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Extensive bloom of a N_2-fixing diatom/cyanobacterial association in the tropical Atlantic Ocean

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ABSTRACT: We encountered an extensive bloom of the colonial diatom *Hemiaulus hauckii* along a 2500 km cruise track off the NE coast of South America in autumn 1996. Each diatom cell contained the heterocystous, N_2-fixing cyanobacterial endosymbiont *Richelia intracellularis*. Surface *Richelia* heterocyst (and filament) densities increased from <100 to >10^6 heterocyst l^{-1} in the bloom. Total abundance ranged from 10^6 heterocyst m^{-2} outside the bloom to over 10^{10} heterocyst m^{-2} within the bloom. Rates of primary production averaged 1.2 g C m^{-2} d^{-1}, higher than typical for oligotrophic open ocean waters. N_2 fixation during the bloom by the *Richelia/Hemiaulus* association added an average of 45 mg N m^{-2} d^{-1} to the water column. The relative importance of NH_4^+ uptake over the course of the bloom increased from 0 to 42% of total N uptake by the *Hemiaulus/Richelia* association. N_2 fixation by *Richelia* exceeded estimates of 'new' N flux via NO_3 diffusion from deep water and, together with additional N_2 fixation by the cyanobacterium *Trichodesmium*, could supply about 25% of the total N demand through the water column during the bloom. Suspended particles and zooplankton collected within the bloom were depleted in ^{15}N, reflecting the dominant contribution of N_2 fixation to the planktonic N budget. The bloom was spatially extensive, as revealed by satellite imagery, and is calculated to have contributed about 0.5 Tg N to the euphotic zone. Such blooms may represent an important and previously unrecognized source of new N to support primary production in nutrient-poor tropical waters. Furthermore, this bloom demonstrates that heterocystous cyanobacteria can also make quantitatively important contributions of N in oceanic water column environments.

KEY WORDS: N_2 fixation · Cyanobacteria · *Richelia* · Symbiont · Diazotroph · N cycling · *Hemiaulus*

INTRODUCTION

Nitrogen fixation by planktonic marine cyanobacteria can be an important source of 'new' N to the euphotic zone of nutrient-poor, open-ocean waters (Fogg 1982). The only cyanobacteria previously thought to fix N_2 at significant rates are members of the filamentous, free-living genus *Trichodesmium* (Capone et al. 1997). *Trichodesmium* spp. do not possess heterocysts, the specialized cells that protect nitrogenase from oxygen inactivation (Carpenter 1983). Heterocystous forms of cyanobacteria, which can often reach high densities in freshwater lakes and contribute substantially to the N budgets of these systems, are not conspicuous in the ocean (Paerl in press).

Richelia intracellularis, an endosymbiont that occurs within several marine diatom species, is the only heterocystous cyanobacterium regularly encountered in oceanic plankton (Sournia 1970). The endosymbiont, when found within some *Rhizosolenia* species, can be seen with transmitted light microscopy and has been reported in various tropical ocean areas (e.g. Venrick 1974, Villareal 1992). Mague et al. (1977) first reported nitrogenase activity associated with *Richelia* that inhabited diatoms of the genus *Rhizosolenia* obtained in plankton concentrates in the Central North Pacific

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Ocean, and Villareal (1990) later confirmed this observation. Endosymbiotic *Richelia* also occur within other genera, such as *Hemiaulus*, but are much more difficult to visualize without epifluorescence microscopy (Villareal 1994). Recently, *Hemiaulus* and its N_2-fixing endosymbiont have been implicated as contributors to the formation of some Mediterranean sapropels (Kemp et al. 1999).

During a research cruise in the southwest North Atlantic Ocean, we observed an extensive bloom of the diatom *Hemiaulus hauckii* containing the endosymbiont *Richelia intracellularis*, which added significant new N to this oligotrophic ecosystem. Such blooms may occur with regularity and may therefore be important in balancing the apparent deficiency in N inputs relative to sinks in oceanwide elemental budgets (Sambrotto et al. 1993, Michaels et al. 1996, Gruber & Sarmiento 1997).

METHODS

Samples were collected on a cruise aboard the RV 'Seward Johnson' between 10 October and 8 November 1996 (see Fig. 1). Seawater samples for nutrients, plankton counts and analysis of suspended particulate C and N (isotopic abundance and mass) were collected with a CTD/rosette system equipped with 10 l Niskin bottles.

Abundance of *Richelia intracellularis*. Plankton concentration was measured by direct microscopic counts. At each depth sampled, the entire content of a Niskin bottle was gravity filtered through a 47 mm diameter Nuclepore filter (10 µm pore size) in a Swinex in-line filter holder attached by tubing to the draincock of the sample bottle. *R. intracellularis* heterocyst density was counted at 100× magnification with a Zeiss epifluorescence microscope using green excitation (BP 510-560, FT 580, LP 590).

Plankton concentrates. *Hemiaulus hauckii* cells were collected by gently towing a small 12.5 cm diameter, 20 µm mesh plankton net for about 5 to 10 min at 1 m depth. The net contents were then filtered through 202 µm mesh netting to remove *Trichodesmium* colonies and subsequently diluted with filtered surface seawater to provide a sufficient volume for the primary productivity, N_2 fixation and ^{15}N uptake determinations. A subsample of the preparation was removed and counted for heterocyst density using a Sedgwick-Rafter chamber at 100× magnification by epifluorescence. Microscopic counting confirmed that no whole *Trichodesmium* colonies were present in the sample.

Nitrogen isotopic analysis. Discrete volumes collected from the depths sampled by the CTD/rosette were examined for suspended particles. Samples were collected onto precombusted GF/F filters by gentle vacuum filtration. Samples were then dried and stored over desiccant for analysis ashore. *Trichodesmium* and *Hemiaulus* were obtained for isotopic analysis from surface bucket samples and net tows as described above. Samples containing 20 colonies of *Trichodesmium*, 100 ml of a suspension of *Hemiaulus* concentrated by gentle filtration, or a mix of the two were collected on precombusted GF/C filters, then dried and stored for analysis ashore. Zooplankton were collected by towing a meter net (333 µm mesh size) diagonally through the upper 100 m of the water column. Animals were separated into size fractions using a graded series of Nitex sieves. Samples from each size fraction were quickly frozen for later analysis. Once ashore, zooplankton samples were dried and ground to a fine powder, which was then subsampled for isotopic analysis.

All natural abundance measurements were made by continuous-flow isotope ratio mass spectrometry using either a VG Prism II (Harvard) or a Europa 20-20 mass spectrometer (CBL). The 2 instruments were intercalibrated with a variety of organic and inorganic standards, and a number of samples were split and analyzed on both instruments as a check on data quality. We conservatively estimate that the overall analytical precision of our isotopic measurements is better than ±0.3‰.

$^{14}CO_2$ fixation. CO_2 fixation was measured by ^{14}C-incorporation. Seawater samples were collected in Niskin bottles from depths chosen to simulate light levels of 100, 50, 25, 10, 1 and 0.1% of surface irradiance. Water was prefiltered through a 105 µm Nitex mesh into 600 ml clear-polycarbonate bottles and spiked with a 5 µCi addition of $H^{14}CO_3^-$. Bottles were placed on deck in a flowing seawater bath under neutral density screens to simulate the light levels of the depth from which samples were collected. After incubation, samples were filtered through GF-75 filters (Poretics Corp., 0.7 mm nominal pore size), and their activity was determined aboard ship using a scintillation counter. Daily rates of photosynthesis were calculated by extrapolating measured rates over a 10 h light day.

Primary productivity determinations were also performed on surface concentrates of *Hemiaulus*. For primary productivity measurements, a 22.5 ml sample was placed in small polycarbonate bottles with additions of about 1 µCi $^{14}CO_2$. Samples were incubated on deck at ambient surface water temperatures at either 100% (Stns 13, 17, 19 & 28) or 50% (Stns 23, 27, 29) of surface irradiance. A light response series was also performed at Stn 19.

Nitrogenase activity. Rates of nitrogenase activity were determined on the *Hemiaulus* concentrates; 10 ml of the concentrate was assayed using the acetylene

reduction technique (Capone 1993). Fifteen 14 ml serum bottles were incubated for 6 to 9 h, bracketing midday, in a flowing seawater bath on deck with neutral density screens simulating 100, 50, 25, 10, and 1% of surface irradiance. Small (100 µl) samples for C_2H_4 production were taken from each vial at several points over the time course. Rates were converted to N_2 fixed using a 4:1 ratio of C_2H_2 reduced to N_2 fixed (Postgate 1982). To estimate water column rates of N_2 fixation, the rate per heterocyst from the concentrated incubation was scaled by the concentration of heterocysts in the water column. Daily rates were extrapolated on the basis of a 10 h light day.

^{15}N uptake. Rates of N uptake were also measured on the *Hemiaulus* concentrates. Uptake of NH_4^+, NO_3^-, urea, glutamate, and a mixed amino acid substrate were measured using ^{15}N tracer techniques as outlined in Glibert & Capone (1993) using highly enriched (>98%) ^{15}N substrates (Cambridge Isotope Laboratories, Inc.). Here, 100 ml of *Hemiaulus* concentrates, obtained as described above, was measured into acid-cleaned, 125 ml polycarbonate incubation bottles. Uptake experiments were initiated by adding a ^{15}N substrate (NH_4^+, NO_3^-, urea, glutamate or a commercial mix of amino acids).

Because many of the nutrients in the surface waters of the study sites were typically at the limit of detection, nominal additions of 0.03 µM ^{15}N l^{-1}, the limit of analytical detection for NH_4^+ and NO_3^-, were used. NH_4^+ was detected in surface waters at about 0.1 to 0.3 µM, but was generally elevated to about 1 µM in assay vials because of manipulation. Thus, this addition was <10% of the ambient concentration. For other N sources, 0.03 µM additions therefore represented an increase of ≥100% of the ambient nutrient pool. The latter situation may have caused stimulation of uptake systems, so uptake rate determinations for nitrate, glutamate, mixed amino acids and urea are tentative as they may overestimate actual uptake rates. The duration of each incubation was about 2 h. ^{15}N incubations were terminated by gently filtering (<125 mm Hg) the contents of the incubation bottles onto pre-combusted (450°C for 2 to 4 h) GF/F filters. Filters were rinsed 3 times with low N filtered seawater to remove any tracer that was not taken up by cells. Time 0 controls were also measured to correct for ^{15}N label adsorption to the filters. These controls were set up exactly like the experimental incubations, but the contents of these bottles were filtered immediately after the ^{15}N addition. Sample and time 0 filters were frozen and returned to the Chesapeake Biological Laboratory for analysis.

Sample ^{15}N enrichment and total particulate nitrogen mass were measured by mass spectrometry on a Europa Scientific ANCA-SL 20-20 IRMS (Isotope Ratio Mass Spectrometer) against a peptone standard. The instrument was calibrated and tuned before each sample run. A reference sample set was analyzed with each set of 21 samples. Reference samples were reproducible to within 0.0001 at.%. Reference standards were run every 5th sample to verify instrument performance over the course of sample runs. Uptake rates were calculated using the equations outlined by Glibert & Capone (1993).

Nutrients and chlorophyll. Nutrient concentrations were determined by a Technicon AAII Autoanalyzer using standard procedures adapted to autoanalyzer for NO_2^-, NO_3^-, NH_4^+ and PO_4^{-3} (Whitledge et al. 1981). Chlorophyll *a* concentrations were measured fluorometrically (Holm-Hansen & Reimann 1978).

Satellite image. The satellite image of chlorophyll concentration for 1996 was derived from the Ocean Color and Temperature Sensor (OCTS) sensor using the Japanese OCTS Version 3 algorithm. OCTS was a visible and infrared multi-spectral radiometer developed by NASDA, the Japanese space agency, to measure global ocean color and sea surface temperature. The sensor was on board the ADEOS spacecraft and was in operation from 1 November 1996 to 29 June 1997. The satellite image of chlorophyll concentration for 1997 was derived from the SeaWiFS sensor using the NASA SeaWiFS Project OC2 algorithm. SeaWiFS is a visible-band, multi-spectral radiometer on board Orbital Sciences Corporation's SeaStar spacecraft and has been operational since September 1997.

RESULTS

The cruise track proceeded in a southeast direction from an initial station east of the Bahamas (27.18° N, 72.87° W), to a point off the northeast coast of Brazil (7.18° N, 42.22° W), and then returned on a parallel northwest track slightly west of the first leg (Fig. 1A). At the first several stations, surface salinities were typical of the SW Sargasso Sea, about 36 psu (Fig. 2C). However, beginning at Stn 6 through Stn 30, surface salinities were, on average, about 34 psu, with substantial variability, and with occasional excursions below 30 psu (Fig. 2C). Surface NO_3^-, NO_2^- and PO_4^{3-} were near or below our limit of detection.

Richelia intracellularis was observed at all stations sampled on the cruise, though we defined the bloom proper as those regions in which *Richelia* concentrations (also approximately equal to *Hemiaulus hauckii* cell and *Richelia* filament densities) exceeded 10×10^3 heterocysts l^{-1} (Fig. 3). We define the bloom as extending between Stns 11 and 29. The total lineal distance (Stns 16 to 25) over which high concentrations of *Hemiaulus/Richelia* were encountered was about

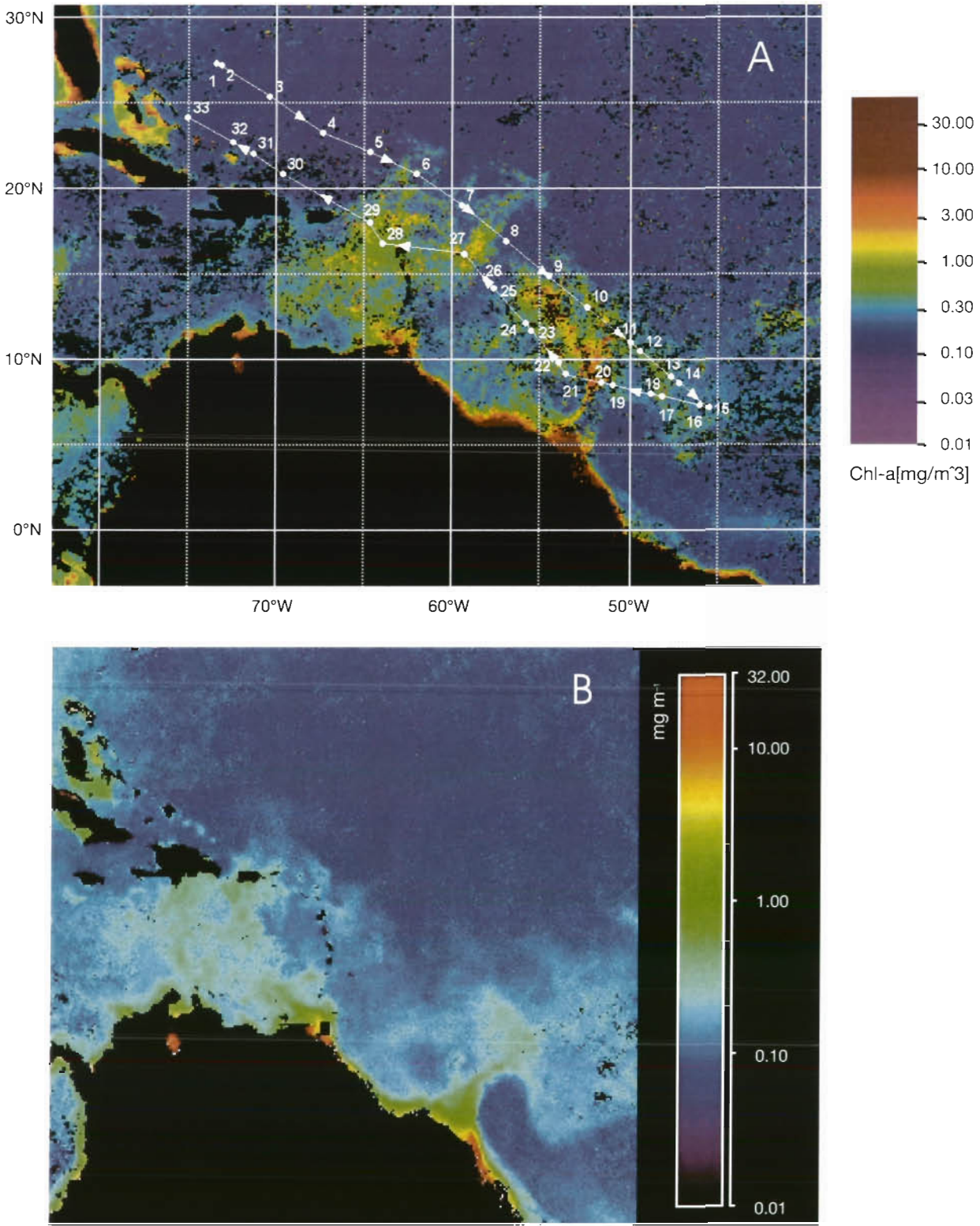

Fig. 1. (A) Cruise track for RV 'Seward Johnson' 9612 showing station locations along the track overlain on (A) a monthly composite image of surface chlorophyll concentration derived from OCTS for November 1996. More cloud covered pixels in the monthly composite have been filled in producing a clearer map. (B) A monthly composite image of surface chlorophyll concentration from the SeaWiFS sensor for November 1997. Both the Orinoco outflow and the Amazon River plume can be seen as elevated chlorophyll concentrations, although the distinct northwestward extending filament seen in 1996 is missing

2500 km. At stations leading up to the bloom (i.e. 3 to 10), the average density in the upper 30 m was 39 heterocysts l^{-1}. On the outward SE leg of the cruise on 21 October at Stn 11, concentrations increased to a mean of 285 × 10^3 heterocysts l^{-1} in the upper 30 m (Fig. 3) and to over 10 × 10^9 heterocysts m^{-2} (Fig. 2). The bloom was observed at the next 2 stations of this leg (Stns 12 and 13), but appeared to have dissipated at our most SE point, Stn 15 (Figs. 1 to 3). On the return NW transect, very high concentrations of *Hemiaulus* and *Richelia* were observed at 11 of the next 15 stations, including stations directly east of (Stns 16 to 20, 23 to 25 October 1996, respectively) and at the northern end of the lesser Antilles, in the vicinity of St. Martens (Stns 26 to 29, 28 October to 2 November 1996, respectively). Thus, the bloom appeared to be advected to the NW during the cruise period. Both the weekly OCTS composite for 3 to 10 November (not shown) and the monthly composite for November 1996 (Fig. 1) show elevated plumes of chlorophyll extending NE from the eastern Caribbean into the tropical Atlantic. In addition, the Amazon River plume can be seen extending northeastward in a separate and distinct patch. We surmise that we first encountered the bloom as part of the Amazon River plume (Stns 11 to 13 and 16 to 23), while a second component of the bloom in the vicinity of the lesser Antilles was possibly associated with the Orinoco River outflow (Stns 27 and 28). Examination of SeaWiFS imagery for November 1997 did not reveal elevated concentrations of chlorophyll, except for the plume of the Amazon extending NE into the Atlantic (Fig. 1B).

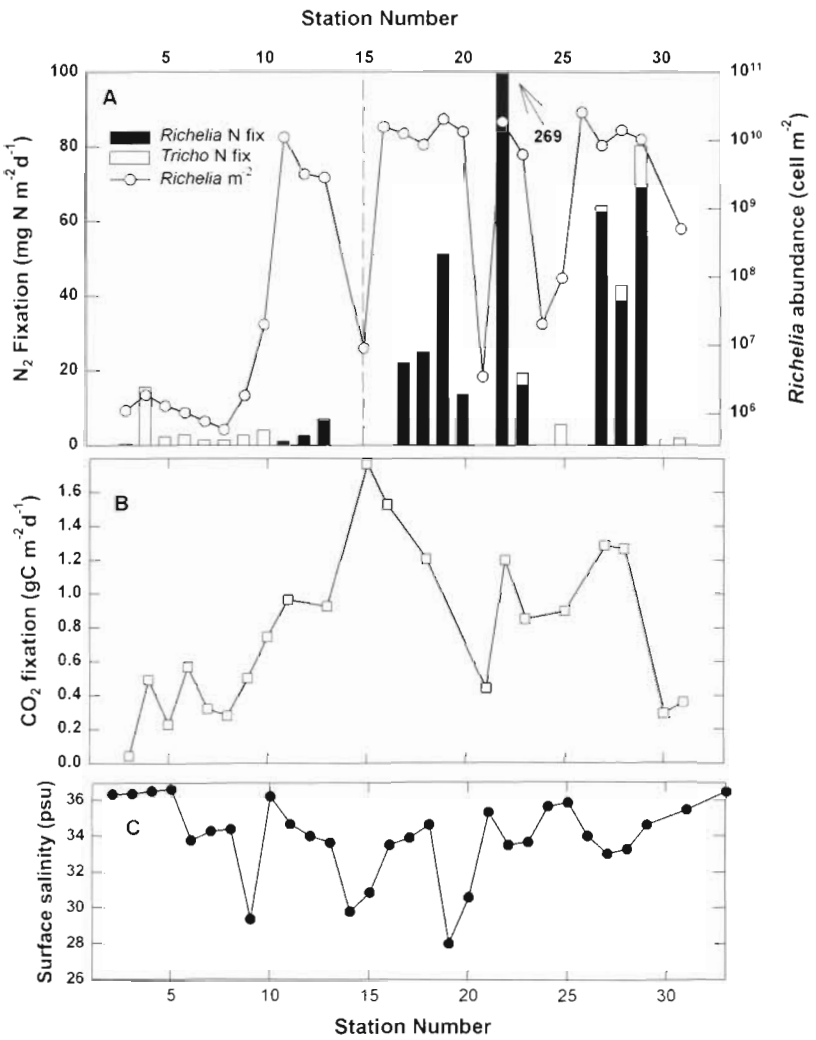

Fig. 2. (A) Depth-integrated concentrations of *Hemiaulus/Richelia* and of N_2 fixation by both *Richelia* and *Trichodesmium*, (B) depth-integrated CO_2 fixation and (C) surface salinity at stations along the cruise track. Station 15 represents our turning around point from the SE to NW leg of the cruise

Surface concentrations in excess of 10^6 heterocysts l^{-1} were noted at Stns 18, 19, 20 and 22 with a maximum at Stn 26 of 1.6 × 10^6 heterocysts l^{-1} (Fig. 3). In general, highest concentrations occurred nearest the surface and decreased with depth (Figs. 3 & 4), with the bulk of biomass in the upper 30 to 40 m. The mean concentration of *Richelia* at bloom stations and within the upper 30 m was 348 (±71) × 10^3 heterocysts l^{-1} (mean ± SE, n = 17). Surface chlorophyll *a* concentrations averaged 0.60 ± 0.12 mg m^{-3} (n = 16) compared to stations outside the bloom, which were almost 10-fold less (Table 1). We also encountered significant patchiness in the abundance of *R. intracellularis* within the bloom: 4 stations (15, 21, 24, 25) along the return transect had relatively low *Richelia* densities. Satellite data show that the Amazon River plume formed distinct filaments, and our cruise track transected through these filaments, likely contributing to the patchy nature of the *Richelia* densities (Fig. 1A).

Within the *Richelia/Hemiaulus* bloom area, depth-integrated photosynthesis averaged 1.12 ± 0.1 g C m^{-2} d^{-1} (mean ± SE, n = 9, calculated assuming 10 h of photosynthesis per day) (Fig. 2B). At stations outside the bloom, primary production averaged 0.42 ± 0.065 g C m^{-2} d^{-1} (n = 12) (Table 1, Fig. 2). With some limited exceptions (e.g. Stn 15), rates of planktonic CO_2 fixation paralleled the population densities of *Richelia* (Fig. 2). We speculate that the dis-

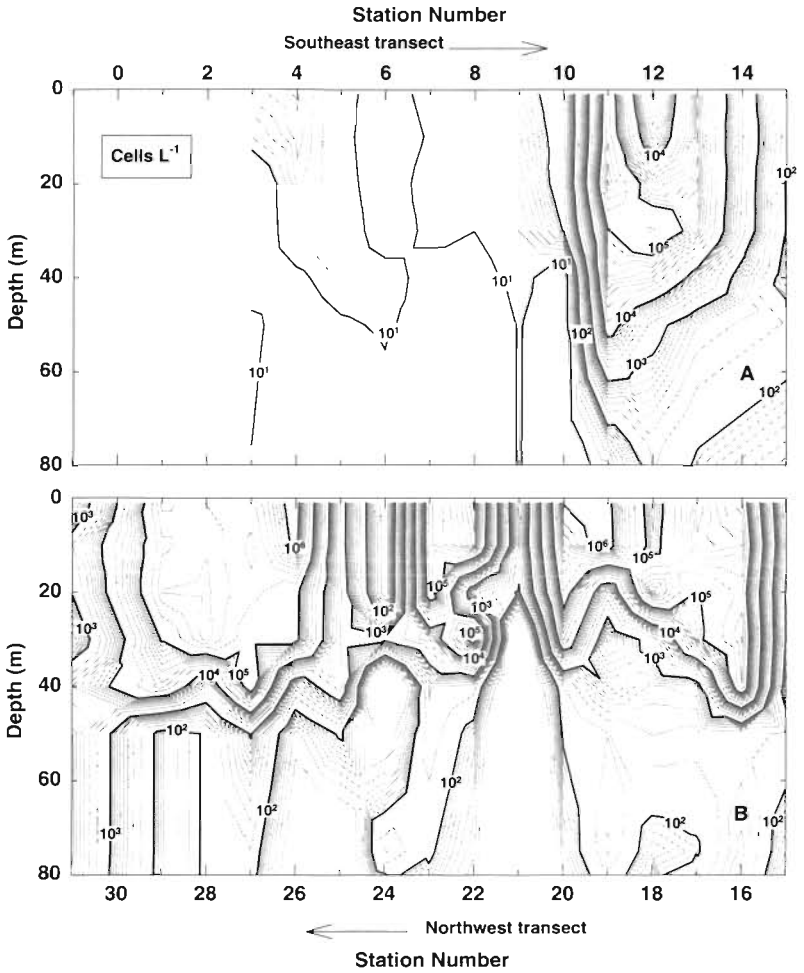

Fig. 3. Contour map of *Hemiaulus/Richelia* densities along 2 cruise transects

parity at Stn 15 may have been a result of sampling from different CTD casts, and water masses, for *Richelia* counts versus $^{14}CO_2$ uptake.

Rates of N_2 fixation also roughly tracked the population density of *Hemiaulus* (Figs. 2 & 5). The highest rates of N_2 fixation typically occurred at the surface (0 to 1 m), where it averaged 0.2 ± 0.07 mg N m^{-3} h^{-1} (mean ± SE, n = 12). Areal rates of N_2 fixation by the *Richelia* endosymbionts were exceptionally high and, for the bloom stations, averaged 45 ± 20 mg N m^{-2} d^{-1} (mean ± SE, n = 13). The colonial diazotrophic cyanobacterium *Trichodesmium* spp. was also present at some of the bloom stations (Fig. 2). The average N_2 fixation rate for *Trichodesmium* from all the stations at which we encountered it averaged 3.6 ± 1.0 mg N m^{-2} d^{-1} (mean ± SE, n = 16), which is comparable to reported rates of N_2 fixation by *Trichodesmium* spp. under non-bloom conditions in tropical waters (Capone et al. 1997).

Samples of *Trichodesmium* and *Hemiaulus* collected in and around the bloom have low $\delta^{15}N$ values, −2.15‰ and −1.24‰, respectively (Table 2). Samples of bulk suspended particles collected at the surface and zooplankton collected in the upper water column also show a significant $\delta^{15}N$ depletion within the bloom (Fig. 5). Along our cruise track, the $\delta^{15}N$ of suspended particles at the surface varied between −1.7 and +2.5‰, with a mean of −0.86 ± 0.23‰ (mean ± SE, n = 9) within the bloom. A value of +2.50‰ was recorded at a single station (Stn 23) where much lower (sub-bloom) *Richelia intracellularis* abundances were observed. The $\delta^{15}N$ of all size fractions collected was markedly lower within the bloom proper than at Stn 21, where the abundance of *R. intracellularis* was much lower (Fig. 5). The mean isotopic difference between the smallest size fraction of zooplankton sampled (>250 µm) and bulk suspended particles was 2.95 ± 0.28‰ (mean ± SE, n = 7). In general, zooplankton $\delta^{15}N$ values tended to increase with animal size and trophic level. All size fractions collected at Stn 23 were enriched in ^{15}N relative to zooplankton collected elsewhere on our transect.

Among the various nitrogenous substrates examined, only NH_4^+ appeared to be of quantitative significance to the N nutrition of the bloom organisms

Table 1 Comparisons of average surface concentration and depth-integrated chlorophyll *a* and CO_2 fixation, at pre-bloom, non-bloom and bloom stations

	Stns	Chlorophyll *a*					Carbon fixation				
		Surface (mg m^{-3})	SE	Depth-integrated (mg m^{-2})	SE	n	Surface (mg m^{-3} h^{-1})	SE	Depth-integrated (mg m^{-2} d^{-1})	SE	n
Pre-bloom	3–10	0.07	0.01	12	0.9	9	0.57	0.09	398	78	8
Non-bloom	3–10, 21, 25, 31, 33	0.09	0.01	15	1.5	14	0.58	0.06	428	65	12
Bloom	11–19, 27–29	0.60	0.12	41	5.0	16	2.14	0.23	1226	95	9

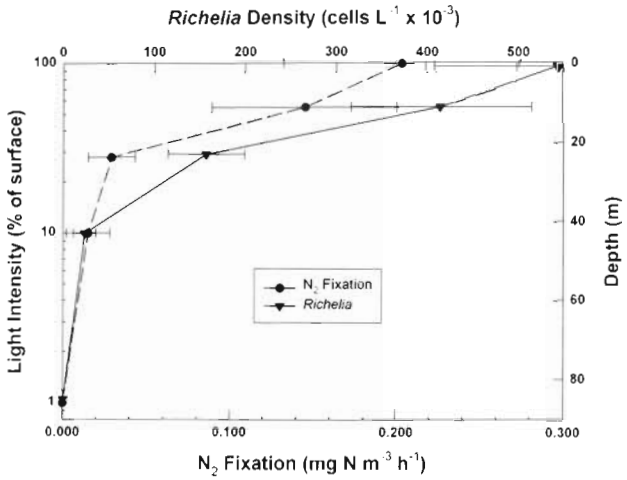

Fig. 4. Mean vertical distribution of *Hemiaulus hauckii* and rate of N_2 fixation for Stations 11 to 29 within the bloom

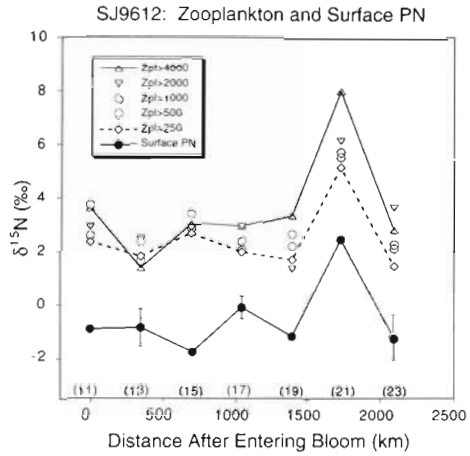

Fig. 5. $\delta^{15}N$ of suspended particles and size-fractionated zooplankton along the cruise track after entry into the bloom. Station numbers are shown in parentheses above the horizontal axis. Trend lines are shown for suspended particles as well as the smallest (>250 µm) and largest (>4000 µm) size fractions of zooplankton. At Stn 13, the >4000 µm size fraction contained abundant gelatinous zooplankton, leading to an anomalously low $\delta^{15}N$ in that size fraction

(Table 3). The absolute rates of NH_4^+ uptake and the relative importance of NH_4^+ to overall N uptake by *Hemiaulus* each appeared to increase from near 0% on the outbound leg and near the extreme SE penetration to 42% along our return cruise track (Table 3). Rates of NO_3^- and urea uptake were low throughout the bloom and usually undetectable. Rates of amino acid uptake were relatively low (from 1 to 3% of the total, except for 11% at Stn 23) over the course of the bloom.

DISCUSSION

Diatom blooms with *Richelia*, such as those we observed, may have been overlooked in the past largely because microscopic examination of phytoplankton species is infrequent on research cruises, and moreover because the endosymbiont can only be seen in *Hemiaulus* with epifluorescence microscopy. Epifluorescence is typically only used on cruises with picoplankton and immunofluorescence research components, and larger diatoms such as those we observed are usually not examined (Villareal 1994).

Relatively high concentrations of a *R. intracellularis/Rhizosolenia* association were previously reported in the Pacific Ocean (Venrick 1974). However, observed concentrations of the endosymbiotic cyanobacterium in this earlier study did not exceed 15×10^3 l^{-1}, i.e. were much lower than concentrations observed in association with the *Hemiaulus* bloom. *Hemiaulus/Richelia* densities of about 100 cells l^{-1} were reported by Villareal (1991), comparable to densities we found outside the bloom (Fig. 2). Hulbert (1976) reported concentrations of up to 10^5 cells l^{-1} in the oceanic blooms of *Hemiaulus* he encountered. *R. intracellularis* has previously been shown to fix N_2 in its association with *Rhizosolenia* in the Central North Pacific Ocean (Mague et al. 1977) and elsewhere (Villareal 1992). Villareal (1991) has demonstrated that the *Hemiaulus/Richelia* association is also diazotrophic. However, the quantitative importance of these associations in oceanic N cycling has not been assessed.

Table 2. Isotopic composition of *Trichodesmium*, concentrated suspensions of *Hemiaulus*, or concentrated suspensions of a mix of the 2 diazotrophs isolated from near-surface net tows

Sample type	No. of stations sampled		$\delta^{15}N$ (‰)
Trichodesmium (20 colonies)	13	Mean	−2.15
		SE	0.09
		n	36
Hemiaulus (100 ml concentrated suspension)	4	Mean	−1.24
		SE	0.25
		n	12
Trichodesmium & *Hemiaulus* mix (100 ml concentrated suspension)	3	Mean	−1.95
		SE	0.47
		n	6
Overall summary	20	Mean	−1.93
		SE	0.11
		n	54

Table 3. Rates of N_2 fixation and uptake ot various combined N sources in concentrated surface suspensions of *Hemiaulus/Richelia* isolated from near-surface net tows om October and November 1996, and relative to the sum of total N uptake

Date	Time	Stn	N_2 fixation ($\mu g\ N\ m^{-3}\ d^{-1}$)	NH_4^+	NO_3^-	Urea	Glu	Mixed amino acids	Sum	Total N fixed (%)	Total NH_4^+ uptake (%)
				($\mu g\ N\ m^{-3}\ d^{-1}$)							
21 Oct	07:30/09:00	11	24	0	0	0	0	nd	24	100	0
	10:30	12	3								
22 Oct	08:00/09:30	13	367	28	1	3	2	10	411	89	7
	11:30	13	177								
24 Oct	08:30	17	1954								
25 Oct	08:00	19	3650	147	8	16	40	123	3984	92	4
	08:00	20	1710								
26 Oct	15:51	22	7110								
27 Oct	08:30/09:30	23	950	120	0	1	6	16	1094	87	11
28 Oct	19:00	25[a]	nd	965	0	0	31	128	2705	?	36
29 Oct	09:00/12:00	27	2210	1694	11	12	36	100	4063	54	42
1 Nov	12:00	28	1320								
2 Nov	08:30	29	6500								
Average			2595	591	4	6	23	76	2451	81	20
SE			769	324	2	3	8	26	742	9	8
n			10	5	5	5	5	5	5	4	5

[a]Assumed average of N_2 fixation from days bracketing this station for Sum

Phytoplankton nitrogen demand

As noted, depth-integrated rates (typically to about 100 m) of phytoplanktonic primary production within the *Hemiaulus/Richelia* bloom were several-fold higher than those typically reported for tropical open-ocean waters and are more comparable to values typical during the annual spring bloom. CO_2 uptake rates averaged 1.15 ± 0.05 g C m^{-2} d^{-1} at bloom stations. By comparison, typical open-ocean productivity rates for the SW Atlantic are about 0.14 to 0.30 g C m^{-2} d^{-1} during the period of summer stratification (Malone et al. 1993, Michaels et al. 1994) at Bermuda and from 0.33 to about 0.80 g C m^{-2} d^{-1} during the spring bloom in that region. Production at Barbados averages 0.38 g C m^{-2} d^{-1} (Beers et al. 1968). At our stations outside the bloom, production averaged 0.43 g C m^{-2} d^{-1} (Table 1).

Depth-integrated rates of N_2 fixation were also exceptionally high at stations within the *Hemiaulus/Richelia* bloom, far exceeding previously reported inputs of N by N_2 fixation in open-ocean, oligotrophic environments, which typically range from 1 to 3 mg N m^{-2} d^{-1} for populations of *Trichodesmium* in the tropics under non-bloom conditions (Capone et al. 1997). The few reports of rates of N_2 fixation during surface blooms of *Trichodesmium* indicate that the input of N by bloom N_2 fixation can be greatly amplified during blooms of this species as well (Carpenter & Capone 1992, Capone et al. 1998).

Total N demand based on depth-integrated phytoplanktonic C fixation (using a Redfield ratio of 6.6) in the bloom was about 174 mg N m^{-2} $d^{-1}$of which N_2 fixation could supply about 25% (based on average N_2 fixation rates). In non-upwelling regions of the tropical and subtropical North Atlantic, the input of 'new' N through the upward flux of NO_3^- into the euphotic zone is thought to range from 0.42 to 2.1 mg N m^{-2} d^{-1} (McCarthy & Carpenter 1983). Thus N_2 fixation in this bloom exceeded NO_3^- flux as an input of 'new' N to the euphotic zone.

We also directly compared rates of N demand based on C fixation by surface concentrates of the *Hemiaulus/Richelia* association with concurrently determined rates of N_2 fixation and NH_4^+ assimilation (Table 4). For the *Hemiaulus/Richelia* concentrates, N_2 fixation could supply the bulk of the daily extrapolated N demand. As noted for the proportion of total N uptake, the contribution of NH_4^+ to N demand also increased with time (Table 4).

Stable isotope data provide an independent and integrative measure of the contribution of N_2 fixation to the organic matter in the upper water column (Wada & Hattori 1976, Carpenter et al. 1997). They confirm that N_2 fixation was the major source of N supporting the growth of the *Hemiaulus/Richelia* association (Table 2). The similarity between the mean $\delta^{15}N$ of *Hemiaulus* collected in net tows and the mean $\delta^{15}N$ of bulk suspended particles collected at the surface within the bloom implies that the near-surface organic

Table 4. Calculation of N demand in October and November 1996 based on $^{14}CO_2$ uptake in *Hemiaulus/Richelia* concentrates (<202 µm, corrected to in situ densities) assuming a 6.6 ratio of C:N, relative to supply by N_2 fixation, NH_4^+ and total N uptake by *Hemiaulus/Richelia* (Table 3)

Date	Time	Stn	N demand (µg N m^{-3} d^{-1})	N fixation (%)	NH_4^+ uptake (%)	Total N supply (%)
21 Oct	07:30/09:00	11	0.1	16.6	0	16.6
	10:30	12				
22 Oct	08:00/09:30	13	0.8	49	4	55
	11:30	13				
24 Oct	08:30	17	5.3	37		
25 Oct	08:00	19	1.2	300	12	327
	08:00	20				
26 Oct	15:51	22	6.4	110		
27 Oct	08:30/09:30	23	1.2	80	10	92
28 Oct	19:00	25				
29 Oct	09:00/12:00	27	6.5	34	26	63
1 Nov	12:00	28	3.7	36		
2 Nov	08:30	29	8.5	76		
Average			4.2	90	13	134
SE			1.0	31	5	65
n			8.0	8	4	4

nitrogen pool was overwhelmingly dominated by recently fixed N. Zooplankton $\delta^{15}N$ values provide additional evidence that N_2 fixation contributed significantly to the N budget of this system (Fig. 5). The value of about 3‰ difference in $\delta^{15}N$ signature between bulk suspended material and the smallest zooplankton fraction (Fig. 5) is very similar to the average trophic shift of ca 3.5‰ typically found in marine and other ecosystems (Minagawa & Wada 1984, Montoya et al. 1990, 1992), which implies that nitrogen derived either directly or indirectly from N_2 fixation was a major component of the diet of the small zooplankton. Our isotopic data indicate that the bloom had persisted long enough for the isotopic signature of N_2 fixation to propagate into the food web. Since the biomass turnover time for large zooplankton is typically on the order of weeks to months, our data are consistent with a rather long-lived bloom in this region. Since we calculate that only about 25% of the overall N demand associated with CO_2 fixation within the bloom can be met by N_2 fixation, our isotopic data imply that recycled pools of N (e.g. NH_4^+) must also be isotopically light as a result of the recycling of recently fixed N.

When we first encountered the bloom, N_2 fixation dominated the combined N utilization by the *Hemiaulus/Richelia* complex. Over our cruise track, NH_4^+ utilization increased. Combined N uptake in surface concentrates, primarily as NH_4^+, represented about 42% of total N utilization after 8 d (Table 3) and 26% of N demand. This increases to about 63% the fraction of the estimated total N demand based on daily CO_2 fixation supplied. Mulholland & Capone (in press) found that for cultures and natural populations of *Trichodesmium*, the N demand associated with cell growth and CO_2 fixation could be satisfied when NH_4^+ uptake was included in estimates of total N utilization.

Causes and controls on bloom

Because the *Hemiaulus hauckii* bloom occurred in the vicinity of the Amazon River plume, it is tempting to speculate on the role that the plume may have played in initiating and sustaining the bloom. The relatively high silicate and iron concentrations in Amazon water (Ryther et al. 1967, Milliman & Boyle 1975, Moore et al. 1986) may have been a factor in promoting growth of the diatom and endosymbiont, respectively. The bloom was observed as far as 1130 km NE (Stn 16) and 2463 km NW (Stn 29) of the mouth of the Amazon River (Figs. 1 & 3). The OCTS and SeaWiFS imagery indicated the bloom we encountered was directly associated with the Amazon River plume and, further north, may even be associated with the Orinoco River outflow. The Amazon plume typically can be detected throughout the bloom area (Froelich et al. 1978, Borstad 1982, Moore et al. 1986, Muller-Karger et al. 1988). Between summer and midwinter, the Amazon outflow is entrained into the North Brazil Current, and between 5° N and 10° N it is retroflected eastward in the North Equatorial Countercurrent (Moore et al. 1986). Through midwinter and spring the water flows NW toward the Caribbean Sea. Remote sensing data from the 3rd trimester of the year typically show a region of elevated chlorophyll level between ca 10° N and 10° S in the tropical Atlantic, extending between the African and South American continents (Muller-Karger et al. 1988). Some of the blooms observed in the tropical Atlantic Ocean which variously have been attributed to thermocline tilting, curl of wind stress or eddy upwelling (Longhurst 1993) may well have been promoted by nitrogen enrichment resulting from N_2 fixation by *Richelia* and/or *Trichodesmium*. In the Caribbean, it has been suggested that the elevated chlorophyll seen in ocean color imagery in the fall is primarily due to colored dissolved organic matter transported by the Orinoco River (Muller-Karger et al. 1988). Our sea-truth observations show that the

oceanic extent of these areas of 'elevated chlorophyll' were algal and contained *Richelia* and/or *Trichodesmium* that would sustain the blooms through N_2 fixation. Anecdotal communications received from dive operators in the Lesser Antilles soon after the cruise also indicated that a brown algal bloom had entered the Caribbean proper (Carpenter pers. obs.).

Biogeochemical implications

Our observations indicate that *Hemiaulus/Richelia* blooms, when and where they occur, can reach very high densities, cover vast areas of the sea surface and contribute substantially to new N inputs. The density of *Hemiaulus/Richelia* we observed in this bloom far exceeds earlier reports. The bloom was very large, judging both from the lineal distance on the cruise over which we encountered it, and satellite coverage. Assuming a bloom width, along our 2500 km cruise track, of 5° to 10°, total areal extent of the bloom would amount to 1.4 to 2.8×10^6 km^2. The approximate area of the Amazon plume of elevated chlorophyll in the OCTS image extends about 7.5° high by about 5° wide while the plume NE of the Leeward Islands is at least $5° \times 5°$ for a total of about 0.8×10^6 km^2 (Fig 1A). The *Hemiaulus/Richelia* bloom is similar in spatial extent to a large surface bloom of the diazotrophic cyanobacterium *Trichodesmium* which we encountered in the Arabian Sea in May 1995 and which was estimated to cover about 2×10^6 km^2 of sea surface (as determined by AVHRR imagery) (Capone et al.1998).

While it is impossible to attribute all of the elevated chlorophyll concentrations seen in the satellite imagery (Fig. 1A) to the *Hemiaulus/Richelia* bloom alone, our sea-truth observations suggest this to be the case east of the Leeward Islands (63° W, Table 1). This is also corroborated in part by satellite imagery from the same month in the following year (Fig. 1B) that shows a similar expanse of elevated chlorophyll in the eastern Caribbean, possibly attributable to the Orinoco River outflow, but not the bloom on the Atlantic side of the Leeward Islands (east of 63° W) as observed in 1996 (Fig. 1A). If we assume an area of 1×10^6 km^2, our average depth-integrated rate of N_2 fixation for the bloom stations (45 mg N m^{-2} d^{-1}) and a bloom duration of 10 d, this 1 event could account for a total input of 0.45 Tg N.

If dense accumulation of diatoms with diazotrophic endosymbionts are not atypical for this region, then this bloom may constitute an important, previously unrecognized source of 'new' nitrogen to support primary production in the tropical Atlantic Ocean. C and N budgets for the North Atlantic Ocean indicate a deficit in the inputs of 'new' N supplied via the upward flux of NO_3^- from deep water to the euphotic zone (Sambrotto et al. 1993, Michaels et al. 1996, Gruber & Sarmiento 1997). N_2 fixation by blooms of diatoms containing the endosymbiont, *Richelia intracellularis*, as well as those of the planktonic non-heterocystous cyanobacterium *Trichodesmium* spp. (Capone et al. 1997) are likely important 'missing terms' contributing to this apparent imbalance in current elemental budgets of the open ocean. On a global scale, the presence of endosymbiotic diazotrophs in high numbers in such surface blooms may also be significant in reconciling existing imbalances in global marine nitrogen budgets.

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