Limnol. Oceanogr., 52(5), 2007, 2249–2259

Trichodesmium in the western Gulf of Mexico: $^{15}N_2$-fixation and natural abundance stable isotope evidence

Carolyn M. Holl[1]
School of Biology, Georgia Institute of Technology, Atlanta, Georgia 30332

Tracy A. Villareal
Marine Science Institute, The University of Texas at Austin, Port Aransas, Texas 78373

Christopher D. Payne[2]
School of Biology, Georgia Institute of Technology, Atlanta, Georgia 30332

Tonya D. Clayton[3]
Center for Coastal Physical Oceanography, Old Dominion University, Norfolk, Virginia 23505

Cassandra Hart
Marine Science Institute, The University of Texas at Austin, Port Aransas, Texas 78373

Joseph P. Montoya
School of Biology, Georgia Institute of Technology, Atlanta, Georgia 30332

Abstract

Surface aggregations of the diazotrophic cyanobacterium *Trichodesmium* were encountered at nearly every station along a 300-km transect in the northwestern Gulf of Mexico during July 2000. $^{15}N_2$-fixation rate measurements for all treatments and locations ranged from 1.3×10^{-4} nmol N trichome^{-1} h^{-1} to 2.6×10^{-2} nmol N trichome^{-1} h^{-1}, with a mean of $3.4 \times 10^{-3} \pm 7.6 \times 10^{-4}$ nmol N trichome^{-1} h^{-1} (mean ± SE, $n = 45$). *Trichodesmium* C-fixation, measured by the incorporation of ^{13}C, ranged from 1.3×10^{-2} nmol C trichome^{-1} h^{-1} to 4.5×10^{-1} nmol C trichome^{-1} h^{-1}, with a mean of $1.2 \times 10^{-1} \pm 9.3 \times 10^{-2}$ nmol C trichome^{-1} h^{-1} (mean ± SE, n = 44). Both $^{15}N_2$-fixation and ^{13}C-fixation rates attenuated with decreasing light intensity from a maximum at 50% surface intensity. Areal *Trichodesmium* $^{15}N_2$-fixation rates, based on a 10-h N_2-fixation day, enumerated vertical trichome abundance and corrected for estimated in situ light intensity ranged from 47.2 μmol N m^{-2} d^{-1} to 118.6 μmol N m^{-2} d^{-1}, with a mean of 84.5 ± 17.7 μmol N m^{-2} d^{-1} (mean ± SE, $n = 4$). The light natural abundance isotopic signatures of dissolved inorganic nitrogen, particulate nitrogen, and zooplankton nitrogen within these surface populations are consistent with our limited biological rate measurements and indicate that recently fixed N is moving into the food chain to promote secondary production in these nutrient-impoverished waters. Although our sample size was relatively small, natural abundance isotope data indicate that as much as 60% of macrozooplankton C and N was derived from *Trichodesmium* in the oligotrophic regions of the Gulf of Mexico.

[1] To whom correspondence should be addressed (choll@oceanicinstitute.org). Present address: Oceanic Institute, Waimanalo, Hawai'i 96795.

[2] Present address: Earth and Ocean Sciences Department, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada.

[3] Present address: St. Petersburg, Florida.

Acknowledgments

We would like to thank S.B. Joye, D.G. Capone, J. McCarthy, and an anonymous reviewer for insightful comments about this manuscript; P. Davoodi, J. Bayles, and J. Landrum for help in the lab, and the captain and crew of the R/V *Longhorn* for their support at sea.

This work was partially supported by National Science Foundation (NSF) grants OCE 997178 to T.A.V., and OCE9977528 and OCE0425583 to J.P.M. T.D.C. received support from NASA Global Change Fellowship. C.M.H. received partial stipend support from NSF-Integrative Graduate Education and Research Traineeship (IGERT) grant DGE0114400.

This is contribution no. 1281 from the University of Texas Marine Science Institute.

Both geochemical estimates and empirical measurements show that N_2-fixation is a major source of new nitrogen (N) supporting primary production in the oligotrophic ocean. *Trichodesmium* spp., the best-studied marine colonial cyanobacteria (Capone et al. 1997), are considered an important source of new N to the oligotrophic ocean. *Trichodesmium* spp. are common in the oligotrophic gyres of the world ocean, yet there is great variation in abundance among these gyres. Dense blooms can form when conditions are suitable: high sea-surface temperature, high light intensity, low nutrients, and quiescent seas all encourage surface bloom formation (Eleuterius et al. 1981; Capone et al. 1998). N_2-fixation rates in both bloom and non-bloom abundances have been measured in the Atlantic (Goering et al. 1966), Pacific (Karl et al. 1997), and Indian Oceans (Lugomela et al. 2002; Jyothibabu et al. 2003), as well as in the Caribbean (Carpenter and Price 1977), China (Saino 1977; Chen et al. 2003), Sargasso (Orcutt et al. 2001), and Arabian Seas (Capone et al. 1998). These studies have determined that *Trichodesmium* is an important

source of N to nutrient-poor regions of the global ocean. This new N may significantly amplify primary production and the consequent export of carbon (C) to the deep ocean.

Trichodesmium has been studied for decades in natural populations and more recently in laboratory cultures (Ohki et al. 1986; Mulholland and Capone 2001). Though the importance of new N input by *Trichodesmium* to many subtropical and tropical gyres has been established, few studies of this cyanobacterium have been conducted in the Gulf of Mexico. Though *Trichodesmium* is frequently present at high abundance (Carpenter 1983*a* and refs. therein; Eleuterius et al. 1981), only one other study has reported N_2-fixation rates by *Trichodesmium* in the Gulf of Mexico (Mulholland et al. 2006).

Within the Gulf of Mexico, one locus of study has been the West Florida shelf, where *Trichodesmium* biomass increased 100-fold after wet deposition of nutrients from Saharan dust (Lenes et al. 2001). The increase in dissolved organic N (DON) concentration after a West Florida shelf *Trichodesmium* bloom has been linked to harmful algal blooms of the dinoflagellate *Karenia brevis* (Walsh and Steidinger 2001; Mulholland et al. 2006). In the Mississippi Sound (north-central Gulf of Mexico), an extensive bloom of *Trichodesmium* was associated with a combination of high sea-surface temperature, high light intensity, and increased salinity as well as low nutrients and low wind activity (Eleuterius et al. 1981). Summer *Trichodesmium* blooms and the resultant increased nutrient load have been implicated in the enhancement of heterotrophic bacterial respiration rates below the euphotic zone (Biddanda and Benner 1997). These studies have shown that *Trichodesmium* is important to nutrient cycling in the coastal Gulf of Mexico; however, we do not know the extent to which *Trichodesmium* can supply new N to the extensive offshore portion of this oligotrophic region as there have been few rate measurements reported.

Here we describe the abundance and contribution of *Trichodesmium* to the supply of new N in the western Gulf of Mexico in July 2000. We measured N_2-fixation rates and pigment concentrations along the transect as well as the stable isotopic signature of organic and inorganic N to assess the effect of this new N on the planktonic food web. Our results show that N_2-fixation by *Trichodesmium* in the Gulf of Mexico is equal to and in many cases exceeds N_2-fixation rates measured in other oligotrophic gyres.

Materials and methods

We collected samples and carried out experiments in the northwestern Gulf of Mexico during a cruise on the R/V *Longhorn* in July 2000 (Fig. 1). A conductivity-temperature-depth (CTD)-rosette system was used to collect water samples for isolation of particles and for nutrient analysis. Zooplankton were collected in diagonal tows through the upper 100 m of the water column using a 1-m-diameter net with a 220-μm mesh size. *Trichodesmium* colonies were collected at the surface by hand-casting a 30-cm-diameter net with a 64-μm mesh size while the ship was drifting.

Hydrography—The 24-bottle CTD-rosette system equipped with a Sea-Bird 911-Plus, a SeaTec FL0500 fluorometer, and a Datasonics altimeter was used to measure vertical profiles of temperature, salinity, and chlorophyll fluorescence at each of the 10 stations along the cruise track. Typical profiles extended from the surface to 250 m with 24 samples taken at equal intervals throughout the profile. Deeper profiles, extending to 1,840 m and 1,200 m, were completed at Station (Sta.) 2 and 4, respectively.

Nutrients, chlorophyll, and Trichodesmium *abundance*—Samples for measurement of dissolved nutrients and chlorophyll *a* were taken from the CTD-rosette immediately after recovery. Nutrient samples (nitrate, phosphate, and silicate) were immediately frozen for later analysis ashore on a Lachat QuikChem FIA 8000 nutrient analyzer. Chlorophyll was measured fluorometrically using a non-acidification technique (Welschmeyer 1994) after extraction in 100% methanol.

Trichodesmium abundance was determined by three methods. At all stations, vertical profiles of discrete water samples (250 mL) were collected directly from Niskin bottles immediately after recovery of the CTD-rosette and preserved in acid Lugol's iodine (Throndsen 1978). The contents of 100 mL of sample were settled and counted within 2 months on an inverted microscope (Hasle 1978). At Sta. 4, the contents of a 12-liter Niskin bottle (nominal volume 10 liters) were filtered through a 10-μm pore size, 47-mm polycarbonate filter. The filter was then examined with a stereoscope at sea and *Trichodesmium* colonies counted. Using this method, free trichomes could be seen but were not enumerated. Whereas colonies were occasionally touching or overlapping, the maximum number observed (32) did not create difficulties in enumeration. We assumed a colony size of 200 trichomes colony^{-1} (Carpenter 1983*b*). At several stations, *Trichodesmium* colony abundance was determined at the surface. Bucket samples were collected, the total volume was determined with a graduated cylinder, and colonies were counted visually.

Stable isotope abundance—At Sta. 1–5, suspended particles were collected for stable isotope analysis by gentle pressure filtration ($\Delta P < 48$ kPa) of at least 10 liters of seawater through a pre-combusted 47-mm Whatman GF/F glass fiber filter (nominal pore size 0.7 μm) and stored frozen. In the lab, filters were dried at 60°C and acid-fumed to remove carbonates. A portion of each filter was then packed into a tin capsule and pelletized for elemental and isotopic analysis by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Carlo Erba NC 2500 elemental analyzer interfaced to a Micromass Optima mass spectrometer.

At selected depths, an aliquot of filtrate from the suspended particle sampling was preserved by acidification (pH 2–3) for isotopic analysis of nitrate ashore. Nitrate was reduced with Devarda's alloy and isolated for isotopic analysis by diffusion (Sigman et al. 1997).

Zooplankton samples from Sta. 1–5 and 8 were thoroughly rinsed with surface seawater to remove any

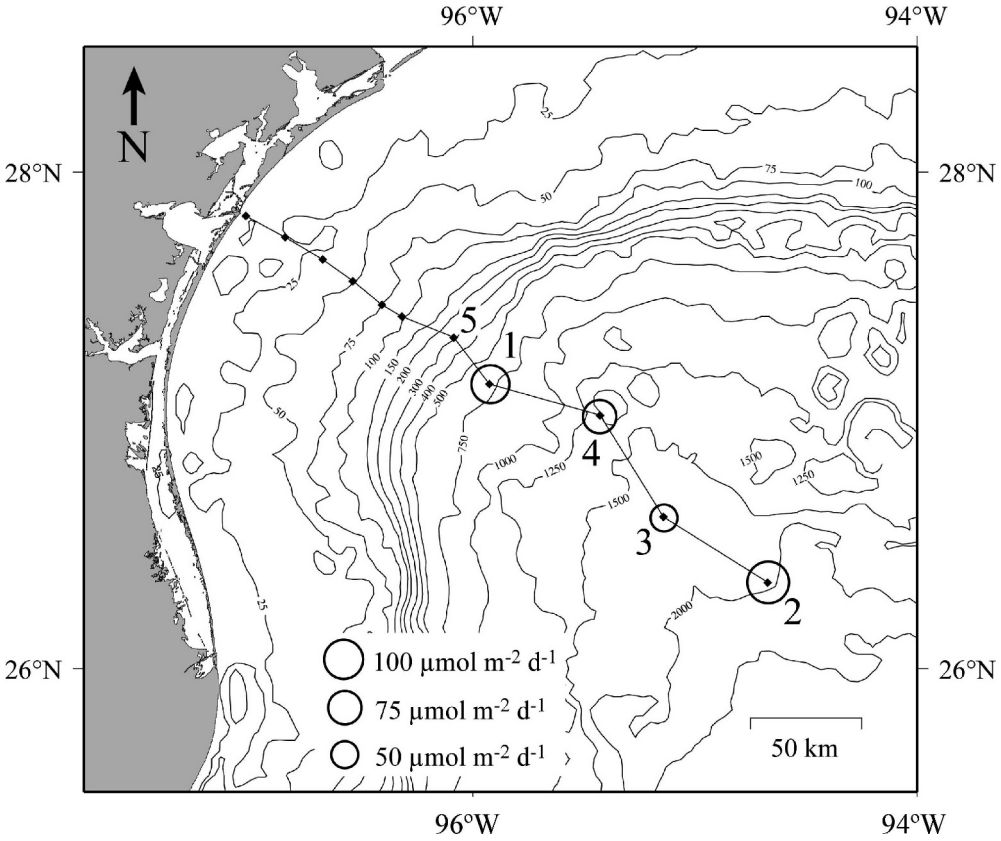

Fig. 1. Station locations and bathymetry for July 2000 cruise. All 10 stations are indicated by small diamonds. Sta. 1–5 are identified with numerical labels. For clarity, the markers for Sta. 6–10 are omitted; these five stations run in sequence from Sta. 5 toward shore. For Sta. 1–4, depth-integrated areal N_2-fixation rates are represented by the open circles. The area of each circle is proportional to the N_2-fixation rate measured at that station.

Trichodesmium caught in the sample and separated into discrete size fractions by passage through a series of Nitex sieves (4,000, 2,000, 1,000, 500, and 250 μm). The size-fractioned samples were frozen for later isotopic analysis ashore. In the laboratory, the samples were dried at 60°C and homogenized by grinding with a mortar and pestle. After grinding, subsamples (ca. 400 μg) were weighed and packed into a tin capsule for elemental and isotopic analysis.

Nitrogen and carbon fixation rate measurements—Trichodesmium colonies were isolated with a plastic inoculating loop and transferred into filtered surface seawater for distribution into 250-mL polycarbonate bottles fitted with silicone septum caps. The bottles were completely filled with filtered surface seawater to exclude bubbles before being sealed. Trace additions of ^{13}C-bicarbonate (0.02 mmoles NaH_2CO_3) and $^{15}N_2$ gas (0.01 mmoles N_2) (Cambridge Isotope; 98 atom% and 99 atom%, respectively) were made to each bottle using a gas-tight syringe. The samples were incubated either under simulated in situ conditions in deck incubators or under in situ conditions on a drifting array (Sta. 4). Shipboard incubations lasted between 6 h and 8 h, and in situ incubations spanned an entire light day. We used a 10-h fixation day as a conservative estimate of *Trichodesmium* N_2-fixation throughout the light day.

To separate positively and negatively buoyant colonies we followed the procedure of Villareal and Carpenter (2003). In short, colonies were randomly isolated as above with an inoculating loop and gently poured into glass Petri dishes. The inverted lid was placed atop the sample to exclude air bubbles and to break the surface tension, and the colonies were allowed to redistribute for 5 min. Negatively buoyant colonies were defined as those that remained on the bottom of the Petri dish after 5 min. The colonies separated by buoyancy were transferred to polycarbonate bottles and treated as described above.

Incubations were terminated by gentle vacuum filtration through pre-combusted 25-mm Whatman glass fiber GF/C filters (nominal pore size 1.2 μm) and stored frozen. In the lab, filters were dried at 60°C and acid-fumed to remove carbonates before being packed into tin capsules for elemental and isotopic analysis by CF-IRMS using a Carlo Erba NC 2500 elemental analyzer interfaced to a Micromass Optima mass spectrometer. N_2-fixation rates were calculated by isotope mass balance as described in Montoya et al. (1996). To calculate CO_2-fixation rates, we estimated the concentration of dissolved inorganic

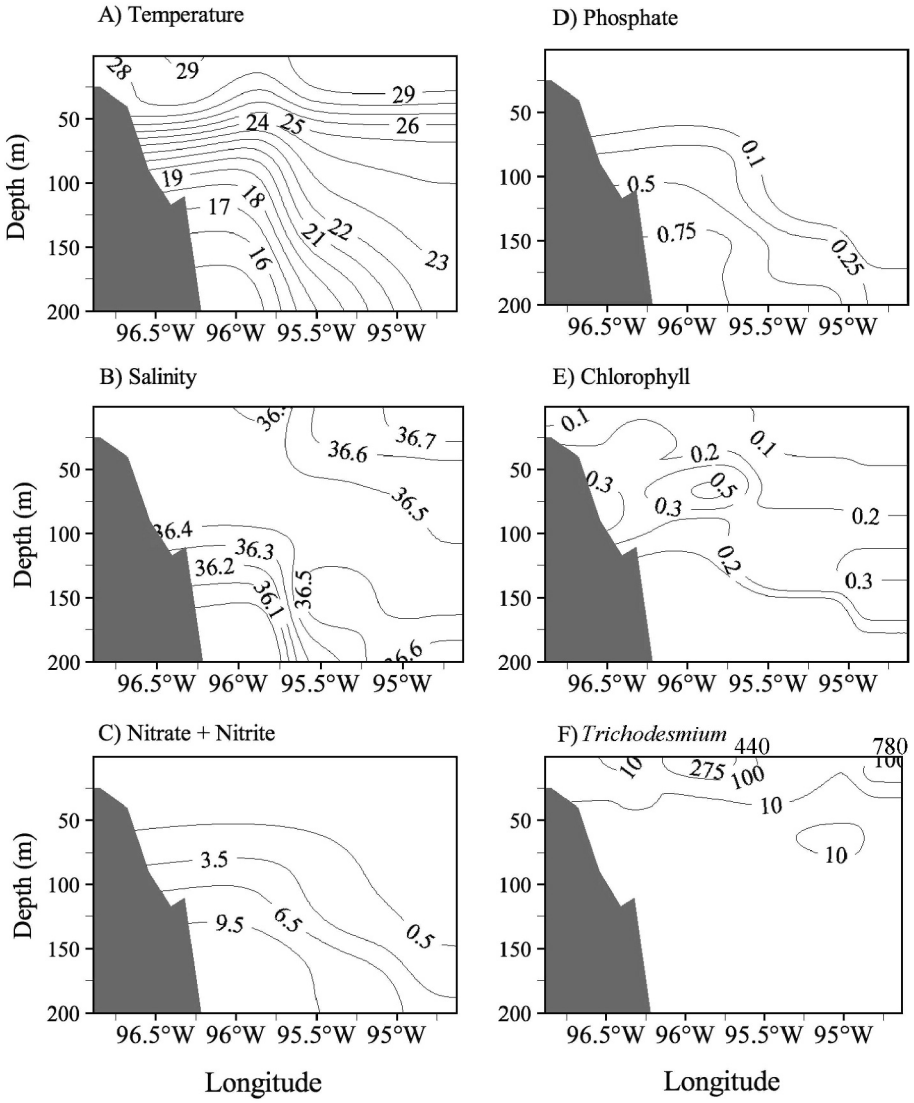

Fig. 2. Hydrographic and biological data along the transect in July 2000. (A) Temperature (°C), (B) salinity, (C) nitrate + nitrite (μmol L^{-1}), (D) phosphate (μmol L^{-1}), (E) chlorophyll (μg L^{-1}), and (F) *Trichodesmium* abundance (trichomes L^{-1}). All *Trichodesmium* counts are from settled water samples only. The numbers above the axis indicate the highest trichome counts noted in two areas of high trichome density.

carbon (DIC) based on salinity (Parsons and Maita 1984) and applied the same isotope mass balance approach.

Results

Hydrography—We observed little variation in sea-surface temperature across the transect. Surface temperatures ranged from 29°C to 31°C with a general trend toward warmer surface temperatures offshore (Fig. 2A). Likewise, temperatures in the upper 50 m of the water column throughout the transect exhibited little variation and ranged from 26°C to 31°C. The mixed layer depth, or the depth over which there was no change in temperature from that at the surface, was generally deeper offshore and was deepest at Sta. 3 at 25 m. The horizontal distribution of properties suggests offshore movement of cooler, coastal water via eddy circulation onto Sta. 1 where a doming of the thermocline occurred at approximately 95.8°W. This doming was accompanied by a slight freshening at the surface (Fig. 2B). This eddy circulation was confirmed by satellite sea surface temperature (SST) (data not shown).

Nutrient and chlorophyll concentrations—Nitrate concentrations ranged from below our limit of detection at the surface at all stations to 30 μmol L^{-1} at 500 m at Sta. 1 and to 25 μmol L^{-1} at 500 m for Sta. 2 and 4 (Fig. 2C). The upper boundary of the nitracline was considerably deeper at the stations farthest from shore. At Sta. 2, 3, and 4 the nitracline started at approximately 125 m, and at Sta. 1 and 5 the nitracline started at approximately 60 m. We did not find measurable NO_3^- shallower than 55 m at Sta. 1 and 5, shallower than 103 m at Sta. 4, or shallower than 125 m at

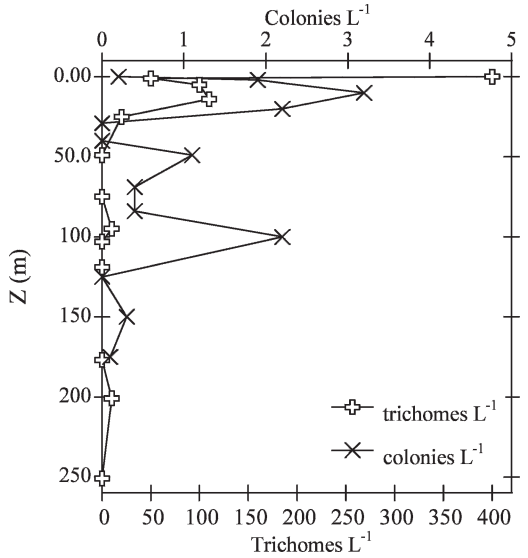

Fig. 3. Vertical profile of *Trichodesmium* abundance at Sta. 4. Colonies L^{-1} enumerated from 10-liter samples and trichomes L^{-1} enumerated from 100-mL samples collected at discrete depths. Note: Y axes offset by −10 m for clarity at 0 m.

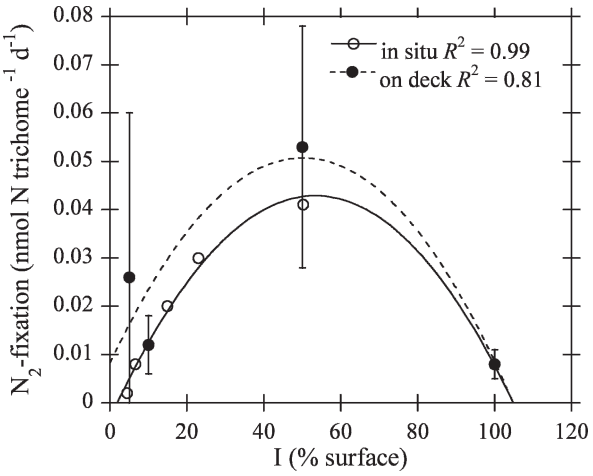

Fig. 4. Trichome-specific *Trichodesmium* N_2-fixation rate (μmol N $trichome^{-1}$ d^{-1}) as a function of light intensity (% surface irradiance) in on-deck and in situ incubations.

Sta. 2 and 3. The phosphocline started only slightly shallower in the water column than the nitracline: 50 m at Sta. 1 and 5, and 90–95 m at Sta. 2, 3, and 4 (Fig. 2D).

Bulk water chlorophyll measurements were made at all stations along the cruise track. Chlorophyll concentrations ranged from a minimum of 0.06 μg L^{-1} at 250 m at Sta. 3 to 1.4 μg L^{-1} at 22 m at Sta. 10 (Fig. 2E). The chlorophyll vertical maximum increased in depth from approximately 65 m at stations closer to shore, where this chlorophyll maximum coincided with a doming of the thermocline, to approximately 125 m at Sta. 2, 3, and 4 (Fig. 2E).

Trichodesmium *abundance—Trichodesmium* was present at 9 of 10 stations (except Sta. 8) along our cruise track, and surface slicks were prominent at Sta. 1–4. The primary approach we used to quantify *Trichodesmium* abundance was the enumeration of trichomes and colonies present in a 100-mL subsample of a 250-mL discrete water sample from vertical profiles using the CTD-rosette. This approach, in combination with surface bucket colony counts at each station, showed the highest trichome abundance at the surface, with values as high as 10^4 trichomes L^{-1} and a mean of $1.5 \times 10^3 \pm 1.1 \times 10^3$ trichomes L^{-1} (mean ± SE, $n = 9$). The highest *Trichodesmium* abundance occurred at Sta. 2 and was 10-fold higher than at any other station. Removing this large outlier yields a mean *Trichodesmium* abundance of 360 ± 157 trichomes L^{-1} (mean ± SE, $n = 8$). Based on the settled water samples, the majority of the *Trichodesmium* biomass was found above 50 m (Fig. 2F).

However, colony enumeration from large-volume samples collected at Sta. 4 produced estimates of trichome abundance approximately three-fold higher than estimates based on 100-mL settled samples (Fig. 3; Table 1). This profile also showed that *Trichodesmium* colonies were distributed throughout the upper 100 m of the water column and down to the upper boundary of the nutricline. In addition, colonies were found as deep as 175 m at this station (Fig. 3).

C- and N_2-fixation—Both N_2-fixation and C-fixation rates attenuated with decreasing light intensity. Maximal rates were observed at 0.50 I_o (50% of surface irradiance)

Table 1. *Trichodesmium* biomass in the northwest Gulf of Mexico, July 2000. Trichomes m^{-2} integrated from surface to 250 m at Sta. 1 through 5 and from surface to 50 m at Sta. 6 through 10.

Sta.	Date	Trichome L^{-1} at surface	Trichome L^{-1} at 10–15 m	Trichome L^{-1} at maximum	Z trichome maximum (m)	Trichome (m^{-2})
1	24 Jul 2000	1,060	300	1,060	0	4.3×10^8
2	25 Jul 2000	10,200	440	10,200	0	2.7×10^8
3	26 Jul 2000	1,000	20	1,000	0	2.8×10^8
4 (0.1 liter)	27 Jul 2000	400	110	400	0	1.8×10^8
4 (10 liter)	27 Jul 2000	400	640	640	10	4.8×10^8
5	28 Jul 2000	400	10	400	0	1.1×10^8
6	28 Jul 2000	0	10	30	35	3.8×10^6
7	28 Jul 2000	10	10	20	30	4.6×10^6
8	28 Jul 2000	0	0	0	–	0
9	29 Jul 2000	10	10	140	20	6.8×10^6
10	29 Jul 2000	0	120	120	10	2.7×10^6

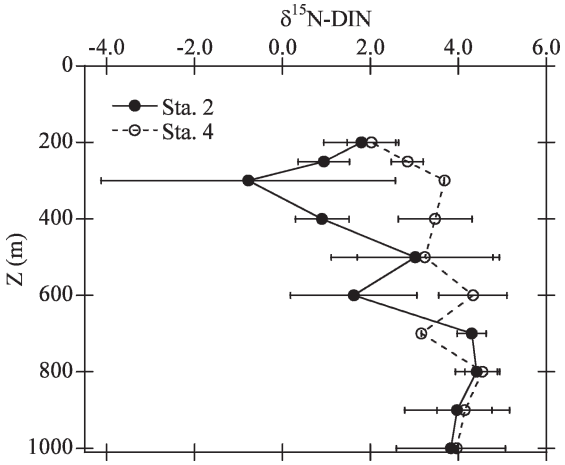

Fig. 5. Vertical profile of δ^{15}N-DIN at Sta. 2 and 4. Error bars represent standard error around the mean of two or more replicate analyses. Where no error bars are shown, only one analysis was completed.

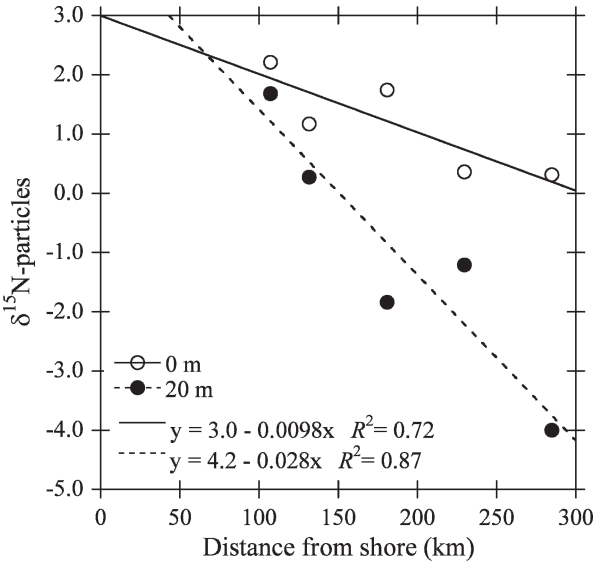

Fig. 6. The δ^{15}N (‰) of surface and 20-m particulate organic matter as a function of distance from shore (km).

(Fig. 4), where N_2- and C-fixation ranged from 8.3×10^{-4} nmol N trichome^{-1} h^{-1} to 1.1×10^{-2} nmol N trichome^{-1} h^{-1}, with a mean of $5.0 \times 10^{-3} \pm 1.2 \times 10^{-3}$ nmol N trichome^{-1} h^{-1} (mean ± SE, $n = 8$) and 5.7×10^{-2} nmol C trichome^{-1} h^{-1} to 2.0×10^{-1} nmol C trichome^{-1} h^{-1}, with a mean of $1.1 \times 10^{-1} \pm 1.8 \times 10^{-2}$ nmol C trichome^{-1} h^{-1} (mean ± SE, $n = 8$), respectively. In on-deck incubations, the *Trichodesmium* N_2-fixation rates showed significant photoinhibition at full surface irradiance (I_o). We assumed a colony size of 200 trichomes colony^{-1} (Carpenter 1983*b*) to compute trichome-specific N_2-fixation rates over the entire range of I. The resulting fixation rate estimates ranged from 1.3×10^{-4} nmol N trichome^{-1} h^{-1} to 2.6×10^{-2} nmol N trichome^{-1} h^{-1}, with a mean of $3.4 \times 10^{-3} \pm 7.6 \times 10^{-4}$ nmol N trichome^{-1} h^{-1} (mean ± SE, $n = 45$). Concurrent C-fixation rates ranged from 1.3×10^{-2} nmol C trichome^{-1} h^{-1} to 4.5×10^{-1} nmol C trichome^{-1} h^{-1}, with a mean of $1.2 \times 10^{-1} \pm 9.3 \times 10^{-2}$ nmol C trichome^{-1} h^{-1} (mean ± SE, $n = 44$).

Although C-fixation rate and particle C : N ratio were not significantly different between positively and negatively buoyant colonies, positively buoyant colonies had a significantly higher N_2-fixation rate ($3.6 \times 10^{-3} \pm 1.4 \times 10^{-3}$ nmol N trichome^{-1} h^{-1} [mean ± SD, $n = 2$]) than negatively buoyant colonies ($5.4 \times 10^{-4} \pm 4.2 \times 10^{-4}$ nmol N trichome^{-1} h^{-1} [mean ± SD, $n = 2$]). Molar C-fixation : N_2-fixation ratios, 9.9 ± 3.9 and 59.1 ± 8.0 (mean ± SD, $n = 2$) for positively and negatively buoyant colonies, respectively, were also significantly different ($p < 0.05$, Student's *t*-test).

Incubations of surface-collected colonies attached to an in situ array deployed at Sta. 4 and allowed to drift for the entire light day showed a similar pattern of decreasing N_2-fixation activity with decreasing light intensity below 0.50 I_o (Fig. 4). Bottles were placed at depths of 5, 15, 30, 50, and 70 m on the array. We assumed that the surface (I_o) N_2-fixation rate was equal to the mean N_2-fixation rate observed at 100% light intensity in our on-deck incubator experiments (Fig. 4). The observed profile shows high light (surface) photoinhibition of N_2-fixation and a maximum rate of N_2-fixation at 50% of surface light intensity, which, depending on the time of day and cloud cover, corresponds roughly to a range from 5-m to 15-m depth.

We used a least-squares parabolic regression of N_2-fixation rate as a function of relative light intensity with our conservative estimate of the vertical distribution of trichome abundance at each station to estimate areal N_2-fixation rates for Sta. 1 through 4 (Fig. 1). The areal fixation rates ranged from 47.2 μmol N m^{-2} d^{-1} to 118.6 μmol N m^{-2} d^{-1}, with a mean of 84.5 ± 17.7 μmol N m^{-2} d^{-1} (mean ± SE, $n = 4$) at our four study stations. The area of the open circle (Fig. 1) is proportional to the areal N_2-fixation rate at each station. We found no trend in N_2-fixation rate with distance from shore. The highest N_2-fixation rates were found at Sta. 2, one of the three deep chlorophyll max stations farthest from shore (Fig. 1).

Stable isotope abundance—Extraction and stable isotopic analysis of dissolved inorganic N (DIN) below 200 m was completed at Sta. 2 and 4. DIN in samples shallower than 200 m was below our limit of detection for this method. The δ^{15}N of the DIN at 200 m was 1.8 ± 0.8‰ (mean ± SE, $n = 3$) and 2.0 ± 0.6‰ (mean ± SE, $n = 3$) at Sta. 2 and 4, respectively. δ^{15}N–DIN at Sta. 2 remained markedly lighter than at Sta. 4 throughout the upper 600 m of the water column, below which the δ^{15}N–DIN at both stations converge on approximately 4.0‰ (Fig. 5).

The δ^{15}N of the suspended particles (>0.7 μm) in surface samples decreased with distance from shore ($\delta^{15}N = 2.99 - 0.01 \times$ kilometers from shore, $R^2 = 0.72$). The same trend was seen in the δ^{15}N of the suspended particles from 20 m ($\delta^{15}N = 4.19 - 0.03 \times$ kilometers from shore, $R^2 = 0.87$) (Fig. 6). Vertical profiles at Sta. 2, 3, and 4 show a δ^{15}N minimum (−4‰ to −2‰) at approximately 20-m depth

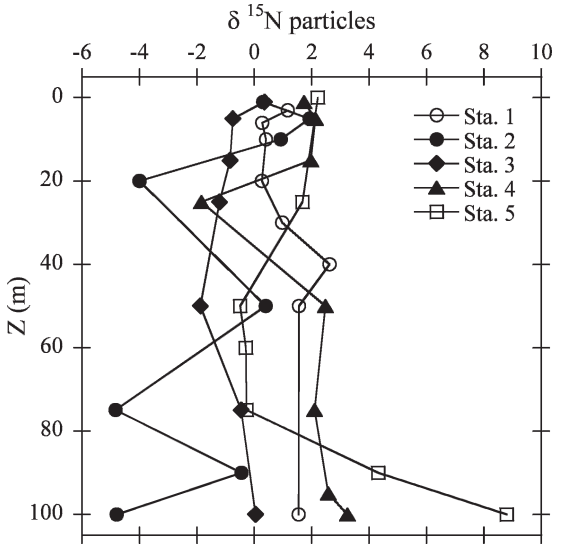

Fig. 7. Vertical profiles of δ^{15}N (‰) of particulate organic matter (>0.7 μm) at Sta.1 through 5.

(Fig. 7). Sta. 1 through 4 have similar δ^{13}C profiles (Fig. 8). At Sta. 5 the δ^{13}C of particulate organic carbon (POC) is markedly higher, although there is no clear trend in δ^{13}C of suspended particles along the transect.

The δ^{15}N of the zooplankton varied along the transect with the largest change in δ^{15}N observed in the smallest (250–500 μm) size fraction (Fig. 9). At Sta. 1, 5, and 8 (within 150 km of shore) the δ^{15}N of the 250–500-μm size fraction varied from 3.7‰ to 4.5‰ with a mean of 3.9 ± 0.4‰ (mean ± SE, $n = 3$). The 250–500-μm size fraction at Sta. 2, 3, and 4 (the offshore stations) varied from 1.6‰ to 2.2‰ with a mean of 2.0‰ ± 0.2‰ (mean ± SE, $n = 3$, $p < 0.05$). Generally, the larger size fractions of zooplankton (500–1,000 μm and 1,000–2,000 μm) had higher δ^{15}N relative to the 250–500-μm size fraction and exhibited the same spatial trends along the transect. There was little

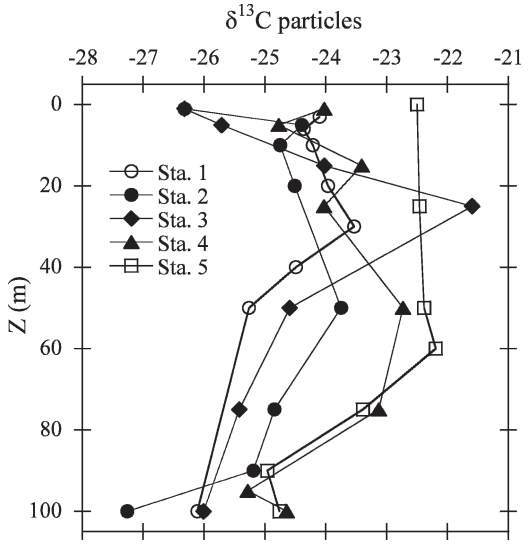

Fig. 8. Vertical profiles of δ^{13}C (‰) of particulate organic matter (>0.7 μm) at Sta. 1 through 5.

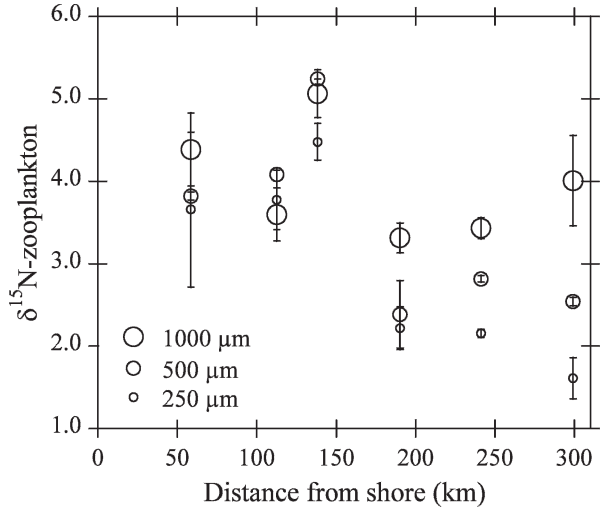

Fig. 9. The δ^{15}N (‰) of the 250-μm, 500-μm, and 1,000-μm zooplankton size fractions as a function of distance from shore (km).

difference between the δ^{13}C of the 250–500-μm and 500–1,000-μm size fractions, which ranged from −18.8‰ to −18.2‰ and from −19.7‰ to −18.5‰, respectively, and no trend with distance from shore (data not shown).

A cross-plot of particulate organic matter (POM), zooplankton, and *Trichodesmium* δ^{15}N as a function of δ^{13}C (Fig. 10) shows two clear groups of zooplankton δ^{15}N. Zooplankton in the 250–500-μm and 500–1,000-μm size fractions from Sta. 1 and 5 had a higher δ^{15}N than the same zooplankton size fractions from Sta. 2, 3, and 4

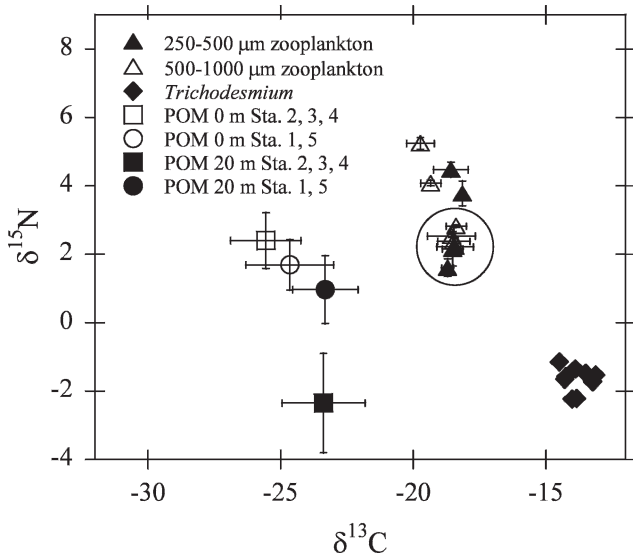

Fig. 10. Cross-plot of δ^{15}N (‰) versus δ^{13}C (‰) of 250–500-μm size-fractioned zooplankton Sta. 1 through 5 (closed triangles); 500–1,000-μm size-fractioned zooplankton (open triangles) Sta. 1 through 5; *Trichodesmium* (closed diamonds); mean surface particulate organic matter (POM) at Sta. 2, 3, and 4 (open square); mean surface POM at Sta. 1 and 5 (open circle); mean POM from 20 m at Sta. 2, 3, and 4 (closed circle); and mean POM from 20 m at Sta. 1 and 5 (closed square). Zooplankton from Sta. 2, 3, and 4 are circled.

(Fig. 10). $\delta^{13}C$ is essentially constant across zooplankton size fractions and stations.

Discussion

Warm, quiescent, nutrient-poor surface waters that favor development of *Trichodesmium* blooms (Eleuterius et al. 1981; Carpenter 1983*a*; Capone et al. 1998) prevail during the summer months in the Gulf of Mexico, making it an ideal environment for this colonial cyanobacterium. Recent models identify the western Gulf of Mexico as a prime area for *Trichodesmium* N_2-fixation (Hood et al. 2002). Although blooms are inherently unpredictable and patchy in distribution, they may contribute to increases in primary production (Capone et al. 1997; Karl et al. 1997), secondary production (Landry et al. 2001), and heterotrophic bacterial production (Biddanda and Benner 1997).

Trichodesmium abundance—Trichodesmium was visibly abundant at each of the stations we sampled (except Sta. 8) and conservative depth-integrated trichome concentrations ranged from 2.7×10^6 trichomes m^{-2} to 4.3×10^8 trichomes m^{-2}, with a mean of $4.1 \times 10^8 \pm 3.0 \times 10^8$ trichomes m^{-2} (mean $\pm$ SE, $n = 9$). For Sta. 1–5, the oligotrophic stations furthest offshore and most removed from potential coastal effects, the mean trichome concentration was $7.3 \times 10^8 \pm 5.4 \times 10^8$ trichomes m^{-2} (mean $\pm$ SE, $n = 5$). A single large-volume profile at Sta. 4 shows the vertical distribution of *Trichodesmium* colonies. The abundance estimate resulting from this profile suggests that the methods we used to compute abundance at all other stations (settled, small-volume samples) produced a severe underestimate of trichome abundance (Fig. 3). Therefore, our trichome abundance estimates should be thought of as highly conservative estimates of *Trichodesmium* biomass and, consequently, N_2-fixation rates.

Published data on the vertical distribution of *Trichodesmium* are limited, and most studies to date have been conducted on surface populations. At Sta. 4, we found an equal number of colonies at 9.8 m and 100 m, and also found trichomes as deep as 200 m (Fig. 3). Although not well documented in the literature, the occurrence of *Trichodesmium* at depth may suggest migratory strategies (Villareal and Carpenter 1990; Villareal and Carpenter 2003) and may have implications for the uptake of DIN by *Trichodesmium* (Holl and Montoya, 2005).

Trichodesmium N_2-fixation rates—N_2-fixation by *Trichodesmium* collected at the surface and incubated in situ declined with depth (Fig. 4), with the highest N_2-fixation rates at the 50% light intensity level. Ideally, *Trichodesmium* would be collected over a range of in situ light levels and then incubated at the corresponding light levels; however, time constraints as well as patchy vertical *Trichodesmium* distribution dictated that we use surface samples for this type of assay. *Trichodesmium* can regulate its buoyancy (Villareal and Carpenter 1990), and in the Gulf of Mexico, ascending colonies (positively buoyant) have significantly lower N : phosphorus (P) ratios than descending (negatively buoyant) colonies, consistent with a vertical migration strategy for P acquisition (Villareal and Carpenter 2003). In addition, *Trichodesmium* may avoid photoinhibition of both C and N_2-fixation by descending away from the surface, thus maximizing N_2-fixation (Carpenter and Price 1977). At greater depths, photosynthesis becomes light-limited, which in turn reduces the energy available for N_2-fixation, leading to the observed decrease in N_2-fixation rate with depth. We found a significant difference in N_2-fixation rate and C-fixation : N_2-fixation ratio between positively and negatively buoyant colonies ($p < 0.05$). However, we found no significant difference in either C-fixation or C : N ratio. We hypothesize that either C-fixation in negatively buoyant colonies was enhanced by incubation at a higher light intensity than the colonies were experiencing when captured, or that fixed N was being released into the dissolved pool by positively buoyant colonies. Our data suggest that any vertical migration by *Trichodesmium* colonies, whether for P acquisition or for avoidance of photoinhibition, affects their N_2- and C-fixation physiology and that efforts should be made to normalize the buoyancy status of colonies collected for incubation.

We used a parabolic regression to describe the relationship between N_2-fixation and light intensity with a maximum located at 50% surface irradiance (Fig. 4). N_2-fixation rates in both our in situ and deck incubations exhibited the same behavior below 50% I_o, therefore validating the use of the parabolic regression from the in situ incubations to compute depth-integrated areal N_2-fixation rates. N_2-fixation was measured by incubating hand-picked colonies, and the resulting N_2-fixation rates were converted to trichome-specific rates using a conversion faction of 200 trichomes per colony (Carpenter 1983*b*). There is high variability in the reported number of trichomes per colony in the literature, ranging anywhere from 10 to 200 trichomes per colony. Our use of the highest estimate of trichomes per colony makes our trichome-specific rates a conservative estimate of *Trichodesmium* N_2-fixation. N_2-fixation rates measured at the four stations farthest removed from potential coastal influences show significant spatial heterogeneity and no trend in N_2-fixation rate with distance from shore (Fig. 1). Vertically integrated rates at these four stations fall well within the range of values reported for *Trichodesmium* in other oligotrophic basins (Table 2). Mulholland et al. (2006) report colony-specific $^{15}N_2$-fixation rates ranging from 2.1 nmol N $colony^{-1}$ d^{-1} to 9.2 nmol N $colony^{-1}$ d^{-1} during a 3-yr period in the Eastern Gulf of Mexico. Incubation conditions and time of year, among other factors, make direct comparisons of field data difficult. However, after conversion of their data to trichome-specific rates, we find our rates to be an order of magnitude higher but within one standard deviation of those reported by Mulholland et al. (2006). The combination of our limited data set with that of Mulholland et al. (2006), suggests that N_2-fixation in the Gulf of Mexico should be considered in models estimating the diazotrophic addition of N to the surface ocean.

Stable isotope natural abundance: dissolved, particulate, and zooplankton—A characteristic marker of diazotrophic

Table 2. Summary of *Trichodesmium* N_2-fixation measurements for oligotrophic waters. Acetylene reduction (AR) estimates are based on a 3 : 1 reduction ratio for conversion to N_2-fixation rates.

Location	Dates	Mean areal rate (μmol N m^{-2} d^{-1})	SE	*n*	Method	Citation
Southwestern N. Atlantic	Nov 1964	41	7.6	19	$^{15}N_2$ uptake	Goering et al., 1966
0–24°N, 45–66°W	May 1965	108	24	17		
Caribbean, 12–22°N		161	20	12	AR	Carpenter and Price 1977
Bermuda Atlantic Time-Series studies	1995–1997	41		14	$^{15}N_2$ uptake	Orcutt et al., 2001
N. Pacific, 21°N, 159°W	1972	134		2	AR	Gunderson 1976
East China Sea, 10–25°N		126	49	32	AR	Saino 1977
Hawaii Ocean Time-Series/station ALOHA	1990–1992	84	43	8	AR	Karl et al., 1997
Arabian Sea, 7–10°N	May 1995	35	7.4	9	AR	Capone et al., 1998
Arabian Sea, 10°N bloom	May 1995	99	25	5	AR,	Capone et al., 1998
Coastal Tanzania (bloom –upper 0.5 m)	1975–1999	59			AR	Lugomela et al., 2002
Gulf of Mexico	July 2000	85	18	4	$^{15}N_2$ uptake	This study

activity is a low δ^{15}N of DIN. In a system dominated by diazotrophy, the δ^{15}N of the organic N pool is lower, resulting in a low δ^{15}N in remineralized nitrogen. Nitrifiers will oxidize this ^{15}N-depleted NH_4^+ and transfer its isotopic signature to the NO_3^- pool, lowering its δ^{15}N in turn. Deep-water NO_3^- in oxic regions typically has a δ^{15}N around 4–6‰ (Liu and Kaplan 1989; Sigman et al. 2000). At Sta. 2 and 4, NO_3^- collected at 200 m had markedly lower δ^{15}N values of 1.8‰ and 2.0‰, respectively (Fig. 5). In general, δ^{15}N-DIN signatures in the upper 600 m at Sta. 2 are lower than at Sta. 4, which corresponds to the higher areal N_2-fixation rate measured at Sta. 2. Below 600 m, δ^{15}N–DIN values converge on the oceanic average. This isotopic marker for diazotrophy in the DIN pool is consistent with a significant diazotrophic source of N in the water column and supports our limited biological rate measurements.

DON leakage or excretion (Bronk and Ward 2000) is also a potentially important sink for newly fixed N and a source of new N to non-diazotrophic phytoplankton. Our particulate N (PN) δ^{15}N vertical profiles show lower values in the upper water column at Sta. 2, 3, and 4 (offshore stations) relative to Sta. 1 and 5 (Fig. 7), and the δ^{15}N of PN at both the surface and 20 m decreased with increasing distance from shore (Fig. 6). These patterns are consistent with local inputs of recently fixed N available for uptake by phytoplankton.

There are few known direct consumers of *Trichodesmium* (O'Neil and Roman 1994). However, the δ^{13}C data from our five offshore study stations imply that a significant fraction of the organic C in small (<500 μm) zooplankton is derived from *Trichodesmium* (Fig. 10) either through direct ingestion or by the incorporation of *Trichodesmium*-derived C via remineralization and the microbial loop. *Trichodesmium* typically has a higher δ^{13}C than bulk suspended particles (Carpenter et al. 1997). In our Gulf of Mexico samples, *Trichodesmium* had a δ^{13}C of −13.86‰, significantly heavier than the bulk suspended particles at our stations, which had a δ^{13}C of approximately −25.00‰ (Figs. 8, 10). These two distinct carbon pools available for zooplankton grazing have characteristic δ^{13}C isotope signatures, which enabled us to employ a two-member mixing model to estimate the contribution of *Trichodesmium* C to zooplankton biomass. A mass balance calculation (Montoya et al. 2002) between the two potential sources of C available to zooplankton, *Trichodesmium* POC and particulate POC, showed that as much as 60% of the C in the zooplankton may come from *Trichodesmium* derived POC and the remaining 40% from bulk particulate POC (Fig. 10).

We did not distinguish zooplankton at the species level, but small pelagic harpacticoid copepods are known to directly ingest *Trichodesmium* and are often found in abundance where *Trichodesmium* is present (Bottger-Schnack and Schnack 1989). Alternately, indirect incorporation of *Trichodesmium*-derived C via the ingestion of viral lysis products from *Trichodesmium* cell breakage (Hewson et al. 2004) could lend zooplankton this C isotopic signature. Our data do not allow us to determine if the zooplankton C isotopic signature came from direct ingestion of *Trichodesmium* or ingestion of *Trichodesmium*-derived C. However, our data are consistent with the hypothesis that *Trichodesmium* C is important to the small (<500 μm and >250 μm) zooplankton found in the oligotrophic north-western Gulf of Mexico. Therefore, in addition to supplying new N to this oligotrophic region, *Trichodesmium* may also be an important source of organic C supporting heterotrophic production.

δ^{15}N tends to increase from food source to consumer (Minagawa and Wada 1984). Therefore, if we use a 3‰ trophic shift (DeNiro and Epstein 1981) in combination with our mixing model, our natural abundance results again suggest that as much as 60% of the zooplankton N at Sta. 2, 3, and 4 is coming from *Trichodesmium*. The smallest zooplankton fractions we collected (250–500 μm and 500–1,000 μm) have lower δ^{15}N in areas where N_2-fixation was measured (Fig. 9). However, the δ^{15}N of the zooplankton at Sta. 1 and 5 is slightly higher than would be predicted by the ingestion of a diet rich in *Trichodesmium* or *Trichodesmium*-derived food sources. Our SST data showed the offshore movement of cooler, coastal water onto Sta. 1, which could have impacted the zooplankton δ^{15}N with the introduction of a terrigenous source of N.

Assuming an average Gulf of Mexico primary production rate of approximately 0.24 g C m^{-2} d^{-1} (Chen et al. 2000) and Redfield stoichiometry, our conservative

estimate of *Trichodesmium* N_2-fixation could support roughly 6% of the primary productivity in the northwestern Gulf of Mexico during the time of this study. Whereas the relative importance of diazotrophic unicellular cyanobacteria in supplying new N to the oligotrophic ocean is becoming increasingly clear (Zehr et al. 2001; Montoya et al. 2004), isotopic evidence suggests that *Trichodesmium* remains an important supplier of new N to the areas in which it is found (Montoya et al. 2002, Capone et al. 2005). The capacity of *Trichodesmium* to fix N has been studied in many tropical and subtropical regions of the world ocean, yet our data are the first published *Trichodesmium* N_2-fixation rates in the Western Gulf of Mexico even though *Trichodesmium* is common to the region. Our sampling, though spatially limited, produced areal N_2-fixation rates for the Gulf of Mexico comparable to N_2-fixation rates measured in other oceanic basins (Table 2). Because the Gulf of Mexico is easily accessible and typically has extensive seasonal surface aggregations of *Trichodesmium*, it provides an ideal location for field studies of *Trichodesmium* biology and its role in oceanic biogeochemistry.

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Received: 14 June 2006
Accepted: 2 April 2007
Amended: 22 May 2007