

Nitrogen fixation along a north–south transect in the eastern Atlantic Ocean

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Abstract

Nitrogenase activity, indicative of N₂ fixation, was measured in the surface waters along a north–south transect in the eastern Atlantic Ocean, from Texel (The Netherlands, 53°N) to Cape Town (South Africa, 35°S) using a sensitive on-line, near real-time acetylene reduction assay. From the beginning of January to the end of February 2000 nitrogenase activity was detected in varying rates, but only between 14°N and 13°S latitudes. Dark incubations yielded an average activity of 2.2 (± 2.4) μmol m⁻² d⁻¹ N, but light increased the activity to 3.7 (± 2.9) μmol m⁻² d⁻¹ N. However, nitrogenase activity in the light was sensitive to O₂ doubling to 7.6 (± 12.7) μmol m⁻² d⁻¹ N when the incubation was anaerobic. In the area where N₂ fixation occurred, phosphate concentrations were fourfold lower than in the area where N₂ fixation was absent, while silicate levels were higher. The water temperature in the area with N₂ fixation was 28°C, while in the adjacent area the temperature was 3°C lower, which might have prevented the proliferation of diazotrophic cyanobacteria. Action spectra revealed that chlorophyll *a*, phycocyanin, and phycoerythrin are the light-harvesting pigments supporting nitrogenase activity. In one area in the northern latitudes, potential nitrogenase activity was highest during daytime, which is characteristic for *Trichodesmium*. In areas with a high potential nitrogenase activity, surface waters were dominated by a phycoerythrin-containing cyanobacterium. Since nitrogenase activities were highest at night, these cells may have been unicellular cyanobacteria like *Crocospaera*.

Nitrogen is an important element that represents 7–10% of the dry weight of living organisms. The source of nitrogen for phytoplankton growth can be either inorganic or organic. Part of the nitrogen supply originates from recycling of mineralized organic matter, e.g., as NH₄⁺, amino acids, or urea (Zehr and Ward 2002). The concentration of NO₃⁻ in the deep water layers in the oceans is higher, and when this NO₃⁻ is transported to the surface by upwelling or deep vertical mixing events it can support growth of phytoplankton (Ward et al. 1989). Next to sedimentation, a process exporting nitrogen-rich material to the deep ocean, a considerable part of NO₃⁻ is denitrified to N₂. Dinitrogen is ubiquitous but unavailable as nitrogen source to most organisms, with the exception of a few specialized prokaryotes that can fix and assimilate it. Among phytoplankton only a few species of cyanobacteria

contain nitrogenase, the enzyme necessary to convert N₂ into ammonium. These N₂-fixing cyanobacteria are likely to compensate for losses by denitrification and sedimentation and close the oceanic biological nitrogen cycle (Zehr and Ward 2002).

Global flux calculations on the basis of measured local denitrification and N₂ fixation rates revealed that sources and sinks of nitrogen in the ocean are in disequilibrium. Estimated losses by denitrification always exceeded those for N₂ fixation (Middelburg et al. 1996). Toggweiler (1999) conceived that N₂ fixation would eventually compensate for any shortage in nitrogen, resulting in phosphate as the ultimate limiting nutrient, to control biomass. By using the N* anomaly, the excess of NO₃⁻ relative to PO₄³⁻, rather than local rate measurements, Gruber and Sarmiento (1997) argued that N₂ fixation must be higher than the calculations on the basis of measured rates and extrapolations of blooms of the dominant diazotroph, *Trichodesmium* spp. According to the most recent calculations (see

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Gruber 2004), the global nitrogen budget of the oceans is in balance. This author used the extensive databases of measured concentrations of nitrate and phosphate, derived from them the anticipated rates of N_2 fixation, and identified “hotspots” of oceanic N_2 fixation. A major hotspot of N_2 fixation is located in the subtropical western North Atlantic Ocean (Sargasso Sea). Recent publications confirmed the presence of significant inputs of N by N_2 fixation in the tropical North Atlantic Ocean, and it was suggested that N_2 fixation may be the most important source of new nitrogen in oligotrophic oceans (Subramaniam et al. 2001; Falcon et al. 2004; Capone et al. 2005). Thus far, N_2 -fixing cyanobacteria and N_2 fixation have not been observed in the pelagic environment of the temperate or cold regions of the oceans (LaRoche and Breitbarth 2005). The only reports of significant N_2 fixation rates in temperate regions come from the brackish Baltic Sea (e.g., Larsson et al. 2001).

Within the geographical regions being identified as N_2 -fixing areas, *Trichodesmium* spp. are the dominant diazotrophs. *Trichodesmium* spp. are nonheterocystous, filamentous N_2 -fixing cyanobacteria (Capone et al. 1997). They contain gas vesicles that provide them with buoyancy, allowing them to position optimally in the water column (Walsby 1978; Villareal and Carpenter 2003). *Trichodesmium* spp. occur as colonies (puffs or tufts) or as single trichomes. These organisms form extensive surface blooms in the (sub)tropical oceans worldwide and are thought to be responsible for a large part of the observed rates of N_2 fixation. The geographical distribution of *Trichodesmium* spp. coincides with water temperatures well above 20°C (LaRoche and Breitbarth 2005; Westberry et al. 2005).

Existing models of N_2 fixation only consider *Trichodesmium* as the sole N_2 -fixing organism (Hood et al. 2004; Westberry et al. 2005). A reason for this bias on *Trichodesmium* may be that most of the literature on oceanic N_2 fixation concerns this organism, which seems to originate from its capability to form surface blooms as a result of its buoyancy. High surface concentrations can be detected by satellites and make shipboard measurements possible without concentration of the biomass (Subramaniam et al. 2001). Even during nonbloom conditions, *Trichodesmium* can be collected using plankton net tows of several miles at preset depths and subsequently concentrate biomass of thousands of liters of seawater. However, sampling by using plankton nets will miss single trichomes of *Trichodesmium* and also the small unicellular N_2 fixers.

The second group of heterocystous N_2 -fixing cyanobacteria that could play an important role in the oceans is the group of symbiotic species that live intracellularly in, or as epiphytes on, large planktonic diatoms. A well-known example is the heterocystous cyanobacterium *Richelia* living symbiotically inside the diatom *Rhizosolenia* (Carpenter et al. 1999). These N_2 -fixing symbiotic associations are presently also only known from the (sub)tropical oceans.

More recently, a variety of *nifH*, one of the structural genes of nitrogenase belonging to unicellular cyanobacteria, has been detected in the Pacific and Atlantic oceans. The analysis and quantification of their transcripts has

shown that these organisms may be of considerable importance for N_2 fixation (Zehr et al. 2001; Falcon et al. 2004; Church et al. 2005). Increased sensitivity of modern isotope-ratio mass spectrometric techniques allows the detection of N_2 fixation in as little as 1 liter of seawater under nonbloom conditions (Montoya et al. 1996; Voss et al. 2004). Montoya et al. (2004) measured high rates of N_2 fixation in the Pacific Ocean by using the $^{15}N_2$ technique and attributed these rates predominantly to (small-sized) unicellular cyanobacteria. However, little is known about their global geographical distribution, abundance, and contribution to oceanic N_2 fixation.

To date, few measurements of nitrogenase activity have been made, mainly in the Atlantic Ocean (Hood et al. 2004). Moreover, the analyses that were carried out were geographically mainly in the (sub)tropical Sargasso and Caribbean seas. N_2 fixation is supposed to be absent in the eastern tropical Atlantic Ocean and only few observations of diazotrophic organisms have been published thus far (Carpenter 1983; Hood et al. 2004). The rationale for the supposed absence of N_2 fixation in the eastern North Atlantic region is that upwelling of cold, nitrate-rich water at the West African coast in combination with the westerly direction of the great ocean currents is not favorable for the proliferation of N_2 -fixing cyanobacteria. However, a recently developed model shows that N_2 fixation may be important during the period August–November in the eastern part of the tropical Atlantic Ocean and some reports are published on the presence of *Trichodesmium* spp. in these regions (Tyrrell et al. 2003; Hood et al. 2004). It was our aim to measure nitrogenase activity in the surface waters during a north–south transect along the eastern Atlantic Ocean. This transect started in The Netherlands and ended in South Africa. Once detected, nitrogenase activity was further characterized by using the sensitive on-line technique and laser-based ethylene detection. In addition, the phytoplankton community was inspected for the presence and dominance of unicellular cyanobacteria by applying flow cytometry.

Material and methods

Cruise, sampling stations, sampling methods—The cruise track is shown in Fig. 1. The cruise started on 04 January 2000 in Texel, The Netherlands and ended on 26 February 2000 in Cape Town, South Africa on board the Dutch RV *Pelagia*. Temperature, salinity, and fluorescence measurements (occasionally measured using a conductivity-temperature-depth [CTD] probe) showed that the upper 40 m was completely mixed during the cruise track between Dakar and Walvisbaay (data not shown). Samples for the measurement of N_2 fixation were taken twice a day, one at 10:00 h and the other at 21:30 h. Samples were taken by filling a 20-liter carboy from a continuously flowing, unfiltered seawater supply with its inlet at 3–4 m depth. From this tank, 2.5-liter samples were filtered on GF/F filters (\varnothing 2.5 cm, Whatman). Wet filters (two drops of filtered water were added to the vial to prevent desiccation) were incubated in duplicate at 25°C under the following conditions: aerobic light, anaerobic light, and aerobic dark

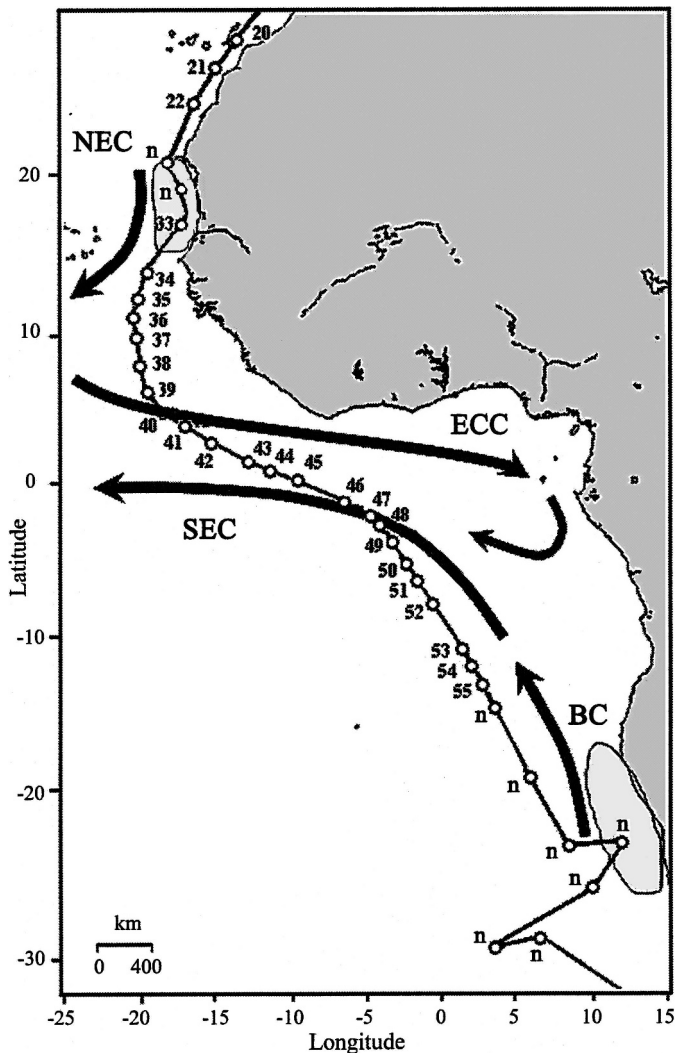


Fig. 1. Cruise track indicating the stations sampled for N₂ fixation measurements and the direction of the great ocean currents. The North Equatorial Current (NEC), South Equatorial Current (SEC), Counter Equatorial Current (CEC), and the Benguela Current (BC) are indicated by arrows and the upwelling areas are shaded in light grey. The stations marked by their number showed acetylene reduction activity (N₂ fixation). The stations indicated with an “n” did not have measurable acetylene reduction activity.

in gastight vials (8 mL) with 10% acetylene in the gas phase. Light was provided by cold white fluorescent tubes at an incident saturating photon irradiance of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Anaerobic conditions were obtained by flushing with N₂. Dark conditions were obtained by wrapping the vials in two layers of aluminum foil. Nitrogenase activity was measured through the reduction of acetylene to ethylene (acetylene reduction assay, ARA) (Hardy et al. 1968). By incubating the GF/F filters we were able to use the sensitive ARA on large water volumes without running into experimental problems due to high gas volume: water volume ratios (Breitbarth et al. 2004). Previously, we demonstrated that the measurement of nitrogenase activity by incubation of biomass on filters is preferred over

incubation in suspension because of the slow diffusion of gasses (Staal et al. 2001). Since it was not our aim to quantify the actual N₂ fixation, we incubated the samples under constant artificial light and temperature. This allowed us to compare different stations, since weather conditions did not influence the measurements. Incubation on filter, in combination with the use of clean acetylene (low ethylene background of <0.2 parts per million [ppm]) allowed sensitive measurement of acetylene reduction. This high sensitivity enabled N₂ fixation measurements in typical oligotrophic water like the open Atlantic Ocean, which is characterized by a low plankton biomass.

Incubations lasted 10–12 h. The gas phase of the vials was sampled with a 500- μL gastight syringe (Hamilton) and manually injected into a gas chromatograph (GC14A, Shimadzu) equipped with a flame ionization detector (FID) (Staal et al. 2001). The temperatures of injector, detector, and oven were set at 90°C, 120°C, and 55°C, respectively. The carrier gas was helium at the highest purity available at a flow rate of 10 mL min⁻¹. The supply of H₂ and air for the FID were 30 and 300 mL min⁻¹, respectively. The column was a 25-m-long wide-bore fused silica (0.53 mm inner diameter) packed with Porapak U (Varian-Chrom-pack). To convert acetylene reduction to N₂ fixation a conversion factor of 4 (C₂H₂ to N₂ reduced) was applied (Gallon et al. 2002). This gave the rate of N₂ fixation per liter of seawater per hour. These rates were converted to surface area per day (m⁻² d⁻¹) assuming that the bulk of N₂ fixation took place in the top 40 m (mixed layer) or 100 m (euphotic depth) of the ocean.

On-line measurement of nitrogenase activity—In addition to batch incubations, on-line N₂ fixation measurements were carried out by using laser photoacoustic ethylene detection (custom made, Department of Molecule and Laser Physics, University of Nijmegen, The Netherlands). This setup has been described in detail by Zuckermann et al. (1997). Laser-based, photoacoustic (LPA) ethylene detection is two orders of magnitude more sensitive than gas chromatography but the greatest advantage is the high time resolution of 25 s. Therefore this was the method of choice for recording light–response curves and action spectra.

Briefly, the on-line system was composed of a temperature-controlled (cryostat water bath) flow-through cell. The sample cell was made of an aluminum block of 10 × 10 × 2.5 cm (Staal et al. 2001). The sample chamber was milled out in the aluminum block and could hold a 47-mm glass fiber filter supported by a small-gauge stainless steel mesh, below which the space was filled with seawater to prevent desiccation. A Teflon cup was placed in the sample cell to prevent contact of seawater with the aluminum. It was found that seawater in direct contact with aluminum caused the chemical reduction of acetylene to ethylene, producing erroneous results. The cell was closed by a glass window, through which the sample could be illuminated. The flow rate of the gas was 2 L h⁻¹ (dilution rate of 80 h⁻¹). The gas mixing system was composed of three electronic mass flow controllers (Brooks 5850S) and a control unit (Brooks 0152), regulated by a computer.

Table 1. Average (\pm SD) volumetric N_2 fixation rates ($\text{nmol N L}^{-1} \text{d}^{-1}$) along the transect 14°N – 13°S when incubated in the dark, in the light, or under anaerobic conditions. The highest rates were measured at station 38. The value in italics was derived through extrapolation.

	Aerobic dark	Aerobic light	Anaerobic light
Average 14°N – 13°S	0.054 ± 0.059	0.093 ± 0.072	0.191 ± 0.317
Average 14°N – 5°N	0.079 ± 0.088	0.154 ± 0.091	0.366 ± 0.474
Average 5°N – 13°S	0.037 ± 0.016	0.059 ± 0.027	0.080 ± 0.035
Sta. 38	0.268	<i>0.557</i>	1.407

The gases were N_2 , O_2 (each containing 400 ppm CO_2) (Hoek-Loos, The Netherlands), and C_2H_2 (highest purity available, containing less than 200 parts per billion [v/v] C_2H_4) (Messer, Netherlands). Routinely, the gas mixture contained 10% (v/v) acetylene, 20% O_2 , and 70% N_2 . Before entering the incubator the gas was bubbled through distilled water to humidify the gas mixture to prevent desiccation of the sample. After the incubator, the gas was led through scrubbers, taking out the water vapor and CO_2 , since both gasses interfere with the laser-based detection of ethylene.

Light-response curves—The recording of light-response curves was described in Staal et al. (2002), except that the LPA system was used instead of a gas chromatograph. The sample cell was illuminated by a slide projector (Leica 250-W halogen lamp) with a set of 10 neutral density filters (Balzers). The set of neutral density filters was chosen such that exponentially increasing photon irradiance from 0 to $1,600 \mu\text{mol m}^{-2} \text{s}^{-1}$ was obtained. Light-response curves were always preceded by a 15-min dark incubation. Light intensity was automatically changed every 5 min. The light-response curve was run from low to high irradiance and the total recording time of one light-response curve took 65 min. The actual incident photon irradiance (photosynthetic active radiation) was measured by a quantum sensor (Li-Cor 250). Light-response curves were fitted using the rectangular hyperbola model. This model is derived from the Michaelis-Menten model for enzyme kinetics (Staal et al. 2002). With this model three parameters are obtained: light-stimulated N_2 fixation (N_{max}), dark N_2 fixation (N_{d}), and the light affinity coefficient for N_2 fixation (α). From these, three other parameters were calculated, namely: total N_2 fixation N_{tot} ($N_{\text{max}} + N_{\text{d}}$), light saturation coefficient $I_k(N_{\text{max}} : \alpha)$, and $N_{\text{tot}} : N_{\text{d}}$. While the parameters α and N depend on biomass, I_k and $N_{\text{tot}} : N_{\text{d}}$ are independent of biomass. The dimension of I_k is $\mu\text{mol m}^{-2} \text{s}^{-1}$ photons, and has the same meaning as the substrate affinity coefficient in the Michaelis-Menten model; $N_{\text{tot}} : N_{\text{d}}$ is dimensionless. These properties are convenient because they describe exclusively the process in the diazotrophic cell.

Action spectra for nitrogenase activity—The recording of action spectra has been described in detail by Staal et al. (2003b). Briefly, the sample cell was illuminated with monochromatic light (10-nm wavelength width) produced by a xenon lamp (Oriel) and a monochromator (grating 675 grooves mm^{-1} , Bausch and Lomb) giving a minimum of

$20 \mu\text{mol m}^{-2} \text{s}^{-1}$ photons. The monochromator was run using a stepper motor. The action spectra ran from 400 to 720 nm, at 8 nm min^{-1} , and with 0.2-nm steps. Ethylene production at different wavelengths was measured with the on-line LPA trace gas detector.

Chlorophyll analyses—Chlorophyll *a* (Chl *a*) from filtered samples was extracted in the dark overnight in 1.5 mL of 90% ethanol per sample. Absorption was measured at 665 nm using a spectrophotometer. An absorption coefficient of $72.3 \text{ mL mg}^{-1} \text{ cm}^{-1}$ was used to calculate the Chl *a* concentration per sample.

Flow cytometry—Three to four water samples from various depths of the mixed upper layer (<40 m depth) were collected before sunrise using a CTD rosette sampler. The phytoplankton community composition was analyzed using flow cytometry (Beckmann Coulter-XL-MCL) within 1 h after sampling (cf. Veldhuis and Kraay 2004). This technique allows the identification of cyanobacteria and different size classes of eukaryotes (pico- to nanophytoplankton) in a nondisruptive manner, on the basis of cell size and the cellular fluorescence of Chl *a* (>630 nm) and phycoerythrin ($575 \pm 20 \text{ nm}$).

Nutrient analysis—Nutrients were measured using an autoanalyzer, using standard protocols. Seawater samples were GF/F filtered before analysis.

Results

Rates and characteristics of N_2 fixation—Nitrogenase activity was only measurable between the 14°N and 13°S latitudes at this north-south transect (Fig. 1). A low rate of acetylene reduction was also found after 24 h of anaerobic light incubations at Stas. 20–22, close to the Canary Islands. At Stas. 34–53 activity was measured under three different conditions: (1) in the dark in air, (2) at saturating light ($200 \mu\text{mol m}^{-2} \text{s}^{-1}$) in air, and (3) at saturating light in N_2 (anaerobic) (Table 1). The rates of nitrogenase activity measured at the different stations were highly variable (Fig. 2). At all stations the dark-incubated samples showed the lowest activities, indicating that nitrogenase was in part light dependent. With the exception of 3 of 20 stations, anaerobic incubation in the light always yielded considerably higher rates, indicating that O_2 inhibited N_2 fixation. The average nitrogenase activities at the stations between the 14°N and 13°S latitudes were 2.2 ± 2.4 , 3.7 ± 2.9 , and $7.6 \pm 12.7 \mu\text{mol m}^{-2} \text{d}^{-1} \text{ N}$ ($n = 20$) in the dark,

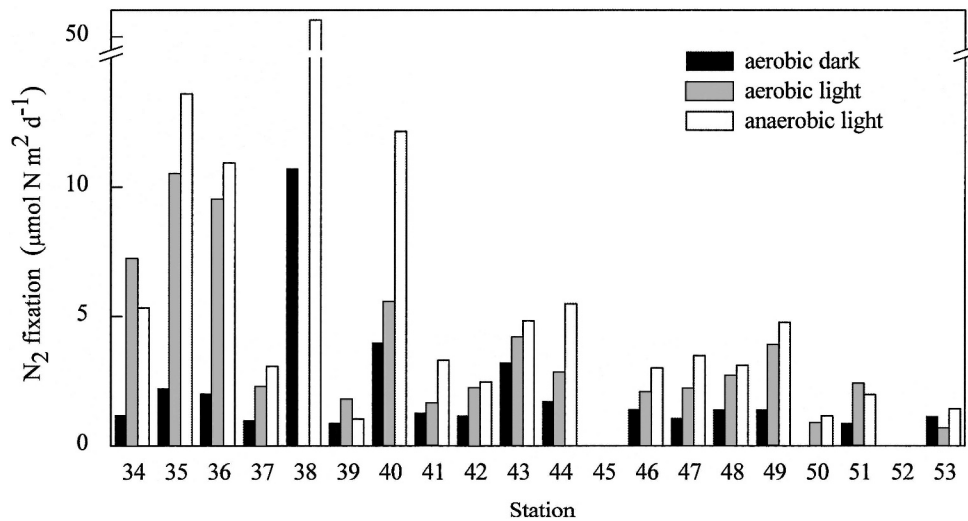


Fig. 2. Rates of N₂ fixation under three incubation conditions and at the different stations. The rates of N₂ fixation were calculated from acetylene reduction by using conversion factor 4. It was assumed that the measured rates were representative for the upper 40 m of the water column (mixed layer) and the daily integrated N₂ fixation rates were converted to surface area (m⁻² d⁻¹). Three different incubations were performed: aerobic dark, aerobic light, and anaerobic light. The light intensity applied was saturating. Incubations started at sunrise (day) or sunset (night) and lasted 12 h to cover the day and night period, respectively.

aerobic, and anaerobic in the light, respectively. Hence, light increased nitrogenase activity by as much as on average 75% compared with the dark incubations, and anaerobic conditions increased activity 100% compared with aerobic conditions (Table 1). The high standard deviations are caused by the large differences in activity between the different stations plus the fact that samples were taken at two different times of the day: in the morning (10:00 h) or after dawn (21:30 h). Stas. 34–40 (the latitudes 14–5°N reveal clearly higher nitrogenase rates than from 5°N to 13°S (Stas. 41–53; Fig. 2). In the northernmost stations (34–40), just south of the Cape Verde Islands, the activities were 3.2 ± 3.5 , 6.2 ± 3.6 , and $14.6 \pm 19.0 \mu\text{mol m}^{-2} \text{d}^{-1} \text{N}$ ($n = 7$) in the dark, aerobic, and anaerobic in the light, respectively. From 5°N to 13°S (stations 41–53) these rates were 1.5 ± 0.6 , 2.4 ± 1.1 , and $3.2 \pm 1.4 \mu\text{mol m}^{-2} \text{d}^{-1} \text{N}$ ($n = 13$), respectively, largely showing the same general pattern with respect to light and O₂. The highest rates were measured at Sta. 38 with $10.7 \mu\text{mol m}^{-2} \text{d}^{-1} \text{N}$ in the dark, and $56.3 \mu\text{mol m}^{-2} \text{d}^{-1} \text{N}$ anaerobically in the light. The rate for the light incubation is estimated as $22.3 \mu\text{mol m}^{-2} \text{d}^{-1} \text{N}$ on the basis of an extrapolation of the dark and anaerobic light values. Because the geographical distance between the two sampling times (at 10:00 h and at 21:30 h, ~260 km), the biomass-corrected activities were compared rather than those based on the water volume. The incubation times of the batch experiments coincided with the light or dark periods. Therefore, the 10:00 h incubations represent N₂ fixation during daytime and those of 21:30 h represent N₂ fixation at night. The potential nitrogenase activity (anaerobic in saturating light) during the daytime exceeded those during

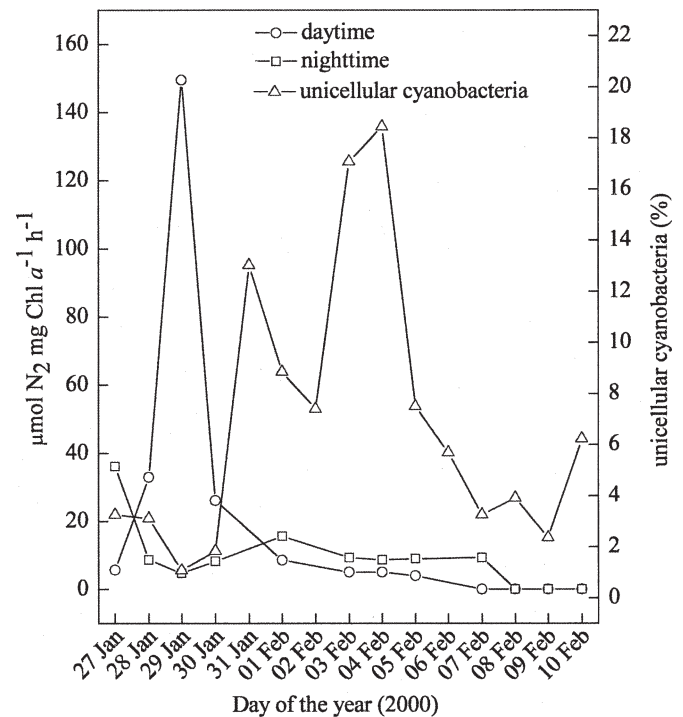


Fig. 3. Potential N₂ fixation rates (anaerobic light incubations) on the days that N₂ fixation was found both during the day and nighttime. N₂ fixation rates are expressed per biomass to correct for differences in biomass between the stations of the day and night measurements. The corresponding contribution of unicellular cyanobacteria (v/v) of the total phytoplankton biomass per measuring day is given.

Table 2. Measured parameters per day. Samples were taken from the aqua flow pump. Nutrient samples were not taken at exactly the same positions as the two different stations, but were always taken within 50 km. NA, not analyzed

Station	N ₂ fixation	Latitude (°N)	Longitude (°E)	Temperature (°C)	Chl <i>a</i> (µg L ⁻¹)	Phosphate (µmol L ⁻¹)	Ammonia (µmol L ⁻¹)	Nitrite (µmol L ⁻¹)	Nitrate (µmol L ⁻¹)	NO _x (µmol L ⁻¹)	Silicate (µmol L ⁻¹)	N:P
34	Yes	13.943	-19.658	22.3	0.436	NA	NA	NA	NA	NA	NA	NA
35	Yes	12.318	-20.268	24.3	0.176	NA	NA	NA	NA	NA	NA	NA
36	Yes	10.984	-20.517	24.4	0.156	NA	NA	NA	NA	NA	NA	NA
37	Yes	9.800	-20.201	26.0	0.166	NA	NA	NA	NA	NA	NA	NA
38	Yes	7.845	-20.000	27.4	0.176	NA	NA	NA	NA	NA	NA	NA
39	Yes	6.134	-19.586	27.7	0.104	NA	NA	NA	NA	NA	NA	NA
40	Yes	4.698	-18.497	27.9	0.218	0.042	0.194	0.026	0.097	0.124	1.349	7.57
41	Yes	3.815	-17.038	28.6	0.187	0.028	0.115	0.021	0.083	0.104	1.212	7.82
42	Yes	2.808	-15.365	28.2	0.197	0.023	0.146	0.024	0.070	0.093	1.300	10.39
43	Yes	1.490	-12.887	28.1	0.135	0.028	0.117	0.008	0.001	0.009	0.971	4.50
44	Yes	0.890	-11.409	28.5	0.145	0.018	0.099	0.007	0.015	0.022	1.065	6.72
45	Yes	0.194	-9.662	28.2	0.145	0.016	0.204	0.016	0.006	0.022	0.937	14.13
46	Yes	-1.135	-6.528	28.0	0.280	0.047	0.108	0.039	0.059	0.098	1.012	4.38
47	Yes	-1.765	-5.043	27.5	0.176	0.038	0.104	0.035	0.068	0.103	0.942	5.45
48	Yes	-2.475	-4.203	27.2	0.290	0.048	0.067	0.022	0.013	0.034	0.765	2.10
49	Yes	-3.843	-3.348	27.7	0.259	0.061	0.147	0.034	0.041	0.075	1.068	3.64
50	Yes	-5.333	-2.417	27.4	0.135	0.117	0.216	0.035	0.016	0.050	1.009	2.27
51	Yes	-6.437	-1.688	27.6	0.104	0.076	0.149	0.050	0.016	0.065	1.736	2.82
52	No	-7.994	-0.658	27.5	0.156	0.064	0.127	0.026	0.036	0.062	2.352	2.95
53	Yes	-10.972	1.321	25.9	0.073	0.111	0.258	0.017	0.040	0.058	1.565	2.85
54	No	-11.975	1.986	25.5	0.041	0.144	0.156	0.021	0.066	0.087	0.985	1.69
55	No	-13.218	2.632	25.1	0.135	0.142	0.154	0.033	0.040	0.073	1.050	1.60
56	No	-14.656	3.364	24.9	0.114	0.186	0.167	0.024	0.044	0.068	0.294	1.26
57	No	-16.363	4.261	24.6	0.114	0.217	0.094	0.025	0.038	0.064	0.372	0.73
58	No	-17.735	4.965	24.3	0.104	0.273	0.072	0.048	NA	0.048	0.334	0.44
59	No	-19.200	5.744	24.1	0.104	0.298	0.086	0.002	0.048	0.050	0.346	0.46

the night at three successive days (Stas. 36–41; 28–30 January) (Fig. 3). At the other sampling days the opposite was found. The potential nitrogenase activity at night exceeded that during the daytime. The ratio of the activities at 10:00 h to 21:30 h was around 0.5 for these days, while it was successively 3, 30, and 3 for Stas. 36–41, at which the highest potential activity was found during the day.

The numerical contribution of unicellular cyanobacteria to the total phytoplankton number (pico- to nanophytoplankton), as measured with the flow cytometer, was highest (up to 80%) for the region coinciding with Stas. 42–53. This region was also characterized by a relatively high night nitrogenase activity. In contrast, a lower percentage of unicellular cyanobacteria (<40%) was found in Stas. 36–41 and here a relatively high nitrogenase activity was observed during the daytime. A low percentage of unicellular cyanobacteria was also characteristic for the region where nitrogenase activity was not detected (stations >54).

With respect to Chl *a* and temperature, the stations with the higher daytime N₂ fixation and the stations with the highest nighttime potential N₂ fixation showed little variation. The average Chl *a* and temperatures were $0.14 \pm 0.03 \mu\text{g L}^{-1}$ Chl *a* and 27.0°C and $0.15 \pm 0.08 \mu\text{g L}^{-1}$ and 26.9°C at the stations with higher daytime and nighttime N₂ fixation, respectively. No relation was found between the potential nitrogenase activity and the Chl *a* content (linear regression analysis, $r^2 = 0.000624$, $p = 0.92$, $n = 18$).

Nutrients and temperature—There were remarkable differences in selective nutrients, Chl *a*, and temperature between the stations where N₂ fixation was detected and those at the southern section (Table 2). As summarized in Table 3, there were no differences in the concentrations of nitrogen in the stations with and without N₂ fixation. Except for ammonium, the concentrations of the other nitrogen compounds were at the detection limit. The Chl *a* content in the stations without N₂ fixation was half of that in the region where N₂ occurred. Phosphate concentration was fourfold higher in the region without N₂ fixation, resulting in very low N:P ratios. The silicate concentration in the region without N₂ fixation was half of that measured in the region with N₂ fixation. The results of an analysis of variance are summarized in Table 4. These differences were accompanied by a decrease in temperature from 27.9°C to 24.9°C. The N:P ratios calculated from the dissolved inorganic nitrogen and phosphate concentrations (Table 2) were well below the Redfield ratio (16) and gradually decreased along the transect to very low values, particularly in the area without N₂ fixation (Fig. 4).

Day-night cycle and light-response curves—On 04 February (Stas. 48 and 49) samples were taken over a day-night cycle. At each sampling time a full light-response curve was recorded. At 08:00 h, around sunrise, N_{tot} , the maximum potential nitrogenase activity, was high, with $\sim 3.0 \mu\text{mol (mg Chl } a)^{-1} \text{ h}^{-1} \text{ C}_2\text{H}_4$, but it decreased during the day to $\sim 0.7 \mu\text{mol (mg Chl } a)^{-1} \text{ h}^{-1} \text{ C}_2\text{H}_4$ at

Table 3. Averages (\pm SD) of temperature, Chl *a*, and nutrients for the stations with and without N₂ fixation.

Chl <i>a</i> and nutrients	Stas. 40–52	Stas. 53–59
	(positive for N ₂ fixation)	(negative for N ₂ fixation)
Temperature (°C)	27.9 \pm 0.4	24.9 \pm 0.6
Chl <i>a</i> ($\mu\text{g L}^{-1}$)	0.187 \pm 0.059	0.098 \pm 0.031
Phosphate ($\mu\text{mol L}^{-1}$)	0.047 \pm 0.028	0.196 \pm 0.070
Ammonium ($\mu\text{mol L}^{-1}$)	0.138 \pm 0.044	0.141 \pm 0.064
Nitrite ($\mu\text{mol L}^{-1}$)	0.026 \pm 0.012	0.024 \pm 0.014
Nitrate ($\mu\text{mol L}^{-1}$)	0.040 \pm 0.032	0.046 \pm 0.010
NO _x ($\mu\text{mol L}^{-1}$)	0.066 \pm 0.037	0.064 \pm 0.014
Silicate ($\mu\text{mol L}^{-1}$)	1.209 \pm 0.421	0.707 \pm 0.497

16:15 h (Fig. 5). Then, at 18:30 h (sunset), nitrogenase activity increased again to $\sim 3.9 \mu\text{mol (mg Chl } a)^{-1} \text{ h}^{-1} \text{ C}_2\text{H}_4$, subsequently increasing further after sunset to $\sim 5.3 \mu\text{mol (mg Chl } a)^{-1} \text{ h}^{-1} \text{ C}_2\text{H}_4$ at 20:15 h and remained high during the night ($\sim 4.4 \mu\text{mol [mg Chl } a]^{-1} \text{ h}^{-1} \text{ C}_2\text{H}_4$ at 22:15 h). The day–night variation of N_{max} largely followed the pattern described above for N_{tot} . However, N_{d} followed a slightly different pattern. It was high in the early morning and remained more or less constant during the first part of the day and then dropped to a low value that was maintained the rest of the day and throughout the night.

The light affinity coefficient was low at sun rise and remained low throughout the day. It increased from sunset onward through the night. Figure 6 depicts the day–night variations of I_{k} and the ratio $N_{\text{tot}}:N_{\text{d}}$. They show a quite different pattern. I_{k} was high early in the morning, decreasing rapidly during the first half of the day, followed by a slower decrease during the rest of the day. It increased slightly after 16:00 h when the minimum was reached. The very high value of I_{k} early in the morning may be an artifact caused by an unusual light–response curve and the actual value is probably much lower. $N_{\text{tot}}:N_{\text{d}}$ represents the amount of nitrogenase activity that is light stimulated. When this ratio is high, nitrogenase is strongly light stimulated and when this ratio is one (i.e., when N_{max} is zero), nitrogenase is energy saturated in the dark and light would not contribute to its activity. As is depicted in Fig. 6, $N_{\text{tot}}:N_{\text{d}}$ reaches a low value during midday. At the end of the afternoon, this ratio suddenly increased to a very high value of close to 10 at around sunset, after which it fell again.

Table 4. Results of the analysis of variance (ANOVA, $\alpha = 0.05$) of the data of Table 3.

	<i>F</i>	<i>P</i>	<i>F</i> _{crit}
Chl <i>a</i>	5.915	0.0229	4.260
PO ₄ ³⁻	55.005	0.0000	4.414
NH ₄ ⁺	0.578	0.4576	4.451
NO ₂ ⁻	0.000	0.9994	4.414
NO ₃ ⁻	0.244	0.6276	4.451
NO _x	0.003	0.9605	4.414
SiO ₃	11.854	0.0029	4.414
N:P	9.417	0.0066	4.414

Light action spectra—High-resolution light action spectra for nitrogenase were recorded at Stas. 45 (0°194'N, 9°662'E) and 50 (5°333'S, 0°417'E). These light action spectra measure nitrogenase activity at different wavelengths of visible light, normalized to the photon flux at these wavelengths. The spectra from both stations clearly show the peaks of Chl *a* (~ 450 and ~ 680 nm) and the phycobiliproteins phycocyanin (~ 620 nm) and phycoerythrin (~ 560 nm) (Fig. 7), but differ in the extent of stimulation per photon from specific wavelengths. Sta. 50 shows a higher photon-normalized activity when compared with Sta. 45, but this may have been due to differences in diazotrophic biomass or their specific activity. No attempt was made to normalize these spectra to Chl *a*, because this parameter is not specific for the N₂-fixing organisms. More important are the relative differences of the two spectra. The light action spectra of Sta. 45 revealed a higher contribution at wavelengths similar to the maximum of absorption by the pigment phycoerythrin when compared with the wavelengths where phycocyanin absorbs. At Sta. 50 the contribution of phycoerythrin was comparably low. Another difference was the ratio of the activity at the red and blue peaks coinciding with the absorption optima of Chl *a*, which was ~ 1 at Sta. 45 and ~ 1.5 at Sta. 50.

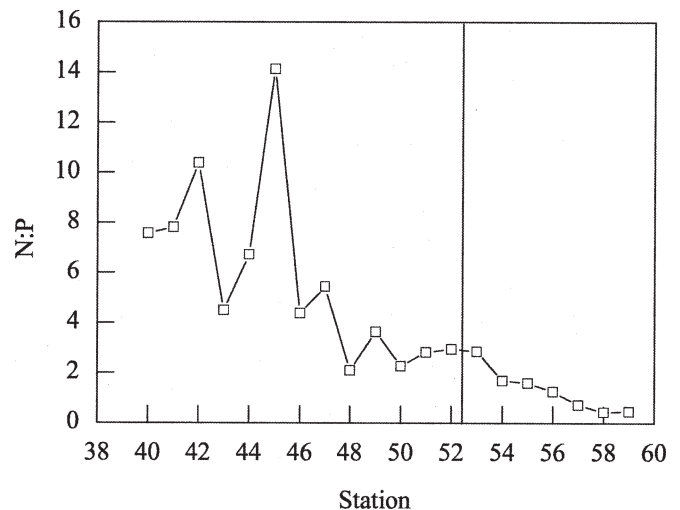


Fig. 4. N:P ratios at Stas. 40–59. The vertical line marks the transition from the area with N₂ fixation (<53) and the area without (>52).

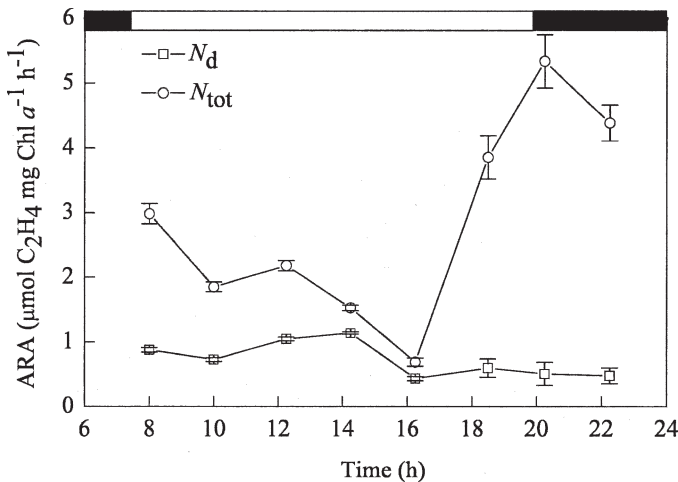


Fig. 5. Variation in N_2 fixation parameters during one day. Parameters were estimated by fitting light–response curves with the rectangular hyperbola model. Error bars indicate the error of the estimated value, given by the fit routine. The N_2 fixation rates at saturating irradiances (N_{tot}) are denoted by circles and the N_2 fixation rates in the dark (N_d) by squares. Bar indicates night (black) and day (white).

Discussion

The eastern Atlantic Ocean has not been subjected to an intensive exploration thus far (Hood et al. 2004). The objective of this study was to identify regions in the eastern Atlantic Ocean along a north–south transect where N_2 fixation is important, to characterize it, and to get a clue of the type of organism involved. Nitrogenase activity was detected at all stations between 14°N and 13°S where the average water temperature was 27°C . In all other stations along the transect nitrogenase activity was absent except for a few stations close to the Canary Islands (20 – 22 ; 30°N) where very low rates of N_2 fixation were detected but only after 24 h of incubation under anaerobic conditions in the light. At the time of the measurements at the Canary Islands the water temperature was 18°C .

Nutrient data are available for Stas. 40–59 (5°N – 23°S) (Table 2), encompassing regions with and without nitrogenase activity. Separating the stations into those with and without N_2 fixation and averaging the nutrient Chl *a* and temperature data, some interesting trends emerged (Table 3). N_2 fixation was clearly accompanied by a considerably higher phytoplankton biomass, as was concluded from the Chl *a* concentrations, which were twice as high as compared with the area without N_2 fixation. This higher biomass evidently led to phosphate concentrations drawn down to very low levels that were four times lower than in the area lacking N_2 fixation. The relatively high phosphate concentrations in the area lacking N_2 fixation were the reason for the extremely low N:P (Fig. 4). Phosphate deficits were used by Deutsch et al. (2007) to identify regions of N_2 fixation in the oceans. In contrast, silicate was much higher in the zone of N_2 fixation. In this area up to 80% of the pico- and nanophytoplankton were unicellular cyanobacteria and it is tempting to hypothesize

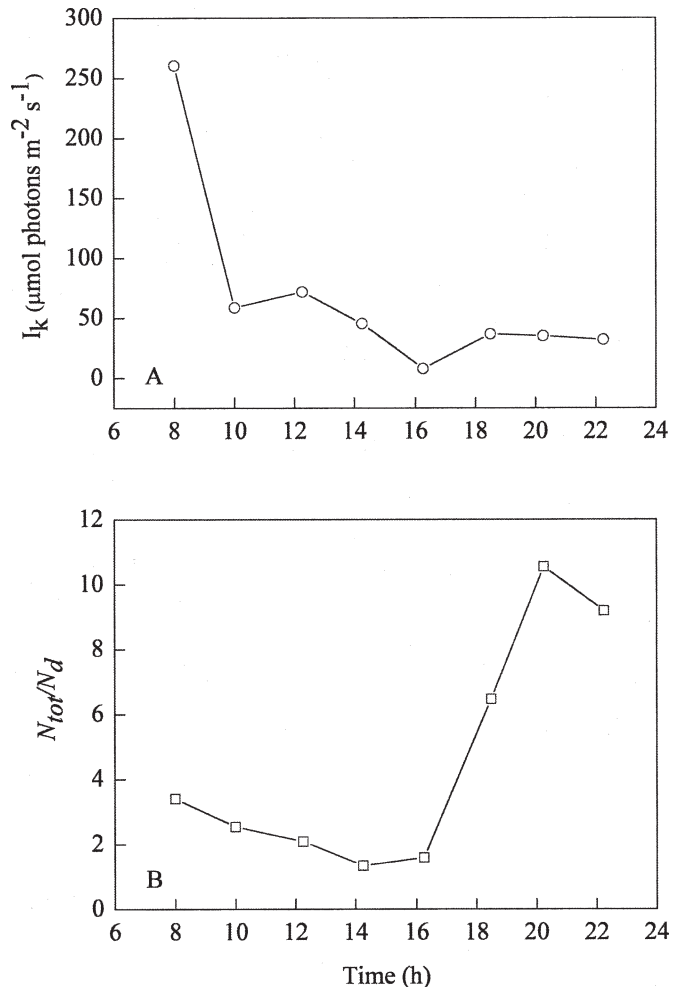


Fig. 6. (A) Variation of the light saturation coefficient (I_k) and (B) the ratio N_{tot}/N_d during one day.

that within this population many of them were diazotrophic cyanobacteria. In the low-silicate region in particular, eukaryotic phytoplankton were present and less than 40% of the pico- and nanophytoplankton were cyanobacteria. The lowered silicate concentration indicated that part of these eukaryotes were probably diatoms with nitrogen as the main limiting nutrient.

The stations where N_2 fixation was absent were located close to an upwelling area (Shillington et al. 2006). The higher N-nutrient concentrations that are expected as a result of this upwelling might have excluded diazotrophic organisms. However, the nitrogen concentrations were not different in the regions with or without N_2 fixation. Therefore it is unlikely that nutrient supply associated with upwelling was the sole reason for the absence of N_2 -fixing organisms. One remarkable difference was the seawater temperature of the regions with and without N_2 fixation. The water temperature in the region lacking nitrogenase activity was 25°C , 3°C lower than in the part of the track with N_2 fixation. The lower water temperature may have been caused by upwelling, entering the area from the south. Upwelling in this area is a wind-driven phenomenon and occurs year round (Shillington et al. 2006).

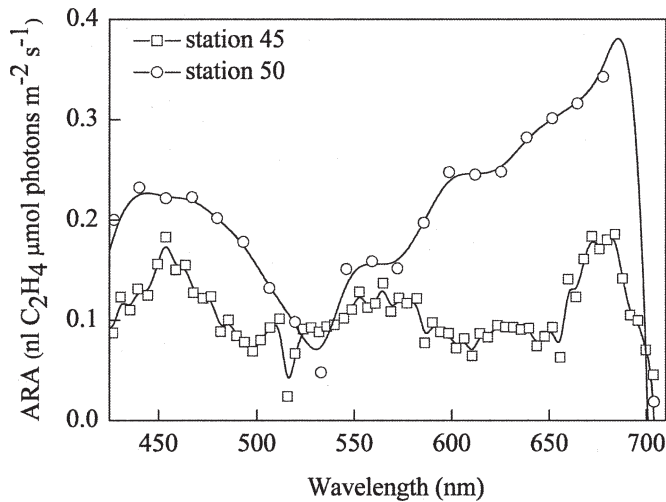


Fig. 7. Action spectra of N₂ fixation recorded at Stas. 45 and 50. Monochromatic light from 400 to 720 nm was produced by a xenon lamp using a monochromator run at 8 nm min⁻¹ with 0.2-nm steps. The minimum monochromatic light intensity was 20 μmol m⁻² s⁻¹ photons. Nitrogenase activity was measured by the acetylene reduction technique using the laser photoacoustic ethylene detector and normalized to the photon flux.

Hitherto, N₂ fixation in the oceans has only been found in regions where the water temperature is above 20°C. During our cruise we sampled many stations where the water temperature was below 20°C (Stas. 5–32, 65–66) or between 20°C and 22°C (Stas. 66–88). None of these stations showed nitrogenase activity. Active populations of *Trichodesmium* have been reported in regions where the temperature exceeds 20°C but with an optimum at 27°C and an upper limit of 34°C (LaRoche and Breitbarth 2005). Also, Lugomela et al. (2002) reported the presence of *Trichodesmium* in coastal waters off Zanzibar only when the water temperature was above 27°C. Mazard et al. (2004) searched for unicellular diazotrophic cyanobacteria in the Indian Ocean and found them only in waters where the temperature exceeded 29°C. An explanation for the crucial effect of temperature for N₂ fixation in the ocean was offered by Staal et al. (2003a). They demonstrated that only at elevated temperature is cell respiration able to maintain the N₂-fixing cell essentially free from O₂. Unicellular cyanobacteria might require even higher temperature than *Trichodesmium* because the higher surface-to-volume ratio of their cells would result in a higher influx of O₂, which is in agreement with the conclusions of Mazard et al. (2004). However, water temperature at Stas. 34–36 was also low (22–24°C, Table 2) and in the same range at Stas. 55–59, but nevertheless supported N₂ fixation. Hence, this calls for additional explanation.

The observed low rates of N₂ fixation near the Canary Islands at a water temperature of 18°C may be attributed to drifted populations. The activities were low and were obtained only after 24 h of incubation under anoxic conditions. Nitrogenase was probably induced during this long incubation, while the organisms did not have to cope with O₂. Drifting of *Trichodesmium* to colder regions has been observed on several occasions, but these populations

were often senescent and did not fix N₂ (LaRoche and Breitbarth 2005).

South of Sta. 42, the cruise continued against the Southern Equatorial Current. The water of this current passes an upwelling area near the coast of Namibia (Benguela Current, Fig. 1) (Shillington et al. 2006), from which it may be supplied with nutrients. This region was characterized by lower nitrogenase activities relative to the more northern stations. Stas. 36–41 were also characterized by high daytime potential N₂ fixation, i.e., of the type of *Trichodesmium*. These stations were situated in or close to the Equatorial Counter Current (ECC), which transports water in an easterly direction and originates from the western site of the Equatorial Atlantic Ocean, a region known for its presence of *Trichodesmium*. Considering the direction of the currents, the water temperature, the presumed nutrient input from the upwelling areas, as well as the structure of the phytoplankton community, a more comprehensive explanation is proposed for the observed differences in N₂ fixation patterns. Because of their faster growth rate and smaller cell size, unicellular cyanobacteria may respond faster to nitrogen deficiency than *Trichodesmium*. This was confirmed by the high contribution of unicellular cyanobacteria in Stas. 41–53. Eventually *Trichodesmium* will become dominant because of its efficient mode of N₂ fixation (spatial and temporal separation of oxygenic photosynthesis and N₂ fixation) and its capacity to regulate buoyancy, providing the organism with a competitive advantage with respect to light. This would be the case in the region of the ECC.

The absence of N₂ fixation in the southern stations (stations >53) may in part be explained by the lower temperature (<27°C) but could also be due to the history of these water masses. These stations are situated in the Benguela Current, and coming from the south its waters were even colder and did not possess any detectable N₂ fixation (data not shown). We hypothesize that these waters need to be inoculated with diazotrophic cyanobacteria from the warmer regions. This might have been the case for the stations <53 that receive water from the Angola Current. The ECC may have been the source of inoculation for Stas. 34–36.

The light-response curves indicate that the rate of N₂ fixation varied during the course of a day. Hence, the batch incubations that lasted the whole light and dark period give the daily integrated rate of N₂ fixation. To compare the rates of N₂ fixation with those published in the literature, the daily integrated rates were extrapolated to the upper 40 m of the water column, which was fully mixed. This is probably a conservative estimate because in this region of the Atlantic Ocean the euphotic zone may extend to 100 m. For instance, N₂ fixation as deep as 100 m was reported for the eastern Atlantic Ocean while the mixing depth extended only to 15–20 m (Voss et al. 2004). Carpenter et al. (2004) reported the highest biomass of *Trichodesmium* at 5–15 m, while we sampled through the inlet of the aqua pump at 3–4 m. However, the fluorescence profiles of the CTD in the top 40 m were homogeneous. For comparison, we also extrapolated the area of integrated N₂ fixation to 100 m (Table 5). Our depth-extrapolated values are within the lower range

Table 5. Comparison of the calculated N_2 fixation in the Eastern Tropical Atlantic Ocean with literature reports of estimated N_2 fixation in the Atlantic and Pacific oceans by presumed unicellular cyanobacteria and *Trichodesmium*. The daily average was obtained from the average of the dark and light incubations. Integration over the mixed layer (40 m) and euphotic depth (100 m) were made assuming that the measured activity was representative for these water columns. The values in italics are extrapolations.

Geographic location	Daily average ($nmol L^{-1} d^{-1} N$)	Integration: mixed layer ($\mu mol m^{-2} d^{-1} N$)	Integration: water column ($\mu mol m^{-2} d^{-1} N$)	Reference
This study				
Eastern Tropical Atlantic Ocean, average 14°N–13°S	0.073 ± 0.068	2.9 ± 2.7	7.3 ± 6.8	This study
Eastern Tropical Atlantic Ocean, average 14°N–5°N	0.113 ± 0.094	4.5 ± 3.8	11.3 ± 9.4	This study
Eastern Tropical Atlantic Ocean, average 5°N–13°S	0.048 ± 0.025	1.9 ± 1.0	4.8 ± 2.5	This study
Sta. 38	0.413	16.5	41.3	This study
Presumed unicellular cyanobacteria				
Tropical North Atlantic	3.6	37–38	94	Falcon et al. 2004
Tropical North Pacific	0.07	2.2	5.5	Falcon et al. 2004
Station ALOHA (North Pacific)	0.38		92	Zehr et al. 2001
Station ALOHA (North Pacific)	0.55	22	32	Dore et al. 2002
Station ALOHA (North Pacific)	0.24–3.6		66 ± 19	Montoya et al. 2004
North Pacific Gyre	17.3 ± 4.8		520 ± 160	Montoya et al. 2004
Arafura Sea (South Pacific)			126 ± 47	Montoya et al. 2004
<i>Trichodesmium</i>				
Tropical North Atlantic			3.7–255	Voss et al. 2004
Tropical North Atlantic			239 ± 38	Capone et al. 2005
Tropical North Pacific			98 ± 40	Capone et al. 1997

recently published for the Atlantic Ocean (Falcon et al. 2004; Voss et al. 2004; Capone et al. 2005) (Table 5).

The measurements showed a clear dependence of N₂ fixation on light. Action spectra indicated the involvement of Chl *a* as well as the phycobiliproteins phycocyanin and phycoerythrin in nitrogenase activity. Since cyanobacteria are the only phytoplankton group possessing these pigments (Veldhuis and Kraay 2000), these action spectra identify cyanobacteria as the diazotrophs. The action spectra of nitrogenase activity were different between stations. It is tempting to speculate on the presence of different types of cyanobacteria. When comparing the quantum efficiencies of the pigments using the results of the action spectra we noticed the following differences. First, the ratio of the photon efficiency at the red and blue peaks of Chl *a* was different. At Sta. 50 this ratio was ~1.5, while at Sta. 45 this was ~1. These ratios are much higher than expected on the basis of the absorption spectrum of Chl *a*, for which the ratio of the red to blue absorption peak is ~0.7. This indicates that other pigments were involved (e.g., carotenoids). The higher the ratio of the quantum efficiencies at the red to blue (675–440 nm) peaks of nitrogenase activity, the greater the presumed contribution of nonphotosynthetic pigments. Second, the organisms at Sta. 45 had a much higher content of phycoerythrin than those at Sta. 50. Although such differences may also be caused by complementary chromatic adaptation or daily fluctuations in action spectra (Staal et al. 2003b), they might represent different pigmented organisms.

The daily patterns of N₂ fixation by the different types of cyanobacteria present in tropical marine environments reveal notable differences. *Trichodesmium* spp. and the related *Katagnymene* spp. fix N₂ only during the day (Chen et al. 1999; Lundgren et al. 2001). In fact, nitrogenase in *Trichodesmium* is degraded and synthesized de novo for each new day. Presumably, there are two groups of N₂-fixing unicellular cyanobacteria that reveal remarkable differences in their expression patterns of *nifH* (Church et al. 2005). Presently, “Group A” cyanobacteria do not have cultured representatives. Remarkably, *nifH* attributed to these cyanobacteria is only expressed during daytime (Church et al. 2005) and, by using size fractionation and ¹⁵N assimilation, Montoya et al. (2004) provided evidence that they were actually fixing N₂. Although we lack further information on the characteristics of N₂ fixation in Group A cyanobacteria, we cannot exclude the possibility that they reveal a pattern of N₂ fixation that is indistinguishable from *Trichodesmium*. Group B cyanobacteria contain cultivated representatives, among which is *Crocospaera watsonii*. These cyanobacteria confine N₂ fixation to the night and behave similarly to *Cyanothece*, another Group B cyanobacterium in which nitrogenase is inactivated and degraded during the light period (Sherman et al. 1998). When the potential nitrogenase activity is highest during the night we conceive that Group B cyanobacteria were dominating. The results strongly hint to Group B unicellular cyanobacteria as the dominant N₂-fixing organisms at 6 of the 9 d that N₂ fixation was detected. Group B *nifH* sequences have been found in the Atlantic Ocean (Falcon et al. 2002). These organisms have been shown

to contribute significantly to local N₂ fixation budgets in the Atlantic Ocean (Falcon et al. 2004). The other 3 d were characteristic for *Trichodesmium* (or Group A cyanobacteria). The flow cytometry data revealed a low contribution of unicellular cyanobacteria to the total phytoplankton volume, indicating that *Trichodesmium* was probably the most important N₂-fixing organism at these stations. We have not encountered blooms of *Trichodesmium* or observed the typical colony forms, but we may have overlooked single trichomes, since these are difficult to sample with flow cytometry because of their buoyancy (Walsby 1978; Villareal and Carpenter 2003), especially at the time of sampling (before sunrise). Unfortunately, we lack information on the community composition or *nifH* diversity in connection with this data set and therefore definite conclusions must await further investigations.

References

- BREITBARTH, E., M. M. MILLS, G. FRIEDRICH, AND J. LA ROCHE. 2004. The Bunsen gas solubility coefficient of ethylene as a function of temperature and salinity and its importance for nitrogen fixation assays. *Limnol. Oceanogr. Meth.* **2**: 282–288.
- CAPONE, D. G., AND OTHERS. 2005. Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. *Glob. Biogeochem. Cycles* **19**: GB2024.
- , J. P. ZEHR, H. W. PAERL, B. BERGMAN, AND E. J. CARPENTER. 1997. *Trichodesmium*, a globally significant marine cyanobacterium. *Science* **276**: 1221–1229.
- CARPENTER, E. J. 1983. Nitrogen fixation by marine *Oscillatoria* (*Trichodesmium*) in the world's oceans, p. 65–104. In E. J. Carpenter and D. G. Capone [eds.], *Nitrogen in the marine environment*. Academic Press.
- , J. P. MONTOYA, J. BURNS, M. R. MULHOLLAND, A. SUBRAMANIAM, AND D. G. CAPONE. 1999. Extensive bloom of a N₂-fixing diatom/cyanobacterial association in the tropical Atlantic Ocean. *Mar. Ecol. Prog. Ser.* **185**: 273–283.
- , A. SUBRAMANIAM, AND D. G. CAPONE. 2004. Biomass and primary productivity of the cyanobacterium *Trichodesmium* spp. in the tropical N Atlantic ocean. *Deep-Sea Res. I* **51**: 173–203.
- CHEN, Y. B., B. DOMINIC, S. ZANI, M. T. MELLON, AND J. P. ZEHR. 1999. Expression of photosynthesis genes in relation to nitrogen fixation in the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101. *Plant Mol. Biol.* **41**: 89–104.
- CHURCH, M. J., C. M. SHORT, B. D. JENKINS, D. M. KARL, AND J. P. ZEHR. 2005. Temporal patterns of nitrogenase (*nifH*) gene expression in the oligotrophic North Pacific Ocean. *Appl. Environ. Microbiol.* **71**: 5362–5370.
- 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**: 163–167.
- DORE, J. E., J. R. BRUM, L. M. TUPAS, AND D. M. KARL. 2002. Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean. *Limnol. Oceanogr.* **47**: 1595–1607.
- FALCON, L. I., E. J. CARPENTER, F. CIPRIANO, B. BERGMAN, AND D. G. CAPONE. 2004. N₂ fixation by unicellular bacterioplankton from the Atlantic and Pacific oceans: Phylogeny and in situ rates. *Appl. Environ. Microbiol.* **70**: 765–770.

- , F. CIPRIANO, A. Y. CHISTOSERDOV, AND E. J. CARPENTER. 2002. Diversity of diazotrophic unicellular cyanobacteria in the tropical North Atlantic Ocean. *Appl. Environ. Microbiol.* **68**: 5760–5764.
- GALLON, J. R., AND OTHERS. 2002. Maximum rates of N₂ fixation and primary production are out of phase in a developing cyanobacterial bloom in the Baltic Sea. *Limnol. Oceanogr.* **47**: 1514–1521.
- GRUBER, N. 2004. The marine nitrogen cycle and atmospheric CO₂, p. 1–48. *In* M. Follows and T. Oguz [eds.], *Carbon-climate interactions*. John Wiley & Sons.
- , AND J. L. SARMIENTO. 1997. Global patterns of marine nitrogen fixation and denitrification. *Glob. Biogeochem. Cycles* **11**: 235–266.
- HARDY, R. W. F., R. D. HOLSTEN, E. K. JACKSON, AND R. C. BURNS. 1968. The acetylene-ethylene assay for N₂ fixation: Laboratory and field evaluation. *Plant Physiol.* **43**: 1185–1207.
- HOOD, R. R., V. J. COLES, AND D. G. CAPONE. 2004. Modeling the distribution of *Trichodesmium* and nitrogen fixation in the Atlantic Ocean. *J. Geophys. Res. Oceans* **109**: C06006.
- LAROCHE, J., AND E. BREITBARTH. 2005. The importance of the diazotrophs as a source of new nitrogen in the ocean. *J. Sea Res.* **53**: 67–91.
- LARSSON, U., S. HAJDU, J. WALVE, AND R. ELMGREN. 2001. Baltic Sea nitrogen fixation estimated from the summer increase in upper mixed layer total nitrogen. *Limnol. Oceanogr.* **46**: 811–820.
- LUGOMELA, C., T. J. LYIMO, I. BRYCESON, A. K. SEMESI, AND B. BERGMAN. 2002. *Trichodesmium* in coastal waters of Tanzania: Diversity, seasonality, nitrogen and carbon fixation. *Hydrobiol.* **477**: 1–13.
- LUNDGREN, P., E. SÖDERBÄCK, A. SINGER, E. J. CARPENTER, AND B. BERGMAN. 2001. *Katagnymene*: Characterization of a novel marine diazotroph. *J. Phycol.* **37**: 1052–1062.
- MAZARD, S. L., N. J. FULLER, K. M. ORCUTT, O. BRIDLE, AND D. J. SCANLAN. 2004. PCR analysis of the distribution of unicellular cyanobacterial diazotrophs in the Arabian Sea. *Appl. Environ. Microbiol.* **70**: 7355–7364.
- MIDDELBURG, J. J., K. SOETAERT, P. M. J. HERMAN, AND C. H. R. HEIP. 1996. Denitrification in marine sediments: A model study. *Glob. Biogeochem. Cycles* **10**: 661–673.
- MONTOYA, J. P., C. M. HOLL, J. P. ZEHR, A. HANSEN, T. A. VILLAREAL, AND D. G. CAPONE. 2004. High rates of N₂ fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean. *Nature* **430**: 1027–1031.
- , M. VOSS, P. KAHLER, AND D. G. CAPONE. 1996. A simple, high-precision, high-sensitivity tracer assay for N₂ fixation. *Appl. Environ. Microbiol.* **62**: 986–993.
- SHERMAN, L. A., P. MEUNIER, AND M. S. COLON-LOPEZ. 1998. Diurnal rhythms in metabolism: A day in the life of a unicellular, diazotrophic cyanobacterium. *Photosynth. Res.* **58**: 25–42.
- SHILLINGTON, F. A., C. J. C. REASON, C. M. DUNCOMBE RAE, P. FLORENCHIE, AND P. PENVEN. 2006. Large scale physical variability of the Benguela Current Large Marine Ecosystem (BCLME), p. 47–68. *In* V. Shannon, G. Hempel, P. Malanotte-Rizzoli, C. Moloney and J. Woods [eds.], *Large marine ecosystems*. V. 14. Elsevier.
- STAAL, M., F. J. R. MEYSMAN, AND L. J. STAL. 2003a. Temperature excludes N₂-fixing heterocystous cyanobacteria in the tropical oceans. *Nature* **425**: 504–507.
- , S. TE LINTEL-HEKKERT, F. HARREN, AND L. J. STAL. 2001. Nitrogenase activity in cyanobacteria measured by the acetylene reduction assay: A comparison between batch incubation and on-line monitoring. *Environ. Microbiol.* **3**: 343–351.
- , P. HERMAN, AND L. J. STAL. 2002. Comparison of models describing light dependence of N₂ fixation in heterocystous cyanobacteria. *Appl. Environ. Microbiol.* **68**: 4679–4683.
- , L. J. STAL, S. TE LINTEL HEKKERT, AND F. J. M. HARREN. 2003b. Light action spectra of N₂ fixation by heterocystous cyanobacteria from the Baltic Sea. *J. Phycol.* **39**: 668–677.
- SUBRAMANIAM, A., C. W. BROWN, R. R. HOOD, E. J. CARPENTER, AND D. G. CAPONE. 2001. Detecting *Trichodesmium* blooms in SeaWiFS imagery. *Deep Sea Res. II* **49**: 107–121.
- TOGGWEILER, J. R. 1999. Oceanography—an ultimate limiting nutrient. *Nature* **400**: 511–512.
- 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. *J. Plankton Res.* **25**: 405–416.
- VELDHUIS, M. J. W., AND G. W. KRAAY. 2000. Application of flow cytometry in marine phytoplankton research: Current applications and future perspectives. *Sci. Mar.* **64**: 121–134.
- , AND ———. 2004. Phytoplankton in the subtropical Atlantic Ocean: Towards a better assessment of biomass and composition. *Deep-Sea Res I* **51**: 507–530.
- VILLAREAL, T. A., AND E. J. CARPENTER. 2003. Buoyancy regulation and the potential for vertical migration in the oceanic cyanobacterium *Trichodesmium*. *Microb. Ecol.* **45**: 1–10.
- VOSS, M., P. CROOT, K. LOCHTE, M. MILLS, AND I. PEEKEN. 2004. Patterns of nitrogen fixation along 10°N in the tropical Atlantic. *Geophys. Res. Lett.* **31**: L23S09.
- WALSBY, A. E. 1978. Properties and buoyancy providing role of gas vacuoles in *Trichodesmium* Ehrenberg. *Br. Phycol. J.* **13**: 103–116.
- WARD, B. B., K. A. KILPATRICK, E. H. RENGER, AND R. W. EPPLEY. 1989. Biological nitrogen cycling in the nitracline. *Limnol. Oceanogr.* **34**: 493–513.
- WESTBERRY, T. K., D. A. SIEGEL, AND A. SUBRAMANIAM. 2005. An improved bio-optical model for the remote sensing of *Trichodesmium* spp. blooms. *J. Geophys. Res. Oceans* **110**: C06012.
- ZEHR, J. P., AND B. B. WARD. 2002. Nitrogen cycling in the ocean: New perspectives on processes and paradigms. *Appl. Environ. Microbiol.* **68**: 1015–1024.
- , AND OTHERS. 2001. Unicellular cyanobacteria fix N₂ in the subtropical North Pacific Ocean. *Nature* **412**: 635–638.
- ZUCKERMANN, H., M. STAAL, L. J. STAL, J. REUSS, S. TE LINTEL HEKKERT, F. HARREN, AND D. PARKER. 1997. On-line monitoring of nitrogenase activity in cyanobacteria by sensitive laser photoacoustic detection of ethylene. *Appl. Environ. Microbiol.* **63**: 4243–4251.

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