High numbers of *Trichodesmium* and diazotrophic diatoms in the southwest Indian Ocean

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[1] We observed high cell numbers of large (>50 μm) nitrogen-fixing phytoplankton to the south and east of Madagascar in February 2005. The distribution of the nitrogen-fixing (diazotrophic) taxa found was markedly different: *Trichodesmium* was most abundant (500–1000 trichomes L^{-1}) in the waters at the southern tip of Madagascar; while high numbers of diazotrophic diatoms (125–500 cells L^{-1}) were limited to the southeast of Madagascar. Using literature values of nitrogen-fixation per cell, we estimate potential rates of nitrogen-fixation for *Trichodesmium* (south of Madagascar) of 1–5 mmol N m^{-2} d^{-1} and for diazotrophic diatoms (east of Madagascar) of 0.4–2.4 mmol N m^{-2} d^{-1}. These cell-based estimates highlight the potential for significant nitrogen-fixation in the southwest Indian Ocean. High numbers of diazotrophic diatoms in subtropical waters to the east of Madagascar may have important implications for the biogeochemistry of the austral phytoplankton bloom in this region. **Citation:** Poulton, A. J., M. C. Stinchcombe, and G. D. Quartly (2009), High numbers of *Trichodesmium* and diazotrophic diatoms in the southwest Indian Ocean, *Geophys. Res. Lett.*, *36*, L15610, doi:10.1029/2009GL039719.

1. Introduction

[2] Planktonic organisms which fix atmospheric N_2 (diazotrophs) have a critical role in oceanic production and the marine nitrogen cycle [*Capone et al.*, 2005; *Mahaffey et al.*, 2005; *Dore et al.*, 2008]. One of the most conspicuous diazotrophic species is the colony-forming cyanobacterium, *Trichodesmium*, which is found throughout tropical and subtropical oceans and often forms large-scale surface blooms [*Capone et al.*, 2005; *Mahaffey et al.*, 2005; *Dore et al.*, 2008]. Another important diazotroph is the cyanobacterium, *Richelia intracellularis* (hereafter *Richelia*), which is an endosymbiont of several diatom genera (e.g., *Hemiaulus* and *Rhizosolenia*) [*Venrick*, 1974; *Villareal*, 1990, 1991, 1992]. The role of *Richelia* in marine biogeochemistry is poorly understood due to a lack of measurements of its distribution, abundance or N_2-fixing potential [*Mahaffey et al.*, 2005]. However, time-series observations from the subtropical North Pacific have recognised the importance of *Richelia*/diatom associations in the annual export of organic matter and biogenic silica from low latitude ecosystems [*Scharek et al.*, 1999a, 1999b; *Dore et al.*, 2008]. Both *Trichodesmium* and *Richelia* are thought to release fixed nitrogen as either ammonium or dissolved organic nitrogen, which is then available to the nutrient-starved non-diazotrophic components of the community [*Letelier and Karl*, 1996; *Villareal*, 1990, 1991, 1992; *Mulholland and Bernhardt*, 2005]. In low latitude ecosystems, *Richelia*/diatom blooms are a mechanism for rapid opal and organic carbon export [*Scharek et al.*, 1999a, 1999b], while *Trichodesmium* fuels upper ocean microbial production [*Dore et al.*, 2008].

[3] The aim of this study was to assess the abundance of large (>50 μm) diazotrophic phytoplankton during a hydrographic survey of the waters to the south and east of Madagascar (Figure 1) [*Quartly*, 2006]. We observed high numbers of two diazotrophic taxa in the area: *Trichodesmium* and the diatom *Rhizosolenia* spp., the latter of which we observed to contain several trichomes of *Richelia* (hereafter termed *Richelia*/*Rhizosolenia*). From measurements of the abundance of these two diazotrophs, in combination with published values of their N_2-fixation per cell, we estimate potential rates of N_2-fixation for these taxa in the southwest Indian Ocean. These observations represent the first in situ information on the abundance and distribution of diazotrophs around Madagascar, and highlight the potential role of N_2-fixation in the biogeochemistry of the austral phytoplankton bloom.

2. Methods

[4] Water samples for the analysis of large (>50 μm) diazotrophic phytoplankton were collected during a hydrographic survey (28/01/05–18/02/05) of the Madagascar Ridge and Madagascar Basin onboard the RRS *Discovery* (Figure 1) [*Quartly*, 2006]. Water samples were collected from either the ship's non-toxic underway seawater supply (inlet depth 5 m) every 2–4 hrs during transit, or from a surface (5 m) Niskin bottle from a small number (n = 24) of CTD casts. Underway samples were collected every 1–2 hrs for measurements of chlorophyll-*a* (n = 322) and macronutrients (nitrate, phosphate, silicate; n = 334), and every 2–4 hrs for large (>50 μm) diazotrophs (n = 100).

[5] Diazotroph abundance was measured on large volume (10 L) water samples, which were slowly concentrated down to ~20 mL by gentle removal of seawater through a 50 μm nylon mesh, and preserved with 2% acidic Lugol's solution in 25 mL glass vials. The abundance of *Trichodesmium* colonies, individual trichomes and diatom cells (L^{-1}) were determined in the full preserved volume using a 25 mL Bogorov tray and binocular microscope [after *Tyrrell et al.*, 2003]. Colonies of *Trichodesmium* were converted into trichome numbers assuming each colony consisted of 200 trichomes [e.g., *Letelier and Karl*, 1996; see also *Carpenter et al.*, 2004]. The most prominent diatoms in the samples were of the genera *Rhizosolenia*,

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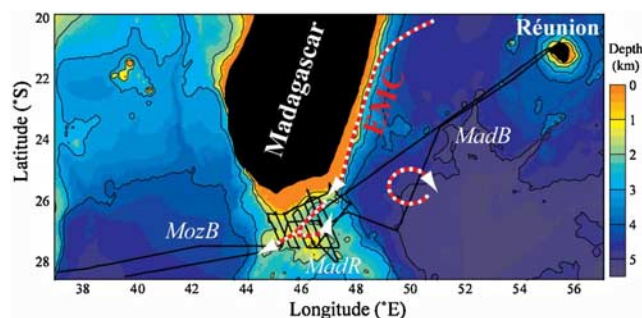
Figure 1. Cruise track superimposed on regional bathymetry and surface currents. Sampling locations are: MozB, Mozambique Basin; MadR, Madagascar Ridge; and MadB, Madagascar Basin. Red/white stripped arrows show major currents in the region, with the East Madagascar Current (EMC) from atlas and divergence south of Madagascar and cyclonic eddy determined from ship-borne measurements [*Quartly*, 2006].

with cell dimensions of $200-800$ μm by $40-60$ μm (n = 36), and each diatom cell contained $1-2$ trichomes of *Richelia*. As some of the diatom cells were smaller than the mesh size used (50 μm), we may have slightly underestimated their abundance. The size and appearance of the diatom cells under the light microscope resembled the larger form of *Rhizosolenia clevei* (var. *clevei*) [*Tomas*, 1997], although the full taxonomic affiliation cannot be confirmed at this time. No other diatrophic diatom genera (e.g., *Hemiaulus*) were observed in the samples, and the (>50 μm) diatom community in the Madagascar Basin was solely comprised of *Rhizosolenia*.

[6] We have estimated the potential magnitude of N_2-fixation associated with *Trichodesmium* from trichome abundance in a similar way to *Tyrrell et al.* [2003]: assuming a nitrogen content of 0.7 nmol N per trichome, a nitrogen-specific N_2-fixation rate of 4 μmol N (mmol trichome N)$^{-1}$ h^{-1}, a 12 hr period of N_2-fixation, and a mixed layer depth of 50 m. For *Richelia*/*Rhizosolenia*, we estimated the potential magnitude of N_2-fixation using *Richelia* cell numbers and the measurements of *Villareal* [1990, 1991, 1992]: we assumed a ethylene production rate of 9.5 pmol ethylene (trichome)$^{-1}$ h^{-1}, a 3:1 acetylene reduction to N_2-fixation ratio [*Mahaffey et al.*, 2005], a 12 hr period of N_2-fixation, a 50 m mixed layer and 2 *Richelia* trichomes per *Rhizosolenia* cell.

[7] Water samples for nitrate (+ nitrite), phosphate and silicate concentrations (μmol kg^{-1}) were collected in 25 mL vials, stored at 4°C and analysed onboard within 24 hrs. Macronutrient concentrations were measured using an auto-analyser following *Sanders and Jickells* [2000]. Measurements of chlorophyll-*a* (Chl) were made on water samples (250 mL) filtered through Fisherbrand MF300 (pore size 0.7 μm) glass fibre filters, extracted in 10 mL 90% acetone (24 hrs, dark, 4°C) and analysed using a TD-700 fluorometer with *Welschmeyer* [1994] filters. The proportion of Chl in the >5 μm size-fraction was measured on 5 μm pore size polycarbonate filters. The Chl contribution of the different diazotrophic taxa were estimated assuming 0.10 ng Chl per *Trichodesmium* trichome [*Carpenter et al.*, 2004] and 0.14 ng Chl per *Rhizosolenia* cell [based on our mean cell dimensions of 474×47 μm, a cell carbon content of

14 ng C cell^{-1} [after *Kovala and Larrence*, 1966] and a carbon to Chl ratio of 100].

3. Results

3.1. Regional Oceanography

[8] The East Madagascar Current (EMC) flows down the eastern coast of Madagascar along the narrow continental shelf (Figure 1) [*Tomczak and Godfrey*, 1994; *Quartly et al.*, 2006]. The interaction between EMC, Madagascar Ridge and local wind patterns cause year-round upwelling around the southern tip of Madagascar [*Tomczak and Godfrey*, 1994; *Quartly et al.*, 2006]. Where the coast veers west, at the southern end of Madagascar, the path of the EMC is poorly constrained (see Figure 1). Although the mean EMC flow is to the west, some of the surface water is caught by eddies and turned cyclonically to the north along the west coast of Madagascar, or anti-cyclonically to flow east at ~28°S [*Tomczak and Godfrey*, 1994; *Quartly et al.*, 2006]. Such flow patterns are particularly evident in ocean colour imagery (Figure 2) [*Quartly and Srokosz*, 2004].

[9] A large phytoplankton bloom (>1 mg Chl m^{-3}) has been observed in austral summer from satellite images of the subtropical southwest Indian Ocean, in close proximity to Madagascar [*Longhurst*, 2001; *Srokosz et al.*, 2004; *Uz*, 2007; *Wilson and Qiu*, 2008]. The bloom develops close to Madagascar in January–February, and persists until April in the Madagascar Basin. The Madagascar bloom exhibits considerable interannual variability in terms of intensity and size, and was only evident as disconnected filaments of elevated Chl during 1998, 2001, 2003, 2005 and 2007 [*Longhurst*, 2001; *Srokosz et al.*, 2004; *Uz*, 2007; *Wilson and Qiu*, 2008].

[10] The Chl concentrations found during our study were between 0.1 and 0.3 mg m^{-3}, with elevated values limited to the Madagascar Basin and over the Madagascar Ridge (Figure 2a). These Chl measurements confirm the constrained extent of the 2005 austral bloom [*Uz*, 2007], with only moderate increases ($0.2-0.3$ mg m^{-3}) in Chl relative to the low values found in the surrounding subtropical waters (~0.1 mg m^{-3}) (Figure 2a). Our size-fractionated Chl measurements show that ~$60-80$% of the Chl in the Madagascar Basin was associated with cells larger than 5 μm in diameter, while only ~$30-40$% of Chl was found in this fraction elsewhere in the study region (e.g., Madagascar Ridge).

[11] Nitrate and phosphate concentrations in the study area were generally low (<0.3 μmol kg^{-1} for nitrate, <0.1 μmol kg^{-1} for phosphate) and close to the detection limits of the macromolar technique. Mesoscale variability in nitrate and phosphate concentrations were observed, with elevated concentrations associated with localised upwelling and eddies [*Tomczak and Godfrey*, 1994; *Quartly et al.*, 2006]. Silicate concentrations were typically $1-2$ μmol kg^{-1} (Figure 2b), although conspicuous patches of depleted silicate (<1 μmol kg^{-1}) were observed in the Madagascar Basin. These patches of low silicate were associated with filaments of high Chl (Figures 2a and 2b) in the Madagascar Basin.

3.2. Diazotroph Distribution

[12] Although *Trichodesmium* was found throughout the study area, high trichome numbers (>500 L^{-1}) were limited to waters over the Madagascar Ridge (Figure 2c). Peaks in
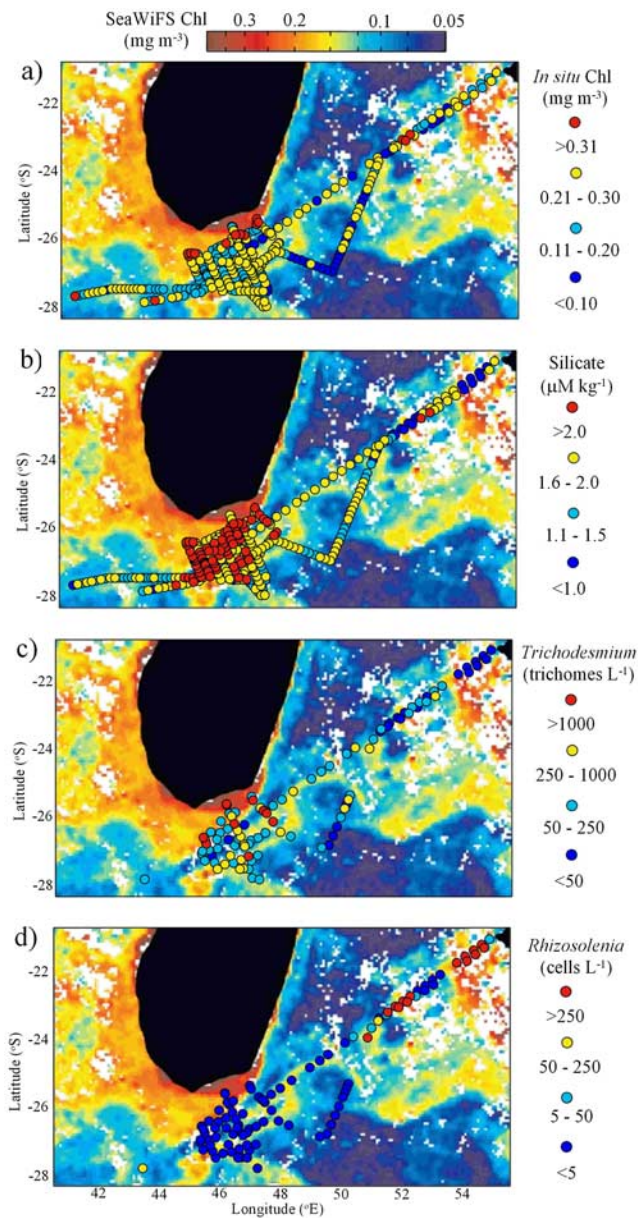
Figure 2. Cruise track and bubble plots of surface properties superimposed on composite SeaWiFS image (6th to 19th February, 2005): (a) chlorophyll (mg m^{-3}), (b) silicate (μM kg^{-1}), (c) *Trichodesmium* (trichomes L^{-1}), and (d) *Rhizosolenia* (cells L^{-1}).

trichome abundance (>1,000 L^{-1}) were generally found close inshore or on the eastern edge of the Madagascar Ridge (Figure 2c). Trichome numbers in the Madagascar Basin were typically <250 L^{-1} (i.e., ~1 colony L^{-1}). The average abundance of *Trichodesmium* in our study was 312 trichomes L^{-1} (range 5–2960 trichomes L^{-1}), which is of a similar magnitude to those reported in the literature. For example, *Karl et al.* [1995] reported an average of 46 trichomes L^{-1} in the subtropical North Pacific; *Tyrrell et al.* [2003] reported an average of 300 trichomes L^{-1} for eight cruises through the eastern tropical Atlantic Ocean (0–15°N); and *Carpenter et al.* [2004] reported averages of 222 L^{-1}, 292 L^{-1} and 2250 L^{-1} for three cruises in the western tropical Atlantic Ocean (0–25°W).

[13] In contrast to the distribution of *Trichodesmium*, significant cell numbers (>5 cells L^{-1}) of *Rhizosolenia* were limited to the Madagascar Basin, where cell densities were generally >250 cells L^{-1} (Figure 2d). The highest cell numbers of *Rhizosolenia* observed were 514 and 620 cells L^{-1}. Areas of high *Rhizosolenia* abundance were associated with silicate concentrations <1 μmol kg^{-1} (Figure 2b) and filaments of elevated Chl (Figure 2a).

[14] Estimates of the Chl associated with the different diazotrophic taxa indicate that *Trichodesmium* may account for 10–50% of the Chl over the Madagascar Ridge, but only 5–10% in the Madagascar Basin. The contribution of Chl from *Rhizosolenia* showed the opposite pattern: 10–50% in the Madagascar Basin and <2% over the Madagascar Ridge. This indicates that *Rhizosolenia* was an important component of the high Chl filaments at the time of sampling, although other phytoplankton (<50 μm) were also present (i.e., nanoflagellates and cyanobacteria).

[15] Assuming that each *Rhizosolenia* cell contained 2 *Richelia* trichomes, we estimate *Richelia* abundances of 250–1000 L^{-1} (or 125–500 $\times$ 10^5 m^{-2} over a 50 m mixed layer). *Richelia* abundances have also been reported in the subtropical North Pacific, where it was found in association with *Rhizosolenia hebetata* var. *semispina* and *Guinardia cylindrus* by *Venrick* [1974], and in association with *R. clevei* var. *communis* (small form of *R.clevei*) [see *Tomas*, 1997] and *Hemiaulus hauckii* by *Scharek et al.* [1999a, 1999b]. *Venrick* [1974] reported upper ocean (60 m) *Richelia* abundances of 1–9.1 $\times$ 10^5 m^{-2} for non-bloom conditions and 175–2648 $\times$ 10^5 m^{-2} during summer diatom blooms in the subtropical North Pacific.

3.3. Potential Rates of N$_2$-Fixation

[16] The average estimate of potential N$_2$-fixation by *Trichodesmium* was 0.53 mmol N m^{-2} d^{-1} (range <0.01– 5 mmol N m^{-2} d^{-1}), with rates >1 mmol N m^{-2} d^{-1} for the Madagascar Ridge where *Trichodesmium* numbers peaked (Figure 2c). Estimates of potential N$_2$-fixation rates by *Trichodesmium* in the Madagascar Basin were generally <0.5 mmol N m^{-2} d^{-1}. Despite uncertainties in these estimates, the values are similar to those from other oceanic regions [see *Mahaffey et al.*, 2005, and references therein]. For example, *Karl et al.* [1995] estimated rates of ~0.14 mmol N m^{-2} d^{-1} for *Trichodesmium* in subtropical waters of the North Pacific; and *Tyrrell et al.* [2003] estimated rates of 0.20 mmol N m^{-2} d^{-1} for an area of high *Trichodesmium* abundance in the eastern tropical Atlantic Ocean (0–15°N). Our estimates are also similar to direct measurements [see *Mahaffey et al.*, 2005, and references therein]. For example, *Capone et al.* [2005] measured rates of 0.1–0.9 mmol N m^{-2} d^{-1} in the western tropical Atlantic Ocean (0–25°W).

[17] The average estimate of potential N$_2$-fixation by *Richelia* was 0.21 mmol N m^{-2} d^{-1} (range <0.01–2.4 mmol N m^{-2} d^{-1}), with values of 0.4–2.4 mmol N m^{-2} d^{-1} in the Madagascar Basin and <0.01 mmol N m^{-2} d^{-1} over the Madagascar Ridge. Previous estimates by *Venrick* [1974] in the subtropical North Pacific indicated rates of 0.4–0.9 mmol N m^{-2} d^{-1} associated with *Richelia/ Rhizosolenia*, while *Villareal* [1991] estimated rates of 0.3 mmol N m^{-2} d^{-1} in association with *Richelia/Hemiaulus* in the tropical southwest Atlantic Ocean. *Carpenter et al.* [1999] encountered a bloom of *Richelia/Hemiaulus* north-

east of South America, with measured rates of N_2-fixation in excess of 1 mmol N m^{-2} d^{-1} inside the bloom. Although we can only derive rough estimates of the potential amount of N_2-fixation associated with *Richelia/Rhizosolenia* and *Trichodesmium* in the study area, it is clear that significant rates (>1 mmol N m^{-2} d^{-1}) of N_2-fixation may be occurring in the southwest Indian Ocean.

4. Discussion

[18] Production by diazotrophs (*Trichodesmium*) has previously been proposed as a mechanism for the formation of the austral bloom off Madagascar [*Longhurst*, 2001; *Uz*, 2007]. Our observations confirm this association, although diazotrophic diatoms were more abundant than *Trichodesmium* in the 2005 austral bloom. *Richelia*/diatom associations link together N_2-fixation and biogenic silica export, as well as providing an important mediator of new and export production in oligotrophic conditions [*Villareal*, 1990, 1991, 1992; *Scharek et al.*, 1999a, 1999b; *Dore et al.*, 2008]. If *Richelia*/diatom associations do have a significant role in the formation of the austral bloom off Madagascar, potential mechanisms of bloom formation would need to account for both iron and silicate supply. Although high abundances of *Trichodesmium* were generally found over the Madagascar Ridge (Figure 2c), moderate numbers of trichomes (50–250 trichomes L^{-1}) were also found in the Madagascar Basin.

[19] To conclude, measurements of the abundance of *Trichodesmium* and *Richelia/Rhizosolenia* in the southwest Indian Ocean have shown that: (1) *Trichodesmium* trichomes were predominantly found in association with the upwelling over the Madagascar Ridge (Figure 2c); (2) *Richelia/Rhizosolenia* cells were found offshore in association with high Chl filaments in the Madagascar Basin (Figure 2d). High cell densities of *Richelia/Rhizosolenia* within the filaments of the austral Madagascar bloom could indicate a significant role for N_2-fixation in the dynamics of the bloom, and will have implications for the fate of material produced. Further field work is required to confirm our observations and fully test the role of N_2-fixation in the biogeochemistry of the southwest Indian Ocean.

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