0.05$, not significant). Samples >50 μm incubated in the dark provided a small percentage of total N_2 fixation at all stations, with only two exceptions in the upwelling of Cape Silleiro (S06 and S08). Since non cyanobacterial-diatom symbioses where found during the inspection of the samples recovered with the net, we cannot directly ascribe >50 μm dark N_2 fixation to these organisms, but rather relate it to the possible presence of other diazotrophs trapped due to net clogging, diazotrophic gut flora of zooplankton (Proctor, 1997) and/or particle adsorption.

Table 1: Summary of the contribution of different fractions to total N_2 fixation rates in the different cruise phases.

	% contribution to total N_2 fixation		
	<10 μm	**>10 μm**	**>50 μm**
CAIBOX	57.92	41.73	0.35
Cape Silleiro	79.31	19.79	0.90
Cape Ghir	78.05	21.61	0.35
All cruise legs	**66.72**	**32.85**	**0.43**

Pooling all the data, we observed that <10 μm diazotrophs provided up to ~80% of total N_2 fixation in the Iberian and NW African upwelling systems (table 1), ~20% was supplied by >10 μm

diazotrophs, while *Trichodesmium* could only account for <1%. In the more oligotrophic and warm waters of the CAIBOX, < and >10 μm N_2 fixation rates were closer (57.92 and 41.73 %, respectively), but >50 μm activity was still low.

4. Discussion

Diazotrophy has been long assumed to be restricted to >20ºC, oligotrophic waters (Stal, 2009). Although the N_2 fixation rates measured during this study are lower than those predicted by geochemical and modelling studies conducted over the region (summarised in Mahaffey et al., 2005), diazotrophic activity revealed to be broadly distributed and present in areas where it was assumed to be negligible, such as coastal upwelling sites.

Iron is a cofactor of the nitrogenase enzyme complex and therefore limits N_2 fixation (Berman-Frank et al., 2001). The proximity of the Sahara desert and the subsequent input of iron through aeolian deposition makes the NE Atlantic a potential site of high diazotrophic activity (Gruber and Sarmiento, 1997). *Trichodesmium* could dominate diazotrophic activity in the eastern side of the Atlantic due to its high cellular Fe quotas (Kustka et al., 2003). However, our results indicate that unicellular diazotrophs predominate as they provided >50% of total N_2 fixation in all the sampled areas during our cruise (table 1). The abundances of *Trichodesmium* found in our study were very low (<0.5 filaments/l). Our results are comparable to those obtained by other authors in the NE Atlantic (Moore et al., 2009; González-Taboada et al., 2010; Fernández et al., 2010) but not to those by Tyrrell et al. (2003), who reported the absence of *Trichodesmium* at many stations along 20ºW in the same latitudes as our CAIBOX transect. These authors took their samples using Niskin bottles. Besides the fact that the use of bottles result in underestimations of *Trichodesmium* abundance (Chang, 2000), the volume taken in their study (50-100 ml) might not be enough to assess the presence of *Trichodesmium* at sites where its abundance is so low.

Since non diatom-cyanobacteria simbionts were found during the inspection of samples recovered with the 50 μm net, we can attribute the low N_2 fixation rates derived from these

incubations to *Trichodesmium* (<0.2 μmol N m^{-2} d^{-1}, figure 3). The majority of the *Trichodesmium* organisms counted appeared as free trichomes with an average of 100 cells per filament. If we consider a mean N_2 fixation cell-specific rate of ~0.96 nmol N $cell^{-1}$ d^{-1} (rates summarised in Mulholland et al., 2006) we can say that our results indicate much lower cell-specific N_2 fixation (0.15 pmol N $cell^{-1}$ d^{-1} on average). The abundances found in this work were always below 0.5 filaments/l and only peaked at frontal sites, such as the AF or Cape Ghir filament edge (figure 3(c)). This low abundance and activity suggest that the *Trichodesmium* populations found had drifted from elsewhere rather than actively growing *in situ*.

Recently, molecular approaches permitted the identification of planktonic diazotrophic unicellular cyanobacteria (Zehr et al., 2001). These organisms appear to be widely distributed and actively fixing N_2 in the oceans yet their contribution to global N cycling is unkown (Falcón et al., 2004; Montoya et al., 2004; Moisander et al., 2010). In our study, the <10 μm fraction provided ~60% and up to 80% of total N_2 fixation (macroscale and mesoscale phases, respectively, table 1). The warmer and oligotrophic waters of the North Atlantic Subtropical Gyre might provide a better habitat for colonial diazotrophs. Indeed, *Trichodesmium* and >10 μm N_2 fixation rates (figures 3(a) and 4(a), respectively) peaked at the AF and maintained high values towards the southern part of the CAIBOX. The drawdown of activity at stations X16 and X17 might be related to high turbulence due to the proximity to the islands, which generates strong submesoscale variability associated with headlands (Mason, 2010).

Few studies have attempted to measure N_2 fixation in upwelling ecosystems. Raimbault and García (2008) measured rates as high as 3.6 nmol N l^{-1} d^{-1} off the Chilean coast. In our case of study, N_2 fixation rates in upwelling sites only reached a maximum of 0.24 nmol l^{-1} d^{-1} off Cape Silleiro (figure 4(b)). Interestingly, unicellular diazotrophy clearly dominated at both upwelling sites (>80% of total N_2 fixation), although in different ways. Dark-incubated <10 μm diazotrophs dominated at Cape Silleiro over light incubations in the off-upwelling stations (S01-S03), suggesting a major contribution of group B cyanobacteria, which temporally separate carbon fixation (during daylight) and N_2 fixation (at night) to avoid nitrogenase deactivation. Their rates

diminished towards the upwelling-affected area (stations S05-S08) and >10 μm light activity increased. Only station S08 presented a clear predominance of <10 μm in-the-light diazotrophy. The situation at Cape Ghir was somewhat contrary to that off Cape Silleiro. The first drifter experiment (head of the filament, stations G12-G22) and the coastal station GM2 presented a dominance of in-the-dark activity. Dark N_2 fixation rates remained similar in the second drifter experiment and filament-edge station GT2, but high rates of <10 μm light activity appeared (~0.1 nmol N l^{-1} d^{-1}). These might be attributed to group A cyanobacteria (UCYN-A), which occur at lower temperatures than other diazotrophs such as *Trichodesmium* (Moisander et al., 2010). These organisms lack the genes necessary for the oxygen-evolving photosystem II and for carbon fixation. Hence, contrarily to group B unicellular cyanobacteria, UCYN-A are able to fix N_2 during daylight avoiding nitrogenase deactivation (Zehr et al., 2008). Certainly, molecular biology studies performed in our cruise have revealed a clear dominance of UCYN-A diazotrophs in the <10 μm fraction (Agawin et al., in preparation).

With this study we have demonstrated that unicellular diazotrophy is broadly extended over the subtropical NE Atlantic and dominates over the classical protagonism of *Trichodesmium*. Although generally low, the rates of N_2 fixation found in upwelling sites were comparable to open ocean ones. The bulk of direct N_2 fixation studies in the North Atlantic have focused on its western basin. The extrapolation of this region's rates to the whole North Atlantic have yielded areal values ranging from 160 to 3600 μmol N m^{-2} d^{-1} (see summary in Mahaffey et al., 2005). On the eastern side of the basin, several studies have identified an excess of N and low $\delta^{15}N$ values, arguing a significant contribution of N_2 fixation to export production (Mahaffey et al., 2003; Hansell et al., 2004; Álvarez and Álvarez-Salgado, 2007; Reynolds et al., 2007; Bourbonnais et al., 2009). Besides the N* and $\delta^{15}N$ approaches, indirect estimations of N_2 fixation over our region of study yield fairly similar values. Álvarez and Álvarez-Salgado (2007) estimated a rate of excess N development of 0.24 mol N m^{-2} y^{-1}, while Bourbonnais et al. (2009) identified a range between 0.056 and 0.26 mol N m^{-2} y^{-1}. If we integrate the average N_2 fixation rate of the CAIBOX cruise down to the Mixed Layer Depth (MLD) and extrapolate it to a year we obtain a rate of 0.003 ± 0.001 mol N m^{-2} y^{-1}.

Differences between geochemically and biologically derived N_2 fixation estimates have been attributed to the existing delay between the process and its signal in the water column, and the interannual variability of both (Bates and Hansell, 2004). The excess N signal repeatedly found over the North Atlantic is likely to be greatly influenced by atmospheric N inputs given that the basin is surrounded by industrialised countries (Zamora et al., 2010). Atmospheric N typically presents low $\delta^{15}N$ values and high N:P ratios and therefore its detection among diazotrophic signatures is troublesome (Hansell et al., 2007). Our results suggest that diazotrophic activity is much lower than reported by geochemical studies, but more widely distributed than previously thought and dominated by unicellular organisms.

5. Conclusions

- Albeit considered to be the principal diazotroph in the ocean, the activity and abundance of *Trichodesmium* in the subtropical NE Atlantic is low. The small cell-specific rates estimated in this study and the accumulation of these organisms in frontal areas suggest that, although present, they are mostly inactive in these waters.
- Unicellular diazotrophs revealed to be the major contributors to total N_2 fixation in the area of study. Remarkably, these organisms dominated in the two upwelling areas sampled, providing up to 80% of total N_2 fixation.

References

Altabet, M. A. (1988), Variations in nitrogen isotopic composition between sinking and suspended particles: implications for nitrogen cycling and particle transformation in the open ocean, *Deep Sea Research Part A, Oceanographic Research Papers*, *35*(4), 535-554.

Álvarez, M., and X. A. Álvarez-Salgado (2007), Biogeochemical budgets in the eastern boundary current system of the North Atlantic: Evidence of net heterotrophy and nitrogen fixation, *Limnology and Oceanography*, *52*(4), 1328-1335.

Barton, E. D., et al. (1998), The transition zone of the Canary Current upwelling region,

Progress In Oceanography, *41*(4), 455-504.

Bates, N. R., and D. A. Hansell (2004), Temporal variability of excess nitrate in the subtropical mode water of the North Atlantic Ocean, *Marine Chemistry*, *84*(3-4), 225-241.

Berman-Frank, I., J. T. Cullen, Y. Shaked, R. M. Sherrell, and P. G. Falkowski (2001), Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*, *Limnology and Oceanography*, *46*(6), 1249-1260.

Bourbonnais, A., M. F. Lehmann, J. J. Waniek, and D. E. Schulz-Bull (2009), Nitrate isotope anomalies reflect N_2 fixation in the Azores Front region (subtropical NE Atlantic), *Journal of Geophysical Research: Oceans*, *114*.

Bronk, D. A., P. M. Glibert, and B. B. Ward (1994), Nitrogen Uptake, Dissolved Organic Nitrogen Release, and New Production, *Science*, *265*(5180), 1843-1846.

Capone, D. G. (1993), Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure, in *Handbook of methods in aquatic microbial ecology*, edited by P. F. Kemp, B. F. Sherr, E. B. Sherr and J. J. Cole, pp. 621-631, Lewis Publishers, Boca Raton, Fla.

Capone, D. G., J. A. Burns, J. P. Montoya, A. Subramaniam, C. Mahaffey, T. Gunderson, A. F. Michaels, and E. J. Carpenter (2005), Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean, *Global Biogeochemical Cycles*, *19*.

Chang, J. (2000), Precision of different methods used for estimating the abundance of the nitrogen-fixing marine cyanobacterium, *Trichodesmium*, *Journal of Experimental Marine Biology and Ecology*, *245*(2), 215-224.

Codispoti, L. A. (2007), An oceanic fixed nitrogen sink exceeding 400 Tg N^{-1} vs the concept of homeostasis in the fixed-nitrogen inventory, *Biogeosciences*, *4*(2), 233-253.

Davis, C. S., and D. J. McGillicuddy, Jr. (2006), Transatlantic Abundance of the N_2-Fixing Colonial Cyanobacterium *Trichodesmium*, *Science*, *312*(5779), 1517-1520.

Deutsch, C., J. L. Sarmiento, D. M. Sigman, N. Gruber, and J. P. Dunne (2007), Spatial coupling of nitrogen inputs and losses in the ocean, *Nature*, *445*(7124), 163-167.

Dugdale, R. C. (1967), Nutrient limitation in the sea: dynamics, identification, and

significance, *Limnology and Oceanography*, *12*(4), 685-695.

Dugdale, R. C., J. J. Goering, and J. H. Ryther (1964), High Nitrogen Fixation Rates in the Sargasso Sea and the Arabian Sea, *Limnology and Oceanography*, *9*(4), 507-510.

Falcón, L. I., E. J. Carpenter, F. Cipriano, B. Bergman, and D. G. Capone (2004), N_2 Fixation by Unicellular Bacterioplankton from the Atlantic and Pacific Oceans: Phylogeny and In Situ Rates, *Applied Environmental Microbiology*, *70*(2), 765-770.

Falkowski, P. G. (1997), Evolution of the nitrogen cycle and its influence on the biological sequestration of CO_2 in the ocean, *Nature*, *387*(6630), 272-275.

Fernández, A., B. Mouriño-Carballido, A. Bode, M. Varela, and E. Marañón (2010), Latitudinal distribution of *Trichodesmium* spp. and N_2 fixation in the Atlantic Ocean, *Biogeosciences Discussions*, *7*(2), 2195-2225.

González-Taboada, F., R. González-Gil, J. Höfer, S. González, and R. Anadón (2010), *Trichodesmium* spp. population structure in the eastern North Atlantic subtropical gyre, *Deep Sea Research Part I: Oceanographic Research Papers*, *57*(1), 65-77.

Gruber, N. (2004), The dynamics of the marine nitrogen cycle and its influence on atmospheric CO_2, in *The ocean carbon cycle and climate*, edited by M. Follows and T. Oguz, pp. 97-148, Kluwer Academic, NATO ASI Series, Dordrecht.

Gruber, N., and J. L. Sarmiento (1997), Global Patterns of Marine Nitrogen Fixation and Denitrification, *Global Biogeochemical Cycles*, *11*.

Hansell, D. A., N. R. Bates, and D. B. Olson (2004), Excess nitrate and nitrogen fixation in the North Atlantic Ocean, *Marine Chemistry*, *84*, 243-265.

Hansell, D. A., D. B. Olson, F. Dentener, and L. M. Zamora (2007), Assessment of excess nitrate development in the subtropical North Atlantic, *Marine Chemistry*, *106*(3-4), 562-579.

Karl, D., A. Michaels, B. Bergman, D. Capone, E. Carpenter, R. Letelier, F. Lipschultz, H. Paerl, D. Sigman, and L. Stal (2002), Dinitrogen fixation in the world's oceans, *Biogeochemistry*, *57-58*(1), 47-98.

Kustka, A. B., A. Sañudo-Wilhelmy, E. Carpenter, D. Capone, and J. Burns (2003), Iron requirements for dinitrogen- and ammonium-supported growth in cultures of *Trichodesmium*

(IMS 101): Comparison with nitrogen fixation rates and iron:carbon ratios of field populations, *Limnology and Oceanography*, *48*(5), 1869-1884.

Mahaffey, C., A. F. Michaels, and D. G. Capone (2005), The conundrum of marine N_2 fixation, *American Journal of Science*, *305*(6-8), 546-595.

Mahaffey, C., R. G. Williams, G. A. Wolff, N. Mahowald, W. Anderson, and M. Woodward (2003), Biogeochemical signatures of nitrogen fixation in the eastern North Atlantic, *Geophysical Research Letters*, *30*.

Mason, E. (2010), High-resolution modelling of the Canary Basin oceanic circulation, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria.

Michaels, A., D. Karl, and D. Capone (2001), Element Stoichiometry, New Production and Nitrogen Fixation, *Oceanography*, *14*(4), 68-77.

Michaels, A., D. Olson, J. Sarmiento, J. Ammerman, K. Fanning, R. Jahnke, A. Knap, F. Lipschultz, and J. Prospero (1996), Inputs, losses and transformations of nitrogen and phosphorus in the pelagic North Atlantic Ocean, *Biogeochemistry*, *35*(1), 181-226.

Michaels, A. F., N. R. Bates, K. O. Buesseler, C. A. Carlson, and A. H. Knap (1994), Carbon-cycle imbalances in the Sargasso Sea, *Nature*, *372*(6506), 537-540.

Moisander, P. H., R. A. Beinart, I. Hewson, A. E. White, K. S. Johnson, C. A. Carlson, J. P. Montoya, and J. P. Zehr (2010), Unicellular Cyanobacterial Distributions Broaden the Oceanic N_2 Fixation Domain, *Science*, *327*(5972), 1512-1514.

Montoya, J. P., M. Voss, and D. Capone (2007), Spatial variation in N_2-fixation rate and diazotroph activity in the Tropical Atlantic, *Biogeosciences*, *4*(3), 369-376.

Moore, M. C., et al. (2009), Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability, *Nature Geoscience*, *2*(12), 867-871.

Mulholland, M. R., P. W. Bernhardt, C. A. Heil, D. A. Bronk, and J. M. O'neil (2006), Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico, *Limnology and Oceanography*, *51*(4), 1762-1776.

Paulmier, A., and D. Ruiz-Pino (2009), Oxygen minimum zones (OMZs) in the modern ocean, *Progress In Oceanography*, *80*(3-4), 113-128.

Proctor, L. (1997), Nitrogen-fixing, photosynthetic, anaerobic bacteria associated with pelagic copepods, *Aquatic Microbial Ecology*, *12*, 105-113.

Raimbault, P., and N. Garcia (2008), Evidence for efficient regenerated production and dinitrogen fixation in nitrogen-deficient waters of the South Pacific Ocean: Impact on new and export production estimates, *Biogeosciences*, *5*(2), 323-338.

Reynolds, S. E., R. L. Mather, G. A. Wolff, R. G. Williams, A. Landolfi, R. Sanders, and E. M. S. Woodward (2007), How widespread and important is N_2 fixation in the North Atlantic Ocean?, *Global Biogeochemical Cycles*, *21*(4).

Staal, M., S. t. L. Hekkert, G. J. Brummer, M. Veldhuis, C. Sikkens, S. Persijn, and L. Stal (2007), Nitrogen fixation along a north-south transect in the eastern Atlantic Ocean, *Limnology and Oceanography*, *52*(4), 1305-1316.

Stal, L. J. (1988), Nitrogen fixation in cyanobacterial mats, *Methods in Enzymology*, *167*, 474-490.

Stal, L. J. (2009), Is the distribution of nitrogen-fixing cyanobacteria in the oceans related to temperature?, *Environmental Microbiology*, *11*(7), 1632-1645.

Stramma, L., G. C. Johnson, J. Sprintall, and V. Mohrholz (2008), Expanding Oxygen-Minimum Zones in the Tropical Oceans, *Science*, *320*(5876), 655-658.

Torres, R., E. D. Barton, P. Miller, and E. Fanjul (2003), Spatial patterns of wind and sea surface temperature in the Galician upwelling region, *Journal of Geophysical Research C: Oceans*, *108*(C4), 3130.

Tyrrell, T. (1999), The relative influences of nitrogen and phosphorus on oceanic primary production, *Nature*, *400*(6744), 525-531.

Tyrrell, T., E. Marañón, A. J. Poulton, A. R. Bowie, D. S. Harbour, and E. M. S. Woodward (2003), Large-scale latitudinal distribution of *Trichodesmium* spp. in the Atlantic Ocean, *Journal of Plankton Research*, *25*(4), 405-416.

Voss, M., P. Croot, K. Lochte, M. Mills, and I. Peeken (2004), Patterns of nitrogen fixation along 10ºN in the tropical Atlantic, *Geophysical Research Letters*, *31*(23), L23S09.

Zamora, L. M., A. Landolfi, A. Oschlies, D. A. Hansell, H. Dietze, and F. Dentener (2010), Atmospheric deposition of nutrients and excess N formation in the North Atlantic,

Biogeosciences, *7*(2), 777-793.

Zehr, J. P., J. B. Waterbury, P. J. Turner, J. P. Montoya, E. Omoregie, G. F. Steward, A. Hansen, and D. M. Karl (2001), Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean, *Nature*, *412*(6847), 635-638.

Zehr, J. P., S. R. Bench, B. J. Carter, I. Hewson, F. Niazi, T. Shi, H. J. Tripp, and J. P. Affourtit (2008), Globally Distributed Uncultivated Oceanic N2-Fixing Cyanobacteria Lack Oxygenic Photosystem II, *Science*, *322*(5904), 1110-1112.