Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic

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Abstract

Deep-water nitrate is a major reservoir of oceanic combined nitrogen and has long been considered to be the major source of new nitrogen supporting primary production in the oligotrophic ocean. $^{15}N : {}^{14}N$ ratios in plankton provide an integrative record of the nitrogen cycle processes at work in the ocean, and near-surface organic matter in oligotrophic waters like the Sargasso Sea is characterized by an unusually low ^{15}N content relative to average deep-water nitrate. Herein we show that the low $\delta^{15}N$ of suspended particles and zooplankton from the tropical North Atlantic cannot arise through isotopic fractionation associated with nutrient uptake and food web processes but are instead consistent with a significant input of new nitrogen to the upper water column by N_2 fixation. These results provide direct, integrative evidence that N_2 fixation makes a major contribution to the nitrogen budget of the oligotrophic North Atlantic Ocean.

Recent biological and geochemical studies have produced greatly increased estimates of the importance of biological N_2 fixation in supporting new production in oligotrophic areas of the ocean (Carpenter and Romans 1991; Lipschultz and Owens 1996; Michaels et al. 1996; Capone et al. 1997; Gruber and Sarmiento 1997; Karl et al. 1997). A number of lines of evidence have indicated that N_2 fixation makes a large contribution to the nitrogen budget of the Sargasso Sea, including extrapolation of small-scale biological observations (e.g., Carpenter and Romans 1991), direct estimates of in situ N_2-fixation rates (Capone et al. 1997; Capone 2001), and biogeochemical studies of elemental ratio anomalies (Lipschultz and Owens 1996; Michaels et al. 1996; Gruber and Sarmiento 1997). Any assessment of the contribution of N_2 fixation to the nitrogen budget of oligotrophic waters must integrate over a range of temporal and spatial scales, bridging the gap between small-scale, short-term biological measurements and the much larger scales implicit in biogeochemical studies. The marine nitrogen cycle is closely coupled to the global carbon cycle through primary production, and the quantity of oceanic new production supported by N_2 fixation remains a significant uncertainty in carbon cycle studies to date (Michaels et al. 2001).

The natural abundance of the stable isotopes of nitrogen in oceanic waters can provide critical evidence of the importance of N_2 fixation as a source of new nitrogen to the open ocean. The depletion of ^{15}N that characterizes near-surface organic matter in oligotrophic waters like the Sargasso Sea is one of the most robust patterns of nitrogen isotopic variation in marine systems (Wada and Hattori 1976; Minagawa and Wada 1986; Carpenter et al. 1997). These low $^{15}N : {}^{14}N$ ratios have been attributed to the effect of food-web processes that preferentially export ^{15}N from the upper water column in fecal pellets while retaining ^{14}N in the surface layer as recycled NH_4^+ (Checkley and Miller 1989). On the other hand, planktonic diazotrophs such as *Trichodesmium* are widely distributed in oligotrophic waters (Capone et al. 1997) and, through N_2 fixation, produce organic matter that is isotopically depleted relative to average oceanic combined nitrogen (Wada and Hattori 1976; Minagawa and Wada 1986; Carpenter et al. 1997). This isotopic contrast can provide a powerful tool for evaluating the impact of recently fixed nitrogen on oceanic ecosystems, although trophic and food web processes must also be accounted for in any attempt to use nitrogen isotope abundances to evaluate the impact of N_2 fixation on the ecosystem.

In this article, we report an extensive set of nitrogen isotope measurements for zooplankton and suspended particles collected on two cruises to the oligotrophic North Atlantic Ocean. In the context of existing knowledge of isotopic frac-
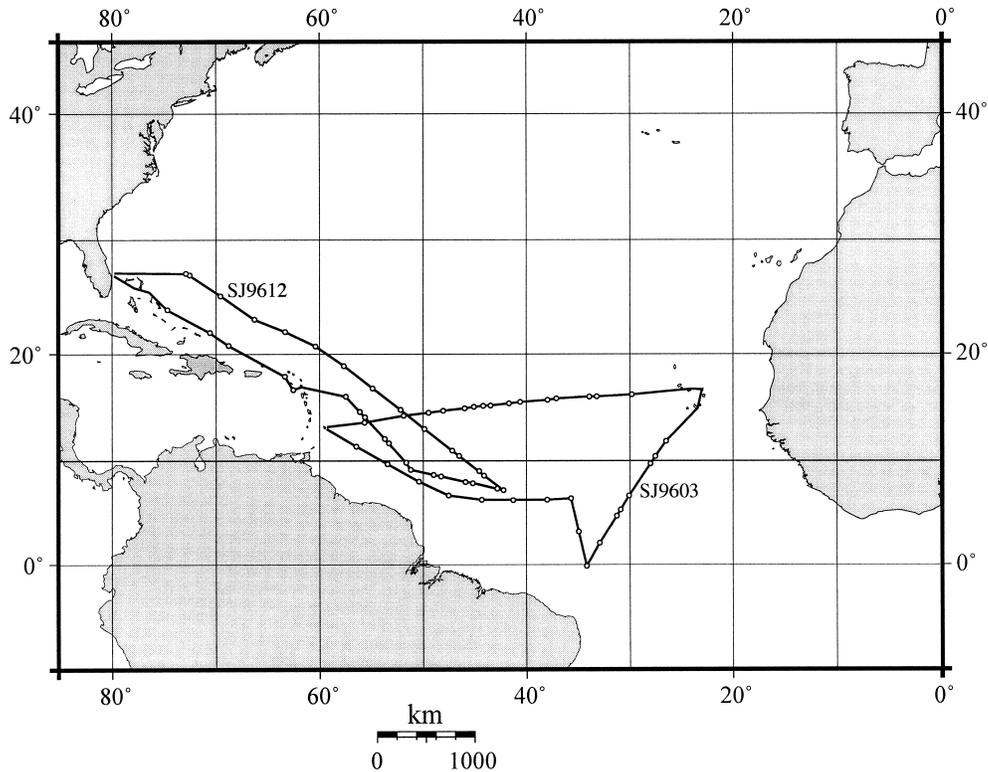
Fig. 1. North Atlantic cruise tracks for SJ9603 (28 March–25 April 1996) and SJ9612 (10 October–8 November 1996).

tionation processes in marine ecosystems, these data allow us to evaluate critically the role of food web processes and N_2 fixation in generating the low $\delta^{15}N$ values characteristic of the oligotrophic subtropical and tropical waters of the North Atlantic and to make a first-order estimate of the contribution of N_2 fixation to the planktonic food web in those waters.

Materials and methods

Samples of seawater and plankton were collected on R/V *Seward Johnson* cruises SJ9603 (28 March–25 April 1996) and SJ9612 (10 October–8 November 1996) to the tropical and subtropical Atlantic (Fig. 1). See Web Appendix 1 at http://www.aslo.org/lo/toc/vol_47/issue_6/1617a1.pdf for station locations. A CTD (conductivity-temperature-depth)–rosette system was used to obtain water samples through the upper water column. Suspended particles were collected by gentle vacuum filtration (200 mm Hg vacuum) of 4–8 liters of seawater through precombusted (450°C for 2 h) 45-mm GF/F filters that were dried at 60°C and stored over desiccant for analysis ashore. For isotopic analysis, filters containing particle samples were trimmed, then cut into quadrants or halves that were pelletized in tin capsules.

Trichodesmium was collected by gently towing a 30-cm diameter, 60-μm mesh size plankton net just below the surface for 5–10 min. Individual colonies were manually isolated with plastic inoculating loops, transferred to precombusted (450°C for 2 h) 25-mm GF/C filters, then dried at

60°C and stored over desiccant. Samples of *Hemiaulus* that contained endosymbiotic *Richelia* were obtained from surface bucket samples and by gently towing a 12.5-cm, 20-μm mesh size plankton net at 1-m depth. Bucket and net samples were passed through 202-μm Nitex mesh to remove *Trichodesmium* colonies, after which they were diluted with filtered surface seawater and filtered under gentle vacuum (<200 mm Hg) onto precombusted (450°C for 2 h) 25-mm GF/C filters, which were dried at 60°C and stored over desiccant. Diazotroph samples were prepared for isotopic analysis in the same way as samples of suspended particles. Diazotroph abundance was quantified at sea by direct microscopic counts (Zeiss epifluorescence microscope, green excitation, 100× magnification) of 47-mm Nuclepore filters (10-μm pore size) prepared by gravity filtration of the entire contents of a 10-liter Niskin bottle.

Zooplankton were collected in diagonal tows of a meter net (333-μm mesh size) through the upper 100 m of the water column. Animals were separated into size fractions by passage through a graded series of Nitex sieves (4,000, 2,000, 1,000, 500, and 250 μm) and were quickly frozen at sea. Once ashore, zooplankton samples were dried at 60°C and ground to a fine powder, which was then subsampled and pelletized in tin capsules for isotopic analysis.

At selected stations, GF/F-filtered seawater samples were transferred to high-density polyethylene bottles and preserved by addition of concentrated HCl to a final pH of 2–2.5. Nitrate was collected for isotopic analysis by reduction to ammonia, which was then extracted from solution by dif-
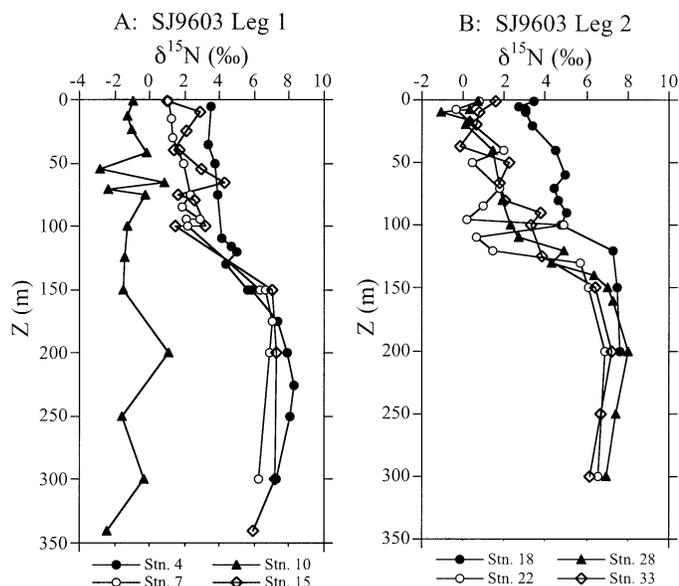
A: SJ9603 Leg 1
$\delta^{15}N$ (‰)

B: SJ9603 Leg 2
$\delta^{15}N$ (‰)

Fig. 2. $\delta^{15}N$ of suspended particles collected at representative stations on (A) leg 1 and (B) leg 2 of cruise SJ9603. Sta. 10 of leg 1 was located at the equator.

fusion and trapping on an acidified GF/F filter (Sigman et al. 1996). After extraction, each acidified filter was transferred to a tin capsule and pelletized for isotopic analysis.

All natural abundance measurements were made by continuous-flow isotope-ratio mass spectrometry (CF-IRMS) using one of three instruments: a VG Prism II in the Department of Earth and Planetary Sciences at Harvard, a Europa 20–20 at the Chesapeake Biological Laboratory, or a Micromass Optima at Georgia Tech. All three mass spectrometers were interfaced to elemental analyzers for online combustion and purification of sample nitrogen and carbon. The carbon isotope data will be reported elsewhere. The three CF-IRMS systems were intercalibrated by running a variety of isotopically characterized organic (peptone, acetanilide, histidine, and glycine) and inorganic standards (KNO_3 and NH_4Cl) on all three systems. In addition, a number of samples were split and analyzed on multiple instruments as a check on data quality. All isotope abundances are expressed as $\delta^{15}N$ values relative to atmospheric N_2. Each analytical run included a size series of elemental (acetanilide) and isotopic (peptone) standards, which provided a check on the stability of the instrument and allowed us to remove the contribution of any analytical blank to our isotopic measurements. In almost all cases, this blank was trivially small relative to the samples run and a blank correction was unnecessary. We conservatively estimate that the overall analytical precision of our isotopic measurements is better than $\pm0.3‰$.

Results

SJ9603 leg 1 (28 March–14 April 1996)—The first leg of this cruise extended southeast from Barbados through the tropical Atlantic to the equator, then northeast to the Cape Verde Islands (Fig. 1). At most stations on this transect, suspended particles in the mixed layer had a $\delta^{15}N$ between 1‰

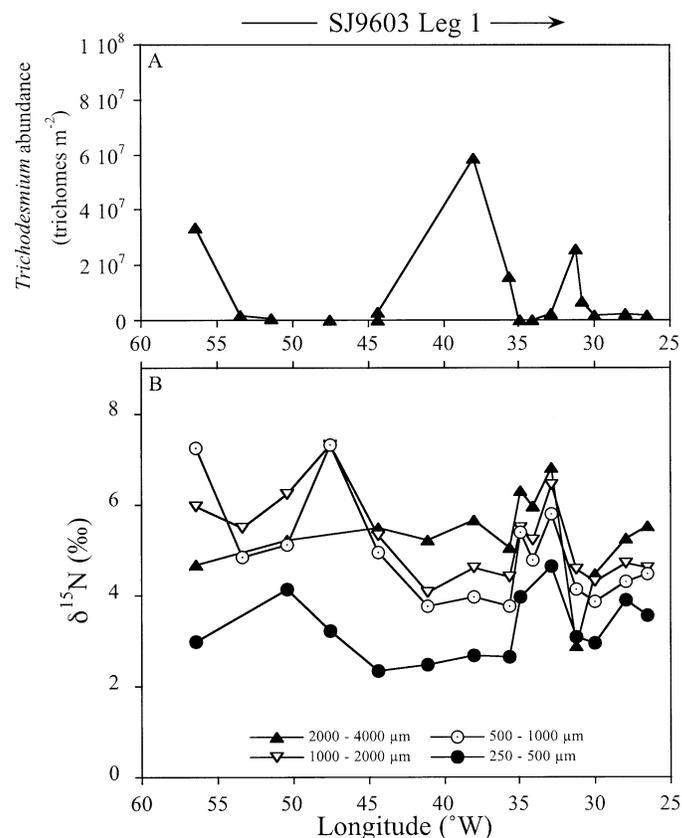Fig. 3. (A) *Trichodesmium* abundance and (B) $\delta^{15}N$ of zooplankton during leg 1 of cruise SJ9603.

and 2‰, increasing to values around 7‰–8‰ at depths of 200–300 m (Fig. 2A). The notable exception to this pattern was Sta. 10, located at the equator (34°10′W), where particles had uniformly low $\delta^{15}N$ values between −3‰ and 1‰ throughout the upper 350 m of the water column.

Along this transect, we encountered a wide range of concentrations of planktonic diazotrophs in the upper water column (Fig. 3A). Colonies of *Trichodesmium* spp. were relatively abundant at the start of this leg, then decreased to the southeast to Sta. 5 (44°18′W). The eastward run at roughly 6°15′N (Sta. 6–8) passed through a region of high trichome abundances centered around roughly 40°W. *Trichodesmium* abundances then dropped to very low values (3.6×10^5 trichomes m^{-2}) near the equator around 34°W. On the northeastward run away from the equator, *Trichodesmium* abundances showed a small peak around 5–6°N then declined toward the eastern end of the transect.

Zooplankton collected during this leg ranged in $\delta^{15}N$ from values ~7‰ at the western end of the transect and near the equator (34°W) to values approaching 2‰ in the middle portion of the basin (Fig. 3B). The $\delta^{15}N$ of zooplankton varied with size fraction, generally increasing with animal size (Fig. 4A). All size fractions showed the same general pattern of spatial variation, although the relationship between $\delta^{15}N$ and size fraction showed some variation among stations. The isotopic spread among zooplankton size fractions at a single station ranged from 2.3‰ to 4.2‰, with generally smaller
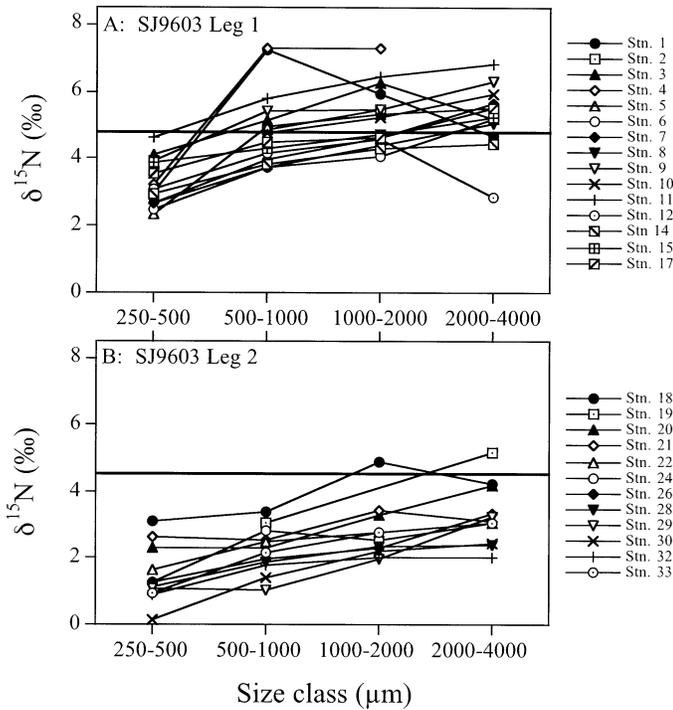
Fig. 4. δ^{15}N of zooplankton as a function of size fraction at stations (A) on the first leg and (B) on the second leg of cruise SJ9603. The horizontal line in each panel represents a typical δ^{15}N for deep-water nitrate.

Fig. 5. (A) *Trichodesmium* abundance and (B) δ^{15}N of zooplankton during leg 2 of cruise SJ9603.

spreads in the eastern portion of the transect extending from the equator northeast toward Cape Verde.

SJ9603 leg 2 (16–25 April 1996)—The second leg of this cruise extended from Cape Verde to Barbados (Fig. 1) and was characterized by a pronounced increase in *Trichodesmium* abundance from east to west (Fig. 5A). The δ^{15}N of suspended particles in the upper water column reflected this increase in *Trichodesmium* abundance, with upper water-column values at the easternmost station (Sta. 18) ranging between 3‰ and 4‰ and increasing to 7‰–8‰ at depth (Fig. 2B). Stations farther west had lower δ^{15}N values in near-surface particles, with values ranging from -1‰ to $\sim$2‰ in the upper 100 m, then once again increasing to values $\sim$7‰–8‰ at depths of 200–300 m (Fig. 2B).

Zooplankton also showed a broad change in δ^{15}N across the basin, with all size fractions decreasing in δ^{15}N by 2‰–3‰ from east to west (Fig. 5B). At the stations sampled, the δ^{15}N of zooplankton generally increased with animal size, with an isotopic spread across size fractions at a single station of 0.9‰–2.3‰ (Fig. 4B).

SJ9612 leg 1 (10–23 October 1996)—The first leg of this cruise extended southeastward from Florida and passed through the southwest Sargasso Sea off the Bahama Bank to a point southeast of Barbados (Fig. 1). Suspended particles were generally depleted in ^{15}N, with values ranging between -2‰ and 2‰ in the upper 100 m of the water column (Fig. 6A). At depth, the δ^{15}N of particles approached 6‰–7‰ around 200 m, although the profiles did not converge as

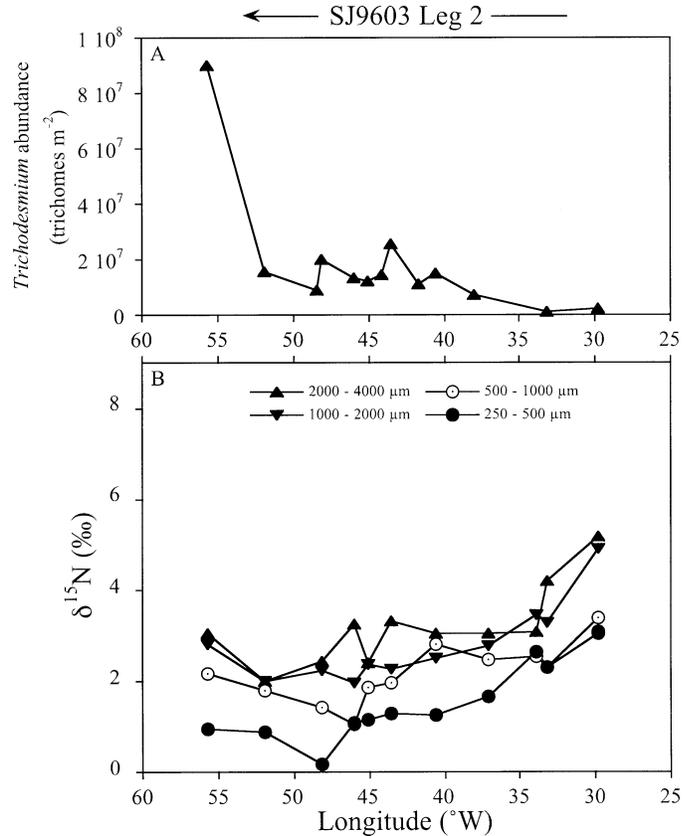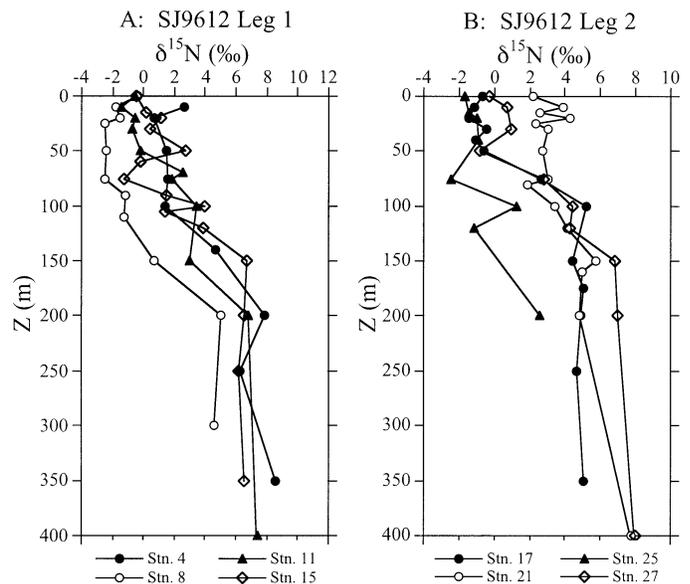Fig. 6. δ^{15}N of suspended particles collected at representative stations on cruise SJ9612. Sta. 21 of leg 2 was the only station sampled that lacked both *Trichodesmium* and *Richelia*.
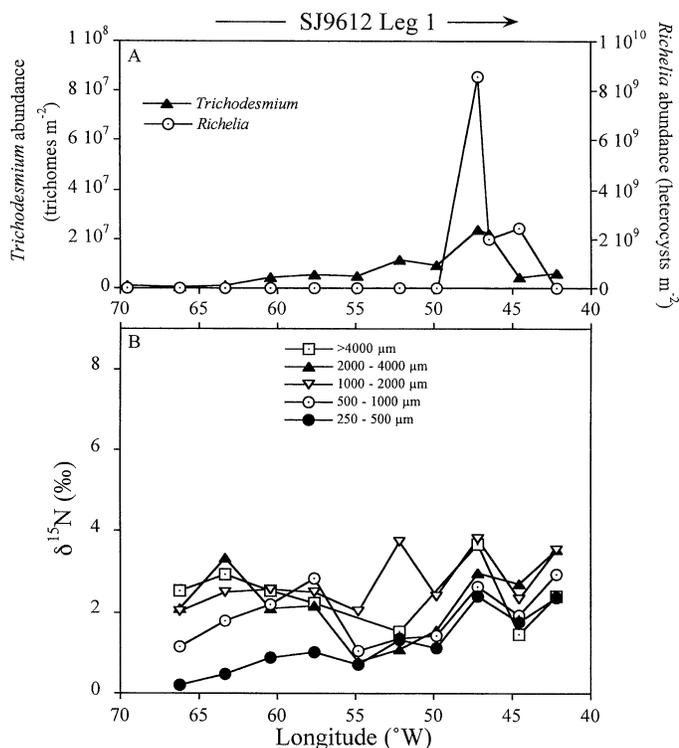
Fig. 7. (A) Diazotroph (*Trichodesmium* and *Richelia*) abundance and (B) δ^{15}N of zooplankton during leg 1 of cruise SJ9612. The *Hemiaulus/Richelia* bloom was concentrated at the surface, and *Richelia* abundances are integrated through the upper 30 m of the water column.

Fig. 8. δ^{15}N of zooplankton as a function of size fraction at stations on the (A) first leg and (B) second leg of cruise SJ9612. The horizontal line in each panel represents a typical δ^{15}N for deep-water nitrate.

closely at depth as the profiles from cruise SJ9603. *Trichodesmium* was abundant throughout much of this transect (Fig. 7A). On the second half of this leg (Fig. 7A), we also encountered a major bloom of the large chain-forming diatom *Hemiaulus hauckii,* which contained abundant heterocystous endosymbiotic cyanobacteria belonging to the genus *Richelia* (Carpenter et al. 1999).

Zooplankton from this transect showed rather low δ^{15}N values throughout, ranging between 0.2‰ and 3.6‰ (Fig. 7B). Of interest, a number of stations (Sta. 8–10) in the middle of the transect (~50–54°W) showed little isotopic separation between the smallest size fraction (250–500 μm) and animals >2,000 μm; at these stations, intermediate-size fractions, notably the 1,000–2,000-μm animals, had the highest δ^{15}N (Fig. 7B). In general, zooplankton on this transect showed a weak association between size and δ^{15}N (Fig. 8A), although the isotopic spread among size fractions at any individual station was comparable to that on the second leg of SJ9603, ranging between 1.2‰ and 2.8‰.

SJ9612 leg 2 (23 October–8 November 1996)—The second leg of this cruise followed a generally northwestward track roughly paralleling the first leg but displaced to the west (Fig. 1). During this leg, we continued to encounter very dense and spatially patchy surface aggregations of *Hemiaulus/Richelia* (Fig. 9A). As on leg 1, *Trichodesmium* was abundant through much of leg 2 (Fig. 9A), although it was absent from several of the stations, notably Sta. 21
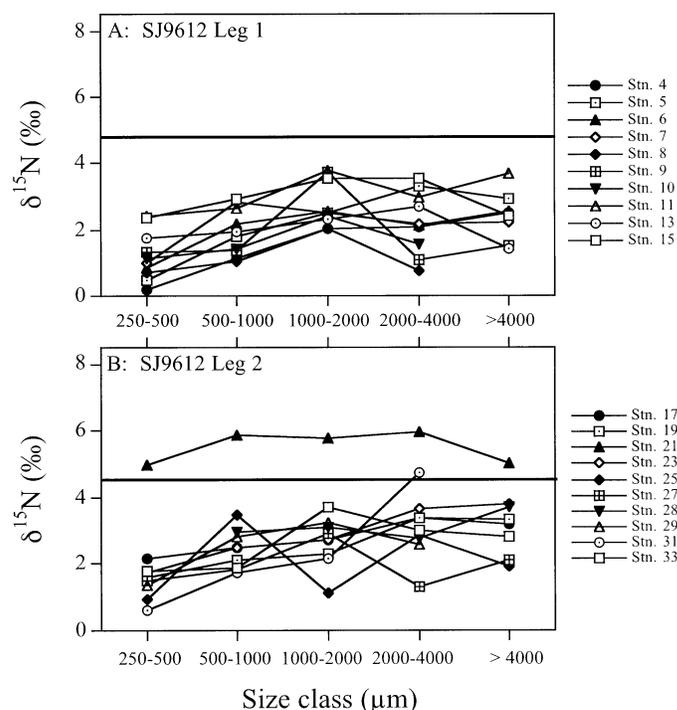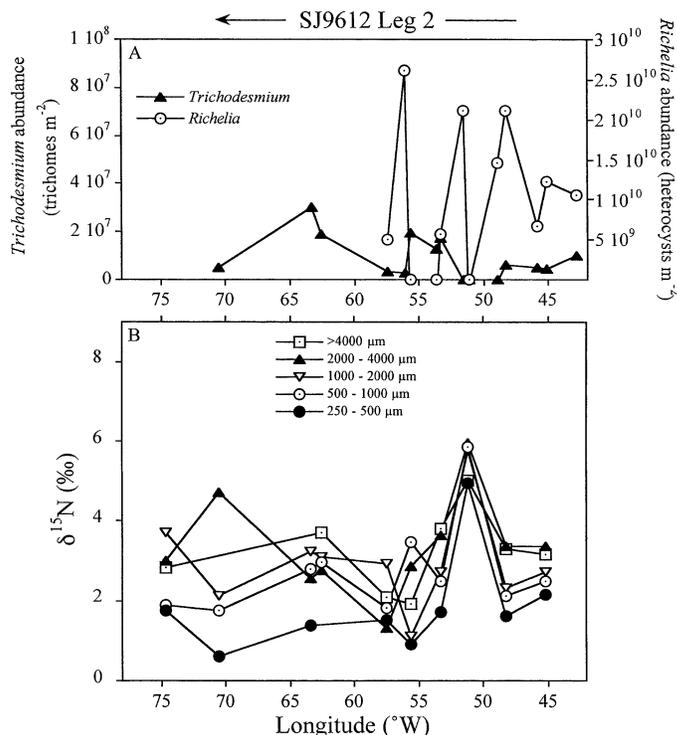

Fig. 9. (A) Diazotroph (*Trichodesmium* and *Richelia*) abundance and (B) δ^{15}N of zooplankton during leg 2 of cruise SJ9612.

Table 1. Summary of isotopic end members for the upper water column. $\delta^{15}N$ measurements of *Trichodesmium* are for samples collected on cruises SJ9603 and SJ9612. Data for *Richelia/Hemiaulus* are for samples collected on cruise SJ9612 and published elsewhere. Relatively few samples were obtained for NO_3^- isotopic measurement on these cruises, and data are available only for two stations of cruise SJ9612.

Sample	N	$\delta^{15}N$ (‰)	SE (‰)	Notes
SJ9603 *Trichodesmium*	20	−1.08	0.13	Isolated colonies
SJ9612 *Trichodesmium*	36	−2.15	0.09	Isolated colonies (Carpenter et al. 1999)
SJ9612 *Richelia/Hemiaulus*	12	−1.24	0.25	Concentrated suspension (Carpenter et al. 1999)
Shallow NO_3^- (<120 m)	7	2.90	0.66	Cruise SJ9612, Sta. 6 and 17
Deep NO_3^- (>120 m)	12	4.78	0.27	Cruise SJ9612, Sta. 6 and 17

(51°10′W). Suspended particles were isotopically depleted in the mixed layer at most stations sampled, with $\delta^{15}N$ values ranging between −2.5‰ and 2‰, then increasing with depth to values around 6‰–7‰ (Fig. 6B). Station 21, the only station on leg 2 that lacked both *Trichodesmium* and *Richelia* (Fig. 9A), was an exception to this pattern, with near-surface $\delta^{15}N$ values between 2‰ and 4‰ (Fig. 6B).

Zooplankton from this transect showed generally low $\delta^{15}N$ values between 0.6‰ and 4.7‰ with the exception of Sta. 21 (51°10′W), where all size fractions were markedly enriched in ^{15}N relative to other stations sampled (Fig. 9B). At Sta. 27, in the middle of the transect (57°28′W), the smallest zooplankton (250–500 μm) were isotopically indistinguishable from animals in the 2,000–4,000-μm size class, a pattern also seen in the middle portion of leg 1 of this cruise (Fig. 7B). In keeping with the pattern observed earlier in the cruise, the isotopic spread among zooplankton size classes on leg 2 was rather small at most stations (1‰–2‰), with a weak association between animal size and $\delta^{15}N$ (Fig. 8B).

SJ9612 nitrate isotopic composition—We measured the $\delta^{15}N$ of NO_3^- at Sta. 6 of leg 1 and Sta. 17 of leg 2 of this cruise. At these two stations, our deep-water values of $\delta^{15}NO_3^-$ averaged 4.8‰, whereas NO_3^- in the upper thermocline had a lower $\delta^{15}N$, averaging 2.9‰ (Table 1).

Discussion

The $\delta^{15}N$ of plankton reflects the inputs of N supporting production as well as the biological processing that may alter isotopic abundances within the ecosystem. As a result, the $\delta^{15}N$ of upper ocean organic matter provides an integrative, in situ tracer of the movement and transformation of nitrogen in the water column. The strong isotopic contrast between deep-water nitrate, which has an average $\delta^{15}N$ of ~4.5‰ over broad reaches of the ocean (Table 1, Liu and Kaplan 1989; Sigman et al. 1997), and diazotroph biomass, which typically has a $\delta^{15}N$ near −1‰–−2‰ (Table 1), provides a useful metric for assessing the regional importance of N_2 fixation. In the open ocean, consumption of upwelled nitrate and N_2 fixation represent fundamentally different mechanisms for supporting biological production with very different implications for the nitrogen cycle and the functioning of the biological pump (Michaels et al. 2001). To a first approximation, we expect the overall isotopic composition of an ecosystem to reflect the relative importance of these different sources of combined nitrogen in supporting production. The majority of stations sampled during these two cruises were characterized by low $\delta^{15}N$ values in both surface suspended particles and zooplankton, a pattern that implies a significant input of nitrogen to the ecosystem through N_2 fixation.

This approach is potentially complicated by the isotopic alterations that accompany common biological processes. For example, phytoplankton uptake of nitrate discriminates against ^{15}N by 5‰–10‰ (Montoya and McCarthy 1995; Waser et al. 1998), producing biomass depleted in ^{15}N relative to the available nitrate while increasing the $\delta^{15}N$ of the residual nitrate. This fractionation mechanism has been invoked to explain temporal variations in the $\delta^{15}N$ of upper water-column particles during the North Atlantic spring bloom (Altabet et al. 1991) and spatial variations in $\delta^{15}N$ in the equatorial Pacific and the Southern Ocean (Altabet and François 1994). In cases where all of the nitrate supplied to the upper water column is converted into biomass, there is no net expression of isotopic fractionation, and the pool of biomass produced will have the same isotopic composition as the original pool of nitrate upwelled. In oligotrophic waters, upwelled nitrate is typically drawn down to the limit of detection by biological uptake in the mixed layer, so the isotopic fractionation associated with nitrate uptake cannot, by itself, produce the low $\delta^{15}N$ values that characterize organic matter in those waters.

Other biological processes may also be important determinants of the $\delta^{15}N$ of oceanic systems, and at least two distinct factors may contribute to the low $\delta^{15}N$ of these waters: (1) animal trophic and excretory processes preferentially export ^{15}N from the upper water column, leading to a low $\delta^{15}N$ in the residual combined N and (2) N_2 fixation may inject significant quantities of ^{15}N-depleted combined nitrogen into the system. These mechanisms are not mutually exclusive, and our data allow us to evaluate their relative importance in controlling the isotopic composition of the upper ocean.

Animal processes, N_2 fixation, and the $\delta^{15}N$ of the upper ocean—Animals play a major role in cycling and transporting organic matter in the ocean; their feeding behavior and physiology are critical components of secondary production and the movement of elements through the food web. A wide variety of animals from terrestrial as well as aquatic habitats are enriched in ^{15}N by ~1.5‰–4.5‰ relative to their diet,

with an overall average enrichment of ~3.5‰ for animals in general (DeNiro and Epstein 1981; Minagawa and Wada 1984; Mullin et al. 1984; Wada et al. 1987; Fry 1988; Montoya et al. 1990, 1992). The biochemical pathways leading to this consistent isotopic enrichment in heterotrophs are not fully understood, although it is clear that catabolic and excretory processes play a critical role in preferentially retaining ^{15}N within animal tissues (Bada et al. 1989; Gannes et al. 1997).

At steady state, the isotopic composition of the nitrogen entering an animal through feeding must equal the isotopic composition of the outputs as dissolved and solid excreta (Fig. 10). Only a few measurements of the isotopic composition of excreta are available for marine organisms. Fecal material typically appears to be slightly enriched in ^{15}N relative to the food consumed (Checkley and Entzeroth 1985; Altabet and Small 1990; Montoya et al. 1992) and therefore depleted relative to the animal's tissues. This is consistent with the mechanisms of digestion and food absorption by most animals, which appear to provide limited opportunities for isotopic fractionation. Zooplankton are primarily ammonotelic, and deamination reactions are known to discriminate against ^{15}N, producing isotopically depleted NH_4^+ (Macko et al. 1986; Bada et al. 1989). In zooplankton, the net effect of this excretory fractionation is to produce NH_4^+ that is depleted in ^{15}N by ~2.7‰ relative to the substrate being catabolized (Checkley and Miller 1989). Acting over time, these excretory processes lead to a progressive enrichment of the residual pool of amino nitrogen within an animal's body.

On an ecosystem level, the same animal processes act to retain ^{14}N preferentially in dissolved form within the upper water column, where it can be recycled into the biota while exporting ^{15}N preferentially to subsurface waters in sinking organic matter. In combination, these animal feeding and excretory processes will tend to lower the average $\delta^{15}N$ of organic matter in the upper water column, as shown schematically in Fig. 10. Within the mixed layer, arrows represent trophic processes that move nitrogen up the food web along with losses of ingested nitrogen. Each transfer of nitrogen represented by an arrow will tend to bring the $\delta^{15}N$ of the target pool nearer that of the source pool of nitrogen.

Although trophic processes can lead to a significant difference between the $\delta^{15}N$ of deep NO_3^- and the surface biota, the animal processes that preferentially export ^{15}N out of the surface require that at least some of the zooplankton have a $\delta^{15}N$ higher than that of deep NO_3^-. We observed this pattern at a number of stations on the first leg of cruise SJ9603, notably at the western end of the transect (between roughly 56° and 47°W) and in the equatorial region around 34°W (Fig. 3B). Similarly, the easternmost station (Sta. 18) of the second leg of this cruise was characterized by a rather high $\delta^{15}N$ in near-surface particles and all size fractions of zooplankton (Figs. 2B, 5B). At all but one of these stations (Sta. 1), the abundance of diazotrophs was low in the water column, and our isotopic indicators are consistent with a strong dominance of upwelled nitrate in supporting new production. Finally, we also observed elevated $\delta^{15}N$ values in both particles and zooplankton at Sta. 21 on cruise SJ9612. This was the only station on either cruise at which diazotrophs were
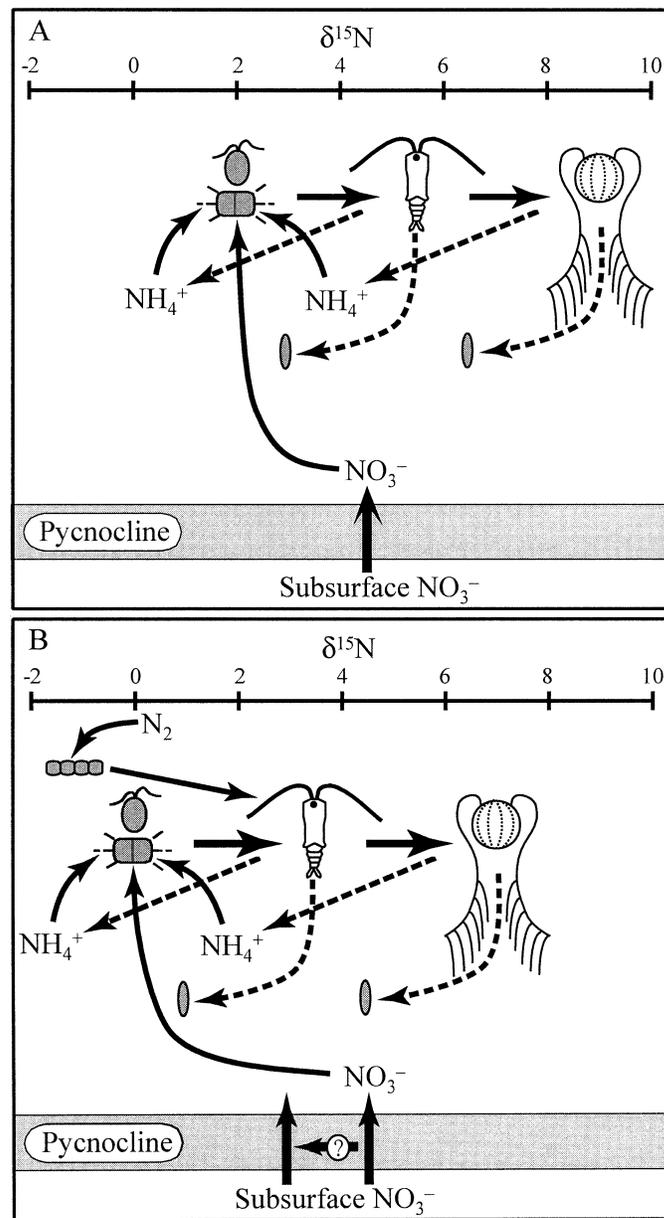
Fig. 10. Schematic of the major processes controlling the isotopic composition of organic nitrogen in the upper water column of the oligotrophic ocean in the (A) absence and (B) presence of significant inputs of new nitrogen through N_2 fixation. Solid arrows represent phytoplankton growth on dissolved inorganic nitrogen and trophic processes that transfer nitrogen up the food web; broken arrows represent transfer of nitrogen into dissolved and solid wastes. For each of the processes shown, the transfer of nitrogen represented by an arrow will tend to bring the $\delta^{15}N$ of the target pool nearer that of the source pool of nitrogen. See text for additional details.

completely absent, and the resulting $\delta^{15}N$ values were among the highest we found anywhere, despite the close spatial proximity of other stations with significant diazotroph biomass and correspondingly low $\delta^{15}N$ in plankton.

Our conceptual model of the upper water-column N cycle provides a useful test of the role of food web processes in

generating the low $\delta^{15}N$ we observed in the upper water column. At steady state, the mass and isotope fluxes in and out of the upper ocean must balance such that the mean $\delta^{15}N$ of the organic nitrogen exported from the upper water column is equal to the weighted average $\delta^{15}N$ of the new nitrogen entering the system. In the absence of significant N_2 fixation (Fig. 10A), animal export processes will produce suspended particles with a $\delta^{15}N$ lower than that of the deep-water NO_3^-, sinking particles that have a $\delta^{15}N$ equal to that of the upwelled NO_3^-, and bulk zooplankton that have a $\delta^{15}N$ similar to or higher than that of subsurface NO_3^-. As noted above, this pattern occurred at only a few of the stations sampled on these two cruises.

Our isotopic data thus allow us to rule out food-web processes as the dominant cause of the low $\delta^{15}N$ values that characterize the oligotrophic Atlantic. In oligotrophic oceanic waters, where riverine and atmospheric inputs to the upper water column are relatively small (Galloway et al. 1996; Prospero et al. 1996), biological N_2 fixation appears to be the only plausible mechanism for introducing significant quantities of ^{15}N-depleted combined nitrogen into the water column. In addition, N_2 fixation is compatible with both the pattern and the magnitude of the isotopic depletion of PN and zooplankton that we observed (Fig. 10B).

N_2 fixation produces biomass with a low $\delta^{15}N$ between $-1‰$ and $-2‰$ (Table 1), ultimately lowering the $\delta^{15}N$ of all organic pools (Fig. 10B). We observed this distinctive isotopic pattern on both cruises at all stations with significant diazotroph populations (Figs. 2–9). At these stations, near-surface suspended particles and zooplankton have a $\delta^{15}N$ well below that of typical subsurface nitrate ($\delta^{15}N = \sim4.5‰$) and is therefore too low to act as a direct source of sinking organic matter to balance the isotope budget for the surface layer if upwelled nitrate is the dominant source of new nitrogen for primary production (Figs. 4, 8). To close the isotope budget, sinking particles must have a $\delta^{15}N$ equal to the weighted average of the inputs of new nitrogen through N_2 fixation and upwelling of NO_3^- (Fig. 10B). Furthermore, the magnitude of the ^{15}N depletion in Sargasso Sea plankton relative to deep-water nitrate indicates that this low-$\delta^{15}N$ source of nitrogen makes a substantial contribution to the N budget of the oligotrophic North Atlantic Ocean.

Importance of N_2 fixation—The magnitude of the diazotroph contribution to the oligotrophic North Atlantic is clearly large, and a simple isotopic mixing model can be used to estimate the relative contributions of N_2 fixation and upwelling of deep-water NO_3^- to the biomass in the upper water column. This is an integrative approach that reflects the turnover time of organic matter in the upper ocean as well as that of NO_3^- in the upper thermocline. The relative contribution of diazotroph nitrogen and deep-water nitrate to the bulk suspended particles in the upper water column can be calculated as

$$\% \text{ diazotroph N} = 100 \times \left(\frac{\delta^{15}N_{particles} - \delta^{15}NO_3^-}{\delta^{15}N_{diazotroph} - \delta^{15}NO_3^-} \right) \quad (1)$$

where we use $\delta^{15}NO_3^- = 4.5‰$ and $\delta^{15}N_{diazotroph} = -2‰$ (Table 1) to estimate the importance of diazotroph nitrogen to

the suspended particles. This calculation is sensitive to the values of $\delta^{15}N_{diazotroph}$ and $\delta^{15}NO_3^-$ used. Our choice of $\delta^{15}N_{diazotroph} = -2‰$ reflects the low end of the range we measured in our study. This is a conservative choice that minimizes the estimated diazotrophic contribution to suspended particles. From an ecosystem perspective, our choice of a deep-water value for $\delta^{15}NO_3^-$ (4.5‰) has the effect of incorporating the nitrate in the upper thermocline into the isotope budget of the mixed layer. If we instead use a low value of $\delta^{15}NO_3^- = 3‰$, our estimates of the new diazotroph contribution to the suspended particles are reduced by $\sim38\%$, reflecting the recycling of recently fixed nitrogen between the upper water column and the upper thermocline nitrate pool.

Our estimates of the diazotroph contribution to bulk surface particulate nitrogen on the first leg of cruise SJ9603 ranged from 13% at our westernmost station (Sta. 4) to $\sim30\%$–40% in the region of the *Trichodesmium* blooms encountered around 6°N (Sta. 7 and 15, Table 2). The estimated diazotroph contribution to near surface particles ranged from a low of 4% of the total at the eastern end of leg 2 to roughly half of the total N content in the western portion of the transect. In contrast, diazotrophs were clearly a dominant source of N, supporting primary production in the upper water column during cruise SJ9612, accounting for well over half of the N in the water column at most of the stations sampled in the southwest North Atlantic.

Our results can be compared with direct, ^{14}C-based measurements of primary production by *Trichodesmium* and other phytoplankton carried out during our cruises. On average, *Trichodesmium* accounted for 14% of total primary production during cruise SJ9603 and 10% of total primary production during cruise SJ9612 (Carpenter et al. pers. comm.). Within the bloom encountered on cruise SJ9612, the total rate of N_2 fixation by *Trichodesmium* and the *Hemiaulus/Richelia* association accounted for $\sim25\%$ of the community N demand (Carpenter et al. 1999). Our ^{15}N budget indicates a much higher contribution of diazotrophs to overall biological production on both these cruises. This difference may reflect the longer effective integration time of our stable isotope measurements and the difficulty of extrapolating from discrete experiments to larger spatial and temporal scales. In addition, other diazotrophs (e.g., Zehr et al. 2001) may also make a significant contribution to the N budget of these waters.

Extending this calculation to the zooplankton is complicated by our relatively poor knowledge of the physiological processes that lead to the trophic enrichment in zooplankton and the net effect of trophic processes in lowering the $\delta^{15}N$ of the upper water column. Nonetheless, we can use the high $\delta^{15}N$ values measured at and near the equator, where diazotroph abundances were very low, as a benchmark for zooplankton $\delta^{15}N$ values in a system supported largely by subsurface NO_3^-. For each size fraction of zooplankton, we used the mean $\delta^{15}N$ of animals at Sta. 9, 10, and 11 of cruise SJ9603 as a reference value, then estimated the relative contribution of diazotroph nitrogen and deep-water nitrate to zooplankton (zpl) biomass as

Table 2. Estimated contribution of N_2 fixation to the organic N in particles and zooplankton at representative stations from the two cruises. The diazotroph contribution is calculated using Eqs. 1 and 2 respectively, and the reference $\delta^{15}N$ values used in these calculations are shown in the first row of the table. See the text for further details.

Station	Particles 0–100 m	Zooplankton (μm)			
		250–500	500–1,000	1,000–2,000	2,000–4,000
Reference $\delta^{15}N$ values (‰)	4.5	4.3	5.3	5.7	6.4
SJ9603 (%)					
Stn. 04	13	17	0	0	ND
Stn. 07	42	26	19	14	8
Stn. 15	32	6	14	13	13
Stn. 18	4	19	26	10	25
Stn. 22	55	42	39	38	39
Stn. 28	50	50	47	43	47
Stn. 33	44	53	43	38	40
SJ9612 (%)					
Stn. 04	46	65	57	48	51
Stn. 08	100	57	58	48	67
Stn. 11	62	30	37	25	40
Stn. 15	60	30	33	28	34
Stn. 17	62	34	39	38	36
Stn. 25	94	54	25	60	42
Stn. 27	52	44	48	36	60

$$\% \text{ diazotroph N} = 100 \times \left(\frac{\delta^{15}N_{zpl} - \delta^{15}N_{reference\ zpl}}{\delta^{15}N_{diazotroph} - \delta^{15}N_{reference\ zpl}} \right) \quad (2)$$

At most stations, the diazotroph contribution to zooplankton biomass is smaller than the contribution to the suspended particles and decreases with increasing animal size, reflecting dilution of diazotroph N through the food web by other sources of N (Table 2). Because the reference zooplankton used in Eq. 2 may reflect some inputs of recently fixed nitrogen, the values shown in Table 2 are a conservative estimate of the role of diazotroph nitrogen in supporting zooplankton biomass production. In fact, measurements of the $\delta^{15}N$ values of individual amino acids isolated from zoo-

Table 3. Change in $\delta^{15}N$ with animal size fraction. For each station, we estimated the mean change in $\delta^{15}N$ between size fractions by linear regression. These station estimates were then grouped by cruise leg and by cruise. A one-way analysis of variance comparing the two cruises was significant ($F_1 = 12.217$; $P = 0.0011$).

Cruise leg	N	Mean change in $\delta^{15}N$ per size fraction (‰)	SE (‰)
SJ9603			
All samples	27	0.65	0.07
Leg 1	15	0.71	0.12
Leg 2	12	0.59	0.06
SJ9612			
All samples	20	0.31	0.07
Leg 1	10	0.24	0.07
Leg 2	10	0.37	0.11

plankton collected at selected stations of leg 2 of cruise SJ9603 are consistent with a higher diazotroph contribution, approaching 100% at times, to the zooplankton in the western part of the transect (McClelland et al. pers. comm.).

Although diazotrophs clearly make a significant contribution to both primary and secondary production over broad stretches of the oligotrophic North Atlantic Ocean, the mechanisms for moving diazotroph N into the planktonic food web are not at all clear. As was noted above, several stations in the vicinity of the *Hemiaulus*/*Richelia* bloom encountered during cruise SJ9612 showed unusually low $\delta^{15}N$ values in the larger size classes of zooplankton (e.g., Sta. 8 and 11). These data imply a significant direct transfer of diazotroph N to large as well as small zooplankton around the bloom, presumably through direct grazing on the large diatoms. In contrast, a variety of observations and studies have shown that very few animals graze directly on *Trichodesmium* (Hawser et al. 1991, 1992), which implies that nitrogen fixed by *Trichodesmium* primarily enters the food web through the microbial loop and recycling processes (Capone et al. 1994, 1997; Letelier and Karl 1996; Capone 2001).

Diazotrophy and food-web structure—The isotopic separation between size fractions of zooplankton at a single station reflects the overall trophic structure of the planktonic food web. Zooplankton collected during cruise SJ9603 show a relatively consistent increase in $\delta^{15}N$ of ~0.6‰–0.7‰ from one size fraction to the next (Fig. 4, Table 3). The primary exception to this pattern of increasing $\delta^{15}N$ and trophic position with animal size occurred at the eastern end of leg 1 of this cruise (Fig. 3B), where the two intermediate size fractions of zooplankton had a higher $\delta^{15}N$ than the largest size fraction. Because animals appear to concentrate ^{15}N

in their tissues primarily as a result of excretory losses of isotopically depleted NH_4^+, trophic enrichment in ^{15}N reflects the net effect of losses through excretion as nitrogen is moved up through the food web. Thus, the relatively constant scaling of $\delta^{15}N$ with size fraction during this cruise implies that the efficiency of nitrogen transfer up the food web is similar throughout much of the oligotrophic North Atlantic.

The zooplankton from SJ9612 showed a much smaller average increase in $\delta^{15}N$ with animal size (Fig. 8, Table 3), which suggests that nitrogen moves efficiently through the food web to larger zooplankton in this region. A one-way analysis of variance (ANOVA) showed significant differences between cruises in the scaling of $\delta^{15}N$ with size fraction (Table 3), a pattern that implies a fundamental difference in the structure of the food web between the two regions sampled.

A number of stations from cruise SJ9612 showed an inversion similar to the one at the eastern end of leg 1 of cruise SJ9603. Of interest, this isotopic inversion is especially pronounced in the middle portion of both legs of SJ9612, where the two largest and the smallest size fractions are nearly indistinguishable isotopically and quite depleted in ^{15}N relative to the intermediate size fractions. This area was also characterized by an extensive bloom of diatoms containing endosymbiotic diazotrophs, and the low $\delta^{15}N$ values in the large size fractions may reflect a direct or preferential transfer of N from diatoms to the larger zooplankton. This isotopic inversion is not as apparent in regions with high *Trichodesmium* abundance, reinforcing the notion that N fixed by *Trichodesmium* may follow multiple pathways into the food web, including remineralization processes and the microbial loop.

Isotopically depleted nitrate and N_2 fixation—The distribution of N* (nutrient ratio) anomalies (Gruber and Sarmiento 1997) in the North Atlantic clearly suggests that the Sargasso Sea mode water (the 18° water) includes a substantial quantity of nitrate arising from the remineralization and nitrification of organic nitrogen recently fixed by surface diazotrophs. The core of the high N* feature in the North Atlantic extends from ~10°N to ~40°N (Gruber and Sarmiento 1997) and thus includes most of the latitudinal range sampled on our two cruises.

Although relatively few data are currently available for the North Atlantic, our measurements of the $\delta^{15}N$ of deep-water NO_3^- (Table 1) are consistent with other measurements that suggest a global average $\delta^{15}N$ of ~4‰–5‰ for deep-water NO_3^- (Liu and Kaplan 1989; Sigman et al. 1997). In regions where N_2 fixation is an important term in the N budget, sinking organic matter will inject diazotroph N into the thermocline, lowering the $\delta^{15}N$ of the subsurface NO_3^- pool and providing a strong linkage between N_2 fixation in the upper water column and the $\delta^{15}N$ of NO_3^- in the upper thermocline. The relatively low $\delta^{15}N$ values we observed for upper thermocline NO_3^- (Table 1) thus provide an integrative record of the relative contributions to the bulk NO_3^- pool of sinking diazotroph organic matter (through remineralization) and deep-water nitrate (through upward transport).

The low $\delta^{15}NO_3^-$ values we found in this study (Table 1)

are still higher than the bulk of the PN and zooplankton samples from these two cruises. This reinforces our conclusion that trophic processes alone do not provide a sufficient explanation for the isotopic depletion of organic matter in these waters. The low $\delta^{15}NO_3^-$ values that characterize the upper thermocline of the oligotrophic Atlantic reduce the isotopic mismatch between upwelled nitrate and the mixed layer organic matter but do not eliminate the discrepancy entirely.

Spatial variation in $\delta^{15}N$—This is the first broad-scale survey of nitrogen isotopes in North Atlantic plankton, and our data show a significant role for diazotrophs in supplying nitrogen to support biological production throughout much of the basin. In the Pacific (Wada and Hattori 1976; Minagawa and Wada 1984; Saino and Hattori 1987; Wada and Hattori 1991) and the Gulf of Mexico (Macko et al. 1984), the $\delta^{15}N$ of mixed-layer plankton shows an inverse correlation with the abundance of *Trichodesmium* in the surface layer, a pattern that is broadly consistent with our data from the North Atlantic. Although a perfect anticorrelation between diazotroph abundance and zooplankton $\delta^{15}N$ is unlikely given the different timescales implicit in measurements of diazotroph biomass and activity (days) and zooplankton isotopic composition (weeks), our data do show low PN and zooplankton $\delta^{15}N$ values where *Trichodesmium* and/or *Hemiaulis*/*Richelia* are abundant (Figs. 3, 5, 7, and 9). Because we have no evidence that zooplankton fractionation and export processes differ systematically across the basin, this large-scale pattern implies that the importance of N_2 fixation in the elemental budget of the subtropical Atlantic is greater in the western portion of the basin. The available data on N_2 fixation rates by planktonic diazotrophs show the highest specific rates in the Caribbean and southwestern Atlantic (Capone et al. 1997), a pattern that matches well with our isotopic data.

One other interesting spatial feature is evident in our zooplankton isotope measurements. Our station at the equator (SJ9603 Sta. 10) provides a strong contrast with our other data, because the $\delta^{15}N$ of suspended particles is quite low, ranging between −2.8‰ and −0.2‰ with little evident vertical structure in the upper 100 m (Fig. 2A). This pattern is consistent with the effects of isotopic fractionation during the consumption of nitrate by phytoplankton following a mixing and/or advective injection of subsurface nutrients into the surface layer (Montoya and McCarthy 1995). In contrast, the zooplankton collected at Sta. 10 have among the highest $\delta^{15}N$ values measured on these two cruises, as do zooplankton collected just north of the equator at Sta. 9 and 11 (Fig. 3B, ~33–35°W). These data suggest a significant degree of decoupling between phytoplankton and zooplankton production in the equatorial region of the Atlantic, which perhaps reflects inefficient transfers of nitrogen from primary producers to zooplankton. Alternately, if upwelling is episodic, then temporal decoupling may occur as phytoplankton consume upwelled NO_3^-, leading to an initial decrease in the $\delta^{15}N$ of suspended particles due to isotopic fractionation during uptake followed by an increase in $\delta^{15}N$ as consumption of NO_3^- proceeds to completion (Altabet et al. 1991). This transient change in $\delta^{15}N$ at the base of the food web will be passed on to the animals as they in turn

Table 4. Comparison between stations sampled on the two cruises. The first pair (SJ9603 Sta. 32 and SJ9612 Sta. 9) were separated by 71 km, whereas the second pair (SJ9603 Sta. 33 and SJ9612 Sta. 25) were separated by 58 km.

Size fraction (μm)	SJ9603-32 vs. SJ9612-09 Sta. 32	Sta. 09	$\Delta\delta^{15}N$	Size fraction (μm)	SJ9603-33 vs. SJ9612-25 Sta. 33	Sta. 25	$\Delta\delta^{15}N$
250–500	0.88	1.32	−0.44	250–500	0.95	0.92	0.03
500–1,000	1.80	1.36	0.44	500–1,000	2.18	3.46	−1.28
1,000–2,000	2.00	3.74	−1.74	1,000–2,000	2.79	1.12	1.67
2,000–4,000	2.02	1.07	0.95	2,000–4,000	3.04	2.86	0.18

consume and incorporate PN into biomass, but the longer turnover time for zooplankton nitrogen will lead to different kinetics in the isotopic responses of PN and zooplankton.

Between-cruise comparisons—Although our two cruises followed quite different tracks, samples collected near two crossing points allow us to evaluate temporal changes in the $\delta^{15}N$ of plankton in these waters. Stations separated by 60–70 km and ~7 months showed rather similar ranges of $\delta^{15}N$ values for the suite of zooplankton sampled, with interesting differences in the distribution of ^{15}N across size fractions (Table 4). The two pairs of stations are broadly similar, with generally low $\delta^{15}N$ values in all size classes of zooplankton relative to the eastern end of the basin (e.g., SJ9603 Sta. 18) or the nearby station that lacked diazotrophs in the upper water column (SJ9612 Sta. 21). The lowest $\delta^{15}N$ values are similar on the two cruises (0.9‰–1.3‰), but the two stations sampled on SJ9612 show higher maximal $\delta^{15}N$ values (3.5‰–3.7‰). The two stations sampled on SJ9603 both showed a monotonic increase in $\delta^{15}N$ with animal size, although the two largest size fractions are quite similar isotopically at both stations. In contrast, the two stations sampled on SJ9612 show maximal $\delta^{15}N$ values in intermediate size classes.

A pairwise (between-cruise) comparison of zooplankton size fractions collected near the crossing points showed differences of ≤0.5‰ in four cases, whereas the other two size fractions differed as much as 1.7‰ (Table 4). The sign of the difference between samplings was not consistent, which suggests that these differences in $\delta^{15}N$ reflect variation about the same mean state of the ecosystem. The overall similarity in isotopic composition of zooplankton collected on cruises separated by roughly a half year confirms that the low $\delta^{15}N$ of planktonic organic matter is a robust feature of the oligotrophic North Atlantic and that it reflects the long-term mean state of the ecosystem rather than the influence of a transient event coincident with one or the other of our cruises.

The $\delta^{15}N$ of suspended particles and size-fractionated zooplankton collected from a broad span of stations from the tropical and subtropical Atlantic tends to be inversely related to diazotroph abundance in the upper water column. The $\delta^{15}N$ of suspended particles and zooplankton from much of the subtropical and tropical North Atlantic is too low to generate an export flux to balance the isotopic inputs associated with vertical transport of nitrate into the mixed layer. This provides a strong constraint on the mechanisms that may give rise to the low $\delta^{15}N$ values that characterize organic

matter in the oligotrophic North Atlantic. These plankton isotopic data as well as geochemical budgets (N* calculations) are all consistent with the hypothesis that the isotopic and elemental budgets of the upper water column of these, and presumably other, oligotrophic waters include large inputs of isotopically depleted organic matter through N_2 fixation by planktonic diazotrophs.

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