



PERGAMON

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

SCIENCE @ DIRECT®

Deep-Sea Research I 50 (2003) 849–861

DEEP-SEA RESEARCH  
PART I

[www.elsevier.com/locate/dsr](http://www.elsevier.com/locate/dsr)

# Relating low $\delta^{15}\text{N}$ values of zooplankton to $\text{N}_2$ -fixation in the tropical North Atlantic: insights provided by stable isotope ratios of amino acids

J.W. McClelland<sup>1</sup>, C.M. Holl, J.P. Montoya\*

*School of Biology, Georgia Institute of Technology, 310 Ferst Drive, Atlanta GA 30332-0230, USA*

Received 27 June 2002; received in revised form 24 March 2003; accepted 28 March 2003

## Abstract

Recent evaluations of the global nitrogen budget include greatly increased estimates of  $\text{N}_2$ -fixation in oceanic waters. Low stable N isotope ratios in planktonic food webs of tropical and subtropical oceans have been used as one indication of the importance of  $\text{N}_2$ -fixation. Interpretation of bulk stable N isotope ratios can, however, be confounded when the source and process information that they contain cannot be separated clearly. In this paper, we use stable N isotope ratios of amino acids to help separate source and trophic effects associated with changes in bulk stable N isotope ratios of zooplankton across the tropical North Atlantic. Patterns in stable N isotope ratios of amino acids along a transect from the Cape Verde Islands to Barbados identify a change in N source supporting zooplankton production, and virtually no change in the trophic position of zooplankton size classes from the eastern to the western side of the tropical North Atlantic. Furthermore, comparison of stable N isotope ratios of amino acids in zooplankton with those in *Trichodesmium* suggests that diazotrophs are the source of the low stable N isotope ratios at the western end of the transect. The evidence provided by stable N isotope ratios of amino acids supports the interpretation of large-scale patterns in bulk stable N isotope ratios that  $\text{N}_2$ -fixation indeed makes a major contribution to the global N budget.

© 2003 Elsevier Science Ltd. All rights reserved.

*Keywords:* Oceanic; Nitrogen fixation; Trophic; Zooplankton; Food web; Stable isotopes

## 1. Introduction

The role of  $\text{N}_2$ -fixation in oceanic waters has received increasing attention over the past several years. Early estimates indicated that it was only a

minor source of nitrogen in marine environments (Capone and Carpenter, 1982; Howarth et al., 1988). Within the past decade, however, additional data on the abundances and  $\text{N}_2$ -fixation rates of diazotrophs (Carpenter and Romans, 1991) and biogeochemical studies of elemental ratio anomalies (Gruber and Sarmiento, 1997; Karl et al., 1997) have produced greatly increased estimates of  $\text{N}_2$ -fixation in oligotrophic waters. These new estimates of  $\text{N}_2$ -fixation have prompted a critical reevaluation of global nitrogen budgets. While

\*Corresponding author. Tel.: +1-404-385-0479.

E-mail address: [joseph.montoya@biology.gatech.edu](mailto:joseph.montoya@biology.gatech.edu) (J.P. Montoya).

<sup>1</sup>Current address is The Ecosystems Center, MBL, Woods Hole, MA 02543, USA.

anthropogenic and natural N<sub>2</sub>-fixation on the continents have been viewed as the largest sources of nitrogen to the biosphere (Mackenzie et al., 1993), oceanic N<sub>2</sub>-fixation may in fact exceed them both (Carpenter and Romans, 1991; Galloway et al., 1996; Gruber and Sarmiento, 1997). Links between N<sub>2</sub>-fixation and oceanic capacity as a source or sink of CO<sub>2</sub> relative to the atmosphere make new estimates of N<sub>2</sub>-fixation relevant to global carbon budgets as well (Falkowski et al., 1998; Broecker and Henderson, 1998).

While N<sub>2</sub>-fixation rates in oceanic waters are almost surely higher than originally thought, estimates for whole ocean basins are still accompanied by substantial uncertainty. Extrapolating measurements of diazotroph abundances and N<sub>2</sub>-fixation rates to the basin scale is hampered by temporal and spatial variability in these parameters (Carpenter and Romans, 1991). Use of the large-scale distributions of nitrate and phosphate to estimate N<sub>2</sub>-fixation (based on  $N^*$  anomalies; Gruber and Sarmiento, 1997) eliminates the scaling problems associated with extrapolation of individual measurements of patchy distributions, but is nonetheless limited by incomplete knowledge of the factors that influence N:P ratios. Effects of atmospheric deposition, flux and lability of dissolved organic matter, and active transport of nutrients from below the euphotic zone by vertically migrating organisms are all poorly characterized (Gruber and Sarmiento, 1997). Moreover, constants used for N:P uptake and remineralization ratios and watermass end-member concentrations of nitrate and phosphate in the calculation of  $N^*$  may need modification. Hence, additional tools are needed to help constrain estimates of N<sub>2</sub>-fixation at the ocean basin scale.

Examination of stable N isotope ratios in constituents of oceanic waters may provide one such tool. Deep-water nitrate has a  $\delta^{15}\text{N}$  value of  $4.8\text{‰} \pm 0.2$  around the globe (Sigman et al., 2000), while oceanic diazotrophs typically have  $\delta^{15}\text{N}$  values from  $-2\text{‰}$  to  $-1\text{‰}$  (Wada and Hattori, 1976; Carpenter et al., 1997; Montoya et al., 2002). This clear difference in end-member values for 'new' nitrogen entering the euphotic zone provides a framework for assessing the importance

of N<sub>2</sub>-fixation: in the absence of other significant sources of 'new' N or fractionating losses, a weighted average of the  $\delta^{15}\text{N}$  values in the different N pools of the euphotic zone will reflect the relative contributions of N from diazotrophs and deep-water nitrate. Furthermore, tracking changes in the  $\delta^{15}\text{N}$  of any one N pool through time or space will identify changes in contributions to this pool by N<sub>2</sub>-fixers. Like  $N^*$ , the information that  $\delta^{15}\text{N}$  values provide is time-integrated (Montoya et al., 2002). The signal from a new input of nitrogen to the euphotic zone will be maintained through recycling until another source of nitrogen replaces it, or until the cumulative effect of fractionating losses washes it out. Atmospheric deposition may contribute substantial N to some offshore regions (Paerl et al., 1999). In other regions losses from denitrification enrich the  $^{15}\text{N}$  content of residual N pools (Liu and Kaplan, 1989). Where these processes are important alongside inputs from deep-water nitrate and N<sub>2</sub>-fixation, stable N isotope data become ambiguous. Nonetheless, regions such as the pelagic North Atlantic where denitrification and atmospheric deposition are of minor importance are well suited for the use of stable N isotope ratios to examine large-scale patterns of N<sub>2</sub>-fixation.

Low  $\delta^{15}\text{N}$  values in biota and inorganic nitrogen pools have been attributed to the influence of N<sub>2</sub>-fixation in various oceanic regions around the world (Wada and Hattori, 1976; Minagawa and Wada, 1986; Liu et al., 1996; Capone et al., 1998; Carpenter et al., 1999; Montoya et al., 2002). For example, Montoya et al. (2002) reported decreases in stable N isotope ratios of zooplankton across the tropical and subtropical Atlantic that correlated with an increase in abundance of the N<sub>2</sub>-fixing cyanobacterium *Trichodesmium* (Fig. 1), and demonstrated that these decreases could not plausibly be explained by isotopic fractionation during food web processes and export. While the results of this and other studies are consistent with the influence of N<sub>2</sub>-fixation on the pelagic nitrogen cycle, direct evidence linking nitrogen in diazotrophs to nitrogen in other components of the pelagic food web has been elusive.

The stable isotope ratios of individual compounds can provide very specific information

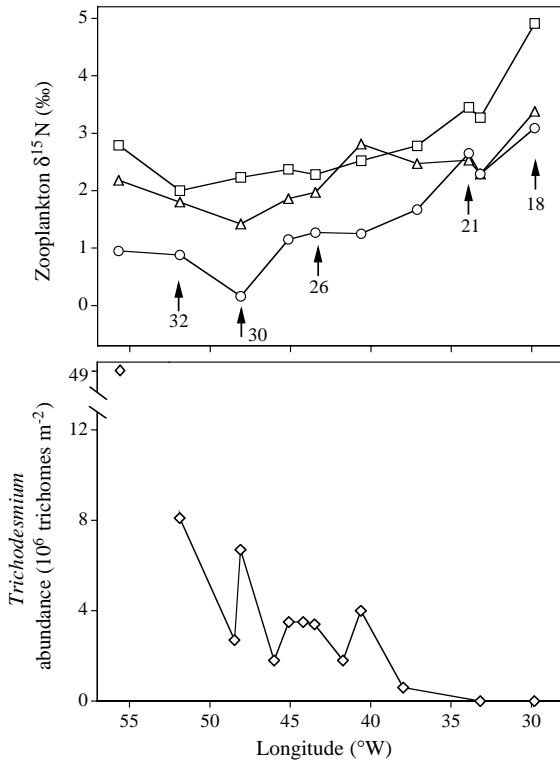


Fig. 1.  $\delta^{15}\text{N}$  values of zooplankton (top panel) and *Trichodesmium* abundance (bottom panel) versus longitude along the Cape Verde Islands to Barbados leg of cruise SJ9603. Zooplankton and *Trichodesmium* data were extracted from Montoya et al. (2002), and *Trichodesmium* data were provided by E. Carpenter. Abundances of *Trichodesmium* were depth integrated from the surface to 20 m. Symbols in the top panel represent 250–500 (circles), 500–1000 (triangles), and 1000–2000 (squares)  $\mu\text{m}$  size fractions of zooplankton. Arrows indicate stations where  $\delta^{15}\text{N}$  values of amino acids were examined.

about the diet and physiology of organisms (Hare et al., 1991; Uhle et al., 1997; McClelland and Montoya, 2002), and about the origins of complex mixtures of organic matter (Rieley et al., 1991). The large sample size needed for biochemical separations and isotopic analysis initially constrained the range of compounds that could be examined. This was particularly true for N, which is less abundant than C in organic matter. With the development of Gas Chromatography/Combustion/Isotope Ratio Mass Spectrometry (GC/C/IRMS) techniques, however, it is now possible to measure compound-specific stable isotope ratios

on very small samples (Macko et al., 1997; McClelland and Montoya, 2002).

In feeding experiments and comparisons among different size fractions of zooplankton McClelland and Montoya (2002) showed that moderate trophic increases in bulk  $\delta^{15}\text{N}$  values are underlain by large changes in some amino acids and little or no change in others. The trophic relationships of individual amino acids between food source and consumer were generally consistent, with phenylalanine and glutamic acid showing particularly robust relationships. The  $\delta^{15}\text{N}$  value of phenylalanine showed no significant change between food source and consumer, while glutamic acid consistently changed by about 7‰ (Table 1). This difference in response makes it possible to separate information about N sources at the base of the food web from information about trophic level. Stable N isotope values of phenylalanine provide information about the base of the food web directly, while the difference in  $\delta^{15}\text{N}$  between glutamic acid and phenylalanine provides a direct measure of the trophic effect (Table 1). Phytoplankton in the experiments had  $\Delta\delta^{15}\text{N}$  (glu-phe) values of about 4‰, while herbivorous zooplankton had  $\Delta\delta^{15}\text{N}$  (glu-phe) values of about 11‰.

The findings of McClelland and Montoya (2002) provide a zooplankton-specific template for interpretation of the field data we present here. Stable N isotope ratios of amino acids are used to separate source and trophic effects associated with changes in bulk stable N isotope ratios of

Table 1  
Relationships among  $\delta^{15}\text{N}$  values of individual amino acids compared between and within plankton

Comparisons	Values (‰)
<b>Trophic</b>	
Zooplankton $\delta^{15}\text{N}(\text{phe})$ –food $\delta^{15}\text{N}(\text{phe})$	0
Zooplankton $\delta^{15}\text{N}(\text{glu})$ –food $\delta^{15}\text{N}(\text{glu})$	7
Zooplankton $\Delta\delta^{15}\text{N}(\text{glu-phe})$ –food $\Delta\delta^{15}\text{N}(\text{glu-phe})$	7
<b>Internal</b>	
Phytoplankton $\Delta\delta^{15}\text{N}(\text{glu-phe})$	4
Herbivorous zooplankton $\Delta\delta^{15}\text{N}(\text{glu-phe})$	11

Note: The combined pool of glutamine and glutamic acid is represented by glu, while phenylalanine is represented by phe. Data are summarized from McClelland and Montoya (2002).

zooplankton across the tropical North Atlantic. In particular, the influence of  $N_2$ -fixation on the  $\delta^{15}N$  values of zooplankton is considered.

## 2. Methods

### 2.1. Zooplankton sampling

The zooplankton analyzed in this study were collected on a transect between the Cape Verde Islands and Barbados during R./V. *Seward Johnson* cruise SJ9603 (28 March–25 April 1996) to the tropical and subtropical Atlantic (Fig. 2). Collections were made with diagonal tows of a meter net (333  $\mu m$  mesh size) through the upper 100 m of the water column. Animals were separated into size fractions by passage through a series of Nitex sieves (2000, 1000, 500, and 250  $\mu m$ ). The specific size fractions used were a function of the sieves available at the time of the study. Samples were rinsed copiously during the separation process to break up and remove any *Trichodesmium* colonies caught in the sieves. Size fractions were frozen for

later analysis. Once ashore, samples were dried at 60°C and ground to a fine powder which was then subsampled for isotopic analysis. Bulk stable N isotope values of zooplankton were measured from 10 stations along the cruise track, while stable N isotope values of individual amino acids in zooplankton were measured from five of these stations (Fig. 2). Examination of amino acids focused on the 250–500  $\mu m$  size fractions of zooplankton, which were dominated by copepods at all stations. Zooplankton abundance and productivity were not quantified on cruise SJ9603.

### 2.2. Lab cultures

In the lab, *Trichodesmium* was grown in batch and continuous culture at 26°C under a photon flux of approximately 128  $\mu mol m^{-2} s^{-1}$  with a 12 h:12 h light:dark cycle. We used a nitrogen-free artificial seawater medium with trace metal and vitamin concentrations as described by Chen et al. (1996) and a phosphate concentration of 10  $\mu M$ . Batch cultures were grown in 2.8 l pyrex Fernbach flasks that were swirled gently by hand each day

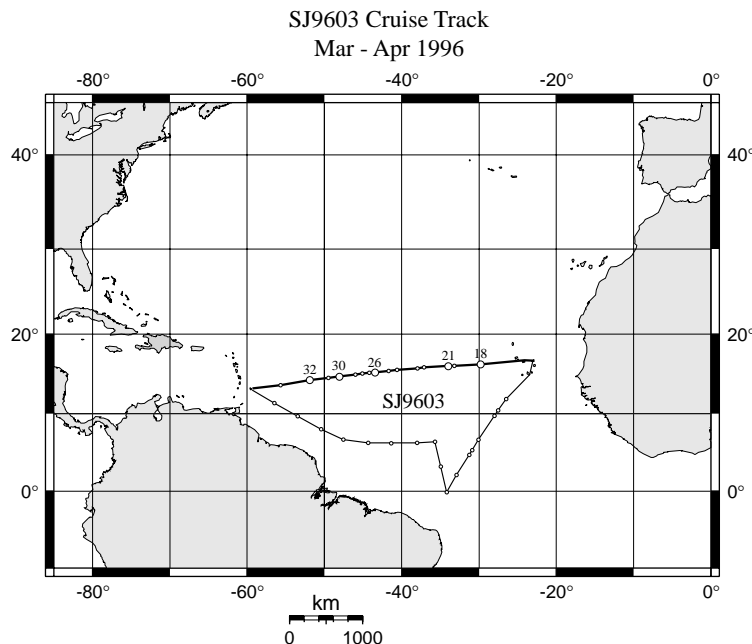


Fig. 2. Cruise track for R./V. *Seward Johnson*, 28 March–25 April 1996 (SJ9603). Data presented in this paper are from leg 2, Cape Verde Islands to Barbados.

and sampled when biomass concentrations reached approximately  $40 \text{ mg l}^{-1}$  dry weight (dw). The continuous culture was maintained at a volume of 1.5 l and a dilution rate of  $0.491 \text{ d}^{-1}$ , maintaining a biomass concentration of approximately  $30 \text{ mg l}^{-1}$  dw. Under these conditions, growth of *Trichodesmium* appeared to be limited by phosphate availability. Gentle mixing was provided by a magnetic stirrer and a stream of filtered air which also maintained a small positive pressure inside the culture vessel to minimize contamination and facilitate the overflow of the culture through a riser tube. The continuous culture was sampled at steady-state, as reflected by 5 days of constant in vivo chlorophyll fluorescence in the culture overflow. For both culture types, samples were collected by passing 1 l of culture through a precombusted ( $450^\circ\text{C}$  for 2 h) 47 mm GF/F filter under gentle vacuum. The filters were dried at  $60^\circ\text{C}$  and stored over desiccant until prepared for isotopic analysis.

### 2.3. Sample preparation

Samples were prepared for GC/C/IRMS analysis of amino acids by hydrolysis followed by purification and derivatization to produce *N*-pivaloyl-*i*-propyl (NPP)-amino acid esters (Metges et al., 1996). In brief, 5 mg of dried sample was placed in a  $16 \times 100$  mm glass tube with a PTFE-lined cap and hydrolyzed with ultra pure 6 M hydrochloric acid for 24 h at  $110^\circ\text{C}$ . The hydrolysate was evaporated to dryness at  $55^\circ\text{C}$  under a stream of  $\text{N}_2$  and the residue was re-dissolved in 2 ml of 0.01 N HCl along with  $400 \mu\text{l}$  of 2.5 mM  $\alpha$ -aminoadipic acid (internal standard) in 0.10 N HCl. This solution was then purified by filtration ( $0.65 \mu\text{m}$  Durapore<sup>®</sup> filter) followed by cation exchange chromatography (Dowex<sup>®</sup> 50WX8-400 ion exchange resin) in a 5 cm column prepared in a Pasteur pipette. Amino acids were eluted with 4 ml of 2 M ammonium hydroxide. The amino acid solution was evaporated to dryness under a stream of  $\text{N}_2$  at  $80^\circ\text{C}$ . Finally, the purified amino acids were derivatized to NPP-amino acid esters (Metges et al., 1996). No blank effects are introduced during the purification and derivatization procedure.

Derivatization of amino acids to NPP esters is a multi-step process. Details of this process are given in Metges et al. (1996). Nonetheless, the following description provides some general information about the chemistry involved. Amino acids are first reacted with a thionylchloride solution in *i*-propanol, which esterifies the carboxy terminus of the amino acids. The esterified product is dried under a gentle stream of  $\text{N}_2$  and then dissolved in pyridine and reacted with pivaloylchloride. This reaction acylates the amino terminus of the amino acids. When the reaction is complete, methylene chloride is added and the mixture is passed over a silica gel column (5 cm in a Pasteur pipette) to remove excess acylating reagents and other impurities. The filtrate is dried under a gentle stream of  $\text{N}_2$  and re-dissolved in ethyl acetate for introduction to the gas chromatograph.

This preparation scheme allows analysis of stable N isotope ratios of 18 common amino acids plus  $\alpha$ -aminoadipic acid (internal standard). Glutamine and glutamic acid are analyzed as a single combined peak, as is the case for asparagine and aspartic acid. Tryptophan, cystine/cysteine and arginine are not compatible with this method. Sample preparation is not necessarily quantitative (though any losses must be non-fractionating) and thus the method is not used to determine absolute amount of different amino acids. Final preparations are dissolved in ethyl acetate, and contained 1–2 nanomoles of each amino acid derivative per  $0.5 \mu\text{l}$  of solution (target injection volume). Under the GC/C/IRMS conditions described below this quantity of N produces peaks that are optimal for stable N isotope determinations of the individual amino acids.

### 2.4. Sample analysis

The stable isotopic composition of nitrogen in NPP derivatives of amino acids were analyzed by GC/C/IRMS using a Micromass Isoprime mass spectrometer interfaced to a Hewlett-Packard 6890 gas chromatograph through a combustion furnace ( $850^\circ\text{C}$ ), reduction furnace ( $500^\circ\text{C}$ ), and liquid nitrogen cold trap. Analyses of all samples were repeated 3–5 times. Mixes of amino acid derivatives from samples and standard mixtures were

injected into the GC, separated on an HP-Ultra 2 column (50 m × 0.32 mm i.d., 0.5 μm film thickness), combusted, reduced, and finally passed through the cold trap to remove water and CO<sub>2</sub> before introduction to the mass spectrometer. Nitrogen isotope ratios for each amino acid in a mix were measured sequentially. Each sample run was preceded by two pulses of reference N<sub>2</sub> and followed by 3 pulses of reference N<sub>2</sub>, whose isotopic composition was calibrated against a variety of organic standards (peptone, histidine, acetanilide) by continuous flow isotope ratio mass spectrometry (CFIRMS) using a Carlo Erba NC 2500 elemental analyzer interfaced to a Micromass Optima mass spectrometer. This system was also used to analyze the stable isotopic composition of N in bulk samples of plankton.

Gas chromatography conditions were set to optimize peak separation and shape. Injections were done splitless at 280°C, and contained 0.3–0.6 μl of sample. The GC temperature program for each run was as follows: initial temperature 120°C for 10 min; ramp up at 3°C min<sup>-1</sup> to 200°C, dwell

for 5 min; ramp up at 1°C min<sup>-1</sup> to 215°C, dwell for 1 min; ramp up at 7°C min<sup>-1</sup> to 300°C, dwell for 8 min. Carrier gas (helium) flow through the GC column was 1 ml min<sup>-1</sup> for the first 38.3 min of each run, then increased to 1.3 ml min<sup>-1</sup> for the remainder. The flame ionization detector was held at 300°C.

All isotope abundances are expressed as δ<sup>15</sup>N values relative to atmospheric N<sub>2</sub>: δ<sup>15</sup>N = [(R<sub>sample</sub>/R<sub>standard</sub>) - 1] × 10<sup>3</sup> where R is the isotope ratio <sup>15</sup>N:<sup>14</sup>N. GC/C/IRMS analyses of standard mixtures of amino acids showed that values for aspartic acid, glutamic acid, proline, tyrosine, and valine were within 0.5‰ of the expected value; leucine, lysine, methionine, and phenylalanine were within 1.0‰ of the expected value; and alanine, glycine, isoleucine, and threonine were within 1.5‰ of the expected value. The aggregate difference between measured and expected values for the amino acids listed above was 0.1 ± 0.8‰ (SD), confirming that no consistent bias was introduced by the derivatization and analysis procedure. The only apparent exception

Table 2

δ<sup>15</sup>N of bulk samples and amino acids from 250 to 500 μm zooplankton collected on cruise SJ9603 and from cultures of *Trichodesmium* grown in nitrogen-free medium

	250–500 μm zooplankton					Tricho. cultures
	Station 32	Station 30	Station 26	Station 21	Station 18	
Bulk sample	0.9	0.2	1.3	2.6	3.1	-1.7 ± 0.4
<i>Amino acids</i>						
Alanine	6.6 ± 0.4	5.3 ± 0.3	8.0 ± 0.1	9.8 ± 0.6	8.5 ± 0.1	-3.2 ± 0.6
Aspartic acid	2.9 ± 0.3	0.8 ± 0.0	5.5 ± 0.2	6.6 ± 0.4	7.3 ± 0.2	2.9 ± 0.6
Glutamic acid	6.3 ± 0.4	5.5 ± 0.2	8.5 ± 0.1	9.7 ± 0.1	10.3 ± 0.1	-1.4 ± 0.6
Glycine	-0.9 ± 0.2	-0.7 ± 0.3	0.2 ± 0.1	1.1 ± 0.4	2.8 ± 0.1	-2.1 ± 0.1
Isoleucine	2.7 ± 0.3	2.8 ± 0.5	6.0 ± 0.2	5.6 ± 0.6	6.8 ± 0.4	-0.7 ± 0.3
Leucine	2.8 ± 0.4	3.2 ± 0.2	4.9 ± 0.2	5.9 ± 0.8	5.5 ± 0.2	-0.3 ± 0.5
Lysine	-3.3 ± 0.4	-4.1 ± 0.3	-1.5 ± 0.2	-1.6 ± 0.3	-0.4 ± 0.2	-2.8 ± 1.3
Methionine	-2.0 ± 0.2	-2.6 ± 0.0	-1.6 ± 0.5	-0.7 ± 1.2	0.6 ± 0.5	-4.4 ± 0.4
Phenylalanine	-4.8 ± 0.1	-5.6 ± 0.5	-3.2 ± 0.3	-1.4 ± 0.3	-2.2 ± 0.5	-3.6 ± 0.4
Proline	1.9 ± 0.2	1.3 ± 0.5	4.5 ± 0.1	7.3 ± 0.6	5.7 ± 0.3	-1.6 ± 0.3
Serine	-5.5 ± 0.1	-4.3 ± 0.7	-2.9 ± 0.5	-3.5 ± 0.5	-2.7 ± 0.1	-8.9 ± 1.2
Threonine	-7.6 ± 0.2	-6.5 ± 0.8	-7.6 ± 0.5	-8.6 ± 0.3	-6.8 ± 0.3	-0.6 ± 0.9
Tyrosine	-3.1 ± 0.9	-4.0 ± 0.3	-1.5 ± 0.6	-1.0 ± 0.8	-1.2 ± 0.9	-3.5 ± 0.2
Valine	2.1 ± 0.0	6.3 ± 0.3	4.4 ± 0.3	5.9 ± 0.8	5.1 ± 0.5	-0.5 ± 0.6

Note: Bulk values are from standard CFIRMS analysis (standard deviation typically ±0.2‰) and amino acid values are from GC/C/IRMS analysis. Values for amino acids in zooplankton are means ±1 SE from 3 to 5 analyses of each sample. Values for *Trichodesmium* are means ±1 SE for three cultures (two batch cultures and one continuous culture), each analyzed three times. Aspartic acid includes asparagine. Glutamic acid includes glutamine.

was with serine, which was clearly lower than expected (average  $-2.7\%$ ). Analytical error associated with isotope measurements of bulk material was typically  $\pm 0.2\%$  (SD).

### 3. Results

Stable N isotope ratios of amino acids in zooplankton spanned a wide range at each station (Table 2). In all cases, the magnitude of the range was similar (average range  $\pm 1$  SD;  $15.7 \pm 2.2\%$ ) and encompassed the bulk  $\delta^{15}\text{N}$  values. Threonine had the lowest  $\delta^{15}\text{N}$  value at each station (Table 2). The amino acid with the highest  $\delta^{15}\text{N}$  value varied between stations, though glutamic acid had one of the two highest  $\delta^{15}\text{N}$  values at all stations (Table 2).

Batch and continuous cultures of *Trichodesmium* had very similar stable N isotope ratios, and thus data from the two types of cultures were combined for presentation (Table 2). The range of  $\delta^{15}\text{N}$  values for amino acids in *Trichodesmium* was smaller (11.8%) than that of the zooplankton, but similarly encompassed its associated bulk  $\delta^{15}\text{N}$  value. Serine and aspartic acid had the lowest and highest  $\delta^{15}\text{N}$  values in *Trichodesmium*, respectively. The  $\delta^{15}\text{N}$  values of threonine and glutamic acid, on the other hand, were firmly in the middle of the range for *Trichodesmium*.

For the most part, the  $\delta^{15}\text{N}$  values of individual amino acids show decreases similar to those of bulk zooplankton from eastern to western stations along the Cape Verde Islands to Barbados transect (Fig. 3). This is demonstrated by growing differences in  $\delta^{15}\text{N}$  values ( $\Delta\delta^{15}\text{N}$ ) of bulk material as well as individual amino acids between station 18 and stations farther west (Fig. 3): values for station 21 straddle the zero line representing station 18, station 26 has values that are the same or lower than the zero line, and stations 30 and 32 have values that are lower yet. Threonine provides an exception to the general pattern, as it remains nearly constant across stations (Fig. 3). Also, aspartic acid and valine at station 30 appear anomalous, given that all other amino acids show shifts that are much closer to those of the bulk material (Fig. 3).

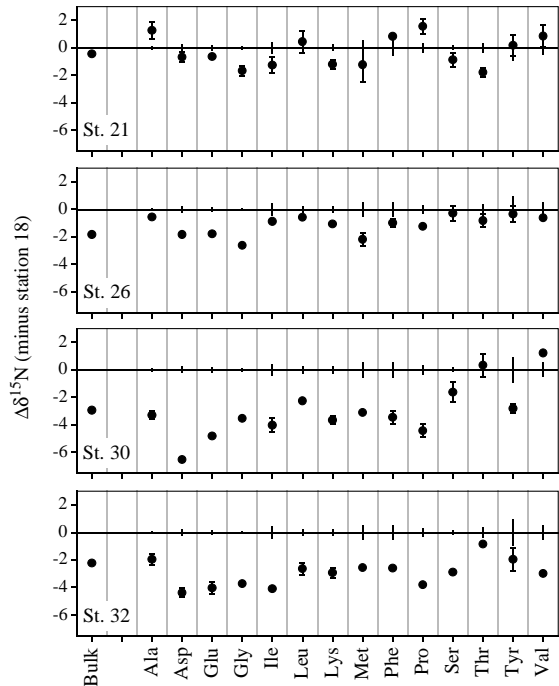


Fig. 3. Difference ( $\Delta\delta^{15}\text{N}$ ) between stable isotope values of zooplankton at the eastern side of the Cape Verde Islands to Barbados transect (station 18) and stable isotope values of zooplankton at increasingly westward stations (top to bottom panels) along the transect. The zero line in each panel represents station 18.  $\Delta\delta^{15}\text{N}$  of bulk values are on the left side of each panel. Values for individual amino acids are listed in alphabetical order. All amino acid values are averages from 3 to 5 analyses. Standard errors for station 18 are represented on the zero line, while standard errors for the other stations are on the symbols.

At stations 30 and 32, where  $\delta^{15}\text{N}$  values of zooplankton were lowest, bulk samples from the 250–500  $\mu\text{m}$  size fraction were, on average, about 2‰ enriched in  $^{15}\text{N}$  relative to bulk *Trichodesmium* (Fig. 4). Average values for glycine, isoleucine, leucine, methionine, and proline showed  $^{15}\text{N}$  enrichments that were similar to the enrichment observed in bulk material (increases within  $\pm 1.5\%$  of bulk). Average values for alanine, aspartic acid, glutamic acid, serine, and valine showed  $^{15}\text{N}$  enrichments that were substantially larger than the bulk material. Within this group,  $^{15}\text{N}$  enrichments in alanine and glutamic acid were most pronounced. There were also a number of amino acids that had  $\delta^{15}\text{N}$  values that were the same as,

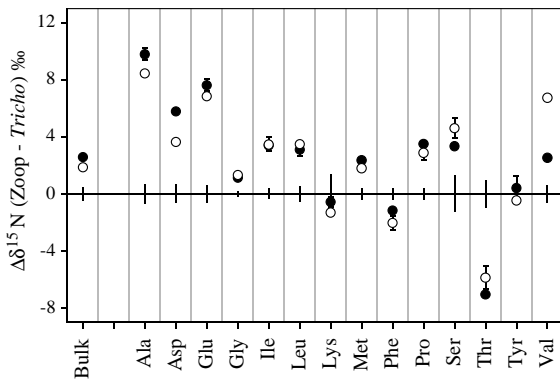


Fig. 4. Difference ( $\Delta\delta^{15}\text{N}$ ) between stable isotope values of *Trichodesmium* from lab cultures and 250–500  $\mu\text{m}$  zooplankton from stations 30 (open symbol) and 32 (closed symbol) of cruise SJ9603. The zero line represents *Trichodesmium*.  $\Delta\delta^{15}\text{N}$  of bulk values are on the left side of the panel. Values for individual amino acids are listed in alphabetical order. All amino acid values are averages from 3 to 5 analyses. Standard errors for *Trichodesmium* are represented on the zero line, while standard errors for zooplankton are on the symbols.

or lower than those of *Trichodesmium*. Within this group, average values for lysine, phenylalanine, and tyrosine differed by less than 2‰ from those of *Trichodesmium*, while average values for threonine were substantially lower.

#### 4. Discussion

Nitrate, particulate organic matter, and zooplankton  $\delta^{15}\text{N}$  values that are low relative to deep-water nitrate have been attributed to the influence of  $\text{N}_2$ -fixation in the oceanic waters of tropical and subtropical regions around the world (Wada and Hattori, 1976; Liu et al., 1996; Capone et al., 1998; Carpenter et al., 1999; Montoya et al., 2002). Yet, establishing a definitive link to  $\text{N}_2$ -fixation has been elusive. Atmospheric deposition contains inorganic nitrogen with low  $\delta^{15}\text{N}$  values (Hoering, 1957) and has been identified as a potentially important source of nitrogen in some offshore waters (Paerl et al., 1999). It has also been suggested that low  $\delta^{15}\text{N}$  values in the euphotic zone arise from the removal of isotopically heavy nitrogen during transformation of suspended particulate nitrogen into sinking particulate nitro-

gen (Altabet, 1988) with concomitant excretion of isotopically light ammonia by zooplankton (Checkley Jr. and Miller, 1989). Finally, isotope effects associated with phytoplankton uptake of nutrients (Wada and Hattori, 1978; Montoya and McCarthy, 1995) and N transfer through food webs (Minagawa and Wada, 1984) may confound interpretation of  $\delta^{15}\text{N}$  in particulate organic matter and zooplankton assemblages.

Examination of stable N isotope ratios of amino acids in zooplankton has allowed us to separate the isotopic effects of trophic transfers from variations in the isotopic composition of the nitrogen sources supporting biological production across a broad span of the tropical North Atlantic. Decreases in  $\delta^{15}\text{N}$  values of individual amino acids in 250–500  $\mu\text{m}$  zooplankton across the transect (Fig. 3) corroborate the pattern observed in bulk materials (Fig. 1). However, it is the decrease in phenylalanine (Fig. 5) in particular that unambiguously links decreases in bulk values across the basin to a change in the isotopic composition of the N source at the base of the food web. At the same time, the relatively constant  $\Delta\delta^{15}\text{N}$  (glu-phe) values across the transect (Fig. 5) indicate that the average trophic position of the 250–500  $\mu\text{m}$  zooplankton has changed very little. The variation in  $\Delta\delta^{15}\text{N}$  (glu-phe) across the ocean basin allows for a maximum difference in trophic position among stations of only 0.2 relative to a full trophic step represented by an increase in  $\Delta\delta^{15}\text{N}$  (glu-phe) of 7‰. Moreover, the  $\Delta\delta^{15}\text{N}$  (glu-phe) values between 11.1‰ and 12.5‰ indicate that the biomass in the 250–500  $\mu\text{m}$  size fraction along the transect was dominated by herbivorous zooplankton at all stations (Fig. 5).

Given that the mesh size of the meter net used to collect zooplankton was 333  $\mu\text{m}$  and the smallest sieve used to sort them was 250  $\mu\text{m}$ , zooplankton between 333 and 250  $\mu\text{m}$  were no doubt under-represented in our analyses. This could have resulted in isotope values for the 250–500  $\mu\text{m}$  size fraction that were somewhat enriched in  $^{15}\text{N}$  relative to actual values for 250–500  $\mu\text{m}$  zooplankton. However, the  $\Delta\delta^{15}\text{N}$  (glu-phe) values reflecting the dominance of herbivores at all stations (Fig. 5) suggest that any such enrichment effect must have been very small.

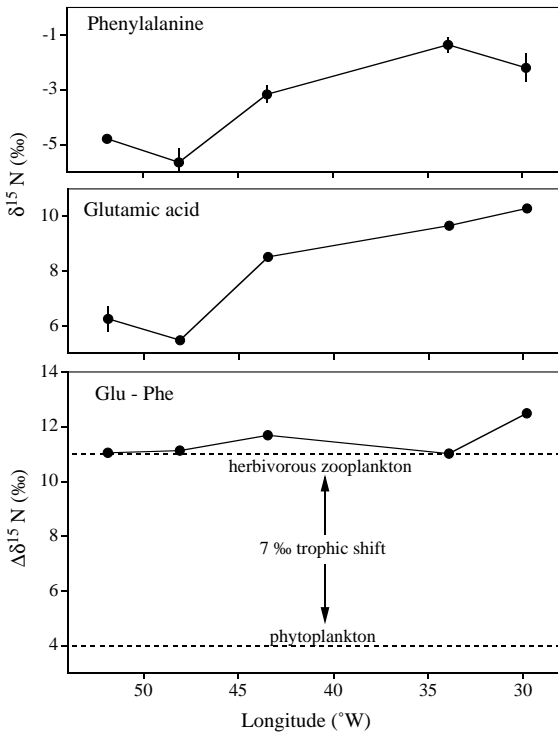


Fig. 5.  $\delta^{15}\text{N}$  values of phenylalanine (top panel) and glutamic acid (middle panel) and  $\Delta\delta^{15}\text{N}$  of glutamic acid minus phenylalanine (bottom panel) as a function of longitude along the Cape Verde Islands to Barbados leg of cruise SJ9603. Values in the top and middle panel are means  $\pm 1$  standard error from 3 to 5 replicate analyses. Values in the bottom panel are the difference between means in the other two panels. The dashed line marks the  $\Delta\delta^{15}\text{N}$  (glu-phe) values identified by McClelland and Montoya (2002) as representative of phytoplankton and herbivorous zooplankton.

Stable isotope analyses of zooplankton in this study focused on discrete size fractions because the available zooplankton samples from cruise SJ9603 were collected and preserved in this way. These samples made it possible to examine N sources to the zooplankton community as a whole. Examination of individual species of zooplankton in future studies would be useful for understanding the underlying variability contributing to overall zooplankton community function.

While examination of amino acid  $\delta^{15}\text{N}$  values in zooplankton alone allows us to identify a change in N source, comparisons with *Trichodesmium* grown in nitrogen-free medium allows us to relate zooplankton with low  $\delta^{15}\text{N}$  values to a pure  $\text{N}_2$ -

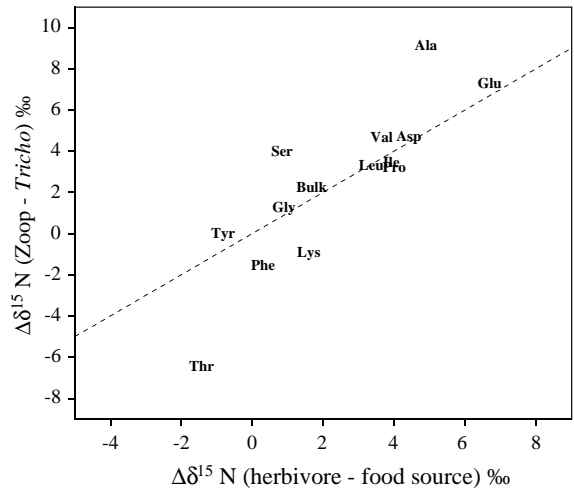


Fig. 6. Comparison of  $\Delta\delta^{15}\text{N}$  (zooplankton—*Trichodesmium*) from Fig. 4 of this paper to  $\Delta\delta^{15}\text{N}$  (planktonic herbivores—food source) as reported in McClelland and Montoya (2002) for laboratory feeding experiments.  $\Delta\delta^{15}\text{N}$  (zooplankton—*Trichodesmium*) values are averages of values at stations 30 and 32. The dashed line indicates a 1:1 relationship.

fixing end-member. The pattern of  $\Delta\delta^{15}\text{N}$  between zooplankton and *Trichodesmium* shown in Fig. 4 is remarkably similar to the pattern identified by McClelland and Montoya (2002) in lab cultures of planktonic herbivores grown on a known food source (Fig. 6). This similarity suggests a strong coupling between diazotrophs and 250–500  $\mu\text{m}$  zooplankton at western stations along the transect.

Although *Trichodesmium* and zooplankton samples collected contemporaneously would also have been very useful for isotopic comparisons, we did not have sufficient material to carry out analyses of individual amino acids on extant *Trichodesmium* samples from the field. Nonetheless, the strong similarity between bulk  $\delta^{15}\text{N}$  values measured in our cultures (Table 2) and in *Trichodesmium* from various field studies spanning the Atlantic and Pacific Oceans (Wada and Hattori, 1976; Carpenter et al., 1999; Montoya et al., 2002) all support our use of lab cultures as an appropriate isotopic end-member for diazotrophy. Values of *Trichodesmium*  $\delta^{15}\text{N}$  from these studies ranged between  $-2.2\text{‰}$  and  $+0.5\text{‰}$ , with values strongly biased toward the negative end of the range. The diatom *Hemiaulus hauckii* containing

the N<sub>2</sub>-fixing symbiont *Richelia intracellularis* had a similarly low  $\delta^{15}\text{N}$  value (Carpenter et al., 1999). The similarity between our culture values and those from field samples suggests that N<sub>2</sub>-fixers in oligotrophic oceans are relying primarily on their diazotrophic abilities to acquire nitrogen for growth.

Cultures of the freshwater diazotroph *Anabaena* grown in N-free medium (Macko et al., 1987) had bulk  $\delta^{15}\text{N}$  values about 1‰ lower than those we measured in cultured *Trichodesmium*. This difference is also evident in the isotope values of individual amino acids. Macko et al. (1987) report amino acid isotope values for *Anabaena* normalized to glutamic acid. Comparison of their values with our glutamic acid-normalized values for *Trichodesmium* revealed that aspartic acid, glycine, lysine, serine, threonine, tyrosine, and valine were all about 2‰ lower, alanine about 1‰ lower, and phenylalanine about 1‰ higher in *Anabaena* than in *Trichodesmium*. Isoleucine and leucine differed by much more, both having glutamic acid-normalized values about 9‰ lower in *Anabaena* than in *Trichodesmium*. Bulk  $\delta^{15}\text{N}$  values for *Anabaena* were also lower than those of any field-collected *Trichodesmium* (Wada and Hattori, 1976; Carpenter et al., 1999; Montoya et al., 2002). The isotopic differences between *Anabaena* and *Trichodesmium* may well reflect fundamental differences in biochemistry related to their occurrence in freshwater versus marine environments, but more study will be needed before a definitive explanation can be offered.

We cannot completely rule out the possibility that inputs of inorganic nitrogen with low  $\delta^{15}\text{N}$  values from atmospheric deposition are the source of low  $\delta^{15}\text{N}$  values of zooplankton at the western end of the transect. However, atmospheric deposition appears to be a relatively minor input to the North Atlantic Ocean beyond the shelf break (Galloway et al., 1996; Prospero et al., 1996), and the most important inputs appear to be south of our study region (Prospero et al., 1996). In addition, this explanation is more contrived and less likely to account for the correlation shown in Fig. 6 than is a close coupling to N<sub>2</sub>-fixation. Assuming no net fractionation effect during phytoplankton uptake, the average stable N

isotope value of atmospheric deposition would have to match that of *Trichodesmium* grown in nitrogen-free medium almost perfectly. The odds of such a coincidence are relatively small given the wide range of observed isotope values for atmospheric deposition: Average values for the  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in rain water at various locations around the world range from about -12‰ to +5‰ (Russell et al., 1998). Measurements of stable N isotope ratios in rain falling on oceanic waters of the tropical North Atlantic would help to further resolve this issue.

Changes in nutrient concentrations can also confound interpretation of stable N isotope data. In particular, fractionation during uptake when nitrate concentrations are high leaves phytoplankton with lower  $\delta^{15}\text{N}$  values in comparison to values for phytoplankton growing under N-limiting conditions (Montoya and McCarthy, 1995). Changes in the availability of other nutrients may also influence N stable isotope ratios of phytoplankton indirectly through physiological changes within cells. However, such effects are more difficult to identify and have yet to be convincingly demonstrated. In any case, fractionation effects from changes in nitrate concentrations are unlikely to be an issue in the present study because nitrate concentrations above the pycnocline were below the limit of detection across the entire E–W transect of cruise SJ9603. Similarly, surface phosphate concentrations were at the limit of detection across our transect and thus do not provide a reasonable correlate with changes in  $\delta^{15}\text{N}$  values. Details of nutrient profiles collected on cruise SJ9603 will be published elsewhere (D.G. Capone, pers. comm.).

While our amino acid data point to a strong link between low  $\delta^{15}\text{N}$  values and an N<sub>2</sub>-fixation source, the particular diazotrophs involved and the pathways of N transfer from diazotrophs to zooplankton cannot be resolved directly with these data. A variety of other marine N<sub>2</sub>-fixers may be important in oligotrophic oceans (Zehr et al., 1998, 2001), and it is possible that the  $\delta^{15}\text{N}$  values of amino acids in these other diazotrophs are similar to those of *Trichodesmium*. It is also possible that phytoplankton using released or regenerated nitrogen derived from a diazotrophic

source have an amino acid  $\delta^{15}\text{N}$  footprint similar to the  $\text{N}_2$ -fixers themselves. However, changes during recycling would be expected to alter the signal to some degree. The remarkable similarity between  $\Delta\delta^{15}\text{N}$  (Zoop-Tricho) and  $\Delta\delta^{15}\text{N}$  (herbivore-food source) shown in Fig. 6 suggests direct consumption of diazotrophs by zooplankton, or at least a tight coupling that results in little fractionation if the pathway of N transfer is indirect. This finding contrasts with the idea that N from *Trichodesmium* reaches zooplankton primarily through the microbial loop (Hawser et al., 1992; Capone et al., 1997).

Hawser et al. (1992) is widely cited as evidence that *Trichodesmium* is toxic to zooplankton grazers. In reality, however, the issue of toxicity in *Trichodesmium* is somewhat more complex. The experiments of Hawser et al. (1992) showed that *T. thiebautii* was toxic, but that *T. erythraeum* was not. Furthermore, *T. thiebautii* was toxic only about 50% of the time. Another study by O'Neil (1998) demonstrated that some zooplankton are actually specialists on *Trichodesmium*, both using the colonies as physical substrate for juvenile development and as a food source. Although our amino acid data do not resolve this issue, they certainly support the possibility that direct grazing on *Trichodesmium* is an important pathway of N transfer to zooplankton.

Regardless of how the low  $\delta^{15}\text{N}$  signal makes it into the 250–500  $\mu\text{m}$  size fraction of zooplankton, it is clear that this signal gets passed on through the rest of the food web. This conclusion is supported by decreases in the  $\delta^{15}\text{N}$  values of larger size fractions which parallel those of the 250–500  $\mu\text{m}$  size fraction across the ocean basin (Fig. 1). The general shift toward higher values from smaller to larger size fractions reflects an increasing proportion of consumers from higher trophic levels. Amino acid data from station 30 demonstrate the trophic effect in further detail: shifts in bulk values are underlain by large changes in some amino acids and little or no change in others (Fig. 7). Biochemical mechanisms that may be driving these changes are discussed in McClelland and Montoya (2002). With regard to the link between diazotrophs and zooplankton, however, it is important to note that the  $\delta^{15}\text{N}$  of

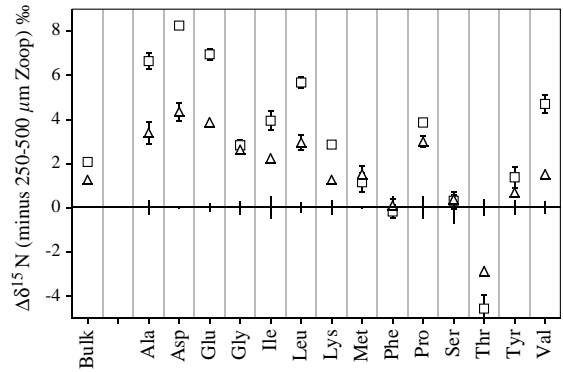


Fig. 7. Differences ( $\Delta\delta^{15}\text{N}$ ) in stable N isotope values among size fractions of zooplankton at station 30. The absolute values ( $\delta^{15}\text{N}$ ) of zooplankton in the 500–1000 and 1000–2000  $\mu\text{m}$  size fractions are reported in McClelland and Montoya (2002). The zero line represents the 250–500  $\mu\text{m}$  size fraction. Triangles represent the difference between the 500–1000 and the 250–500  $\mu\text{m}$  size fractions. Squares represent the difference between the 1000–2000 and 250–500  $\mu\text{m}$  size fractions.  $\Delta\delta^{15}\text{N}$  of bulk values are on the left side of the panel. Values for individual amino acids are listed in alphabetical order. All amino acid values are averages from 3 to 5 analyses. Standard errors for 250–500  $\mu\text{m}$  size fraction are represented on the zero line, while standard errors for the 500–1000 and 1000–2000  $\mu\text{m}$  size fractions are represented on the triangles and squares, respectively.

phenylalanine remains the same in all size fractions (Fig. 7) indicating trophic continuity with the base of the food web. At the same time, the  $\delta^{15}\text{N}$  values of glutamic acid become progressively higher (Fig. 7) reflecting a trophic shift with size fraction.

The patterns in  $\delta^{15}\text{N}$  values of bulk samples and individual amino acids across the tropical North Atlantic identify a change in N source supporting zooplankton production from the eastern to the western side of the basin. Comparison of  $\delta^{15}\text{N}$  values of zooplankton with those of *Trichodesmium* suggests that this change is driven by increasing contributions of nitrogen from diazotrophs to the planktonic food web. The length of time that a stable isotopic imprint of  $\text{N}_2$ -fixation is maintained within zooplankton depends on the rate that the stable isotopic composition of nitrogen recycled in the euphotic zone changes as a result of contributions from deep-water nitrate, flux of particles to the ocean interior, and denitrification. Nonetheless, it is safe to say that episodic injections of ‘new’ nitrogen to the

euphotic zone are recorded in a time-integrated manner in the tissues of zooplankton. This time-integrative quality provides an excellent bridge for extrapolating measurements of highly variable diazotroph abundances and fixation rates to the ocean basin scale. The tight coupling of zooplankton to  $N_2$ -fixation suggested by stable isotope data from the tropical North Atlantic supports a growing body of evidence that  $N_2$ -fixation makes a major contribution to oceanic N budgets.

### Acknowledgements

We thank C.L. Johnson for her work at sea and in the lab, C.F. Reynolds for assistance with amino acid derivatizations, C.D. Payne and K.M. Rathburn for carrying out the isotopic analyses of bulk zooplankton and E.J. Carpenter for providing the *Trichodesmium* abundance data used in Fig. 2. We also appreciate the assistance in operations at sea provided by the officers and crew of the R./V. *Seward Johnson*. The comments of M.L.E. Fogel and F. Lipschultz were a valuable help to us in preparing the manuscript. This project was supported by grants NSF-OCE9633510 and NSF-OCE9819086.

### References

- Altabet, M.A., 1988. Variations in nitrogen isotopic composition between sinking and suspended particles: implications for nitrogen cycling and particulate transformations in the open ocean. *Deep-Sea Research I* 35, 535–554.
- Broecker, W.S., Henderson, G.M., 1998. The sequence of events surrounding Termination II and their implications for the cause of glacial–interglacial  $CO_2$  changes. *Paleoceanography* 13, 352–364.
- Capone, D.G., Carpenter, E.J., 1982. Nitrogen fixation in the marine environment. *Science* 217, 1140–1142.
- Capone, D.G., Zehr, J.P., Paerl, H.W., Bergman, B., Carpenter, E.J., 1997. *Trichodesmium*, a globally significant marine cyanobacterium. *Science* 276, 1221–1229.
- Capone, D.G., Subramaniam, A., Montoya, J., Voss, M., Humborg, C., Johansen, A., Siefert, R., Carpenter, E.J., 1998. An extensive bloom of the  $N_2$ -fixing cyanobacterium, *Trichodesmium erythraeum*, in the Central Arabian Sea. *Marine Ecology Progress Series* 172, 281–292.
- Carpenter, E.J., Romans, K., 1991. Major role of the cyanobacterium *Trichodesmium* in nutrient cycling in the North Atlantic Ocean. *Science* 254, 1356–1358.
- Carpenter, E.J., Harvey, H.R., Fry, B., Capone, D.G., 1997. Biogeochemical tracers of the marine cyanobacterium *Trichodesmium*. *Deep-Sea Research I* 44, 27–38.
- Carpenter, E.J., Montoya, J.P., Burns, J., Mulholland, M.R., Subramaniam, A., Capone, D.G., 1999. Extensive bloom of a  $N_2$ -fixing diatom/cyanobacterial association in the tropical Atlantic Ocean. *Marine Ecology Progress Series* 185, 273–283.
- Checkley Jr., D.M., Miller, C.A., 1989. Nitrogen isotope fractionation by oceanic zooplankton. *Deep-Sea Research I* 36, 1449–1456.
- Chen, Yi.-Bu., Zehr, J.P., Mellon, M., 1996. Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101 in defined media: evidence for a circadian rhythm. *Journal of Phycology* 32, 916–923.
- Falkowski, P.G., Barber, R.T., Smetacek, V., 1998. Biogeochemical controls and feedbacks on ocean primary production. *Science* 281, 200–296.
- Galloway, J.N., Howarth, R.W., Michaels, A.F., Nixon, S.W., Prospero, J.M., Dentener, F.J., 1996. Nitrogen and phosphorus budgets of the North Atlantic Ocean and its watershed. *Biogeochemistry* 35, 3–25.
- Gruber, N., Sarmiento, J.L., 1997. Global patterns of marine nitrogen fixation and denitrification. *Global Biogeochemical Cycles* 11, 235–266.
- Hare, P.E., Fogel, M.L., Stafford Jr., T.W., Mitchell, A.D., Hoering, T.C., 1991. The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. *Journal of Archeological Science* 18, 277–292.
- Hawser, S.P., O'Neil, J.M., Roman, M.R., Codd, G.A., 1992. Toxicity of blooms of the cyanobacterium *Trichodesmium* to zooplankton. *Journal of Applied Phycology* 4, 79–86.
- Hoering, T., 1957. The isotopic composition of the ammonia and the nitrate ion in rain. *Geochimica et Cosmochimica Acta* 12, 97–102.
- Howarth, R.W., Marino, R., Lane, J., Cole, J.J., 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. *Limnology and Oceanography* 33, 669–687.
- Karl, D., Letelier, R., Tupas, L., Dore, J., Christian, J., Hebel, D., 1997. The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. *Nature* 388, 533–538.
- Liu, K., Kaplan, I.R., 1989. The eastern tropical Pacific as a source of  $^{15}N$ -enriched nitrate in seawater off southern California. *Limnology and Oceanography* 35, 820–830.
- Liu, K., Su, M., Hsueh, C., Gong, G., 1996. The nitrogen isotopic composition of nitrate in the Kuroshio Water northeast of Taiwan: evidence for nitrogen fixation as a source of isotopically light nitrate. *Marine Chemistry* 54, 273–292.

- Mackenzie, F.T., Ver, L.M., Sabine, C., Lane, M., Lerman, A., 1993. C N P S global biogeochemical cycles and modeling of global change. In: Wollaster, R., Mackenzie, F. T., Chou, L. (Eds.), *Interactions of C N P and S Biogeochemical Cycles and Global Change*, Vol. 14. Springer, Berlin, NATO ASI Series, pp. 1–61.
- Macko, S.A., Uhle, M.E., 1997. Stable nitrogen isotope analysis of amino acid enantiomers by gas chromatography/combustion/isotope ratio mass spectrometry. *Analytical Chemistry* 69, 926–929.
- Macko, S.A., Fogel (Estep), M.L., Hare, P.E., Hoering, T.C., 1987. Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms. *Chemical Geology* 65, 79–92.
- McClelland, J.W., Montoya, J.P., 2002. Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology* 83, 2173–2180.
- Metges, C.C., Petzke, K., Hennig, U., 1996. Gas chromatography/combustion/isotope ratio mass spectrometric comparison of *N*-acetyl- and *N*-pivaloyl amino acid esters to measure  $^{15}\text{N}$  isotopic abundances in physiological samples: a pilot study on amino acid synthesis in the upper gastrointestinal tract of minipigs. *Journal of Mass Spectrometry* 31, 367–376.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of  $^{15}\text{N}$  along food chains: further evidence and the relationship between  $\delta^{15}\text{N}$  and animal age. *Geochimica et Cosmochimica Acta* 48, 1135–1140.
- Minagawa, M., Wada, E., 1986. Nitrogen isotope ratios of red tide organisms in the East China Sea: a characterization of biological nitrogen fixation. *Marine Chemistry* 19, 245–249.
- Montoya, J.P., McCarthy, J.J., 1995. Isotopic fractionation during nitrate uptake by phytoplankton grown in continuous culture. *Journal of Plankton Research* 17, 439–464.
- Montoya, J.P., Carpenter, E.J., Capone, D.G., 2002. Nitrogen-fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic. *Limnology and Oceanography* 47, 1617–1628.
- O’Neil, J., 1998. The colonial cyanobacterium *Trichodesmium* as a physical and nutritional substrate for the harpacticoid copepod *Macrosetella gracilis*. *Journal of Plankton Research* 20, 43–59.
- Paerl, H.W., Willey, J.D., Go, M., Peierls, B.L., Pickney, J.L., Fogel, M.L., 1999. Rainfall stimulation of primary production in western Atlantic Ocean waters: roles of different nitrogen sources and co-limiting nutrients. *Marine Ecology Progress Series* 176, 205–214.
- Prospero, J.M., Barrett, K., Church, T., Dentener, F., Duce, R.A., Galloway, J.N., Levy II, H., Moody, J., Quinn, P., 1996. Atmospheric deposition of nutrients to the North Atlantic Basin. *Biogeochemistry* 35, 27–73.
- Russell, K.A., Galloway, J.N., Macko, S.A., Moody, J.L., Scudlark, J.R., 1998. Sources of nitrogen in wet deposition to the Chesapeake Bay Region. *Atmospheric Environment* 32, 2453–2465.
- Riele, G., Collier, R.J., Jones, D.M., Eglinton, G., Eakin, P.A., Fallick, A.E., 1991. Sources of sedimentary lipids deduced from stable carbon-isotope analysis of individual compounds. *Nature* 352, 425–427.
- Sigman, D.M., Altabet, M.A., McCorkle, D.C., Francois, R., Fischer, G., 2000. The  $\delta^{15}\text{N}$  of nitrate in the Southern Ocean: nitrogen cycling and circulation in the ocean interior. *Journal of Geophysical Research C* 105, 19599–19614.
- Uhle, M.E., Macko, S.A., Spero, H.J., Engel, M.H., Lea, D.W., 1997. Sources of carbon and nitrogen in modern planktonic foraminifera: the role of algal symbionts as determined by bulk compound specific stable isotopic analyses. *Organic Geochemistry* 27, 103–113.
- Wada, E., Hattori, A., 1976. Natural abundance of  $^{15}\text{N}$  in particulate organic matter in the North Pacific Ocean. *Geochimica et Cosmochimica Acta* 40, 249–251.
- Wada, E., Hattori, A., 1978. Nitrogen isotope effects in the assimilation of inorganic nitrogenous compounds by marine diatoms. *Geomicrobiology Journal* 1, 85–101.
- Zehr, J.P., Mellon, M.T., Zani, S., 1998. New nitrogen-fixing microorganisms detected in oligotrophic oceans by amplification of nitrogenase (*nifH*) genes. *Applied Environmental Microbiology* 64, 3444–3450.
- Zehr, J.P., Waterbury, J.B., Turner, P.J., Montoya, J.P., Omereg, E., Steward, G.F., Hansen, A., Karl, D.M., 2001. New nitrogen-fixing unicellular cyanobacteria discovered in the North Pacific Central Gyre. *Nature* 412, 635–638.