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Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico

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Abstract

During a 3-yr study in the Gulf of Mexico, we measured dinitrogen (N_2) fixation and nitrogen (N) release by *Trichodesmium* and compared these rates with water column N demand and the estimated N necessary to support blooms of *Karenia brevis*, a toxic dinoflagellate that severely affects the West Florida shelf. Net and gross N_2 fixation rates were compared in simultaneous incubations using $^{15}N_2$ uptake and acetylene reduction, respectively. The difference between net and gross N_2 fixation is assumed to be an approximation of the rate of N release. Results demonstrate that *Trichodesmium* in the Gulf of Mexico are fixing N_2 at high rates and that an average of 52% of this recently fixed N_2 is rapidly released. Calculations suggest that observed densities of *Trichodesmium* can provide enough N to support moderately sized *K. brevis* blooms. Based on other studies where $^{15}N_2$ and acetylene reduction were compared directly, it appears that N release from *Trichodesmium* is common but varies in magnitude among environments. In addition, carbon (C) and N-based doubling times for *Trichodesmium* vary among studies and environments. Comparing gross N_2 fixation and C fixation directly, C-based doubling times exceeded N-based doubling times, which suggests an imbalance in elemental turnover or a failure to fully quantify *Trichodesmium* N use.

Blooms of the toxic dinoflagellate *Karenia brevis* occur in the oligotrophic waters of the eastern Gulf of Mexico where known nitrogen (N) sources are insufficient to support the observed biomass accumulations (Steidinger et al. 1998; Walsh and Steidinger 2001; Vargo et al. 2004). Estuarine transport can supply only an estimated 5% to 20% of the N required for the daily growth needs of a moderate population (3×10^5 cells L^{-1}) of *K. brevis* (Vargo et al. 2004). However, large *K. brevis* blooms frequently co-occur or occur subsequent to blooms of the N_2-fixing cyanobacteria, *Trichodesmium* spp. (Walsh and Steidinger 2001). *Trichodesmium* spp. have long been recognized to be both abundant and common in the eastern Gulf of Mexico, forming dense blooms from February to August, often in shallower coastal waters (King 1950). Correlations between the timing and magnitude of blooms of *Trichodesmium* spp. and *K. brevis* in the Gulf of Mexico and the coastal Atlantic have been made based on historical red tide monitoring data, unpublished research results, and anecdotal information (Lenes et al. 2001; Walsh and Steidinger 2001). *Trichodesmium* was originally thought to have a role in *K. brevis* blooms by providing a biological mechanism for concentrating phosphorus in surface waters (Graham et al. 1953), which could be made available to *K. brevis* via decomposition and regeneration (Chew 1953). More recently, it has been hypothesized that *Trichodesmium* provide a source of new N to fuel growth of *K. brevis* (Walsh and Steidinger 2001).

Trichodesmium spp. can fix dinitrogen (N_2) at high rates and are therefore not N limited. Further, *Trichodesmium* release fixed N as dissolved organic N (DON) (Glibert and Bronk 1994), amino acids (Capone et al. 1994), and NH_4^+ (Mulholland and Capone 2001; Mulholland et al. 2004), and the accumulation of NH_4^+ and/or DON has been observed in and around *Trichodesmium* blooms in the

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Pacific (Devassy et al. 1978; Karl et al. 1992, 1997) and the Gulf of Mexico (Lenes et al. 2001; Heil et al. unpubl. data), along the coast of Australia (Glibert and O'Neil 1999), and in aging *Trichodesmium* cultures (Mulholland and Capone 2001). At other times, nutrient concentrations within and around blooms may be depleted because the released N is rapidly taken up by organisms growing on and around colonies (e.g., *see* Sellner 1992) or co-occurring in the water column.

In some coastal systems, blooms of dinoflagellates and diatoms have been observed during and subsequent to *Trichodesmium* blooms (Devassy et al. 1978; Revelante et al. 1982; Furnas and Mitchell 1996). On the West Florida shelf, dense *K. brevis* blooms occur during and subsequent to *Trichodesmium* blooms, and it has been hypothesized that they provide a source of new N to fuel growth of *K. brevis* (Walsh and Steidinger 2001). Here we report the first estimates of N_2 fixation from the Gulf of Mexico and compare them with rates reported for other regions. Simultaneous N_2 and carbon (C) fixation rates are also compared to determine biomass-specific doubling times of *Trichodesmium* in this region. In addition to direct estimates of N release, gross N_2 fixation, estimated using the acetylene reduction method, was compared with net N_2 uptake as an independent measure of N release in the field (Mulholland et al. 2004; Mulholland and Berardt 2005). These rates are then compared to estimates of $C_2H_2 : N_2$ reduction ratios observed in other systems and water column N demand in the Gulf of Mexico in areas where *Trichodesmium* are seasonally abundant and where *K. brevis* blooms routinely occur.

Methods

During cruises on the West Florida shelf in the Gulf of Mexico (Fig. 1) a suite of parameters was measured including (1) N_2 fixation by *Trichodesmium* using the acetylene reduction method and by $^{15}N_2$ uptake, (2) the production of $^{15}NH_4^+$ from $^{15}N_2$ by *Trichodesmium*, (3) regeneration of NH_4^+ by isotope dilution, (4) the distribution of *Trichodesmium* in the water column, and (5) water column uptake of NH_4^+, NO_3^-, urea, and a mixture of amino acids. During 2001, cruises were aboard the R/V *Walton Smith* (July) and the R/V *Suncoaster* (October); in 2002, cruises were aboard the R/V *Walton Smith* (July and October); and, in 2003, cruises were aboard the R/V *Pelican* (June and November). On the June and July cruises, a surface radio-beacon drogue was deployed and used to follow surface populations of *Trichodesmium* spp. for periods of up to 5 d.

For N_2 fixation measurements, *Trichodesmium* colonies were collected from the upper 20 m using a 64-μm, 0.5-m plankton net towed at speeds <0.5 m s^{-1}. Colonies were transferred to filtered (Whatman GF/F, nominal pore size of 0.7 μm) seawater using inoculating loops to rinse the colonies and then transferred to experimental incubation bottles filled with 0.2-μm filtered seawater.

Rates of N_2 fixation were estimated using the acetylene reduction method (Capone 1993), which estimates gross N_2 fixation (Mulholland et al. 2004), and using ^{15}N-labeled N_2, which estimates net $^{15}N_2$ uptake into particulate organic nitrogen (PON) (Montoya et al. 1996; Mulholland et al. 2004). Filtrate from the $^{15}N_2$ incubations was collected to determine rates of production of dissolved $^{15}NH_4^+$ from $^{15}N_2$ (*see following*). Acetylene (C_2H_2) reduction assays were initiated by removing 2 mL of headspace from 24-mL serum vials containing 10 mL of 0.2-μm filtered seawater and 10 colonies of *Trichodesmium* and then adding 2 mL of C_2H_2. Immediately after the C_2H_2 addition, and at increments over the course of the day, 100 μL of headspace was removed and the production of ethylene was measured using a Shimadzu gas chromatograph equipped with a flame ionization detector.

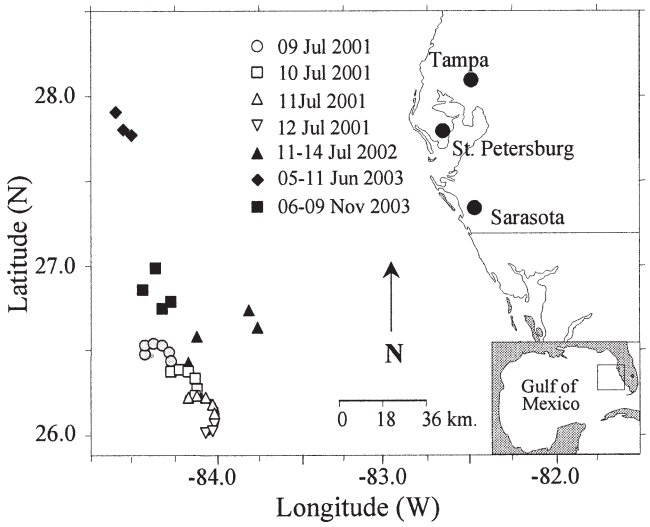

Fig. 1. Map of the study area along the West Florida shelf in the Gulf of Mexico.

Nitrogenase preferentially reduces C_2H_2 as opposed to its natural substrate N_2, and the stoichiometry of these two reactions is different. As a result, one must use a conversion factor to convert acetylene reduction rates to N_2 fixation rates. The theoretical molar ratio of $C_2H_2 : N_2$ reduction is 3 : 1 (mol : mol) (Montoya et al. 1996; Postgate 1998). However, nitrogenase-dependent H_2 release, which is inhibited by C_2H_2, results in deviations from this theoretical stoichiometry (*see Discussion following for details*). We used a 3 : 1 conversion factor and evaluated deviations between N_2 fixation rate estimates using the C_2H_2 reduction method and $^{15}N_2$ uptake. These are compared with previous intercalibrations between the two methods to estimate N release by difference (Mulholland et al. 2004; Mulholland and Bernhardt 2005).

Uptake experiments were initiated by putting tracer additions (<10%) of highly enriched (99%) $^{15}N_2$ into gas-tight bottles (315 or 727 mL) filled with whole water or <0.2-μm filtered seawater containing 50 or 100 colonies of *Trichodesmium* (Mulholland and Capone 2001). Carbon fixation was measured simultaneously by putting tracer additions (<10% or the calculated dissolved inorganic C pool) of $H^{13}CO_3^-$ into the ^{15}N incubations (Mulholland and Capone 2001). After 2 to 6 h, incubations were

terminated by filtration through combusted (450°C for 2 h) GF/F filters. Filtrate was reserved to measure the production of $^{15}NH_4^+$ from recently fixed $^{15}N_2$. Parallel incubations were conducted to measure NH_4^+ uptake and independently measure NH_4^+ regeneration by the isotope dilution method (Glibert et al. 1982). All ^{15}N and ^{13}C samples were analyzed on a Europa 20/20 mass spectrometer equipped with an automated nitrogen and carbon analysis for gas, solids, and liquids (ANCA-GSL) preparation module. Rates of uptake were calculated based on the equations of Montoya et al. (1996) and Orcutt et al. (2001) using a mixing model

$$\text{N uptake} = \frac{(\text{atom \% PON})_{\text{final}} - (\text{atom \% PON})_{\text{initial}}}{(\text{atom \% enrichment N source pool} - \text{atom \% PON})_{\text{initial}} \times \text{time}} \times [\text{PON}] \quad (1)$$

$$\text{C uptake} = \frac{(\text{atom \% POC})_{\text{final}} - (\text{atom \% POC})_{\text{initial}}}{(\text{atom \% enrichment C source pool} - \text{atom \% POC})_{\text{initial}} \times \text{time}} \times [\text{POC}] \quad (2)$$

Daily rates of N_2 fixation were calculated from hourly measurements of acetylene reduction or from $^{15}N_2$ incubations lasting 4 h or more. Ten hours of C and N_2 fixation per day were assumed. N- and C-based doubling times were calculated as in Orcutt et al. (2001).

Production of NH_4^+ was measured with two techniques: (1) by adding ^{15}N-labeled N_2 gas and quantifying the appearance of ^{15}N in the NH_4^+ pool (Mulholland et al. 2004) and (2) by adding ^{15}N-labeled NH_4^+ and measuring isotope dilution over time (Glibert et al. 1982; Mulholland and Bernhardt 2005). The NH_4^+ pool was isolated with solid phase extraction (Dudek et al. 1986). Rates of $^{15}NH_4^+$ production from $^{15}N_2$ were calculated using N_2 as the source pool and assuming that intracellular pools of NH_4^+ and DON were minimal so that release of these compounds occurred prior to assimilation into PON; mixing is between the enriched N_2 and NH_4^+ pools (Glibert and Bronk 1994; Mulholland et al. 2004). Rates of NH_4^+ regeneration determined by isotope dilution were calculated assuming that both isotopic signatures and NH_4^+ concentrations changed over the course of incubations (Eq. 2; Mulholland and Bernhardt 2005). This is a modification of the equations outlined by Glibert et al. (1982) but is consistent with the original model of Caperon et al. (1979).

Water column N uptake was estimated in 1-liter incubations (4–6 h in duration) using additions (0.05 μmol L^{-1} N) of highly enriched (96–99%) tracer compounds: $^{15}NH_4^+$, $^{15}NO_3^-$, and ^{15}N-labeled urea or dissolved primary amines. Uptake rates were calculated using Eq. 1 above.

Nutrient concentrations were measured using an Astoria-Pacific automated nutrient analyzer. Dissolved free amino acid concentrations were measured by high performance liquid chromatography (Cowie and Hedges 1992). Particulate organic C and PON concentrations were measured with the mass spectrometer described above.

Table 1. Maximum hourly rates of N_2 fixation estimated using the C_2H_2 reduction technique and a conversion factor of 3 ± SD from triplicate incubations.

Date	Time of maximum N_2 fixation (h)	Gross N_2 fixation C_2H_2 reduction (nmol N col^{-1} h^{-1})
08 Jul 2001	16:00	1.17±0.02
09 Jul 2001	16:30	1.27±0.31
10 Jul 2001	16:30	1.96±0.37
11 Jul 2001	12:30	1.15±0.31
12 Jul 2001	15:00	1.20±0.25
11 Jul 2002	12:30	2.41±1.77
12 Jul 2002	13:00	0.93±0.54
13 Jul 2002	12:00	0.48±0.20
14 Jul 2002	12:30	1.89±0.57
05 Jun 2003	15:30	0.44±0.08
06 Jun 2003	14:00	0.93±0.10
07 Jun 2003	13:00	0.85±0.22
08 Jun 2003	15:00	0.94±0.24
09 Jun 2003	17:00	0.77±0.26
10 Jun 2003	14:00	1.50±0.12
11 Jun 2003	12:30	2.56±0.12
06 Nov 2003	15:30	0.72±0.15
07 Nov 2003	12:00	0.92±0.45
08 Nov 2003	13:00	1.52±0.46
09 Nov 2003	15:00	1.17±0.49

Water column *Trichodesmium* spp. abundance was estimated by filtering the entire contents of 20-liter Niskin bottles collected along depth profiles onto filters and then counting colonies microscopically.

Results

N_2 fixation, C fixation, and N release by Trichodesmium—During daily C_2H_2 reduction experiments, average maximum hourly rates of gross N_2 fixation ranged from 0.44 to 2.56 nmol N colony^{-1} (col^{-1}) h^{-1} over the entire study period (six cruises over 3 yr; Table 1). The time of day that the maximum N_2 fixation rate was observed varied but was always in the afternoon. The daily pattern in rates, maximum hourly rates, and integrated daily N_2 fixation rate estimates also varied from day to day over a drogue study in 2001, but there was no pattern of increasing or decreasing colony-specific rates over the course of the drogue deployment (Fig. 2A). When we compared rates of N_2 fixation made over the course of the same day using the C_2H_2 reduction technique (gross N_2 fixation) versus $^{15}N_2$ uptake (net N_2 uptake), the pattern and rates of N_2 fixation varied between the two methods (Fig. 2B). Similarly, the timing of the daily N_2 fixation maxima, estimated using the C_2H_2 reduction technique, varied daily during cruises in July 2002 (Fig. 3A), June 2003 (Fig. 3B), and November 2003 (Fig. 3C).

Daily integrated rates of N_2 and C fixation were calculated based on estimates of net $^{15}N_2$ uptake, gross N_2 fixation (C_2H_2 reduction), and $H^{13}CO_3^-$ uptake (Table 2). Net N_2 uptake from $^{15}N_2$ incubations ranged from 2.1 to 9.2 nmol N col^{-1} d^{-1}, while gross N_2 fixation rates ranged from 3.3 to 20.5 nmol N col^{-1} d^{-1} based on

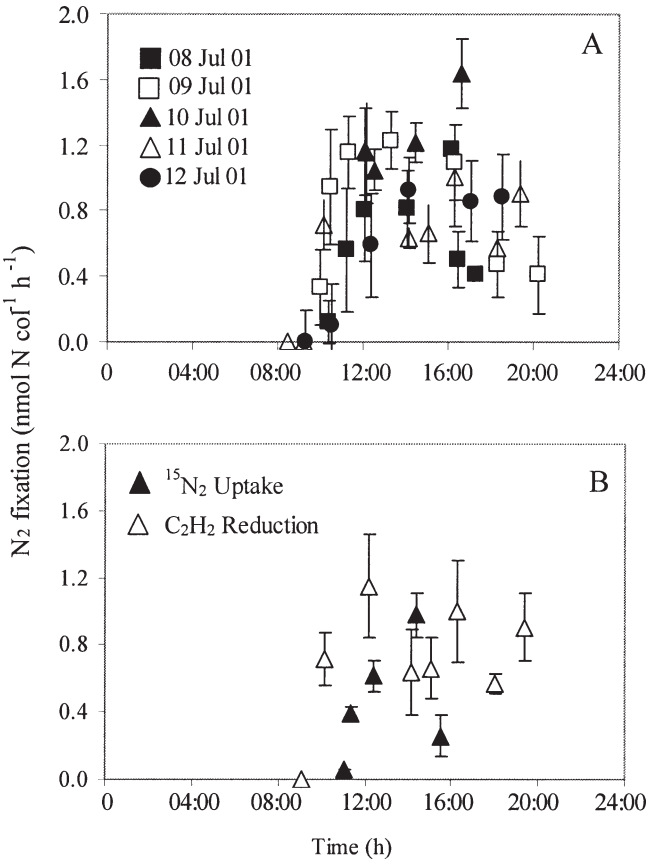

Fig. 2. (A) Rates of gross N_2 fixation (estimated using the C_2H_2 reduction method and a conversion factor of 3) over the course of several days during a cruise on the West Florida shelf in the Gulf of Mexico in July 2001. (B) Paired comparisons of gross N_2 fixation (C_2H_2 reduction) and net N_2 uptake ($^{15}N_2$) rates on 11 July 2001. Error bars are standard deviations from triplicate incubations.

C_2H_2 reduction. Bicarbonate uptake was only measured in 2003, and daily rates ranged from 82 to 281.6 nmol C col^{-1} d^{-1}. Molar ratios of C fixation to gross N_2 fixation (estimated using the C_2H_2 reduction method) ranged from 10.4 to 27.4, in excess of the molar C : N ratio of *Trichodesmium* (average of about five during 2003). Based on daily N_2 and C fixation, N turnover time for *Trichodesmium* ranged from 0.03 to 0.15 d^{-1} over the 3-yr study period, while C turnover in 2003 was estimated to be between 0.16 and 0.24 d^{-1}. This translates into N- and C-specific doubling times for *Trichodesmium* of 4.7 to 27.1 d and 2.9 and 4.2 d, respectively.

Surface abundance of *Trichodesmium* spp. varied from 0.2 to 7.8 colonies L^{-1} during our cruises in July 2001 and 2002. However, these values are closer to background *Trichodesmium* concentrations (0.75 colonies L^{-1}) and low compared with summer averages of 20 colonies L^{-1} (Lenes et al. 2001) and bloom concentrations in excess of 1,000 colonies L^{-1} (Vargo et al. 2004). Surface concentrations of *Trichodesmium* in excess of 6,500 colonies L^{-1} were observed during the ecology and oceanography of harmful algal blooms (ECOHAB) Florida program from 1998 to 2002 (Vargo pers. comm.).

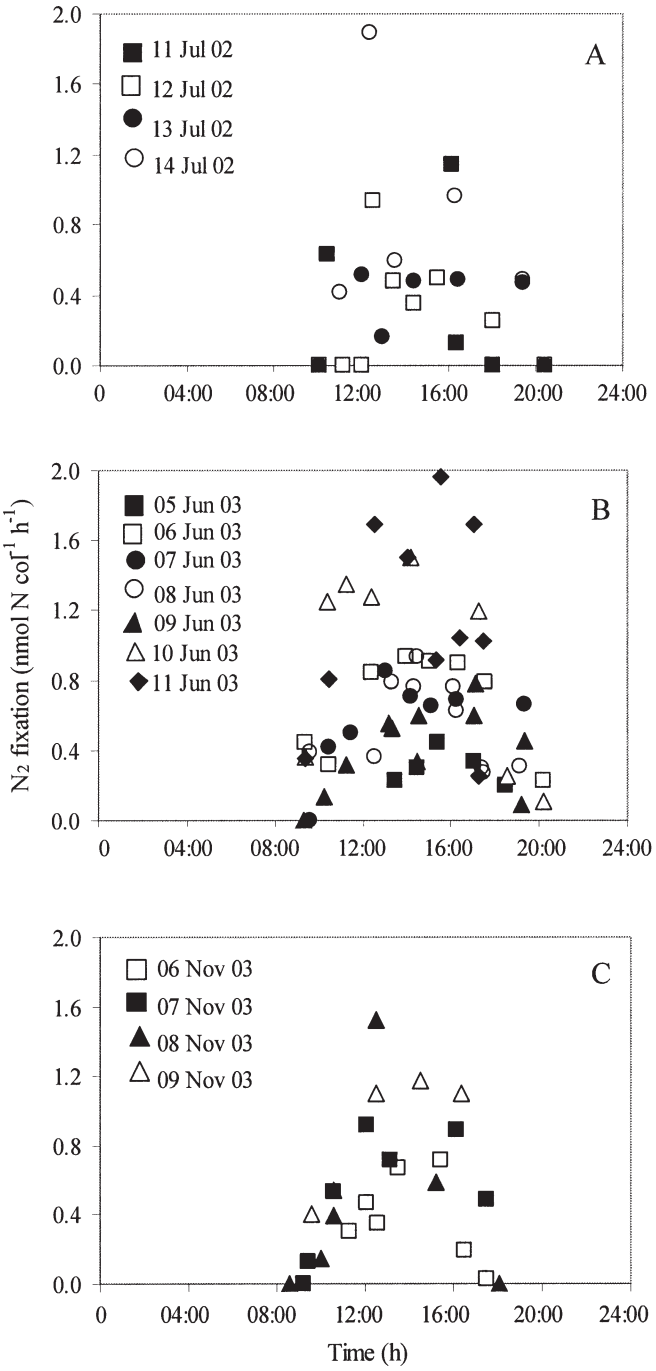

Fig. 3. Rates of gross N_2 fixation (estimated using C_2H_2 reduction and a conversion factor of 3) over daily cycles during (A) July 2002, (B) June 2003, and (C) November 2003.

We also compared net and gross N_2 fixation using $^{15}N_2$ uptake (net) and acetylene reduction (gross) methods, with C fixation over discrete intervals in order to examine variability in the ratio of net : gross N_2 fixation and the ratio of C : N_2 fixation. Rates of gross N_2 fixation always exceeded net N_2 uptake from $^{15}N_2$ incubations, with net N_2 uptake ranging from 19% to 91% of the gross N_2 fixation (Table 3). This suggests substantial loss or release of recently fixed N_2 from cells (9% to 81% release, average

Table 2. Integrated daily estimates of C and N_2 fixation by *Trichodesmium*, estimated turnover of particulate nitrogen (PN) and particulate carbon (PC), and *Trichodesmium* doubling times ± SD. C : N uptake is the ratio of C fixation to gross N_2 fixation measured using the acetylene reduction technique and a conversion factor of 3.

Date	Net $^{15}N_2$ uptake (nmol N col^{-1} d^{-1})	Gross N_2 fixation (nmol N col^{-1} d^{-1})	C fixation (nmol C col^{-1} d^{-1})	C : N uptake molar ratio	PN (μmol N col^{-1})	PC (μmol C col^{-1})	PC : PN molar ratio	N turnover (d^{-1})	N doubling time (d)	C turnover (d^{-1})	C doubling time (d)
08 Jul 2001		7.5			0.15±0.01			0.05	13.8		
09 Jul 2001		10.6			0.14±0.01			0.08	9.2		
10 Jul 2001	2.61	20.5			0.14±0.03			0.15	4.7		
11 Jul 2001	5.68	9.8			0.11±0.01			0.09	7.8		
12 Jul 2001	2.56	8.4			0.10±0.01			0.08	8.3		
11 Jul 2002		11.6						0.13	5.4		
12 Jul 2002	2.06	6.0			0.09±0.01			0.07	10.5		
13 Jul 2002		5.6						0.08	8.7		
14 Jul 2002	3.72	10.0			0.07±0.01			0.14	4.9		
28 Oct 2002	2.86				0.07±0.004						
05 Jun 2003		3.3			0.13±0.01	0.74±0.04	5.9±0.07	0.03	27.1		
06 Jun 2003		7.7			0.10±0.01	0.55±0.05	5.8±0.28	0.08	9.0		
07 Jun 2003		7.3	156.8	21.5	0.16±0.02	0.72±0.04	4.8±0.13	0.05	15.2	0.22	3.2
08 Jun 2003		5.8	116.2	20.0	0.15±0.04	0.70±0.02	4.8±0.06	0.04	17.9	0.17	4.2
09 Jun 2003	5.61	5.7	151.0	26.7	0.16±0.01	0.80±0.03	5.1±0.13	0.04	19.6	0.19	3.7
10 Jun 2003	9.21	11.6	120.2	10.4	0.14±0.01	0.69±0.07	5.0±0.09	0.08	8.4	0.17	4.0
11 Jun 2003	8.60	15.8	281.6	17.9	0.23±0.08	1.16±0.01	4.8±0.01	0.07	10.1	0.24	2.9
06 Nov 2003		4.4	119.7	27.4	0.14±0.01	0.73±0.02	5.1±0.18	0.03	22.2	0.16	4.2
07 Nov 2003	5.48	6.9	129.8	18.8	0.13±0.06	0.63±0.01	4.9±0.05	0.05	13.1	0.21	3.4
08 Nov 2003	2.31	7.2	82.0	11.4	0.09±0.02	0.44±0.04	5.0±0.10	0.08	8.7	0.19	3.7
09 Nov 2003		10.1	125.3	12.4	0.11±0.02	0.64±0.04	4.9±0.10	0.09	7.5	0.20	3.5
Study average									11.6		3.6

Table 3. Direct comparisons of N_2 fixation estimates made using C_2H_2 (gross) and $^{15}N_2$ uptake (net), estimates of N release on a per-colony basis and as a percentage of the gross N_2 fixation, molar ratios of C_2H_2 reduction : $^{15}N_2$ uptake, and C fixation over discrete time intervals.

Date	Time interval (h)	N_2 fixation		N release (nmol N col^{-1})	N release (% of N_2 fixation)	C_2H_2 : $^{15}N_2$ molar ratio	C fixation (nmol C col^{-1})	C : N uptake molar ratio
		C_2H_2 reduction (nmol N col^{-1})	$^{15}N_2$ uptake (nmol N col^{-1})					
08 Jul 2001	12:00–14:00	1.62	1.14	0.48	29.6	4.3		
09 Jul 2001	10:00–12:00	2.10	0.40	1.70	81.0	15.8		
10 Jul 2001	11:00–17:00	9.54	1.98	7.56	79.2	14.5		
11 Jul 2001	09:00–16:00	6.30	1.54	4.76	75.6	12.3		
	10:30–14:30	3.56	2.84	0.72	20.2	3.8		
	10:30–12:30	2.30	0.80	1.50	65.2	8.6		
12 Jul 2001	10:00–17:00	4.83	2.24	2.59	53.6	6.5		
	12:30–17:30	4.45	2.30	2.15	48.3	5.8		
	12:30–14:30	1.86	0.74	1.12	60.2	7.5		
12 Jul 2002	14:00–18:00	1.40	1.04	0.36	25.7	4.0		
14 Jul 2002	14:00–20:00	4.38	2.82	1.56	35.6	4.7		
07 Jun 2003	15:00–16:30	0.55	0.22	0.33	60.1	7.5	23.6	42.7
08 Jun 2003	14:30–16:30	1.52	0.62	0.90	59.2	7.4	23.2	15.3
09 Jun 2003	14:30–17:00	1.50	0.93	0.58	38.3	4.9	37.8	25.2
	15:00–17:00	1.54	1.40	0.14	9.1	3.3	26.8	17.4
10 Jun 2003	10:30–11:30	1.35	0.66	0.69	51.1	6.1	10.2	7.6
	10:30–12:30	2.64	1.62	1.02	38.6	4.9	16.2	6.1
	10:30–14:30	5.64	4.60	1.04	18.4	3.7	48.0	8.5
11 Jun 2003	11:00–13:00	3.38	1.94	1.44	42.6	5.2	65.0	19.2
	14:00–16:00	3.92	2.36	1.56	39.8	5.0	56.4	14.4
06 Nov 2003	14:00–16:00	1.44	0.32	1.12	77.8	13.5	24.0	16.7
07 Nov 2003	12:00–16:00	3.32	2.72	0.60	18.1	3.7	52.0	15.7
08 Nov 2003	11:00–13:00	3.04	0.58	2.46	80.9	15.7	16.4	5.4
Study average					48	7.3		13.8

of 52%; Fig. 4A). Based on differences in gross N_2 fixation and net N_2 uptake, we estimate release rates ranging from 0.14 to 7.56 nmol N col^{-1} (average of 0.59 nmol N col^{-1} h^{-1}; Fig. 4B) over intervals ranging from 1.5 to 7 h. Release was not related to the incubation duration. The molar ratio of C_2H_2 reduction : $^{15}N_2$ uptake varied between 3.3 and 15.8 with an overall average of 7.3 (Table 3).

During 2003, we conducted parallel experiments wherein we measured production of $^{15}NH_4^+$ from $^{15}N_2$ and NH_4^+ regeneration by isotope dilution to compare the two estimates of N regeneration. During these experiments, net $^{15}N_2$ uptake ranged from 0.01 to 2.15 nmol col^{-1} h^{-1}, production of $^{15}NH_4^+$ from $^{15}N_2$ was at the limit of detection, and NH_4^+ regeneration estimated from isotope dilution ranged from 0.01 to 0.72 nmol N col^{-1} h^{-1} (Table 4). Production of NH_4^+ within incubation bottles could be in excess of net $^{15}N_2$ uptake, which suggests substantial release of NH_4^+ during incubations. This analysis does not consider the probability that DON is also being released (e.g., Capone et al. 1994; Glibert and Bronk 1994; Mulholland et al. 2004). Pooling the data, there was no clear pattern suggesting higher release rates at any particular part of the day during these paired comparisons.

Water column N_2 fixation—In addition to *Trichodesmium*, it is now known that there are other unicellular cyanobacterial N_2 fixers that occur in the water column in marine environments (Zehr et al. 2001; Montoya et al. 2004). In this study, we estimated that water column N_2 uptake ranged from 11.1 to 234 pmol L^{-1} h^{-1} (Table 5). If we consider that unicells might fix for 24 h a day (e.g., Montoya et al. 2004), then daily fixation rates range from 0.27 to 5.62 nmol N L^{-1} d^{-1}. This estimate is comparable to that which might be expected for *Trichodesmium* under background conditions if there are 0.75 colonies L^{-1} and using our study averages of 9.4 nmol N col^{-1} d^{-1} (gross N_2 fixation) or 4.8 nmol N col^{-1} d^{-1} (net N_2 uptake).

Water column N uptake—To put these measurements of N_2 fixation in surface waters in context, we compared water column uptake rates of combined N sources (NH_4^+, NO_3^-, urea, and amino acids) during cruises in 2001 and 2002. Total rates of combined N uptake were lowest in July 2001 (30.6 and 53.7 nmol N L^{-1} h^{-1}), and the bulk of the N that was taken up was as NH_4^+ or as NH_4^+ and urea (Table 6). Total measured N uptake was within the range of N demand that could be supported by an average (20 colonies L^{-1}) population of *Trichodesmium* fixing N_2 at the maximal rates we observed (2.56 nmol N col^{-1} h^{-1}). In

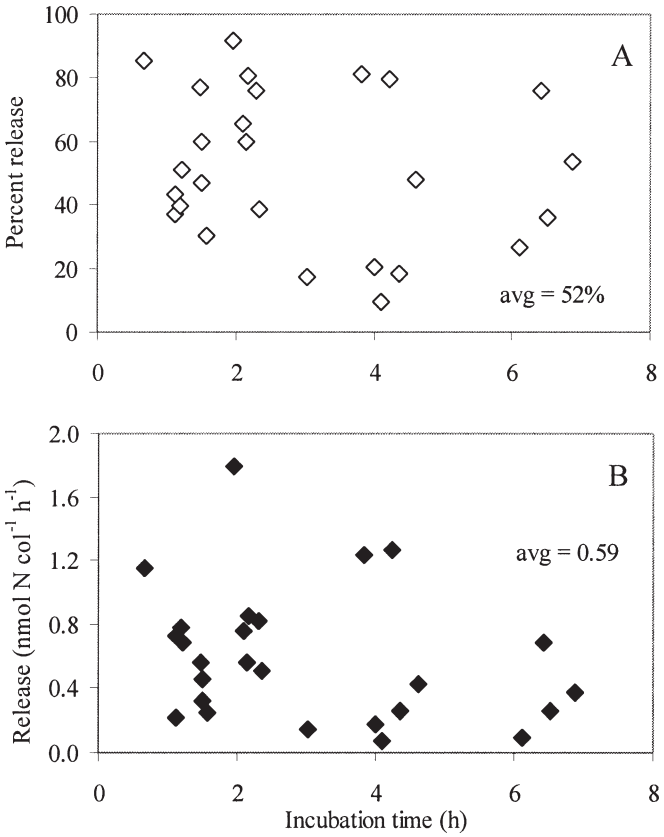

Fig. 4. Pooled estimates of N release estimated by the difference between gross N_2 fixation (C_2H_2 reduction) and net N_2 uptake made over the entire study period (2001 to 2003) (A) expressed as a percentage of recently fixed N_2 and (B) expressed as a colony-specific rate.

contrast, in the fall of 2001, there was a *K. brevis* bloom and total surface N uptake was two orders of magnitude higher than in July 2001 (2,330 and 1,540 nmol N L^{-1} h^{-1}; Table 6). The bulk of that N taken up, 69% to 84%, was as urea. During the summer of 2002, total N uptake was again reduced, and NH_4^+ and urea satisfied the bulk of the N demand. In the fall, N demand increased, and urea was the primary source of N taken up in surface waters.

Discussion

N_2 fixation—These are the first reported measurements of N_2 fixation from the eastern Gulf of Mexico. Results from N_2 fixation studies indicate that the N_2 fixation rates we report for *Trichodesmium* in the Gulf of Mexico (Table 1) are within the range of those reported from studies in other basins (Table 7) and have similar variability. Our calculated N-based doubling times (4.7 to 27 d; Table 2) were also within the ranges reported elsewhere (Table 7). Despite the number of studies now reporting rates of N_2 fixation by *Trichodesmium*, measured per-colony rates can vary by nearly two orders of magnitude, as can N-based doubling times. There are fewer but equally variable (e.g., two orders of magnitude) estimates of C fixation and C-based doubling times for *Trichodesmium* (Table 8). In this study, N-based doubling times (average 11.6 d) exceeded those estimated from C fixation (average 3.6 d), but N-based doubling times varied only by a factor of about three within individual cruises, and C fixation times were within a factor of 1.5. *Trichodesmium* have also been shown to use combined N (*see* table 5 in Mulholland and Capone 1999), and this may result in underestimates of total N turnover. In addition, high N release rates could also result in underestimates of total N turnover. As such, we assert that C-based doubling times are likely to be more robust than those based on estimates of N_2 fixation.

One likely reason for the highly variable rates and doubling times is that the physiological status of natural populations is unknown and the environmental variables controlling rates of N_2 fixation and growth by these organisms are poorly constrained. Additionally, species- or strain-specific differences in the field are poorly described in general, and differences in N_2 fixation rates in field incubations may depend on which species or strains of *Trichodesmium* are present (Carpenter et al. 1993).

Similarly, there is very little information on factors controlling growth rates for these organisms under oligotrophic conditions. Doubling times of hundreds of days are unlikely to produce the surface accumulations observed in nature, and even doubling times on the order of 3 d may be insufficient for producing the large ephemeral aggregations that are often observed. It is therefore likely that physical mechanisms are also involved in producing large surface blooms.

Oceanic concentrations of both phosphorus (P) and iron (Fe) have been proposed to limit growth of *Trichodesmium* and phytoplankton in nature (Karl et al. 1995; Sañudo-Wilhelmy et al. 2001; Mills et al. 2004), but there is little experimental evidence demonstrating relationships between P and Fe turnover and N_2 fixation and doubling times for *Trichodesmium*. During culture studies, N_2 fixation rates are higher in cultures grown at higher P concentrations (Fu and Bell 2003; Mulholland and Bernhardt 2005). In order to better evaluate global rates of N_2 fixation, it will be important to constrain this variability and make models of N_2 fixation regionally specific.

In addition to implications for growth rates, paired comparisons of N_2 and C fixation suggest some stoichiometric imbalance in the cycling of these elements through particulate material. While *Trichodesmium* particles are very close to Redfield stoichiometry with respect to C and N, C is fixed at much higher rates than N_2 (Table 9). This may be because C turnover is faster than N turnover, because N is released at high rates prior to its assimilation, or because cells are rapidly turning over C to achieve other cellular goals such as reducing intracellular oxygen through Mehler reactions (e.g., Kana 1992).

High N release rates would seem to argue for high N turnover; however, if N is released prior to its assimilation into biomass, this would contribute to gross N_2 fixation (e.g., reduction of N_2 to NH_4^+) but not net uptake. In numerous paired comparisons between acetylene reduction and $^{15}N_2$ uptake, ratios of C_2H_2 reduced to N_2 taken up have varied by at least an order of magnitude (Table 10).

Table 4. Simultaneous estimates of $^{15}N_2$ uptake and NH_4^+ regeneration by isotope dilution and $^{15}NH_4^+$ production in 2003, ±SD (NA indicates that there was none available). $^{15}NH_4^+$ production during $^{15}N_2$ incubations was also measured but was always below detection (data not shown).

Date	N_2 fixation $^{15}N_2$ uptake (nmol N col^{-1} h^{-1})	NH_4^+ production isotope dilution (nmol N col^{-1} h^{-1})	Incubation time (h)
05 Jun 2003	0.01±0.005		13:00–14:30
06 Jun 2003	0.05±0.002		09:30–11:00
07 Jun 2003	0.17±0.06		09:45–11:15
	0.37±0.09	0.11±NA	14:40–16:10
08 Jun 2003	0.01±0.01	0.06±NA	09:40–11:20
	0.31±NA		14:30–16:00
09 Jun 2003	0.63±0.40	0.72±NA	14:50–15:20
	0.37±0.14	0.11±0.02	14:50–16:00
	2.15±0.30	0.02±NA	14:50–17:00
	0.70±0.01	0.04±0.001	14:50–19:00
10 Jun 2003	0.20±NA	0.36±0.34	10:30–11:20
	0.66±0.19	0.07±0.002	10:30–12:00
	0.81±0.52	0.01±NA	10:30–13:00
	1.15±0.08	0.20±0.14	10:30–15:00
11 Jun 2003	0.97±0.21	0.07±0.01	10:45–12:00
	1.18±0.88	0.08±0.02	14:00–15:30
06 Nov 2003	0.290±NA	0.28±0.07	13:15–14:45
07 Nov 2003	0.685±0.205	0.20±0.01	12:00–14:15
08 Nov 2003	0.170±0.08	0.12±0.05	12:30–17:00
09 Nov 2003	0.019±0.01	0.02±0.01	11:30–13:45

Table 5. Water column N_2 fixation rates ± SD. NA indicates there were none available.

Date	Depth	N_2 fixation (pmol L^{-1} h^{-1})
10 Jul 2001	surface	38.4±7.0
11 Jul 2001	surface	11.1±NA
12 Jul 2001	surface	74.0±NA
09 Jun 2003	surface	233.7±105.4
07 Nov 2003	surface	39.3±NA
	Chl max	63.0±22.4
08 Nov 2003	surface	174.9±20.7
	surface	158±NA
09 Nov 2003	surface	57.6±1.2
	Chl max	44.2±27.5

Table 6. Rates of combined N uptake (nmol N L^{-1} h^{-1}) ± SD in surface waters of the Gulf of Mexico.

Date	NH_4^+	NO_3^-	Urea	Amino acids	Total
08 Jul 2001	22.7±<0.1	1.5±0.1	3.9±<0.1	2.5±0.1	30.6
09 Jul 2001	25.1±1.6	1.5±0.2	21.6±1.9	5.5±3.9	53.7
30 Sep 2001	163±11.5	26.5±1.6	1,950±52.3	189±1.2	2,329
01 Oct 2001	190±1.0	44.2±0.3	1,060±56.3	246±15.4	1,540
11 Jul 2002	46.7±0.6	1.3±0.4	41.7±4.8	2.1±0.2	91.8
13 Jul 2002	66.6±3.7	1.7±0.3	60.8±5.7	1.1±0.1	130.2
14 Jul 2002	66.2±11.6	1.7±0.1	141±0.4	4.3±<0.1	212.8
27 Oct 2002	86.2±13.1	246±4.5	638±13.5	56.6±1.5	1,027
29 Oct 2002	9.5±4.0	24.9±0.9	214±13.4	5.2±0.3	253.7
30 Oct 2002	6.0±0.4	3.4±0.3	30.5±5.4		39.9

Table 7. N-based turnover and doubling times based on 10 h of N_2 fixation per day for *Trichodesmium* collected from different environments. Times were calculated based on study-specific estimates of N biomass unless noted. Rates were recalculated using molar ratios of 3 : 1 moles C_2H_2 : N_2 reduced where noted. References are found in Web Appendix 1, http://www.aslo.org/lo/toc/vol_51/issue_4/1762a1.pdf.

Location	Reported as	N_2 fixation (nmol N col^{-1} h^{-1})	N_2 fixation (nmol N col^{-1} d^{-1})	PN turnover time (d)	N-based doubling time (d)	Reference
Subtropics						
Sargasso Sea	0.2 ng N (μg algal N)$^{-1}$ h^{-1}	0.30	3.0	42	35	Dugdale et al. (1961)*
Sargasso Sea	0.044 μg N col^{-1} d^{-1}	0.26	2.6	80	56	Carpenter (1973)
W. Sargasso Sea	0.069 pg N cell^{-1} h^{-1}	0.15	1.5	73	51	Carpenter and McCarthy (1975)
N. Pacific	0.27 μg N μg Chl a^{-1} h^{-1}	0.42	4.2	41	29	Mague et al. (1977)†
Sargasso Sea	0.033 pg N cell^{-1} h^{-1}	0.15	1.5	103	71	Carpenter and Price (1977)‡§‖
N. Pacific	504 pmol C_2H_4 μmol N^{-1} h^{-1}	0.05	0.5	298	206	Saino and Hattori (1978)*
Sargasso Sea	0.022 pg N cell^{-1} h^{-1}	0.10	1.0	155	107	McCarthy and Carpenter (1979)‡‖
East China Sea	0.7 pmol C_2H_4 trichome^{-1} h^{-1}	0.09	0.9	161	111	Saino and Hattori (1980)¶
Tanzania Coast	69 nmol L^{-1} C_2H_2 (10^3 trichome)$^{-1}$ d^{-1} (Fig. 5)	0.77	7.7	20	14	Bryceson and Fay (1981)*¶#
Sargasso Sea	0.266 nmol C_2H_4 col^{-1} h^{-1} (min.)	0.18	1.8	86	59	Scranton (1984)*
Sargasso Sea	1.47 nmol C_2H_4 col^{-1} h^{-1} (max.)	0.98	9.8	16	11	Scranton (1984)*
Sargasso Sea	0.94 nmol C_2H_4 col^{-1} h^{-1} (avg.)	0.63	6.3	24	17	Scranton et al. (1987)*††
Sargasso Sea	4.97 pmol C_2H_4 trichome^{-1} h^{-1}	0.73	7.3	21	14	Carpenter et al. (1987)¶
N. Atlantic	4.04 pmol C_2H_4 trichome^{-1} h^{-1}	0.59	5.9	26	18	Carpenter et al. (1987)*
N. Atlantic	32.6 nmol C_2H_4 μg Chl a^{-1} d^{-1} (Fig. 1)	0.09	0.9	175	121	Paerl (1994)*§
N. Atlantic	4.3 nmol C_2H_4 μg Chl a^{-1} h^{-1}	0.14	1.4	110	77	Paerl (1994)*§
N. Atlantic	305 pmol N col^{-1} h^{-1}	0.31	3.1	50	35	Capone et al. (1994)*
N. Atlantic (BATS)**	0.89 nmol C_2H_4 col^{-1} h^{-1} (net tows)	0.59	5.9	35	25	Orcutt et al. (2001)
N. Atlantic (BATS)	1.00 nmol C_2H_4 col^{-1} h^{-1} (SCUBA)	0.67	6.7	32	22	Orcutt et al. (2001)
N. Atlantic (BATS)	0.18 nmol N col^{-1} h^{-1} (net tows)	0.18	1.8	100	69	Orcutt et al. (2001)
N. Atlantic (BATS)	0.70 nmol N col^{-1} h^{-1} (SCUBA)	0.70	7.0	26	18	Orcutt et al. (2001)
N. Atlantic (BATS)	0.39 nmol N col^{-1} h^{-1}	0.39	3.9	51	36	Orcutt et al. (2001)
N. Atlantic (BATS)	32.2 pmol N col^{-1} h^{-1} (min.)	0.03	0.3	559	387	Orcutt et al. (2001)
N. Atlantic (BATS)	796.2 pmol N col^{-1} h^{-1} (max.)	0.80	8.0	23	16	Orcutt et al. (2001)
Gulf of Mexico	3.3 nmol N col^{-1} h^{-1} (min.)	0.28	2.8	51	35	Mulholland et al. (this study)
Gulf of Mexico	20.5 nmol N col^{-1} h^{-1} (max.)	1.71	17.1	8.2	5.7	Mulholland et al. (this study)

Table 7. Continued.

Location	Reported as	N_2 fixation (nmol N col^{-1} h^{-1})	N_2 fixation (nmol N col^{-1} d^{-1})	PN turnover time (d)	N-based doubling time (d)	Reference
Tropics						
Atlantic	1.9 ng N (μg algal N)$^{-1}$ h^{-1}	0.29	2.9	53	36	Goering et al. (1966)‡
Caribbean Sea	0.077 pg N cell^{-1} h^{-1}	0.34	3.4	44	31	Carpenter and Price (1977)‡§‖
Caribbean Sea	0.090 pg N cell^{-1} h^{-1}	0.40	4.0	38	26	Carpenter and Price (1977)‡§‖
Caribbean Sea	2 fg N cell^{-1} h^{-1} (min.)	0.01	0.1	1,700	1,179	Scranton (1983)‡‖
Caribbean Sea	120 fg N cell^{-1} h^{-1} (max.)	0.54	5.4	28	20	Scranton (1983)‡‖
Caribbean Sea	1.3 nmol C_2H_4 col^{-1} h^{-1}	0.87	8.7	18	12	Scranton et al. (1987)‡††
Caribbean Sea	6.45 pmol C_2H_4 trichome^{-1} h^{-1}	0.95	9.5	16	11	Carpenter et al. (1987)*¶
Virgin Islands	99 pg N trichome^{-1} h^{-1}	1.41	14.1	10	7.0	Carpenter and Capone (1992)¶
Blooms worldwide	150 pg N trichome^{-1} h^{-1}	2.14	21.4	6.7	4.6	Carpenter and Capone (1992)¶
Caribbean Sea	0.45 nmol C_2H_4 col^{-1} h^{-1} (*Trichodesmium thiebautii*)	0.30	3.0	55	38	Carpenter et al. (1993)
Caribbean Sea	0.28 nmol C_2H_4 col^{-1} h^{-1} (*Trichodesmium erythraeum*)	0.19	1.9	83	58	Carpenter et al. (1993)
Caribbean Sea	156 pmol N col^{-1} h^{-1}	0.16	1.6	97	68	Capone et al. (1994)*
Caribbean Sea	182 pmol N col^{-1} h^{-1}	0.18	1.8	84	58	Glibert and Bronk (1994)*
Arabian Sea	1.45 pmol trichome^{-1} h^{-1}	0.29	2.9	52	36	Capone et al. (1998)¶‡‡
N. Atlantic (May1994)	0.44 nmol C_2H_4 col^{-1} h^{-1}	0.29	2.9	34	23	Capone et al. (2005)§§
N. Atlantic (Apr 1996)	0.18 nmol C_2H_4 col^{-1} h^{-1}	0.12	1.2	70	48	Capone et al. (2005)§§
N. Atlantic (Oct 1996)	0.44 nmol C_2H_4 col^{-1} h^{-1}	0.29	2.9	20	14	Capone et al. (2005)§§
N. Atlantic (Feb 2001)	0.24 nmol C_2H_4 col^{-1} h^{-1}	0.16	1.6	62	43	Capone et al. (2005)§§
N. Atlantic (Jul 2001)	0.17 nmol C_2H_4 col^{-1} h^{-1}	0.11	1.1	87	60	Capone et al. (2005)§§
N. Atlantic (Apr 2003)	0.14 nmol C_2H_4 col^{-1} h^{-1}	0.09	0.9	106	73	Capone et al. (2005)§§

* Assumed an average N biomass of 2.13 μg N col^{-1} (McCarthy and Carpenter 1979).
† Original paper used a molar ratio of 1.9 : 1 moles C_2H_2 : N_2 reduced (Mague et al. 1977).
‡ Assumed an average of 29,800 cells col^{-1} (Carpenter and McCarthy 1975).
§ Assumed an average of 48 ng Chl *a* col^{-1} for *T. thiebautii* (Carpenter et al. 1993).
‖ Original paper used a molar ratio of 6.3 : 1 moles C_2H_2 : N_2 reduced (Carpenter and Price 1977).
¶ Assumed an average of 200 trichomes col^{-1} (Carpenter 1983; Carpenter and Capone 1992).
# Colony size was 2 to 45 trichomes bundle^{-1} (Bryceson and Fay 1981).
** BATS, Bermuda Atlantic Time-series Study.
†† Colony size was 10 to 30 trichomes col^{-1} (Capone et al. 1998).
‡‡ Original paper used a molar ratio of 4.1 : 1 moles C_2H_2 : N_2 reduced (Scranton et al. 1987).
§§ We used PN values from same cruises reported in Carpenter et al. (2004); for the last three cruises, we used the average N biomass of 1.38 μg N col^{-1} from May 1994.

Table 8. C-based turnover and doubling times based on 10 h of C fixation per day for *Trichodesmium* collected from different environments. Times were calculated based on study-specific estimates of C biomass unless noted. References are found in Web Appendix 1.

Location	Reported as	C fixation (nmol C col^{-1} h^{-1})	C fixation (nmol C col^{-1} d^{-1})	PC turnover time (d)	C-based doubling time (d)	Reference
Subtropics						
N. Pacific	0.38 μg C μg Chl a^{-1} h^{-1}	1.1	10.8	75	52	Mague et al. (1977)
Sargasso Sea	1 μg C 10^{-6} cell^{-1} h^{-1}	2.5	24.8	38	26	Carpenter and Price (1977)*†
Sargasso Sea	4.2 ng C col^{-1} h^{-1}	0.4	3.5	269	186	McCarthy and Carpenter (1979)
N. Atlantic	11.3 μg C μg Chl a^{-1} h^{-1} (Fig. 1)	3.8	37.7	25	17	Paerl (1994)‡
N. Atlantic	1.63 μg C col^{-1} d^{-1}	13.6	135.8	6.1	4.3	Carpenter and Roenneberg (1995)
N. Atlantic (BATS)	17.54 nmol C col^{-1} h^{-1} (net tows)	17.5	175.4	5.4	3.7	Orcutt et al. (2001)*
N. Atlantic (BATS)	13.91 nmol C col^{-1} h^{-1} (SCUBA)	13.9	139.1	6.8	4.7	Orcutt et al. (2001)*
N. Atlantic (BATS)	17.4 nmol C col^{-1} h^{-1} (simultaneous N and C)	17.4	174.0	5.4	3.8	Orcutt et al. (2001)*
Gulf of Mexico	82 nmol C col^{-1} h^{-1} (min.)	6.8	68.3	7.6	5.3	Mulholland et al. (this study)
Gulf of Mexico	281.6 nmol C col^{-1} h^{-1} (max.)	23.5	234.7	5.0	3.5	Mulholland et al. (this study)
Tropics						
Caribbean Sea	2 μg C 10^{-6} cell^{-1} h^{-1}	5.0	49.7	19	13	Carpenter and Price (1977)*†
Kuroshio	3.0 mg C mg Chl a^{-1} h^{-1}	8.5	85	11	7.7	Shimura et al. (1978)§
Kuroshio	5.2 mg C mg Chl a^{-1} h^{-1}	14.7	147.3	6.4	4.4	Shimura et al. (1978)§
Caribbean Sea	0.606 μg C col^{-1} d^{-1} (gross)	4.2	42.1	22	16	Li et al. (1980)
Caribbean Sea	0.426 μg C col^{-1} d^{-1} (net)	3.0	29.6	32	22	Li et al. (1980)
Caribbean Sea	3.9 μg C col^{-1} d^{-1} (*T. thiebautii*)	32.5	325	3.1	2.1	Carpenter et al. (1993)
Caribbean Sea	3.0 μg C col^{-1} d^{-1} (*T. erythraeum*)	25.0	250	3.7	2.6	Carpenter et al. (1993)
Caribbean Sea	10.8 μg C col^{-1} d^{-1}	90.0	900.0	0.9	0.6	Carpenter and Roenneberg (1995)
Caribbean Sea	1.29 μg C col^{-1} d^{-1}	10.8	107.5	7.8	5.4	Carpenter and Roenneberg (1995)
N. Atlantic (May 1994)	0.22 μg C col^{-1} h^{-1}	18.3	183.3	3.5	2.4	Carpenter et al. (2004)
N. Atlantic (Apr 1996)	0.07 μg C col^{-1} h^{-1}	5.8	58.3	8.1	5.6	Carpenter et al. (2004)
N. Atlantic (Oct 1996)	0.09 μg C col^{-1} h^{-1}	7.5	75.0	5.3	3.7	Carpenter et al. (2004)

* Assumed an average C biomass of 11.3 μg C col^{-1} (McCarthy and Carpenter 1979).
† Assumed an average of 29,800 cells col^{-1} (Carpenter and McCarthy 1975).
‡ Assumed an average of 48 ng Chl *a* col^{-1} for *T. thiebautii* (Carpenter et al. 1993).
§ Assumed an average of 34 ng Chl *a* col^{-1} for the central Pacific (Mague et al. 1977).

Because C_2H_2 reduction measures just the reduction step, it is a measure of gross N_2 fixation, while movement of $^{15}N_2$ from the dissolved to the particulate pool measures net N assimilation (*see* Mulholland et al. 2004; Mulholland and Bernhardt 2005). Release of recently reduced N_2 and the difficulty in chemically recovering all possible dissolved pools into which products of N_2 fixation might be released may make intercalibration between the two methods impossible. However, the difference between N_2 reduction (gross N_2 fixation) and net N_2 assimilation has proved

Table 9. Paired comparisons of C and N_2 fixation. References are found in Web Appendix 1.

Location	C : N_2 fixation		Reference
	Range	Average	
Gulf of Mexico	5.4–42.7	13.1	Mulholland et al. (this study)
New Caledonia (lagoon)	9.2–77.7		Mulholland et al. (unpubl. data)
North Atlantic (latitudinal gradient)	13.6–33.3	21.9	Mulholland et al. (unpubl. data)
North Pacific	1.2–2.1		Mague et al. (1977)
Sargasso Sea		16	Carpenter and Price (1977)
Sargasso Sea	1.5–87		McCarthy and Carpenter (1979)
BATS (puffs)	13–437	128	Orcutt et al. (2001)
BATS (tufts)	15–703	198	Orcutt et al. (2001)
N. Atlantic		63	Carpenter et al. (2004); Capone et al. (2005)
N. Atlantic		63	Carpenter et al. (2004); Capone et al. (2005)
N. Atlantic		63	Carpenter et al. (2004); Capone et al. (2005)
Trichodesmium IMS101 (batch)	4.6–132.5	28	Mulholland and Capone (2001)*
Trichodesmium IMS101 (batch)	6.5–15.2	9.6	Mulholland and Capone (2001)†
Trichodesmium IMS101 (continuous)	13.4–20		Mulholland and Bernhardt (2005)‡

* Midday estimate during exponential growth.
† Cumulative estimate over a growth or diel cycle. Ratio increased during stationary phase growth.
‡ Lower at faster growth rates.

promising in culture systems as an index of the release of recently fixed N_2 (Mulholland et al. 2004; Mulholland and Bernhardt 2005). What this means is that measuring an average C_2H_2 : $^{15}N_2$ uptake ratio of six would translate into a release rate of about 50%.

There has long been debate about the ''correct'' calibration factor to use in translating C_2H_2 reduction to N_2 fixation. Most of this discussion has revolved around the role of nitrogenase in H_2 production. Although the theoretical molar ratio of C_2H_2 : N_2 reduction is 3 : 1

Table 10. Results from paired comparisons of C_2H_2 : N_2 measurements. Numbers are reported as molar ratios, and N release is estimated as the observed molar ratio minus the theoretical ratio (3) divided by the observed molar ratio. References are found in Web Appendix 1.

Location	C_2H_2 : N_2 (molar ratio)		Estimated N release (%)	Reference
	Range	Average		
Gulf of Mexico	3.3–15.8	7.3	59	Mulholland et al. (this study)
N. Atlantic (latitudinal gradient)	2.1–7.4	4.2	29	Mulholland et al. (unpubl. data)
Sargasso Sea		6.0	50	Carpenter and McCarthy (1975)
N. Pacific		1.9		Mague et al. (1977)
N. Pacific	3–10			Saino (1977)
Sargasso Sea		6.3	52	Carpenter and Price (1977)
Sargasso Sea		2.9		Scranton (1984)
Caribbean and Sargasso Seas		4.1	27	Scranton et al. (1987)
Caribbean Sea		3.4	12	Glibert and Bronk (1994)
BATS (net tows)		4.9	39	Orcutt et al. (2001)
BATS (SCUBA)		1.4		Orcutt et al. (2001)
BATS		3.0		Orcutt et al. (2001)
N. Atlantic	0.9–7.3	3.6	17	Capone et al. (2005)
Trichodesmium IMS101 (batch)	1.7–9.8	5.6	46	Mulholland et al. (2004)
Trichodesmium IMS101 (continuous)	3.0–22.2	11.4	74	Mulholland and Bernhardt (2005)

(mol:mol) (Montoya et al. 1996; Postgate 1998), because nitrogenase catalyzes the reduction of other compounds, including H^+, in conjunction with N_2 fixation, higher ratios are often assumed (e.g., $>3:1$). Acetylene blocks H_2 production by nitrogenase, but N_2 does not, and so nitrogenase-mediated H_2 production and N_2 fixation can proceed simultaneously in the absence of C_2H_2, thereby altering the stoichiometry of N_2 reduction. For example, if a mole of H_2 is produced with each mole of N_2 reduced, the molar ratio of $C_2H_2 : N_2$ reduction becomes $4:1$ (mol : mol). However, little net H_2 production was observed under environmental conditions (e.g., rates of H_2 production were two to three orders of magnitude lower than N_2 fixation rates in incubations containing *Trichodesmium* and could not account for a substantial deviation from the $3:1$ theoretical ratio; Scranton 1984; Scranton et al. 1987), which suggests either (1) that hydrogen production from nitrogenase is low in nature, justifying a $3:1$ conversion factor, or (2) that cells efficiently recoup H_2 produced during N_2 fixation, arguing for higher ratios and a thorough evaluation of hydrogen cycling within cells. We did not measure H_2 production as part of this study. Despite low rates of H_2 production, Scranton et al. (1987) measured a C_2H_2 reduction : $^{15}N_2$ uptake ratio of $4.1:1$. We contend that in the absence of H_2 production, deviations from the theoretical $3:1$ ratio of $C_2H_2 : N_2$ reduction might also arise from N release. Alternatively, measurements of H_2 production may be underestimated if N_2 fixers recycle or recoup H_2 intracellularly rather than release it into the environment or if production and consumption of H_2 are tightly coupled in the environment (e.g., co-occurring taxa such as *K. brevis* take up H_2). Physiological studies of H_2 production catalyzed by nitrogenase in *Trichodesmium* are hampered by the lack of in vitro assays, and we are unaware of studies examining H_2 uptake by *K. brevis* or other co-occurring taxa.

While there have been observations that *Trichodesmium* release recently fixed N_2 as DON in natural populations (Capone et al. 1994; Glibert and Bronk 1994) and as NH_4^+ in cultures of *Trichodesmium* IMS101 (Mulholland et al. 2004; Mulholland and Bernhardt 2005), it is unclear why cells do this. Previous speculation suggested that this is a mechanism for the extracellular transfer of fixed N between cells that fix N_2 and those that do not have that capability (Mulholland and Capone 1999, 2000). Another possible fate for regenerated N is co-occurring organisms (O'Neil et al. 1996). In the current study, release rates averaged about 52% of the recently fixed N_2 or 0.29 nmol $col^{-1}\ h^{-1}$, and much of this was probably NH_4^+ and DON (including amino acids; Capone et al. 1994). So far in this discussion of N_2 fixation, we have not considered unicellular diazotrophs, which fixed between 0.01 and 0.23 nmol N $L^{-1}\ h^{-1}$ during this study (Table 5). These rates are within the range reported in water collected from 25 m from Sta. ALOHA in the Pacific (0.01 to 0.15 nmol $L^{-1}\ h^{-1}$) but lower than that reported for the pigment maximum (about 120 m) at the same site (0.047 to 1.85 nmol $L^{-1}\ h^{-1}$), during a transect in the North Pacific (0.31 to 1.3 nmol $L^{-1}\ h^{-1}$), or along the north coast of Australia (up to 62 nmol $L^{-1}\ h^{-1}$) (Montoya et al. 2004). The fate of N_2 fixed by unicellular cyanobacteria is unknown, and so we cannot speculate on its contribution to the dissolved pool or to higher trophic levels.

Water column N uptake—To put the observed N_2 fixation and release rates into the context of water column N demand, *Trichodesmium* abundance was between 0.2 and 7.8 col L^{-1} during 2001 and 2002, and this would result in a release of about 0.12 to 4.6 nmol N $L^{-1}\ h^{-1}$ (using an average release rate of 0.59 nmol N $L^{-1}\ h^{-1}$). While background concentrations of *Trichodesmium* during November to April in the eastern Gulf are about 0.75 col L^{-1} (Vargo pers. comm.), summer *Trichodesmium* concentrations are generally much higher, averaging 20 col L^{-1} (11.8 nmol N $L^{-1}\ h^{-1}$ released based on our average release rates). During blooms, densities along the West Florida shelf can reach >1,000 col L^{-1} (average release rate of 590 nmol N $L^{-1}\ h^{-1}$). Based on in situ rates of N uptake by *K. brevis* (Bronk et al. 2004), we calculate an N demand for the large 2001 *K. brevis* bloom off St. Petersburg (up to 8.5×10^6 cells L^{-1}) to be on the order of 0.15 to 1.15 nmol N $L^{-1}\ h^{-1}$. This is well within the range of N release rates measured during our July study. Independently, Vargo et al. (2004) calculated a total N demand of 56 to 267 nmol N $L^{-1}\ d^{-1}$ for a moderate (3×10^5 cells L^{-1}) *K. brevis* population undergoing 0.2 divisions d^{-1}. Based on the current study, if we assume 10 h of N_2 fixation per day and modest *Trichodesmium* populations of 20 col L^{-1}, we calculate an average release of 118 nmol N $L^{-1}\ d^{-1}$, within the range necessary to support a moderate *K. brevis* population. If we invoke the higher *Trichodesmium* densities, *Trichodesmium* can produce ample dissolved N to fuel *K. brevis* population growth.

When we compare these estimates with estimates of surface water N demand during this study (Table 6), *Trichodesmium* can account for a substantial fraction of the water column N demand. During September and October 2001, *K. brevis* was abundant in the surface waters and the N demand was quite high as compared with summer sampling periods when *Trichodesmium* was more abundant. The temporal separation between populations of *K. brevis* and *Trichodesmium* may indicate indirect trophic coupling as well. Urea was the primary source of N during the fall, when *K. brevis* blooms reach their peak. The source of this urea is not known, but we are currently examining the possibility that urea may be regenerated from *Trichodesmium* biomass during its decay (Mulholland et al. unpubl. data). In addition, NH_4^+ and amino acids, including glutamate, were important components of the bulk water column uptake observed in this study and during the *K. brevis* bloom during 2001 (Bronk et al. 2004). These compounds are also common, identifiable release products during *Trichodesmium* growth (Capone et al. 1994; Mulholland et al. 2004; Mulholland and Bernhardt 2005).

While these results do not establish a direct trophic link between the two populations, we conclude that N_2 fixation is quantitatively significant in the eastern Gulf of Mexico and that recently fixed N_2 from *Trichodesmium* is released as dissolved N in forms that can be taken up by natural

populations of phytoplankton that include *K. brevis*. This is the first step toward demonstrating a direct link between N_2 fixation by *Trichodesmium* and the harmful bloom-former *K. brevis*.

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