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Rates of dinitrogen fixation and the abundance of diazotrophs in North American coastal waters between Cape Hatteras and Georges Bank

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Abstract

We coupled dinitrogen (N_2) fixation rate estimates with molecular biological methods to determine the activity and abundance of diazotrophs in coastal waters along the temperate North American Mid-Atlantic continental shelf during multiple seasons and cruises. Volumetric rates of N_2 fixation were as high as 49.8 nmol N L^{-1} d^{-1} and areal rates as high as 837.9 μmol N m^{-2} d^{-1} in our study area. Our results suggest that N_2 fixation occurs at high rates in coastal shelf waters that were previously thought to be unimportant sites of N_2 fixation and so were excluded from calculations of pelagic marine N_2 fixation. Unicellular N_2-fixing group A cyanobacteria were the most abundant diazotrophs in the Atlantic coastal waters and their abundance was comparable to, or higher than, that measured in oceanic regimes where they were discovered. High rates of N_2 fixation and the high abundance of diazotrophs along the North American Mid-Atlantic continental shelf highlight the need to revise marine N budgets to include coastal N_2 fixation. Integrating areal rates of N_2 fixation over the continental shelf area between Cape Hatteras and Nova Scotia, the estimated N_2 fixation in this temperate shelf system is about 0.02 Tmol N yr^{-1}, the amount previously calculated for the entire North Atlantic continental shelf. Additional studies should provide spatially, temporally, and seasonally resolved rate estimates from coastal systems to better constrain N inputs via N_2 fixation from the neritic zone.

Dinitrogen (N_2) fixation supplies new nitrogen (N) to the world's oceans, thereby alleviating N limitation of primary productivity; however, most prior measurements of pelagic marine N_2 fixation rates are primarily from oligotrophic regions, where N-depleted conditions are thought to favor growth of N_2-fixing microorganisms (diazotrophs). Despite the recognition that biological N_2 fixation ultimately controls the input term of the marine N budget and that diazotrophs contribute substantially to new production in oligotrophic gyres (Montoya et al. 2004; Capone et al. 2005; Zehr and Paerl 2008), little is known about the distribution of diazotrophs and their activity in most coastal regions, where 21–30% of the total oceanic primary productivity occurs (Jahnke 2010), and where geochemical and climatological models are poorly resolved (Gruber and Sarmiento 1997; Deutsch et al. 2007). Rates of oceanic N_2 fixation have been primarily reported from oligotrophic tropical and subtropical regions where nutrients (primarily N) are depleted and warm surface temperatures are thought to be favorable for N_2 fixation, at least by *Trichodesmium*, the most commonly studied diazotroph (Carpenter and Capone 2008; Zehr and Paerl 2008). However, evidence now suggests that other groups of oceanic diazotrophic cyanobacteria thrive across a broader range of environments than previously thought (Moisander et al. 2010) and therefore global rates of N_2 fixation may be underestimated. Consistent with this observation, recent geochemical models hint that global marine N_2 fixation has been underestimated by models and field sampling, and determining sources of this missing N may be an important step in resolving oceanic N budgets (Gruber and Sarmiento 1997; Codispoti 2006; Deutsch et al. 2007).

Previous reports suggest that planktonic N_2 fixation is undetectable or insignificant in most estuarine and coastal waters, including those along the North American Mid-Atlantic shelf (Howarth et al. 1988; Zehr and Paerl 2008; Conley et al. 2009). However there are few rate measurements to support this assertion. The diversity of *nifH*, the gene encoding the iron protein in the enzyme nitrogenase that mediates N_2 fixation, has been shown to be far greater in estuarine environments such as the Chesapeake Bay (Zehr et al. 2003; Jenkins et al. 2004; Short et al. 2004) than in oceanic environments (Zehr et al. 2003), which suggests that the genetic capability for N_2 fixation may be greater in estuarine-influenced coastal systems. However, the high estuarine *nifH* diversity may also be due to contributions from organisms transported into these systems from sediments, soils, terrigenous material, and other allochthonous inputs, rather than to populations of autochthonous diazotrophs actively fixing N_2 in these systems. Along the North American Mid-Atlantic shelf, inputs of microbes from several large estuaries, including the Chesapeake Bay,

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can be augmented by the introduction of tropical and subtropical diazotrophs that are advected into coastal waters from the Gulf Stream, where N_2 fixers are known to be abundant (Capone et al. 2005; Carpenter and Capone 2008). Tropical marine N_2 fixers commonly occur in Gulf Stream–influenced coastal waters south of Cape Hatteras (Prufert-Bebout et al. 1993).

Oligotrophic tropical and subtropical marine environments depleted in dissolved inorganic nitrogen (DIN = $(NO_3^- + NO_2^- + NH_4^+)$), and eutrophic fresh and brackish water environments enriched in phosphorus (P), have been thought to be primary sites for N_2 fixation in aquatic systems (Howarth et al. 1988; Zehr and Paerl 2008; Conley et al. 2009). Consistent with this notion, expression of *nifH* genes has been previously observed primarily in oligotrophic waters, including the temperate and subtropical Pacific (Church et al. 2005; Needoba et al. 2007) and the subtropical and tropical Atlantic Ocean (Langlois et al. 2005). Despite the numerous sources of diazotrophic organisms to temperate coastal systems, planktonic N_2 fixation has not been broadly examined in coastal marine systems because nutrient inputs in these systems can be high and this is thought to be a condition that is unfavorable for active N_2 fixation (Nixon et al. 1996; Zehr et al. 2008; Conley et al. 2009). However, recent observations demonstrate that there is active N_2 fixation in NO_3^--replete ($\sim$ 10 μmol L^{-1}) waters below the upper mixed layer in the eastern tropical North Atlantic Ocean (5 stations; Voss et al. 2004), in surface waters in the English Channel (2 stations; Rees et al. 2009), and the Mekong River plume in the South China Sea (22 stations over 2 seasons; Grosse et al. 2010). In addition, diazotrophs and active N_2 fixation have now been found at higher latitudes than was previously thought (Rees et al. 2009; Moisander et al. 2010). In this study, we further broaden the domain of active oceanic N_2 fixation to include coastal ocean regions.

Methods

We measured N_2 fixation rates and diazotroph abundance in coastal waters along the North American Mid-Atlantic continental shelf on oceanographic cruises between 36.5°N and 39°N and −76°W to −74°W aboard the R/V *Hugh Sharp* during summer and autumn 2006, and from Cape Hatteras to Georges Bank (between 35°N to 43°N and −76°W to −65°W) during a cruise aboard the R/V *Delaware II* in August 2009. In order to understand the biogeographical and hydrographic constraints on the distribution of active N_2 fixation and the diazotrophs mediating this process, we simultaneously measured nutrient concentrations and hydrographic parameters at stations distributed within our study area. Surface-water temperature and salinity mapping was also done using the ship's underway sampling system during the R/V *Hugh Sharp* cruises in 2006. In 2009, a weekly average of sea-surface temperature was constructed using Moderate Resolution Imaging Spectroradiometer Terra and Aqua satellite data.

At sampling stations, vertical profiles of temperature and salinity were measured using a conductivity, temperature, and depth sensor mounted to a sampling rosette. Water samples were collected at 2–4 depths using Niskin bottles mounted to the same rosette. Samples were collected from near-surface waters in the upper 6 m and from near the bottom (when the shallow-water column was well-mixed) or the fluorescence maximum at all stations. Additional water samples were collected between these depths or when features were identified during vertical profiling. Whole water was transferred directly to incubation bottles during 2006 and transferred to clean 10-liter carboys during 2009. In 2009, water from the carboys was then dispensed into incubation bottles. Samples destined for nutrient analyses were immediately filtered through 0.2-μm Supor cartridge filters and filtrate was collected into sterile sample bottles and frozen. Frozen samples were transported to Old Dominion University (ODU) for analysis of DIN and dissolved inorganic phosphorus (DIP) as described below. Samples were also collected for analysis of chlorophyll *a* (Chl *a*). These samples were filtered onto glass-fiber (Whatman GF/F) filters (0.7-μm pore size) and frozen in sterile centrifuge tubes, transported to the laboratory at ODU and analyzed using the method of Welschmeyer (1994) within 2 weeks of sample collection during 2006 and within 18 d of sample collection during 2009.

Uptake experiments were initiated by adding tracer additions (< 10%) of highly enriched (99%) $^{15}N_2$ to gas-tight bottles filled with whole water (Montoya et al. 1996; Mulholland et al. 2006). We recognize that this method may underestimate N_2 fixation during short incubations (Mohr et al. 2010); all of the data reported here are from experiments incubated for 24 h in on-deck incubators and so gas solubility was likely to be less problematic. If we allow for equilibration of the gas bubble over the first 8 h of the incubation period, we estimate that N_2 fixation would be underestimated by a factor of about 1.4. Incubation bottles were placed in on-deck incubators equipped with flow-through seawater to maintain near-ambient temperatures. After 24 h, incubations were terminated by filtration through precombusted (450°C for 2 h) GF/F filters. Samples were placed into sterile microcentrifuge tubes, frozen, and transported to the laboratory for analysis. In the laboratory, samples were dried, pelletized into tin disks, and analyzed for total particulate N and carbon (C) and isotopic enrichment on a Europa 20–20 mass spectrometer equipped with an automated nitrogen and carbon analyzer preparation module. Rates of uptake were calculated using a mixing model (Montoya et al. 1996; Orcutt et al. 2001).

Areal rates of N_2 fixation were calculated by integrating our rate measurements from the surface mixed layer and fluorescence maximum over the euphotic zone. This was defined as 1% of photosynthetic active radiation (PAR), and was determined directly using a PAR sensor mounted to the sampling rosette. To estimate integrated N_2 fixation rates over the euphotic zone, we multiplied the average of rate measurements made in the surface mixed layer by its depth and the rate measurements made at the fluorescence maximum and below the surface mixed layer by the remainder of the euphotic depth, and then added the two together. The upper mixed layer depth was calculated

using the Levitus sigma-*t* criterion of 0.125 change from surface.

In the laboratory at ODU, frozen filtrate was thawed and nitrate plus nitrite and DIP concentrations were measured colorimetrically using an Astoria Pacific nutrient analyzer according to manufacturer specifications (Parsons et al. 1984). Detection limits were 0.02 μmol L^{-1} and 0.01 μmol L^{-1}, respectively. Ammonium concentrations were measured manually using the phenol-hypochlorite method (Solarzano 1969), with a detection limit of 0.02 μmol L^{-1}.

Samples for molecular analyses were collected onto precombusted (450°C for 2 h) GF/F filters during 2006 (in duplicate from two depths—surface and chlorophyll fluorescence maximum) and using Sterivex filters (0.2-μm pore size) in 2009. Because of the larger pore size filters in 2006, some of the unicellular cyanobacteria smaller than 0.7 μm may have passed through the filter, which caused an underestimate of *nifH* gene copies in some diazotrophic groups during that year. Samples were immediately frozen and stored in liquid nitrogen until transported to ODU, where they were transferred to −80°C freezers. Samples were shipped overnight on dry ice to University of California at Santa Cruz, where deoxyribonucleic acid (DNA) extraction was carried out using a modified Qiagen Plant kit method (Moisander et al. 2008). Quantitative polymerase chain reaction (qPCR) was carried out using seven sets of Taqman® primer-probe sets (Church et al. 2005; Foster et al. 2007) in 2006 and three primer-probe sets in 2009, and previously published protocols (Short and Zehr 2005, 2007; Foster et al. 2007), to quantify the abundances of the major diazotroph groups in the study area. Diazotroph diversity was investigated by amplifying a partial *nifH* fragment using degenerate primers, and PCR products were cloned and sequenced (Zehr and Turner 2001). Sequences were trimmed and imported to an Arb database aligned using a profile Hidden Markov model (HMMER software) algorithm using a protein family (PFAM) seed alignment. The new translated sequences were aligned to the existing database using the FastAlign feature in Arb (Ludwig et al. 2004). A neighbor-joining tree was constructed with the amino-acid sequences. Bootstrapping was done using Mega 4.1 (Tamura et al. 2007). Unique sequences from each station were submitted to GenBank and their accession numbers are FJ756578–FJ756722.

Results

During the July 2006 shelf cruise between the Chesapeake and Delaware Bay mouths, surface-water temperatures ranged from 21.0°C to 25.5°C, surface salinity ranged from 22.9 to 34.7, and surface Chl *a* concentrations ranged from 0.10 μg L^{-1} to 3.98 μg L^{-1} (Table 1). The highest Chl *a* concentrations (1.88–3.98 μg L^{-1}) and lowest salinities (22.9–26.5) were observed in the Chesapeake Bay Plume (CBP)–influenced region. At shelf stations and those influenced by the Gulf Stream, surface Chl *a* concentrations were lower (0.10–0.42 μg L^{-1}) and salinity was higher (31.0–34.3). Surface DIN and DIP concentrations were

Table 1. Physical and chemical properties of near-surface water samples (upper 2 m) collected at stations along the Mid-Atlantic continental shelf during July 2006 aboard the R/V *Hugh Sharp*. Areal rates of N_2 fixation were estimated based on volumetric rate estimates made near the surface (upper mixed layer) and at the fluorescence maxima or near the bottom (Table 2) at stations with a well-mixed and shallow-water column and integrated over the euphotic zone. MAS indicates Mid-Atlantic shelf stations, GSI indicates Gulf Stream influenced stations, and CBP denotes Chesapeake Bay Plume influenced stations. Standard deviations are in parentheses. Standard deviations of 0.00 indicate that the standard deviation was < 0.01.

Longitude (°W)	Latitude (°N)	Location	Temperature (°C)	Salinity	Chl *a* (μg L^{-1})	$NO_3^-+NO_2^-$ (μmol L^{-1})	NH_4^+ (μmol L^{-1})	DIP (μmol L^{-1})	DIN : DIP molar ratio	N_2 fixation (nmol L^{-1} d^{-1})	N_2 fixation (μmol m^{-2} d^{-1})
−75.298	37.695	MAS 1	21.0	31.7	0.42(0.05)	0.15(0.02)	0.34(0.03)	0.21(0.02)	2.3	3.7(0.3)	83.7
−75.152	37.631	MAS 2	22.8	31.1	0.33(0.04)	0.10(0.00)	0.37(0.02)	0.07(0.00)	6.7	2.0(0.5)	48.8
−75.050	37.519	MAS 3	24.1	31.0	0.24(0.01)	0.10(0.01)	0.39(0.03)	0.05(0.00)	9.8	12.9(0.4)	235.4
−74.683	36.686	GSI 1	23.4	34.3	0.10(0.10)	0.21(0.02	0.35(0.01)	0.03(0.00)	18.7	19.3(0.6)	204.9
−74.863	36.627	GSI 2	23.9	32.0	0.22(0.00)	0.18(0.01)	0.33(0.03)	0.04(0.01)	12.8	10.8(2.1)	162.2
−75.058	36.601	GSI 3	23.4	32.9	0.24(0.04)	0.17(0.01)	0.38(0.02)	0.07(0.01)	7.9	11.7(0.9)	216.4
−75.273	36.532	GSI 4	24.7	34.7	0.23(0.01)	0.26(0.03)	0.37(0.00)	0.05(0.01)	12.6	17.3(1.9)	369.1
−76.019	36.972	CBP 1	22.0	26.5	3.54(0.07)	0.27(0.02)	0.38(0.02)	0.08(0.01)	8.1	22.5(0.8)	152.7
−75.908	36.900	CBP 2	25.2	23.3	3.98(0.00)	0.24(0.00)	0.36(0.03)	0.05(0.01)	12.0	24.5(6.9)	165.8
−75.857	36.803	CBP 3	25.5	22.9	2.78(0.34)	0.18(0.00)	0.34(0.03)	0.05(0.01)	10.4	30.9(7.1)	158.8
−75.766	36.671	CBP 4	23.5	27.4	1.88(0.08)	0.14(0.00)	0.35(0.04)	0.03(0.01)	16.3	35.1(2.1)	302.5
−75.719	36.885	CBP 5	23.9	25.7	2.13(0.19)	0.18(0.01)	0.34(0.03)	0.06(0.01)	8.7	39.0(7.2)	370.2
−74.299	38.042	MAS 4	23.5	31.0	0.20(0.00)	0.10(0.00)	0.36(0.02)	0.03(0.00)	15.3	23.3(0.9)	392.4
−74.384	38.122	MAS 5	22.9	31.5	0.32(0.01)	0.02(0.00)	0.37(0.03)	0.10(0.01)	3.9	3.8(0.9)	153.1

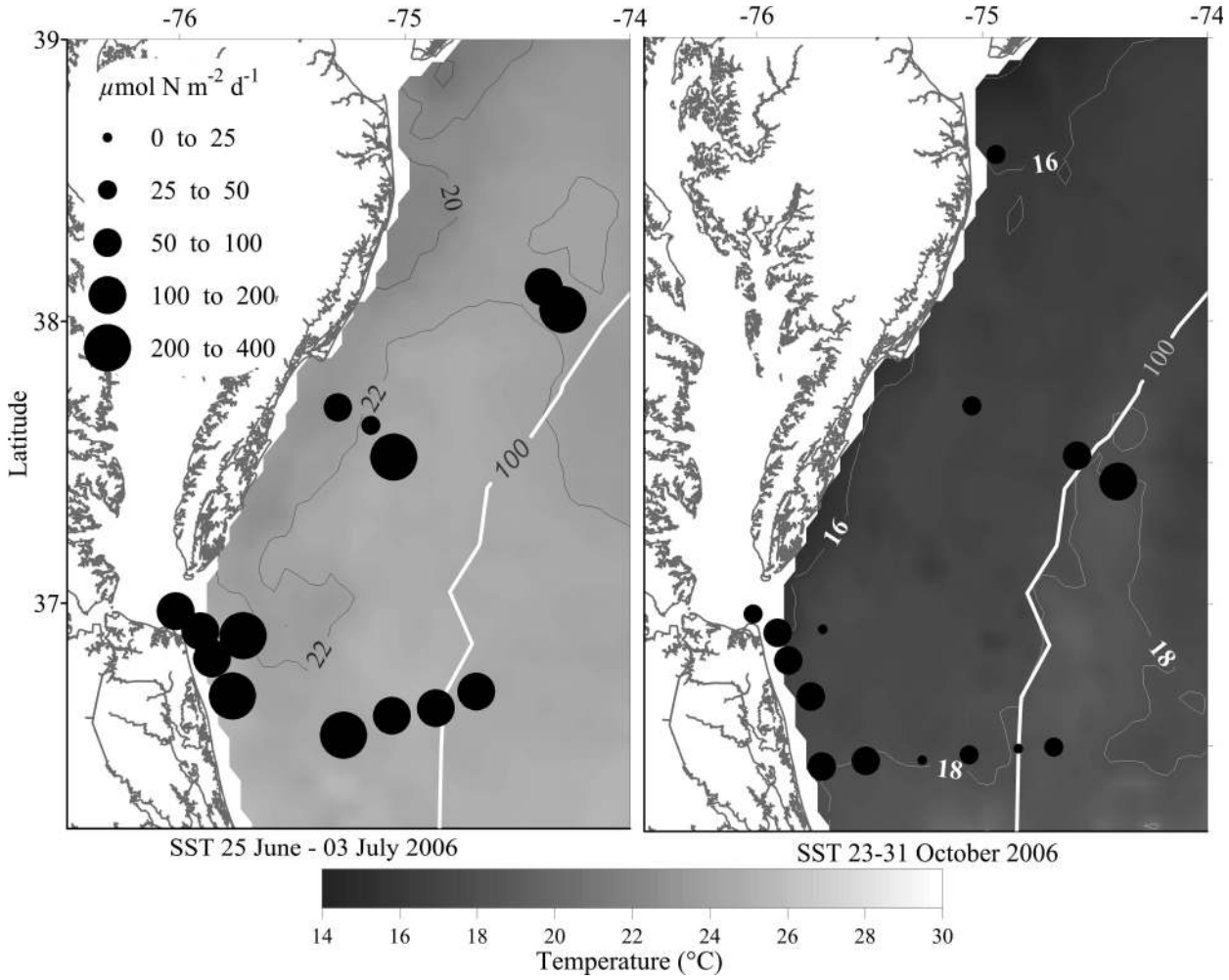

Fig. 1. Areal rates of N_2 fixation (μmol N m^{-2} d^{-1}) in surface waters collected from the Mid-Atlantic shelf waters between Chesapeake and Delaware bays during cruises in (A) July and (B) October–November during 2006, superimposed upon the sea surface temperature (SST) fields observed during the respective field campaigns.

uniform across the study area during July 2006, ranging from 0.40 μmol L^{-1} to 0.65 μmol L^{-1} and from 0.03 μmol L^{-1} to 0.21 μmol L^{-1}, respectively (Table 1). The DIN : DIP ratio ranged from 2.3 to 18.7 and was generally < 16, indicative of N limitation. Average surface N_2 fixation rates ranged from 2.0 to 39.0 nmol L^{-1} d^{-1} and depth integrated N_2 fixation rates ranged from 48.8 μmol m^{-2} d^{-1} to 392.4 μmol m^{-2} d^{-1} over the study area (Table 1; Fig. 1A).

During the same cruise in July 2006, but in samples collected from the fluorescence maximum or near the bottom when the water column was shallow and well-mixed, water temperatures were lower (ranging from 11.0°C to 21.9°C), salinity was higher (ranging from 29.4 to 35.4), and Chl *a* concentrations were higher (ranging from 0.33 μg L^{-1} to 4.20 μg L^{-1}) at all but the CBP-influenced stations (Table 2). DIN concentrations were uniform, ranging from 0.46 to 0.64 μmol N L^{-1} at water depths < 40 m. Higher $NO_3^- + NO_2^-$ concentrations were observed at the two stations where samples were collected from > 40 m. DIP concentrations ranged from 0.01 μmol P L^{-1} to 0.36 μmol P L^{-1} in samples collected from the fluorescence maximum. DIN : DIP ratios were < 16 at all but 2 stations, one where DIP was near the limit of analytical detection (0.01 μmol P L^{-1}) and the other at a deep station where there were high concentrations of $NO_3^- + NO_2^-$. Volumetric rates of N_2 fixation were lower in samples from the fluorescence maximum than in surface-water samples, with average rates ranging from 1.0 nmol L^{-1} d^{-1} to 8.8 nmol L^{-1} d^{-1}. When we compared N_2 fixation rates and temperature, we observed higher N_2 fixation rates at higher temperatures (Fig. 2A). However, the water column was stratified during this period, and so all of the samples with water temperatures < 20°C were collected at the fluorescence maximum, where light levels were also lower.

During the October–November 2006 cruise, surface-water temperatures were lower than in July, ranging from 15.2°C to 21.6°C (Table 3). However, surface salinity and Chl *a* concentrations were similar to those measured in July, ranging from 23.5 μg Chl *a* L^{-1} to 34.6 μg Chl *a* L^{-1} and from 0.46 μg Chl *a* L^{-1} to 5.88 μg Chl *a* L^{-1}, respectively. Higher concentrations of Chl *a* were observed at shelf stations in November compared with July. DIN

Table 2. Physical and chemical properties of water samples collected at the fluorescence maximum, or near the bottom for shallow-water stations (depth of sample collection is indicated), at stations along the Mid-Atlantic continental shelf during July 2006 aboard the R/V *Hugh Sharp*. MAS indicates Mid-Atlantic shelf stations, GSI indicates Gulf Stream–influenced stations, and CBP denotes Chesapeake Bay Plume–influenced stations. Standard deviations are in parentheses. Standard deviations of 0.00 indicate that the standard deviation was < 0.01.

Longitude (°W)	Latitude (°N)	Location	Depth (m)	Temperature (°C)	Salinity	Chl *a* (μg L^{-1})	$NO_3^- + NO_2^-$ (μmol L^{-1})	NH_4^+ (μmol L^{-1})	DIP (μmol L^{-1})	DIN : DIP molar ratio	N_2 fixation (nmol L^{-1} d^{-1})
−75.298	37.695	MAS 1	21	13.0	32.5	2.81(0.36)	0.16(0.02)	0.30(0.03)	0.36(0.04)	1.3	4.1(1.0)
−75.152	37.631	MAS 2	24	12.3	32.7	1.92(0.07)	0.21(0.01)	0.33(0.04)	0.01(0.01)	72.0	2.1(0.03)
−75.050	37.519	MAS 3	29	12.5	32.9	1.08(0.09)	0.19(0.02)	0.33(0.00)	0.08(0.01)	6.9	1.4(0.2)
−74.683	36.686	GSI 1	100	16.5	35.4	0.33(0.08)	2.06(0.15)	0.31(0.01)	0.11(0.02)	22.1	1.0(0.0)
−74.863	36.627	GSI 2	42	14.0	34.3	2.20(0.21)	0.55(0.01)	0.35(0.02)	0.10(0.01)	9.1	1.7(0.5)
−75.058	36.601	GSI 3	25	13.4	33.8	1.54(0.23)	0.21(0.02)	0.37(0.01)	0.09(0.01)	6.7	4.2(1.0)
−75.273	36.532	GSI 4	36	13.5	33.7	1.35(0.55)	0.31(0.03)	0.33(0.03)	0.15(0.01)	4.3	3.2(0.9)
−76.019	36.972	CBP 1	13	17.8	30.2	4.20(0.03)	0.14(0.01)	0.32(0.02)	0.21(0.02)	2.2	5.0(0.1)
−75.908	36.900	CBP 2	15	17.6	30.9	3.86(0.12)	0.20(0.02)	0.35(0.02)	0.11(0.00)	5.2	4.3(0.6)
−75.857	36.803	CBP 3	14	16.6	32.2	3.18(0.10)	0.19(0.02)	0.27(0.01)	0.07(0.00)	6.3	3.5(0.1)
−75.766	36.671	CBP 4	18	13.4	32.8	1.93(0.18)	0.19(0.01)	0.29(0.01)	0.11(0.01)	4.2	2.2(0.1)
−75.719	36.885	CBP 5	18	21.9	29.4	2.05(0.01)	0.21(0.00)	0.30(0.02)	0.05(0.01)	9.7	8.8(0.8)
−74.299	38.042	MAS 4	48	17.8	32.6	0.51(0.05)	0.21(0.02)	0.32(0.03)	0.11(0.00)	5.0	2.6(0.6)
−74.384	38.122	MAS 5	40	11.0	33.0	1.33(0.05)	0.24(0.02)	0.30(0.03)	0.22(0.01)	2.5	3.8(0.5)

Fig. 2. Relationship between volumetric rates of N_2 fixation (nmol L^{-1} d^{-1}) and temperature in samples collected from near the surface and at the fluorescence maximum during cruises in (A) July and (B) October–November during 2006, and in (C) surface-water samples pooled over the summer (July) and autumn (Oct–Nov) cruises.

Table 3. Physical and chemical properties of near-surface water samples (upper 2 m) collected at stations along the mid-Atlantic continental shelf during October–November 2006 aboard the R/V *Hugh Sharp*. Areal rates of N_2 fixation were estimated based on volumetric rate estimates made near the surface (upper mixed layer) and at the fluorescence maxima (below the upper mixed layer) and integrated over the euphotic zone. DBP is Delaware Bay, CBP is Chesapeake Bay Plume, MAS is Mid-Atlantic Shelf, and GSI denotes Gulf Stream–influenced stations. Standard deviations are in parentheses. Standard deviations of 0.00 indicate that the standard deviation was < 0.01.

Longitude (°W)	Latitude (°N)	Location	Temperature (°C)	Salinity	Chl *a* (μg L^{-1})	$NO_3^-+NO_2^-$ (μmol L^{-1})	NH_4^+ (μmol L^{-1})	DIP (μmol L^{-1})	DIN : DIP molar ratio	N_2 fixation (nmol N L^{-1} d^{-1})	N_2 fixation (μmol m^{-2} d^{-1})
−74.933	38.933	DBP	15.5	31.2	2.89(0.30)	1.06(0.06)	0.86(0.10)	0.22(0.02)	8.7	5.6(0.2)	118.5
−75.700	36.900	MAS 1	17.4	30.6	2.17(0.18)	0.42(0.07)	0.73(0.08)	0.23(0.02)	5.0	2.7(0.3)	62.5
−76.017	36.967	CBP 1	15.2	23.6	3.89(0.71)	3.01(0.26)	1.17(0.07)	0.26(0.02)	16.1	8.6(0.2)	82.6
−75.900	36.884	CBP 2	15.2	23.5	5.88(0.18)	2.05(0.49)	0.86(0.09)	0.21(0.01)	13.8	15.7(0.4)	233.4
−75.850	36.800	CBP 3	15.4	24.8	3.99(0.69)	0.96(0.26)	0.45(0.03)	0.19(0.02)	7.5	17.3(2.0)	135.3
−75.750	36.667	CBP 4	17.8	30.9	3.24(0.36)	0.48(0.14)	0.21(0.03)	0.15(0.02)	4.6	20.3(5.9)	253.4
−75.700	36.417	MAS 2a	18.1	30.5	2.96(0.06)	0.75(0.06)	0.40(0.01)	0.19(0.02)	6.1	6.5(0.6)	121.1
−75.700	36.417	MAS 2b	18.0	30.7	2.24(0.13)	0.64(0.14)	0.72(0.01)	0.19(0.00)	7.2	4.0(0.1)	59.6
−75.517	36.434	MAS 3	18.3	31.2	2.01(0.04)	0.28(0.07)	0.48(0.02)	0.11(0.00)	6.9	7.2(6.6)	179.1
−75.250	36.450	MAS 4	18.8	32.3	0.91(0.13)	0.24(0.00)	0.21(0.03)	0.09(0.01)	5.0	2.0(0.2)	65.8
−75.050	36.467	MAS 5	19.2	32.6	0.73(0.03)	0.12(0.00)	0.73(0.07)	0.10(0.01)	8.5	2.3(0.3)	70.0
−74.833	36.483	GSI 1	21.6	34.5	0.46(0.04)	0.21(0.06)	0.07(0.01)	0.02(0.01)	14.2	1.4(1.1)	40.9
−74.667	36.484	GSI 2	21.4	34.6	0.93(0.00)	0.30(0.07)	0.11(0.02)	0.05(0.00)	8.3	1.3(0.1)	51.7
−74.400	37.417	GSI 3	19.6	33.5	0.55(0.02)	0.12(0.00)	0.01(0.00)	0.05(0.01)	2.6	4.1(4.3)	228.5
−74.567	37.517	MAS 6	17.9	33.6	0.64(0.04)	0.66(0.09)	0.07(0.01)	0.14(0.01)	5.2	4.5(1.2)	133.3
−75.034	37.700	MAS 7	17.0	32.2	1.53(0.03)	0.30(0.07)	0.22(0.04)	0.13(0.03)	4.0	4.1(1.2)	91.7

and DIP concentrations in surface waters were higher than those observed in July, ranging from 0.13 μmol L^{-1} to 4.18 and from 0.02 μmol L^{-1} to 0.26 μmol L^{-1}, respectively. The highest nutrient concentrations were observed at plume-influenced stations. DIN : DIP ratios were generally ≤ 16 in surface waters (Table 3). Average volumetric N_2 fixation rates in surface waters ranged from 1.3 nmol L^{-1} d^{-1} to 20.3 nmol L^{-1} d^{-1} during November, lower than the range observed during summer. Depth-integrated N_2 fixation rates ranged from 40.9 to 253.4 μmol m^{-2} d^{-1} during this time period (Table 3; Fig. 1B).

Because the water column was well-mixed during October–November 2006, temperature and salinity were similar in surface and deeper water samples (Tables 3 and 4). Nutrient concentrations and DIN : DIP ratios were also similar at both depths, and DIN : DIP ratios were usually ≤ 16. On average, volumetric rates of N_2 fixation were lower at the fluorescence maximum (range of 0.2–13.9 nmol N L^{-1} d^{-1}) than in surface waters. However, during the autumn cruise, rates of N_2 fixation in samples collected from the fluorescence maximum or near the bottom at plume-influenced stations were higher than those observed during the summer cruise. When we compared N_2 fixation rates and temperature for the autumn cruise, we observed higher N_2 fixation rates at lower temperatures and no distinct grouping of samples from the surface and the fluorescence maximum (Fig. 2B), likely because the water column was well-mixed during this period, and temperatures were similar in samples collected at the surface and the fluorescence maximum. Pooling data from surface-water samples from the summer and autumn cruises, we observed higher rates of N_2 fixation at higher temperatures but also lower N_2 fixation rates during autumn when light intensity was lower than during the July cruise (Fig. 2C).

During the geographically more extensive cruise during August 2009, there was a wider range of surface-water temperatures over the study area; from 11.4°C to 23.1°C for samples collected on Georges Bank and in the Gulf of Maine, and from 21.0°C to 27.0°C along the Mid-Atlantic shelf between 35.745°N and 41.314°N latitude (Table 5). Surface salinities ranged from 29.9 to 33.0, and Chl *a* concentrations ranged from 0.12 μg L^{-1} to 4.54 μg L^{-1}, DIN concentrations ranged from below the limit of analytical detection to 5.90 μmol N L^{-1}, and DIP concentrations ranged from the limit of analytical detection to 1.02 μmol P L^{-1} during July 2009. On average, DIN and DIP concentrations were more than double at the Georges Bank and Gulf of Maine stations (2.46 μmol L^{-1} and 0.51 μmol L^{-1} DIN and DIP, respectively) than at the Mid-Atlantic Shelf stations west of −70°W longitude (0.92 μmol L^{-1} and 0.20 μmol L^{-1} DIN and DIP, respectively). During this cruise, DIN : DIP ratios were < 16 at all but 4 stations where DIP was at or near the limit of analytical detection. Average volumetric rates of N_2 fixation in surface-water samples ranged from 1.0 nmol N L^{-1} d^{-1} to 49.8 nmol N L^{-1} d^{-1} (Table 5).

Water temperatures were cooler and salinity higher at the fluorescence maximum than in surface waters along the Mid-Atlantic Shelf but this was not always the case for

Table 4. Physical and chemical properties of water samples collected at the fluorescence maximum, or near the bottom at stations with shallow, well-mixed water columns (depth of sample collection is indicated), along the Mid-Atlantic continental shelf during October–November 2006 aboard the R/V *Hugh Sharp*. DBP is Delaware Bay, CBP is Chesapeake Bay Plume, MAS is Mid-Atlantic Shelf, and GSI denotes Gulf Stream–influenced stations. Standard deviations are in parentheses. Standard deviations of 0.00 indicate that the standard deviation was < 0.01.

Longitude (°W)	Latitude (°N)	Location	Depth (m)	Temperature (°C)	Salinity	Chl*a* (μg L^{-1})	$NO_3^-+NO_2^-$ (μmol L^{-1})	NH_4^+ (μmol L^{-1})	DIP (μmol L^{-1})	DIN : DIP molar ratio	N_2 fixation (nmol L^{-1} d^{-1})
−74.933	38.933	DBP	9	15.3	31.4	3.81(0.06)	1.15(0.09)	0.99(0.06)	0.20(0.02)	10.7	11.3(0.2)
−75.700	36.900	MAS 1	6	17.3	30.6	2.49(0.16)	0.45(0.06)	0.64(0.06)	0.22(0.01)	4.9	4.9(0.6)
−76.017	36.967	CBP 1	6	16.1	27.8	3.80(0.27)	1.33(0.00)	1.70(0.14)	0.27(0.04)	11.4	4.4(0.2)
−75.900	36.884	CBP 2	6	16.5	28.8	3.28(0.35)	1.21(0.00)	1.41(0.08)	0.26(0.03)	10.3	13.9(7.4)
−75.850	36.800	CBP 3	5	17.3	30.2	3.80(0.29)	0.69(0.06)	0.67(0.07)	0.22(0.02)	6.3	6.0(0.2)
−75.750	36.667	CBP 4	5	17.8	31.1	4.79(0.01)	0.24(0.00)	0.22(0.02)	0.16(0.02)	3.0	10.9(2.3)
−75.700	36.417	MAS 2a	10	18.3	31.4	2.52(0.39)	0.69(0.15)	0.90(0.09)	0.19(0.01)	8.4	5.6(0.01)
−75.700	36.417	MAS 2b	12	19.3	32.7	2.21(0.08)	0.93(0.06)	1.51(0.02)	0.15(0.01)	16.0	1.5(0.01)
−75.517	36.434	MAS 3	10	19.3	32.6	2.16(0.17)	0.24(0.00)	0.38(0.04)	0.11(0.01)	5.6	7.1(4.8)
−75.250	36.450	MAS 4	18	19.9	34.4	1.85(0.10)	1.37(0.32)	0.63(0.04)	0.11(0.02)	18.0	1.9(1.2)
−75.050	36.467	MAS 5	23	20.7	34.4	1.67(0.08)	1.75(0.16)	0.09(0.01)	0.11(0.02)	16.3	1.0(0.1)
−74.833	36.483	GSI 1	20	23.9	36.1	0.63(0.00)	0.27(0.06)	0.06(0.03)	0.03(0.02)	13.2	0.7(0.4)
−74.667	36.484	GSI 2	—	23.9	36.1	0.42(0.05)	3.86(0.82)	0.03(0.01)	0.20(0.01)	19.5	0.2(0.2)
−74.400	37.417	GSI 3	30	21.0	34.6	0.87(0.01)	0.21(0.06)	0.03(0.01)	0.06(0.01)	4.4	0.9(0.2)
−74.567	37.517	MAS 6	27	18.8	34.7	1.97(0.01)	0.80(0.18)	0.07(0.01)	0.11(0.00)	8.3	2.7(0.4)
−75.034	37.700	MAS 7	25	17.0	33.1	1.31(0.01)	0.24(0.00)	0.79(0.00)	0.14(0.02)	7.4	1.8(0.8)

Georges Bank where the water column was well-mixed (Table 6). Water temperatures ranged from 8.4 to 20.9 and salinity ranged from 30.7 to 35.5 at the fluorescence maximum. DIN concentrations ranged from 0.16 μmol N L^{-1} to 9.51 μmol N L^{-1} and DIP concentrations ranged from 0.03 μmol N L^{-1} to 1.91 μmol P L^{-1} in samples collected from the fluorescence maximum. As for surface waters, DIN : DIP ratios were ≤ 16. Average volumetric rates of N_2 fixation at the fluorescence maximum ranged from 0.3 nmol N L^{-1} d^{-1} to 20.3 nmol N L^{-1} d^{-1}, lower than the range observed in surface waters. Depth-integrated N_2 fixation rates in August 2009 ranged from 19.6 μmol N m^{-2} d^{-1} to 837.9 μmol N m^{-2} d^{-1} (Fig. 3; Table 5), with the highest depth integrated N_2 fixation rates measured at a station on Georges Bank.

When we compared N_2 fixation rates with DIN concentrations (Fig. 4A,C) and temperature (Fig. 4B,D), in samples collected in surface water (Fig. 4A,B) and at the fluorescence maximum (Fig. 4C,D) for all three cruises, we observed that the highest rates of N_2 fixation were measured in samples where DIN concentrations were lowest and temperatures were highest; however, there was no significant correlation between N_2 fixation and temperature or DIN concentration for any of the cruises in surface waters or at the fluorescence maximum ($p > 0.05$). We observed no relationship between DIP concentrations and rates of N_2 fixation during individual cruises or in the pooled data.

NifH gene copies were quantified using qPCR and previously used primer-probe sets from samples collected during July 2006 and August 2009. Unicellular N_2-fixing group A cyanobacteria (UCYN-A) was the most abundant diazotroph quantified in the study area during both cruises. UCYN-A *nifH* gene copies ranged from not quantifiable to 3.5×10^7 gene copies L^{-1} (Table 7). The highest numbers of gene copies detected were from samples collected from Mid-Atlantic shelf waters during August 2009. Gene copies from the tropical diazotrophs, *Trichodesmium* and *Richelia–Hemiaulus* associations, were also quantifiable throughout the study area with the exception of stations within the Chesapeake Bay influenced region. Although high rates of N_2 fixation were observed in the CBP area, the number of gene copies retrieved by qPCR was low. Subsequent sequencing of extracted DNA from these stations identified genes from UCYN-A cyanobacteria, and α and γ-proteobacteria (Fig. 5).

Discussion

Although oligotrophic tropical and subtropical marine environments depleted in DIN, and eutrophic fresh and brackish water environments enriched in phosphorus (P), have been thought to be primary sites for N_2 fixation (Howarth et al. 1988; Paerl 2008; Conley et al. 2009), we found active N_2 fixation in this temperate coastal marine system even where DIN concentrations were measurable in surface waters (range was 0.13 μmol L^{-1} to 5.90 μmol L^{-1}; Tables 1, 3, and 5) and at the fluorescence maximum (Tables 2, 4, and 6). In 2006, we found that N_2 fixation rates were often high (Fig. 1; Tables 1–4) and that *nifH*

Table 5. Physical and chemical properties of near-surface water samples (upper 6 m) collected at stations along the Mid-Atlantic continental shelf during August 2009 aboard the R/V *Delaware II*. Areal rates of N_2 fixation were estimated based on volumetric rate estimates made near the surface, in the upper mixed layer, and at the fluorescence maxima and integrated over the euphotic zone. MAS is Mid-Atlantic Shelf between Cape Hatteras and 39°N, MASN is the Mid-Atlantic Shelf between 39°N and 41.5°N but west of −70°W, and GBGM denotes stations on the shelf area east of −70°W on Georges Bank and in the Gulf of Maine. Standard deviations are in parentheses. BLD indicates that the value was below the detection limit, in which case the detection limit was used to calculate the DIN : DIP ratio. Standard deviations of 0.00 indicate that the standard deviation was < 0.01.

Longitude (°W)	Latitude (°N)	Location	Temperature (°C)	Salinity	Chl *a* (μg L^{-1})	$NO_3^- + NO_2^-$ (μmol L^{-1})	NH_4^+ (μmol L^{-1})	DIP (μmol L^{-1})	DIN : DIP molar ratio	N_2 fixation (nmol N L^{-1} d^{-1})	N_2 fixation (μmol N m^{-2} d^{-1})
−70.944	41.314	MASN	21.0	31.4	0.57(0.12)	1.13(0.87)	0.63(0.43)	0.40(0.15)	4.4	1.1(0.1)	30.2
−71.811	41.304	MASN	22.6	—	0.23(0.04)	0.24(0.35)	BDL	0.09(0.07)	2.9	9.2(5.0)	152.3
−72.837	40.059	MASN	24.8	31.4	0.51(0.04)	1.61(0.24)	0.04(0.03)	0.50(0.12)	3.3	2.2(1.4)	53.4
−73.344	39.098	MASN	25.6	31.2	0.22(0.02)	BDL	0.30(0.38)	0.30(0.10)	1.1	21.9(4.8)	215.8
−73.300	38.464	MAS	25.7	—	0.12(0.02)	0.28(0.31)	0.41(0.01)	0.21(0.07)	3.3	11.9(4.6)	778.8
−74.642	37.855	MAS	26.4	31.1	0.19(0.02)	0.04(0.00)	0.37(0.16)	0.14(0.07)	2.9	8.6(1.5)	169.7
−74.974	37.516	MAS	27.0	31.0	0.28(0.02)	0.48(0.16)	1.00(0.66)	BDL	—	—	69.0
−75.180	36.946	MAS	26.7	30.8	0.85(0.10)	0.34(0.04)	0.50(0.04)	BDL	—	1.2(0.3)	19.6
−75.395	35.745	MAS	23.6	32.4	0.30(0.05)	BDL	0.30(0.00)	0.18(0.02)	1.8	5.3(1.5)	35.7
−75.706	36.554	MAS	24.6	30.7	4.54(0.43)	0.19(0.09)	0.90(0.09)	0.03(0.01)	36.3	25.7(15.8)	192.4
−75.477	37.428	MAS	26.0	30.9	2.33(0.33)	0.20(0.04)	0.34(0.12)	BDL	—	49.8(9.5)	351.3
−74.515	39.007	MASN	23.1	30.8	0.57(0.04)	0.27(0.02)	1.62(0.03)	0.55(0.09)	3.4	2.3(0.5)	25.8
−74.512	39.232	MASN	22.3	31.0	—	—	—	—	—	2.6(0.1)	34.3
−73.880	39.516	MASN	26.2	30.4	0.52(0.03)	0.20(0.25)	0.89(0.07)	0.12(0.01)	9.1	3.0(0.5)	56.3
−73.388	40.063	MASN	25.5	29.9	0.89(0.08)	0.11(0.12)	0.54(0.25)	0.19(0.10)	3.4	1.8(0.8)	60.3
−71.740	40.330	MASN	23.5	32.2	0.21(0.05)	0.74(0.11)	0.45(0.06)	0.27(0.03)	4.4	9.1(5.3)	86.9
−69.991	40.164	GBGM	23.1	33.0	0.22(0.01)	0.50(0.29)	2.31(0.47)	0.34(0.34)	8.3	4.5(1.8)	208.0
−69.912	40.641	GBGM	22.6	32.9	0.24(0.03)	0.16(0.20)	1.42(0.03)	0.25(0.02)	6.3	28.6(15.1)	837.9
−69.324	41.160	GBGM	11.4	32.1	1.74(0.12)	5.44(0.09)	0.46(0.01)	0.52(0.01)	11.3	5.6(0.7)	125.6
−68.529	40.607	GBGM	18.8	32.3	0.57(0.03)	1.39(0.43)	1.47(0.19)	0.34(0.09)	8.4	3.7(0.4)	92.9
−68.214	41.404	GBGM	17.3	32.3	4.05(0.21)	0.02(0.00)	1.91(0.03)	0.66(0.14)	2.9	7.0(0.6)	102.6
−67.822	41.268	GBGM	17.0	32.3	3.95(0.72)	0.10(0.11)	3.54(0.12)	0.84(0.18)	4.3	10.6(0.8)	198.7
−67.203	40.984	GBGM	18.0	32.1	1.57(0.04)	BDL	1.28(0.04)	0.49(0.09)	2.7	4.1(0.4)	97.6
−66.955	41.572	GBGM	14.2	32.3	1.92(0.10)	1.65(0.56)	1.98(0.11)	0.78(0.05)	4.7	2.5(0.7)	52.2
−66.442	41.687	GBGM	15.4	32.3	2.47(0.57)	0.39(0.09)	1.01(0.06)	0.57(0.16)	2.5	4.3(0.5)	122.4
−65.736	42.057	GBGM	17.0	32.3	0.49(0.08)	BDL	1.30(0.10)	0.16(0.03)	8.3	3.1(0.5)	66.4
−66.652	42.478	GBGM	16.1	32.2	1.39(0.14)	0.29(0.24)	0.35(0.04)	0.32(0.00)	2.0	1.9(0.6)	51.9
−66.797	41.995	GBGM	17.2	32.2	0.94(0.12)	0.13(0.16)	1.55(0.17)	0.47(0.07)	3.6	7.5(0.01)	207.6
−67.601	42.050	GBGM	16.2	32.3	3.11(0.38)	0.08(0.09)	4.30(0.16)	0.50(0.19)	8.8	2.2(0.4)	47.9
−69.282	42.181	GBGM	20.4	31.2	0.84(0.06)	BDL	2.01(0.14)	1.02(0.67)	2.0	22.2(1.8)	194.3
−69.672	42.498	GBGM	22.1	31.1	0.31(0.07)	0.30(0.40)	1.61(0.11)	0.43(0.37)	4.4	18.3(5.2)	245.5

Table 6. Physical and chemical properties of water samples collected at the fluorescence maximum, or near the bottom for shallow-water stations (depth of sample collection is indicated), along the Mid-Atlantic continental shelf during August 2009 aboard the R/V *Delaware II*. MAS is Mid-Atlantic Shelf between Cape Hatteras and 39°N, MASN is the Mid-Atlantic Shelf between 39°N and 41.5°N but west of −70°W, and GBGM denotes stations on the shelf area east of −70°W on Georges Bank and in the Gulf of Maine. Standard deviations are in parentheses. BLD indicates that the value was below the detection limit, in which case the detection limit was used to calculate the DIN : DIP ratio. Standard deviations of 0.00 indicate that the standard deviation was < 0.01.

Longitude (°W)	Latitude (°N)	Location	Depth (m)	Temperature (°C)	Salinity	Chl *a* (μg L^{-1})	$NO_3^- + NO_2^-$ (μmol L^{-1})	NH_4^+ (μmol L^{-1})	DIP (μmol L^{-1})	DIN : DIP molar ratio	N_2 fixation (nmol N L^{-1} d^{-1})
−70.944	41.314	MASN	14.4	19.0	31.4	1.34(0.01)	0.02(0.00)	0.14(0.11)	0.50(0.07)	0.3	1.1(0.1)
−71.811	41.304	MASN	31	19.8	32.9	1.29(0.01)	0.25(0.04)	BLD	0.56(0.01)	0.5	1.8(0.6)
−72.837	40.059	MASN	30.5	9.8	32.8	4.22(0.00)	1.02(0.00)	BLD	0.29(0.09)	3.6	1.0(0.1)
−73.344	39.098	MASN	30	8.4	33.0	1.87(0.09)	2.54(0.17)	0.91(0.03)	0.77(0.07)	4.5	1.9(0.1)
−73.300	38.464	MAS	39.1	17.6	35.5	2.66(0.55)	BLD	0.28(0.36)	0.21(0.00)	1.4	20.3(10.5)
−74.642	37.855	MAS	28.6	9.9	32.9	1.71(0.04)	0.44(0.00)	0.03(0.01)	0.07(0.00)	6.7	2.7(2.0)
−74.974	37.516	MAS	16.5	20.9	33.1	0.73(0.13)	0.23(0.05)	0.61(0.30)	0.10(0.00)	8.4	2.8(0.1)
−75.180	36.946	MAS	24.9	—	33.1	4.76(0.11)	0.85(0.06)	0.47(0.06)	0.56(0.05)	2.4	0.7(0.8)
−75.395	35.745	MAS	13	18.8	33.3	0.52(0.21)	0.14(0.16)	0.42(0.00)	0.32(0.06)	1.8	1.3(0.3)
−75.706	36.554	MAS	7.1	18.3	32.7	4.79(0.09)	0.31(0.09)	0.25(0.02)	0.44(0.00)	1.3	10.9(2.3)
−75.477	37.428	MAS	10.3	19.4	32.4	1.52(0.08)	0.14(0.16)	0.26(0.01)	0.03(0.02)	13.3	9.1(0.6)
−74.515	39.007	MASN	11.5	17.1	31.6	1.85(0.07)	0.23(0.12)	0.29(0.07)	0.79(0.04)	0.7	1.8(0.0)
−74.512	39.232	MASN	—	15.0	31.6	—	—	—	—	—	2.1(0.5)
−73.880	39.516	MASN	18.8	13.7	32.7	0.52(0.06)	0.16(0.20)	0.30(0.01)	0.19(0.11)	2.4	2.0(0.1)
−73.388	40.063	MASN	24.9	11.2	32.5	2.32(0.05)	0.84(0.06)	0.66(0.03)	0.55(0.03)	2.7	2.4(0.4)
−71.740	40.330	MASN	25.7	13.9	33.4	1.50(0.07)	3.66(0.25)	1.13(0.05)	0.83(0.22)	5.8	1.2(0.6)
−69.991	40.164	GBGM	27.3	18.7	33.8	1.62(0.26)	0.49(0.05)	0.48(0.12)	0.06(0.01)	16.2	0.3(0.1)
−69.912	40.641	GBGM	22.5	12.0	32.7	4.95(0.68)	0.41(0.00)	0.37(0.02)	0.30(0.00)	2.6	5.5(1.0)
−69.324	41.160	GBGM	8.9	11.3	32.1	1.69(0.16)	4.58(0.36)	1.01(0.06)	0.40(0.13)	14.0	2.1(0.4)
−68.529	40.607	GBGM	22.3	16.6	32.3	3.08(0.58)	0.35(0.03)	0.28(0.02)	0.39(0.19)	1.6	4.5(0.2)
−68.214	41.404	GBGM	17.5	17.3	32.3	4.17(0.14)	0.18(0.23)	5.33(0.00)	0.66(0.15)	8.3	4.7(0.5)
−67.822	41.268	GBGM	17.4	16.9	32.3	4.39(0.81)	0.26(0.10)	1.52(0.09)	0.77(0.21)	2.3	12.2(1.2)
−67.203	40.984	GBGM	10.5	16.0	32.2	4.39(0.74)	BLD	1.00(0.08)	0.53(0.06)	1.9	5.6(1.0)
−66.955	41.572	GBGM	15	14.0	32.3	1.85(0.09)	1.59(0.02)	1.25(0.01)	0.78(0.11)	3.6	1.2(0.0)
−66.442	41.687	GBGM	15.1	12.7	32.5	0.97(0.07)	2.22(0.67)	0.60(0.06)	0.64(0.00)	4.4	5.0(1.9)
−65.736	42.057	GBGM	19.7	15.1	32.4	1.34(0.02)	0.13(0.15)	0.39(0.07)	0.16(0.04)	3.3	1.0(0.2)
−66.652	42.478	GBGM	14.6	16.1	30.7	1.50(0.01)	0.64(0.16)	0.50(0.00)	0.28(0.08)	4.1	3.2(0.2)
−66.797	41.995	GBGM	15.3	14.5	32.3	1.63(0.03)	0.54(0.49)	0.46(0.01)	0.53(0.00)	1.9	5.0(0.3)
−67.601	42.050	GBGM	9.5	15.9	32.3	3.38(0.20)	BDL	0.50(0.03)	0.33(0.32)	1.6	1.3(0.3)
−69.282	42.181	GBGM	23.1	11.8	32.2	2.03(0.22)	0.35(0.23)	0.22(0.04)	0.62(0.00)	0.9	2.0(0.0)
−69.672	42.498	GBGM	22.9	13.0	32.3	0.81(0.08)	9.19(1.97)	0.32(0.01)	1.91(0.14)	5.0	3.3(0.3)

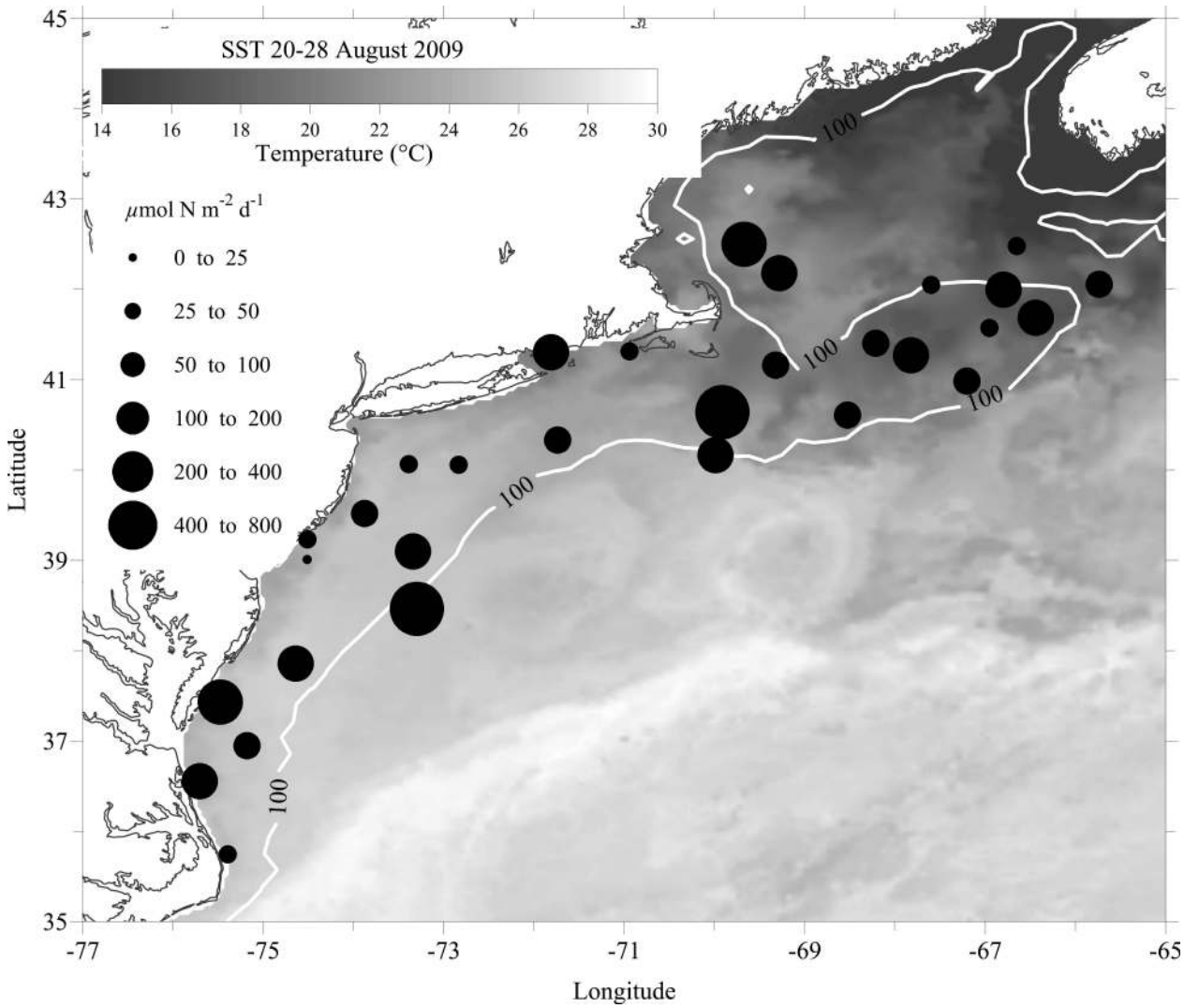

Fig. 3. Areal rates of N_2 fixation (μmol N m^{-2} d^{-1}) in surface waters collected from the Mid-Atlantic shelf waters between Cape Hatteras and Georges Bank in August 2009, superimposed upon a weekly averaged sea surface temperature (SST) field from satellite observations made during the field campaign.

gene copies were abundant (Table 7) in (1) areas influenced by the Chesapeake Bay (CBP) or the Delaware Bay plumes (DBP), (2) Gulf Stream–influenced waters (GSI), and (3) Mid-Atlantic shelf waters (MAS). During our wider survey in 2009, we found that high rates of N_2 fixation (Fig. 3; Tables 5 and 6) and abundant *nifH* gene copies (Table 7) extended into coastal waters north (to almost 43°N) and east (to −65°W) of the Delaware Bay plume, including the highly productive Georges Bank. Expression of *nifH* genes was previously observed primarily in oligotrophic waters in the temperate and subtropical Pacific (Church et al. 2005; Needoba et al. 2007) and the subtropical and tropical Atlantic Ocean (Langlois et al. 2005). Similarly, rates of pelagic marine N_2 fixation have been primarily reported from tropical and subtropical oligotrophic gyres (Table 8; Mahaffey et al. 2005; Carpenter and Capone 2008; Mulholland and Lomas 2008).

N_2 fixation rates in this study were generally higher in the well-lit surface mixed layer than at the depth of the fluorescence maximum, ranging from averages of 2.0 nmol N L^{-1} d^{-1} to 39.0 nmol N L^{-1} d^{-1} during summer (July 2006), 1.3 nmol N L^{-1} d^{-1} to 20.3 nmol N L^{-1} d^{-1} during autumn (Oct–Nov 2006), and 1.0 nmol N L^{-1} d^{-1} to 49.8 nmol N L^{-1} d^{-1} during August 2009 (the limit of analytic detection was $\sim$ 0.1 nmol N L^{-1} d^{-1}). This is consistent with the observation that cyanobacterial diazotrophs (and most cyanobacteria) are predominantly photoautotrophic (Zehr et al. 2008). Rates reported here from the euphotic zone are also within the range or higher than those reported previously for the tropical and subtropical Atlantic Ocean and other areas of the world's oceans (Table 8), but not as high as some of those reported from tropical coastal waters north of Australia (Montoya et al. 2004).

Temperature is thought to limit planktonic N_2 fixation by some marine cyanobacteria (Staal et al. 2003; Breitbarth et al. 2007; Moisander et al. 2010). Consistent with this idea, during cruises on the continental shelf between the

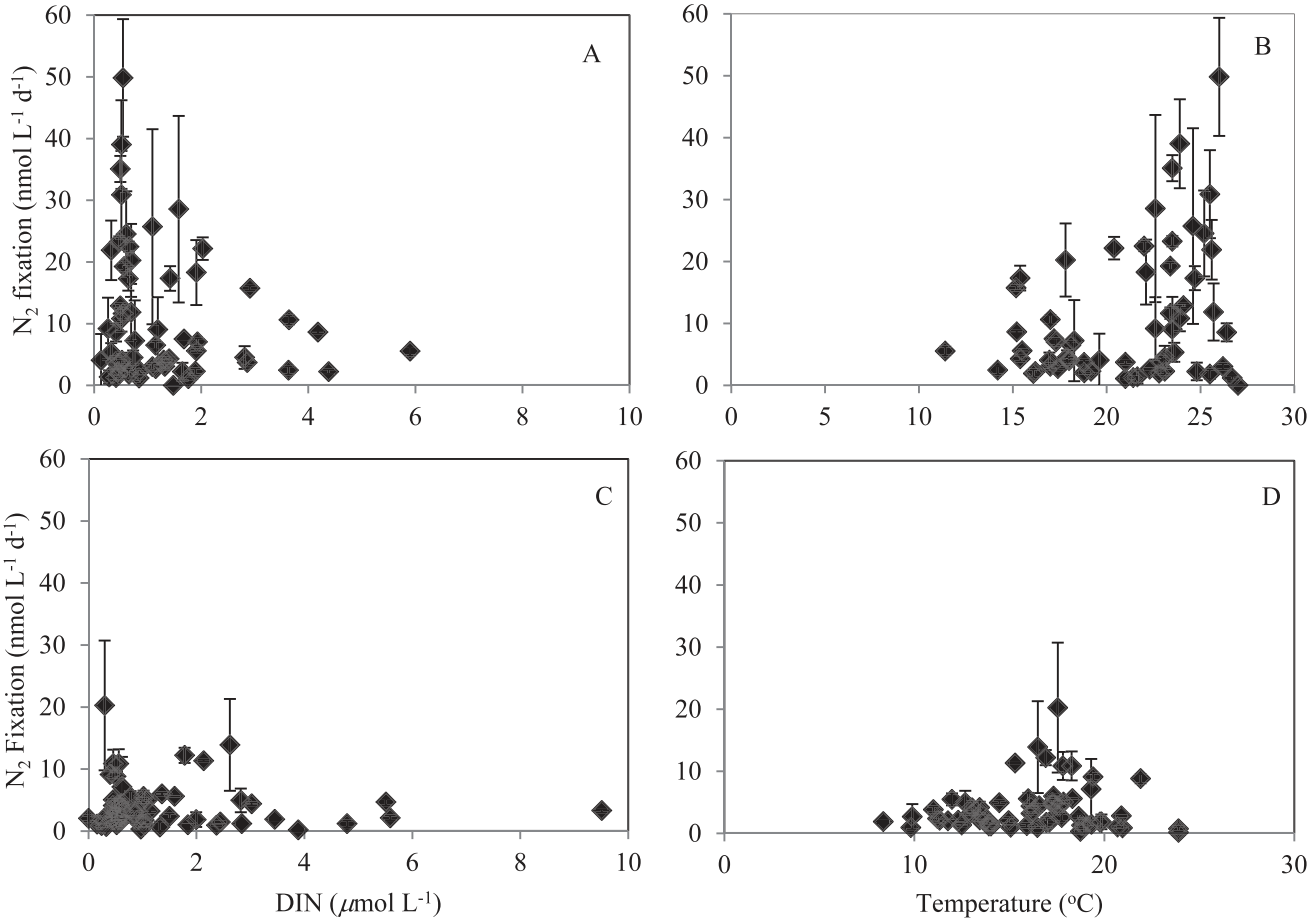

Fig. 4. Relationship between volumetric rates of N_2 fixation (nmol L^{-1} d^{-1}) and (A) DIN concentrations and (B) water temperature, in samples collected from near the surface (upper 5 m), and (C, D) the relationship between these same parameters in samples collected from the fluorescence maximum or near the bottom during all three cruises (summer and autumn 2006 and autumn 2009).

Chesapeake Bay and Delaware Bay plumes, higher rates of N_2 fixation were measured in summer when surface-water temperatures were higher (23.5°C ± 1.2°C) than in autumn, when water temperatures were on average 6°C cooler (17.8°C ± 2.0°C; Figs. 1, 3). Temperature and N_2 fixation rates were positively related in July (Fig. 2A). When water temperatures were below 18°C, N_2 fixation was always < 9 nmol N L^{-1} d^{-1}, and when temperatures were above 18°C, N_2 fixation rates were higher, ranging from 2 nmol N L^{-1} d^{-1} to 39.0 nmol N L^{-1} d^{-1}, consistent with the hypothesis that water temperature plays a role in controlling rates of N_2 fixation. However, all of rate measurements made at water temperatures < 18°C during July were from samples collected at the fluorescence maximum, where PAR may have limited N_2 fixation by phototrophs. In contrast, during October–November, the highest rates of N_2 fixation were measured at stations with lower water temperatures (15–18°C; Fig. 2B). During October–November, surface waters were cooling and water temperatures at the fluorescence maximum were often as warm as, or warmer than, those recorded in surface waters. N_2 fixation rates were usually, but not always, higher in the upper 10 m (Table 3) than in samples collected from deeper than 12 m (Table 4), which suggested that light was an important factor controlling the spatial distribution of N_2 fixation. During our expanded cruise track in 2009, when there was a wider range in surface-water temperatures (11.4°C to 27.0°C), there was no relationship between N_2 fixation rates in surface-water samples and surface-water temperature ($p > 0.05$; Fig. 4). In fact, many of the highest N_2 fixation rates were measured in cooler waters (Fig. 3; Tables 5 and 6). The high rates of coastal N_2 fixation reported here over a broader temperature domain are consistent with those from two coastal stations in the temperate English Channel (~ 50°N latitude), where measured planktonic N_2 fixation rates were 18.9 ± 0.1 nmol N L^{-1} d^{-1} and 20.0 nmol N L^{-1} d^{-1} (water temperatures were 18.8°C and 20.1°C, respectively; Rees et al. 2009).

Although oligotrophic tropical and subtropical marine environments depleted in DIN, and eutrophic fresh and brackish water environments enriched in P, have been thought to be primary sites for N_2 fixation (Howarth et al. 1988; Zehr and Paerl 2008; Conley et al. 2009), we found active N_2 fixation in the presence of DIN and in the absence of P enrichment in this temperate coastal marine system. In this study the DIN : DIP ratio was usually < 16,

Table 7. *NifH* gene copies from samples collected during July 2006 and August 2009 on the western Mid-Atlantic shelf. UCYN-A were most abundant throughout the study area. *NifH* genes from Group C cyanobacteria, *Chaetoceros–Calothrix,* and *Rhizosolenia–Richelia* associations were not detected (nd) in any of the 2006 samples so were not probed in 2009. Group B cyanobacteria *nifH* gene copies were detected but not quantifiable (dnq) during 2006 and so were not used in 2009. *Trichodesmium nifH* gene copies were quantified in all but the CBP samples. *NifH* genes from *Richelia–Hemiaulus* associations were quantifiable in all but the GBGM region.

	(*nifH* copies L^{-1})		
Station	UCYN-A	*Trichodesmium*	*Richelia–Hemiaulus*
Mid-Atlantic Shelf waters (MAS): Jul 2006			
MAS 1; 37.695°N; −75.298°W	1.72×10^4	2.65×10^3	nd
MAS 2; 37.631°N; −75.152°W	dnq	dnq	1.67×10^4
MAS 3; 37.519°N; −75.050°W	6.23×10^5	dnq	2.34×10^3
MAS 4; 38.042°N; −74.299°W	1.63×10^6	nd	1.62×10^3
MAS 5; 38.122°N; −74.384°W	5.40×10^4		nd
Mid-Atlantic Shelf waters (MAS): Aug 2009			
Sta. 37; 36.554°N; −75.706°W	3.5×10^7	nd	4.2×10^3
Sta. 38; 37.428°N; −75.476°W	2.5×10^7	nd	1.6×10^5
Gulf Stream influence (GSI): Jul 2006			
GSI 1; 36.686°N; −74.683°W	1.20×10^6	nd	3.10×10^3
GSI 2; 36.627°N; −74.863°W	1.96×10^5	7.47×10^2	3.72×10^4
GSI 3; 36.601°N; −75.058°W	1.10×10^4	1.27×10^5	1.97×10^4
GSI 4; 36.532°N; −75.273°W	7.65×10^4	7.63×10^2	1.80×10^4
Chesapeake Bay Plume influence (CBP): Jul 2006			
CBP 1; 36.972°N; −76.019°W	1.93×10^4	nd	dnq
CBP 2; 36.900°N; −75.908°W	dnq	nd	nd
CBP 3; 36.803°N; −75.857°W	2.52×10^4	nd	nd
CBP 4; 36.671°N; −75.766°W	3.03×10^3	nd	nd
CBP 5; 36.885°N; −75.719°W	2.84×10^4	nd	nd
Mid-Atlantic Shelf waters (north of 39°N; MASN): Aug 2009			
Sta. 44; 39.516°N; −73.880°W	1.7×10^5	nd	7.9×10^4
Sta. 50; 40.330°N; −71.740°W	8.2×10^4	9.1×10^4	2.9×10^3
Georges Bank and Gulf of Maine (east of −71°N; GBGM): Aug 2009			
Sta. 65; 40.607°N; −68.529°W	6.2×10^5	dnq	nd
Sta. 72; 41.268°N; −67.822°W	2.3×10^2	nd	nd
Sta. 75; 40.984°N; −67.203°W	4.6×10^5	1.1×10^2	nd
Sta. 92; 41.995°N; −66.797°W	4.3×10^5	nd	dnq
Sta. 99; 42.181°N; −69.282°W	8.5×10^5	dnq	nd

suggesting N limitation, but there was no relationship between N_2 fixation rates and the DIN : DIP ratio or the DIN concentration during any of the cruises ($p > 0.05$). Consistent with our results, *nifH* gene expression has been observed in the temperate Pacific (Church et al. 2005; Needoba et al. 2007) and Atlantic (Langlois et al. 2005) oceans and active N_2 fixation has now been observed in NO_3^--replete ($\sim$ 10-μmol L^{-1}) waters below the upper mixed layer in the eastern tropical North Atlantic Ocean (5 stations; Voss et al. 2004), in surface waters in the English Channel (2 stations; Rees et al. 2009), and in the Mekong River plume in the South China Sea (22 stations over two seasons; Grosse et al. 2010). In culture studies, *Trichodesmium* has been shown to be capable of fixing N_2 in the presence of up to 20 μmol N L^{-1} DIN, although rates are reduced above about 5 μmol L^{-1} DIN (Mulholland et al. 2001; Holl and Montoya 2005). Concentrations of DIN in surface waters over the study area were never depleted; average DIN concentrations in July were 0.54 μmol N L^{-1} ($\pm$ 0.09 μmol N L^{-1}) and concentrations ranged from 0.40 μmol N L^{-1} to 2.37 μmol N L^{-1} (Tables 1 and 2). During the October–November cruise, average DIN concentrations were higher (1.4 μmol N L^{-1}; $\pm$ 1.1 μmol N L^{-1}) with concentrations ranging from 0.06 μmol N L^{-1} to 4.18 μmol N L^{-1} (Tables 3 and 4). Over the expanded study area during 2009, DIN concentrations ranged from 0.16 μmol N L^{-1} to 9.51 μmol N L^{-1} (Tables 5 and 6). Based on these data, the absolute depletion of DIN appears unnecessary for active marine N_2 fixation; however, the nutrient thresholds above which N_2 fixation is inhibited remain to be elucidated for most marine diazotrophs.

UCYN-A were the most abundant diazotroph throughout the study area, ranging from the limit of detection to 3.5×10^7 *nifH* gene copies L^{-1} (Table 7), among the highest abundances ever observed (Carpenter and Capone 2008; Moisander et al. 2010). The highest UCYN-A *nifH* abundances were measured at the southernmost coastal stations, where the highest rates of N_2 fixation were also observed in August 2009 (Table 7; Fig. 3; *see* also Table 5). This group was also abundant in samples from stations on Georges Bank and in the Gulf of Maine (latitudes > 40°N), where surface-water temperatures were between 17.0°C and

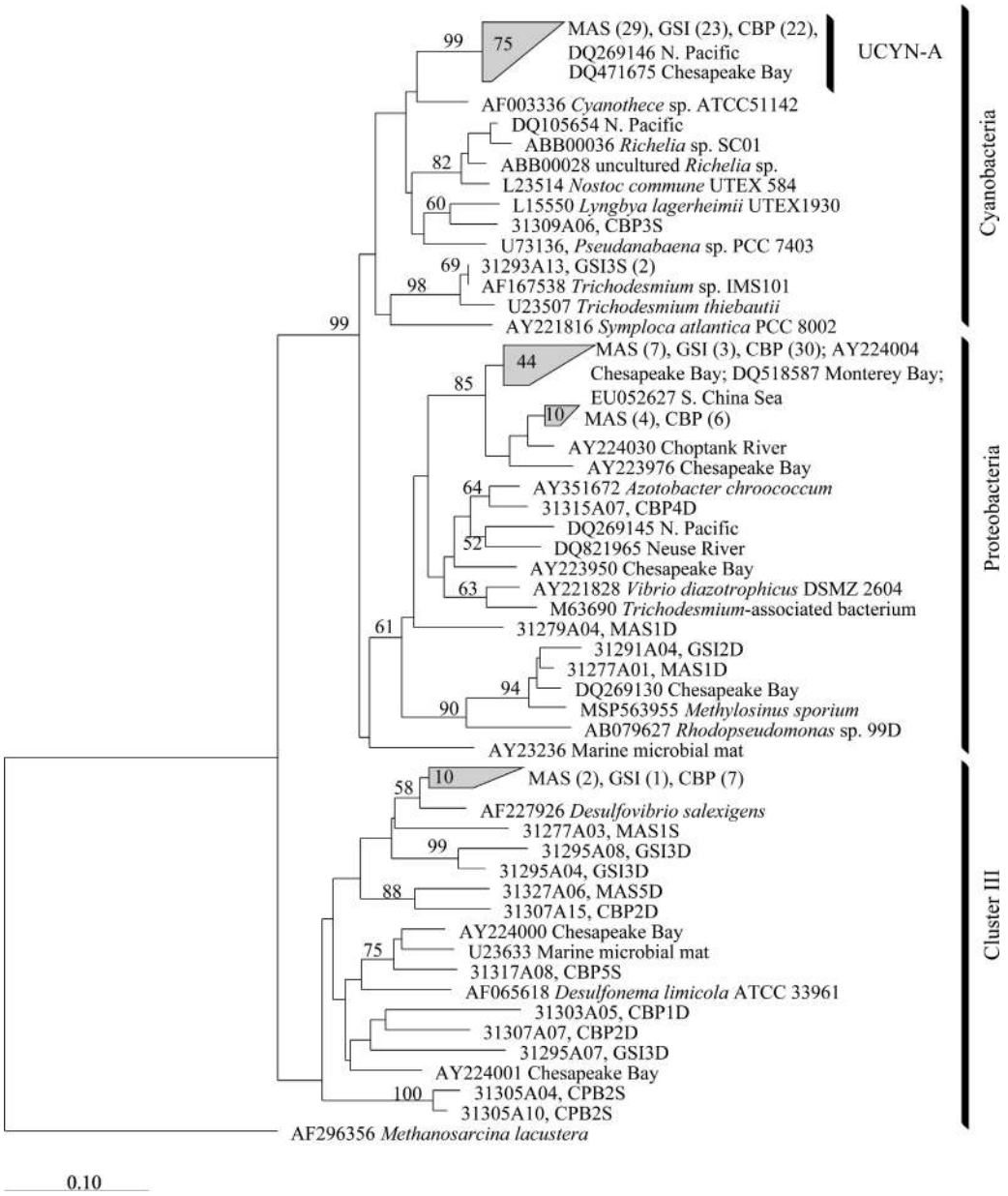

Fig. 5. Neighbor-joining tree of *nifH* amino-acid sequences obtained from samples collected in July 2006. Numbers in the triangles are the number of sequences in those groups. Bootstrap values (500 replicates) > 50% are shown at the respective nodes.

20.6°C, consistent with observations that this organism thrives at cooler water temperatures than other marine diazotrophs (Langlois et al. 2008; Moisander et al. 2010). *NifH* gene sequences clustering with UCYN-A were previously reported from two sites in the temperate English Channel (Rees et al. 2009), which suggests that N_2 fixation in North Atlantic coastal waters may be widespread. Unlike other cyanobacterial N_2 fixers, UCYN-A is a photoheterotroph that is likely symbiotic (Zehr et al. 2008; Tripp et al. 2010) and this has important implications with regards to coupled N and C dynamics in systems where they are abundant.

Trichodesmium nifH genes were detected throughout the study area except in the Chesapeake Bay–influenced region. This is consistent with the recent observation that these diazotrophs may be more widespread than previously thought (Davis and McGillicuddy 2006). *Hemiaulus–Richelia* (diatom–cyanobacterial symbioses) associations were also detected throughout the study area, but the highest concentrations were measured at stations west of −71°W, consistent with their abundance in river plumes and coastal regions with high silicate concentrations (Foster et al. 2007). Although detected, *nifH* gene abundances for *Trichodesmium* and *Richelia* were low or undetectable at many stations on Georges Bank, in the Gulf of Maine, and in the Chesapeake Bay plume–influenced region. Group B (*Crocosphaera*) cyanobacteria were detected but only at very low levels, near the limit of detection, during 2006.

The highest N_2 fixation rates during July 2006 were measured at the CBP-influenced stations where only

Table 8. Ranges of water-column N_2 fixation rates measured in other oceanographic provinces. Rates are presented as hourly rates because it is unclear whether all unicellular diazotrophs exhibit diel periodicity in N_2 fixation (updated from Mulholland et al. 2006; references can be found in the Web Appendix at www.aslo.org/lo/toc/vol_57/issue_4/1067a.pdf).

Date	Location	Depth	N_2 fixation (nmol L^{-1} h^{-1})	Method*	Reference
Atlantic Ocean:					
Spring 2002	Tropical N Atlantic	euphotic zone	~0.147	AR	Falcon et al. 2004
Summer 2001	Tropical N Atlantic	euphotic zone	0.025–0.045	$^{15}N_2$	Falcon et al. 2004
Seasonally 2001–2003	Western tropical N Atlantic	euphotic zone	0–1.2	$^{15}N_2$	Subramaniam et al. 2008
Autumn 2007	Eastern N Atlantic	surface	0–6.3	$^{15}N_2$	Turk et al. 2011
Autumn 2002	Tropical N Atlantic	upper 100 m	up to 3.1	$^{15}N_2$	Voss et al. 2004
Jul 2006	Coastal eastern temperate N Atlantic	surface	0.79–0.83	$^{15}N_2$	Rees et al. 2009
Jun 2006	Tropical N Atlantic	surface	0.02–0.053	$^{15}N_2$	Goebel et al. 2010†
Winter 2000	Subtropical and tropical eastern N Atlantic	3–4 m	0–0.059	AR	Staal et al. 2007
Spring 2007	Eastern N Atlantic	surface	0.300±0.115	$^{15}N_2$	Ibello et al. 2010
Jul 2006	Coastal western N Atlantic	euphotic zone	0.03–1.84	$^{15}N_2$	This study
Oct–Nov 2006	Coastal western N Atlantic	euphotic zone	0.01–1.02	$^{15}N_2$	This study
Aug 2009	Coastal western N Atlantic	euphotic zone	0.02–3.20	$^{15}N_2$	This study
Pacific Ocean:					
Jul 2000	Subtropical N Pacific	25 m	0.010–0.016	$^{15}N_2$	Zehr et al. 2001
Seasonally 2000–2001	Subtropical N Pacific	upper 100 m	0–0.092	$^{15}N_2$	Dore et al. 2002†
Autumn 2002	Subtropical N Pacific	euphotic zone	~0.003	AR	Falcon et al. 2004
2000–2002	Subtropical N Pacific and Kaneohe Bay	25 m and Surface	0.01–0.15	$^{15}N_2$	Montoya et al. 2004
Jun–Jul 2002	Eastern N Pacific	euphotic zone	0.047–1.85	$^{15}N_2$	Montoya et al. 2004
Winter 2007	Subtropical and tropical western N Pacific	euphotic zone	0–0.15	$^{15}N_2$	Shiozaki et al. 2009†
2006–2007	Temperate to equatorial N Pacific	euphotic zone	0.04–1.17	$^{15}N_2$	Shiozaki et al. 2010†
2000–2001	Subtropical N Pacific	25 m	0.014–0.095	$^{15}N_2$	Zehr et al. 2007‡
2004–2007 monthly	Subtropical N Pacific	upper 25 m	0.02–0.46	$^{15}N_2$	Church et al. 2009†
Jul 2005	Subtropical N Pacific	5 m	0.18–0.95	$^{15}N_2$	Fong et al. 2008†
2001–2002	Kane'ohe Bay	surface	0.029–0.048	$^{15}N_2$	Zehr et al. 2007‡
Autumn 1999	Arafura Sea	fluorescence maximum	20–62	$^{15}N_2$	Montoya et al. 2004
Summer 2006	Equatorial Pacific	euphotic zone	0.002–25.42	$^{15}N_2$	Bonnet et al. 2009†
Summer–winter 2004–2005	Western Pacific	—	0.04–0.83	AR	Kitajima et al. 2009†
Oct 2005	Temperate N Pacific	10 m	0.006–0.013	$^{15}N_2$	Needoba et al. 2007†
Jul–Aug	Subtropical N Pacific	upper 50 m	0.001–0.86	^{15}N	Watkins-Brandt et al. 2011†,‡
Autumn 2004	South Pacific	euphotic zone	0–0.15	$^{15}N_2$	Raimbault and Garcia 2008†
Mar–Apr 2007	Western S Pacific	surface	0–4.5	$^{15}N_2$	Moisander et al. 2010
Indian Ocean:					
Oct 2003	Leeuwin Current	euphotic zone	0.005–0.07	$^{15}N_2$	Holl et al. 2007
Mediterranean Sea:					
May 2002	E Mediterranean	16 m	5.38	$^{15}N_2$	Rees et al. 2006§
2003–2004 (12 cruises)	NW Mediterranean	upper 60 m	0.083–0.71	$^{15}N_2$	Garcia et al. 2006†
Jun–Jul 2008	Mediterranean	euphotic zone	0.004–0.075	$^{15}N_2$	Bonnet et al. 2011†
2004–2008	SE Mediterranean	euphotic zone	0–0.013	$^{15}N_2$	Yogev et al. 2011†
Spring 2007	Mediterranean	surface	0.052±0.031	$^{15}N_2$	Ibello et al. 2010
2004	W Mediterranean	euphotic zone	0.083–0.29	$^{15}N_2$	Sandroni et al. 2007†
South China Sea:					
Apr 2007 (dry season)	Mekong River Plume	surface	0.11–22.77	$^{15}N_2$	Grosse et al. 2010
Apr 2007 (wet season)	Mekong River Plume	surface	0.06–5.05	$^{15}N_2$	Grosse et al. 2010

Table 8. Continued.

Date	Location	Depth	N_2 fixation (nmol L^{-1} h^{-1})	Method*	Reference
Baltic Sea:					
Summer 1995–1996	Central Baltic	surface	0.19–5.6	$^{15}N_2$	Ohlendieck et al. 2000
Summer 1998–1999	Baltic Sea	0–25 m	0–7	$^{15}N_2$	Stal et al. 2003
Gulf of Mexico:					
2001–2003	Gulf of Mexico	surface	0.011–0.23	$^{15}N_2$	Mulholland et al. 2006
2003	Gulf of Mexico	fluorescence maximum	0.044–0.063	$^{15}N_2$	Mulholland et al. 2006

* AR is acetylene reduction.
† Converted from daily rate measured over 24 h.
‡ Used averages from control incubations.
§ Report rates from outside the phosphate-enriched area.

modest concentrations of UCYN-A were quantifiable by qPCR (Table 7). UCYN-A-like *nifH* genes were previously found to be expressed at the mouth of the Chesapeake Bay (Short and Zehr 2007). Sequencing results suggest that diazotrophic α- and γ-like Proteobacteria, similar to those found previously in the Chesapeake Bay (Jenkins et al. 2004; Short et al. 2004; Short and Zehr 2007), may also have contributed to the high rates of N_2 fixation in this region (Fig. 5). Expression of *nifH* by γ-Proteobacteria has been shown in other studies (Bird et al. 2005; Langlois et al. 2005), and therefore N_2 fixation by these and other organisms present but not quantified may have contributed to the high volumetric rates of N_2 fixation measured in the CBP during 2006. High precipitation in June 2006 prior to the cruise could have resulted in washout of microbes from the Chesapeake Bay and may have contributed to the high number of sequences similar to Proteobacteria found in the Chesapeake Bay plume during 2006 (Fig. 5). Two sequence types from the English Channel (Rees et al. 2009) had a > 97% identity at the amino acid level with two Proteobacterial sequences found in this study, which suggests that this group of diazotrophs may also be widespread in North Atlantic coastal waters.

Areal rates of N_2 fixation ranged from 40.9 μmol N m^{-2} d^{-1} to 392.4 μmol N m^{-2} d^{-1} for the area between Chesapeake and Delaware bays during 2006 (Fig. 1; Tables 1 and 3), and 19.6 μmol N m^{-2} d^{-1} to 837.9 μmol N m^{-2} d^{-1} over the area between about 35°N to 43°N and −75°W to −65°W during 2009 (Fig. 2; Table 5). These rates are within the range of areal N_2 fixation rates reported for tropical and subtropical regions (range of 3.7–703 μmol N m^{-2} d^{-1}; Carpenter and Capone 2008), the tropical North Atlantic Ocean (average 239 ± 38 μmol N m^{-2} d^{-1}; Capone et al. 2005), and the temperate English Channel (average 350 μmol N m^{-2} d^{-1}; Rees et al. 2009), and represent a substantial new N input into this ocean basin. In the area north of the Delaware Bay (39–41.5°N) and west of −70°W, areal rates of N_2 fixation were on average 79.5 μmol N m^{-2} d^{-1} (range was 30.2–215.8 μmol N m^{-2} d^{-1}), lower than those measured between Delaware Bay and Cape Hatteras (average 230.9 μmol N m^{-2} d^{-1}; range = 19.6–778.8 μmol N m^{-2} d^{-1} between 35°N and 39°N) during 2009. At latitudes > 40°N and east of −70°W, average areal rates of N_2 fixation were 176.8 μmol N m^{-2} d^{-1} (range = 47.9–837.9 μmol N m^{-2} d^{-1}), despite the markedly lower water temperatures in this region (Table 5).

Integrating over a year, we calculate a total N input of about 0.02 Tmol N yr^{-1} from planktonic N_2 fixation in the North American continental shelf waters between Cape Hatteras and Nova Scotia (35–45°N latitude). Although this region represents only about 6.4% of the total North Atlantic continental shelf area (and the North Atlantic includes about 20% of the global continental shelf area), N inputs from N_2 fixation calculated for this region alone are about equal to those calculated previously for the entire North Atlantic continental shelf (0.02 Tmol N yr^{-1}; Nixon et al. 1996). Further, comparable rates of N_2 fixation have been reported from the English Channel on the other side of the basin (Rees et al. 2009) and N_2 fixation rates are likely to be much higher in the coastal waters just south of our study area due to the proximity of the Gulf Stream where *Trichodesmium* and other tropical diazotrophs are known to occur (Prufert-Bebout et al. 1993). Estimates of N inputs from N_2 fixation for the entire North Atlantic basin range from 0.09 Tmol N yr^{-1} to 8.7 Tmol N yr^{-1} (Capone et al. 2005; Mahaffey et al. 2005; Carpenter and Capone 2008). Consequently, it is likely that N_2 fixation for the entire North Atlantic basin has been underestimated due to the exclusion of coastal waters.

Although N_2 fixation is just a small fraction of the total new N input to this coastal system, as compared with riverine flow and atmospheric deposition, it may be increasingly significant in future oceanic N budgets given projections that high CO_2 and temperature may favor N_2 fixation and growth of colonial and unicellular diazotrophs (Hutchins et al. 2009). In order to better estimate N inputs via marine planktonic N_2 fixation at present and in the future, we require a better understanding of where N_2 fixation occurs in the ocean and the physiology of different groups of diazotrophs.

These are the first results reporting planktonic N_2 fixation rates and *nifH* gene abundances from the temperate North American Mid-Atlantic continental shelf ecosystem (between 35°N and 45°N latitude), a region where N_2 fixation was previously thought to be negligible.

Diazotrophs are more abundant on the Mid-Atlantic shelf than anywhere previously measured in the ocean and N_2 fixation rates are comparable to or higher than those

reported for most oceanic systems (Table 8), yet N_2 fixation has not been included in coupled physical–biological models of N cycling the Mid-Atlantic Bight shelf region (Fennel et al. 2006) and coastal systems have been neglected in global marine N_2 fixation budgets in general (Galloway et al. 2004; Codispoti 2006). Findings from this study suggest that temperate coastal systems can have abundant N_2 fixers and that marine N budgets need to consider coastal N_2 fixation activity. Further, we need to reexamine the physiological capacities and limitations of diazotrophic organisms that occupy coastal environments.

Acknowledgments

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