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# TROPHIC RELATIONSHIPS AND THE NITROGEN ISOTOPIC COMPOSITION OF AMINO ACIDS IN PLANKTON

J. W. McClelland and J. P. Montoya[1]

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

*Abstract.* Stable nitrogen isotope ratios of whole organisms and tissues are routinely used in studies of trophic relationships and nitrogen flow through ecosystems, yet changes underlying increases in $\delta^{15}N$ from food source to consumer are not completely understood. In this study, the $\delta^{15}N$ of 16 amino acids in marine planktonic consumers and their food sources were examined using gas chromatography/combustion/isotope ratio mass spectrometry of their N-pivaloyl-i-propyl-amino acid ester derivatives. Moderate increases in bulk $\delta^{15}N$ with trophic position reflect an averaging of large increases in the $\delta^{15}N$ of some amino acids, and little or no change in others. Amino acids showing consistently large increases (e.g., glutamic acid changes by ~7‰ between food and consumer) provide greater scope for defining trophic position than the smaller isotopic changes in bulk material. In contrast, amino acids like phenylalanine show no change in $\delta^{15}N$ with trophic position and therefore preserve information about nitrogen sources at the base of the food web. The ability to acquire information about both trophic level and nitrogen sources at the base of the food web from single samples of consumer tissues offers a powerful new tool for elucidating pathways of N transfer through food webs.

*Key words: amino acids; food webs; nitrogen; plankton; stable isotopes; trophic.*

## Introduction

Stable isotope ratios have been used in ecosystems research since the early 1970s (Peterson and Fry 1987). Pioneering studies used information provided by stable isotope ratios of bulk samples to identify sources contributing to mixtures of particulate organic matter (Haines 1977) and to examine food web relationships (Peterson et al. 1985). An abundance of recent publications makes it clear that these applications of stable isotopes are now a routine component of ecosystems research.

Use of bulk stable isotope ratios to link consumers to food sources relies on the assumption that the isotopic composition of a consumer reflects the weighted mean of the isotopic composition of its food sources (Gannes et al. 1997). This assumption is generally accurate for stable isotope ratios of carbon (Peterson and Fry 1987, Lajtha and Michener 1994). Stable isotope ratios of nitrogen, on the other hand, tend to increase from food source to consumer (Minagawa and Wada 1984). Hence, stable N isotope ratios are most frequently used as trophic position indicators (Peterson and Fry 1987, Lajtha and Michener 1994).

Although there is no doubt that our understanding of food web relationships has been greatly advanced by the use of stable isotope measurements, confounding factors often make data interpretation more ambiguous than we would like. One major problem is that the number of different food sources that can be discerned in a consumer's diet is limited to one more than the number of different isotopes (e.g., C, N, S, O) used. The number of potential food sources to a consumer is frequently far greater than the number of isotopes that can be examined. Another confounding factor is that stable isotope ratios may change during decomposition (Benner et al. 1987, Currin et al. 1995). Finally, factors such as dietary N content (Adams and Sterner 2000) and nutritional stress (Hobson et al. 1993) can lead to changes in consumer stable N isotope ratios.

Such observations have prompted a general call for studies that examine the biochemical and physiological underpinnings for stable isotope ratios of whole organisms and specific tissues (Gannes et al. 1997, Gannes et al. 1998). By elucidating these underpinnings we can more accurately interpret stable isotope data in food web studies. Furthermore, stable isotope analysis of specific compounds may allow differentiation of a greater number of food sources to consumers and provide more detailed trophic level information.

The stable isotope ratios of individual compounds can provide very specific information about the diet and physiology of organisms (Hare et al. 1991, Fogel et al. 1997, Uhle et al. 1997), and about the origins of complex mixtures of organic matter (Rieley 1991). The large sample size needed for bulk 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 pos-

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[1] Corresponding author.

sible to measure compound-specific stable isotope ratios on very small samples (Macko and Uhle 1997, Meier-Augenstein 1999).

One of the targets of GC/C/IRMS is isotopic analysis of individual amino acids. With this approach, fascinating details about carbon sources to blue crabs (*Callinectes sapidus*, Fantle et al. 1999), planktonic foraminifera (Uhle et al. 1997), and estuarine bacteria (Pelz et al. 1998) have been revealed. Food web studies using GC/C/IRMS to examine N isotope values of individual amino acids have not yet (to the best of our knowledge) been published.

In this paper, we examine the stable N isotope ratios of amino acids in planktonic consumers and their food sources. Our primary research objective was to elucidate changes in N-isotope ratios of individual amino acids responsible for increases in bulk N-isotope ratios across trophic levels. Lab comparisons explicitly focused on the trophic relationship between the marine rotifer *Brachionus plicatilis* and the alga *Tetraselmis suecica*. Comparisons among different size fractions of zooplankton from the tropical Atlantic ocean provided a more general example of trophic interactions in a natural environment.

## Methods

### *Lab cultures*

Samples of *B. plicatilis* grown on a diet of *T. suecica* were collected opportunistically from large volume cultures maintained by Terry Snell's research group at the Georgia Institute of Technology. In these cultures, an inoculum of *T. suecica* is added to 240 L of 15 PSU artificial seawater (Instant Ocean; Aquarium Systems, Mentor, Ohio, USA) containing standard f/2 nutrients (Guillard 1975) at an initial density of $1 \times 10^4$ cells/mL and allowed to grow for ~7 d at 25°C under constant fluorescent illumination of ~2000 lux. When algal density reaches $1 \times 10^6$ cells/mL, 140 L of culture are transferred to a new tank and inoculated with the rotifer *Brachionus plicatilis* at an initial density of 0.1 individuals/mL. *B. plicatilis* is then grown up and harvested as a food source for other organisms.

For our study, rotifer density and $\delta^{15}N$ of bulk algae were measured daily. The $\delta^{15}N$ values of bulk rotifers were measured on days five through seven after inoculation. Samples of algae and rotifers were collected for analysis of amino acid $\delta^{15}N$ on days six and seven. On all dates, rotifers were separated from algae by gentle sieving through a 53-μm nylon mesh. Sufficient rotifer biomass for amino acid analysis was provided by 5 L of culture. Rotifers were kept in fresh medium for 1 h to allow gut contents to clear. Samples were then collected on precombusted Whatman GF/F filters (Whatman, Clifton, New Jersey, USA) by gentle vacuum filtration. The large culture volumes allowed us to sample rotifers grown at relatively low densities (2 and 7 individuals/mL on days six and seven respectively), minimizing the effect of regenerated N on the algal isotopic composition.

### *Field samples*

Samples of zooplankton were collected on R/V Seward Johnson cruise SJ9603 (28 March through 25 April 1996) to the tropical and subtropical Atlantic. Collections were done with diagonal tows of 1-$m^2$ net (333-μm 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 (250, 500, and 1000 μm; Sefar America, Briarcliff Manor, New York, USA) and 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. Here we consider samples collected at Station 21 (16°04′ N, 33°55′ W) and Station 30 (14°44′ N, 48°06′ W); data from other stations will be reported elsewhere.

### *Sample preparation*

Samples were prepared for GC/C/IRMS analysis of amino acids by acid hydrolysis followed by 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 × 100 mm glass tube with a PTFE (polytetrafluoroethylene)-lined cap and hydrolyzed with ultra pure 6-mol/L hydrochloric acid for 24 h at 110°C. The hydrolysate was evaporated to dryness at 55°C under a stream of $N_2$ and the residue was redissolved in 0.01-mol/L HCl. This solution was then purified by filtration (0.65-μm Durapore filter; Millipore, Bedford, Massachusetts, USA) followed by cation exchange chromatography (Dowex 50WX8-400 ion exchange resin; Sigma-Aldrich, St. Louis, Missouri, USA) in a 5-cm column prepared in a pasteur pipette. Amino acids were eluted with 4 mL of 2-mol/L ammonium hydroxide. The eluate was evaporated to dryness under a stream of $N_2$ at 80°C. Finally, the purified amino acids were derivatized to NPP-amino acid esters (Metges et al. 1996).

This preparation scheme allows analysis of 18 common amino acids plus α-aminoadipic acid (internal standard) and has the advantage that the derivatization introduces no exogenous nitrogen to the analytes. 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 the method. Final preparations were dissolved in ethyl acetate, and contained 1–2 nmol of each amino acid derivative per 0.5 μL of solution (target injection volume). Standard mixtures of amino acids were run through the entire analytical procedure to confirm the reproducibility of isotope measurements.

### *Isotope 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

TABLE 1. Values of $\delta^{15}$N of bulk samples and amino acids of *B. plicatilis* and *T. suecica*.

| | First sampling | | Second sampling | |
|---|---|---|---|---|
| $\delta^{15}$N source | *T. suecica* (Food source) | *B. plicatilis* (Consumer) | *T. suecica* (Food source) | *B. plicatilis* (Consumer) |
| Bulk sample | −1.9 | 0.1 | −1.7 | −0.2 |
| Amino Acids | | | | |
| Alanine | 0.5 ± 0.7 | 4.8 ± 0.1 | −1.6 ± 0.1 | 4.0 ± 0.4 |
| Aspartic acid | −0.5 ± 0.3 | 4.6 ± 0.2 | −0.6 ± 0.4 | 3.2 ± 0.4 |
| Glutamic acid | −0.4 ± 0.2 | 6.5 ± 0.4 | −0.1 ± 0.3 | 6.4 ± 0.3 |
| Glycine | −4.7 ± 0.4 | −3.2 ± 0.4 | −4.4 ± 0.3 | −4.1 ± 0.8 |
| Isoleucine† | −0.8 ± 0.5 | 3.3 ± 0.1 | −1.3 ± 0.9 | 2.4 ± 0.4 |
| Leucine† | −0.4 ± 0.8 | 2.8 ± 0.1 | −1.5 ± 0.3 | 2.0 ± 0.3 |
| Lysine† | −1.1 ± 0.4 | 0.7 ± 0.4 | −1.8 ± 0.2 | −0.4 ± 1.2 |
| Phenylalanine† | −5.3 ± 0.2 | −4.2 ± 0.2 | −4.3 ± 0.4 | −4.8 ± 1.0 |
| Proline | 0.1 ± 0.3 | 4.3 ± 0.2 | −0.4 ± 0.2 | 3.3 ± 0.3 |
| Serine | −7.8 ± 0.6 | −7.1 ± 0.2 | −7.8 ± 0.3 | −6.9 ± 0.3 |
| Threonine† | −2.4 ± 0.4 | −3.4 ± 0.4 | −2.3 ± 0.1 | −4.1 ± 0.5 |
| Tyrosine | −2.2 ± 0.6 | −3.3 ± 0.6 | −2.5 ± 0.3 | −3.1 ± 0.1 |
| Valine† | 1.8 ± 0.3 | 4.5 ± 0.1 | 1.0 ± 1.5 | 5.6 ± 0.3 |

*Notes:* Samples were collected on two successive days after determining that bulk values were at a steady state. Values for bulk samples are from CFIRMS analysis (standard deviation is typically ± 0.2‰). Values for amino acids are means ± 1 SE from three analyses of each sample by GCIRMS. Aspartic acid includes asparagine. Glutamic acid includes glutamine.

† Amino acids commonly required in animal diets (essential amino acids.)

(Micromass, Manchester, UK) coupled to a Hewlett Packard 6890 gas chromatograph (Hewlett Packard, Wilmington, Delaware, USA). The GC and isoprime were interfaced with a combustion furnace (850°C), reduction furnace (500°C), and liquid nitrogen cold trap. Mixes of amino acid derivatives from samples were injected into the GC, separated on an HP-Ultra 2 column (50 m × 0.32 mm inner diameter, 0.5 μm film thickness; Hewlett Packard, Wilmington, Delaware, USA), combusted, reduced, and finally passed through the cold trap to remove water and $CO_2$ before introduction to the mass spectrometer. Nitrogen isotope ratios for each amino acid in a mix were measured sequentially.

Gas chromatograph 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 to 200°C, dwell for 5 min; ramp up at 1°C/min to 215°C, dwell for 1 min; ramp up at 7°C/min to 300°C, dwell for 8 min. Carrier gas (helium) flow through the GC column was 1 mL/min for the first 38.3 min of each run, then increased to 1.3 mL/min for the remainder. The flame ionization detector was held at 300°C.

The stable isotopic composition of N in bulk sample preparations was analyzed by continuous flow isotope ratio mass spectrometry (CFIRMS) using a Micromass Optima mass spectrometer (Micromass, Manchester, UK) coupled to a Carlo Erba NA 2100 elemental analyzer (CE Instruments, Milan, Italy).

All isotope abundances are expressed as $\delta^{15}$N values relative to atmospheric $N_2$: $\delta^{15}N = ([R_{sample}/R_{standard}] - 1) \times 10^3$, where $R$ is the isotope ratio $^{15}N:^{14}N$. Analytical error associated with isotope measurements of bulk material was typically ±0.2‰ (1 SD). Analytical error associated with isotope measurements of mixtures of purified amino acids run through our derivatization and analytical protocols ranged from ±0.2‰ to ±1.4‰ (1 SD). As a check on our sample processing, we added an internal standard (α-aminoadipic acid) to each sample immediately after the hydrolysis step. All samples and standard mixtures for amino acid analysis were injected and analyzed three times. We report means and standard errors of these replicate analyses.

## RESULTS

### *Lab comparisons*

The stock culture of *T. suecica* had an initial bulk $\delta^{15}$N value of −0.8‰, which gradually decreased to a minimum of −1.9‰ 6 d after the introduction of *B. plicatilis*. On the two successive days when samples were collected for amino acid analyses, *T. suecica* had bulk $\delta^{15}$N values averaging −1.8‰ and amino acid $\delta^{15}$N values ranging from −7.8‰ to +1.8‰ (Table 1). On the same dates, *B. plicatilis* had bulk $\delta^{15}$N values averaging −0.1‰ and amino acid $\delta^{15}$N values ranging from −7.1‰ to +6.5‰ (Table 1). For both *T. suecica* and *B. plicatilis*, differences in amino acid $\delta^{15}$N values between sampling dates were generally <1‰. Exceptions were alanine in *T. suecica* and aspartic acid in *B. plicatilis*, which showed differences nearer to 2‰ between sampling dates. Quantities of methionine in samples from the lab cultures were too small for reliable measurement of isotope ratios.

Bulk $\delta^{15}$N values increased by ~1.7‰ from food source (*T. suecica*) to consumer (*B. plicatilis*), while changes in amino acid $\delta^{15}$N values varied widely (Fig. 1). Isoleucine, leucine, valine, alanine, aspartic acid,

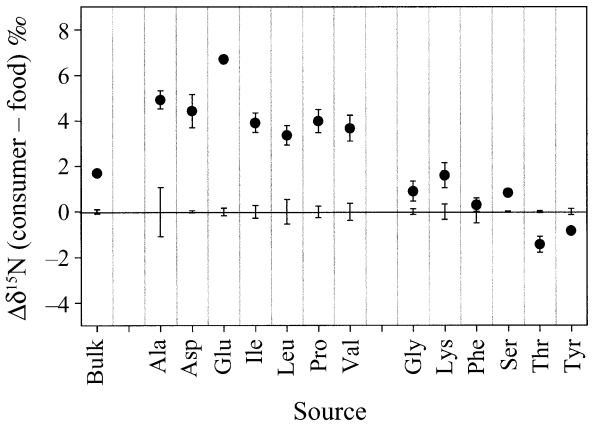


FIG. 1. Differences in stable N isotope values ($\Delta\delta^{15}N$) of bulk material and individual amino acids between consumer (*B. plicatilis*) and food source (*T. suecica*). Points are means from two sampling dates, and error bars reflect the range of values from *B. plicatilis* (on points) and *T. suecica* (on zero line). Amino acids are grouped into those that show changes greater than the bulk material and those that change less than the bulk material. Abbreviations are: Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Ile, isoleucine; Leu, leucine; Pro, proline; Val, valine; Gly, glycine; Lys, lysine; Phe, phenylalanine; Ser, serine; Thr, threonine; Tyr, tyrosine.

glutamic acid, and proline all showed larger changes in $\delta^{15}N$ between food and consumer than did the bulk material, while lysine, phenylalanine, threonine, glycine, serine, and tyrosine all changed less than did the bulk material (Fig. 1). Glutamic acid showed the largest increase (~7‰), while phenylalanine remained unchanged from food source to consumer. Glycine, serine, and tyrosine all differed by <1‰ from food source to consumer.

### *Field comparisons*

The $\delta^{15}N$ values of bulk zooplankton at station 21 ranged from 2.3‰ to 3.4‰, and $\delta^{15}N$ values for amino acids ranged from −8.6‰ to +12.3‰ (Table 2). Bulk $\delta^{15}N$ for the 500–1000 μm size fraction was 0.3‰ lower than that of the 250–500 μm size fraction, while bulk $\delta^{15}N$ for the 1000–2000 μm size fraction was 0.8 ‰ higher than that of the 250–500 μm size fraction. Correspondingly, $\delta^{15}N$ values for amino acids from the 500–1000 μm size fraction were generally the same or slightly lower those of the 250–500 μm size fraction, while $\delta^{15}N$ values for amino acids from the 1000–2000 μm size fraction were generally the same or higher than those of the 250–500 μm size fraction (Fig. 2, bottom panel).

The $\delta^{15}N$ values of bulk zooplankton at station 30 ranged from 0.3‰ to 2.2‰, and $\delta^{15}N$ values for amino acids ranged from −11.0‰ to +12.4‰ (Table 2). Bulk $\delta^{15}N$ values increased by ~1‰ between increasing size fractions, while amino acid $\delta^{15}N$ values (with the exception of threonine) either increased or remained the same (Fig. 2, top panel). The $\delta^{15}N$ of threonine decreased between increasing size fractions.

At both stations 21 and 30, the group of amino acids showing the least change among the different size fractions (Fig. 2) was the same as that identified in the trophic comparison between *B. plicatilis* and *T. suecica* (Fig. 1). Similarly, the group of amino acids showing larger changes between size fractions (Fig. 2) was the same as that identified in the lab comparison (Fig. 1). The more dramatic changes in amino acid $\delta^{15}N$ values among size fractions at station 30 as compared to station 21 correlate with greater differences in bulk values at station 30. Alanine and glutamic acid were among

TABLE 2. Values of $\delta^{15}N$ of bulk samples and amino acids of zooplankton from the tropical North Atlantic.

| | Station 21 (16°04′ N, 33°55′ W) | | | Station 30 (14°44′ N, 48°06′ W) | | |
|---|---|---|---|---|---|---|
| $\delta^{15}N$ source | 250–500 μm | 500–1000 μm | 1000–2000 μm | 250–500 μm | 500–1000 μm | 1000–2000 μm |
| Bulk sample | 2.6 | 2.3 | 3.4 | 0.3 | 1.4 | 2.2 |
| Amino Acids | | | | | | |
| Alanine | 9.8 ± 0.6 | 9.8 ± 0.7 | 12.3 ± 0.4 | 5.3 ± 0.3 | 8.6 ± 0.5 | 11.9 ± 0.4 |
| Aspartic acid | 6.6 ± 0.4 | 6.4 ± 0.1 | 7.6 ± 0.7 | 0.8 ± 0.0 | 5.1 ± 0.4 | 9.0 ± 0.1 |
| Glutamic acid | 9.7 ± 0.1 | 10.0 ± 0.1 | 12.0 ± 0.6 | 5.5 ± 0.2 | 9.4 ± 0.1 | 12.4 ± 0.3 |
| Glycine | 1.1 ± 0.4 | 1.5 ± 0.1 | 1.2 ± 0.3 | −0.7 ± 0.3 | 1.9 ± 0.2 | 2.1 ± 0.3 |
| Isoleucine† | 5.6 ± 0.6 | 4.0 ± 0.2 | 7.0 ± 0.4 | 2.8 ± 0.5 | 5.0 ± 0.1 | 6.7 ± 0.4 |
| Leucine† | 5.9 ± 0.8 | 8.0 ± 0.2 | 8.0 ± 0.7 | 3.2 ± 0.2 | 6.2 ± 0.4 | 8.9 ± 0.3 |
| Lysine† | −1.6 ± 0.3 | −1.8 ± 0.3 | −2.1 ± 0.2 | −4.1 ± 0.3 | −2.8 ± 0.2 | −1.2 ± 0.1 |
| Methionine† | −0.7 ± 1.2 | −0.1 ± 0.7 | −0.1 ± 0.4 | −2.6 ± 0.0 | −1.0 ± 0.4 | −1.4 ± 0.4 |
| Phenylalanine† | −1.4 ± 0.3 | −2.5 ± 0.6 | −2.7 ± 0.4 | −5.7 ± 0.5 | −5.6 ± 0.3 | −5.8 ± 0.3 |
| Proline | 7.3 ± 0.6 | 4.6 ± 0.6 | 4.6 ± 0.1 | 1.3 ± 0.5 | 4.3 ± 0.2 | 5.1 ± 0.2 |
| Serine | −3.5 ± 0.5 | −3.1 ± 0.2 | −3.4 ± 0.7 | −4.3 ± 0.7 | −3.9 ± 0.2 | −4.0 ± 0.4 |
| Threonine† | −8.6 ± 0.3 | −7.9 ± 0.5 | −7.5 ± 0.8 | −6.5 ± 0.8 | −9.3 ± 0.1 | −11.0 ± 0.6 |
| Tyrosine | −1.0 ± 0.8 | −0.5 ± 0.3 | −1.0 ± 0.3 | −4.0 ± 0.2 | −3.3 ± 0.2 | −2.6 ± 0.5 |
| Valine† | 5.9 ± 0.9 | 4.5 ± 0.4 | 7.4 ± 0.4 | 6.3 ± 0.3 | 7.8 ± 0.1 | 11.0 ± 0.4 |

*Notes:* Table entries are reported in units of parts per thousand (‰). Column headings show size fractions. Values for bulk samples are from standard CFIRMS analysis (standard deviation is typically ± 0.2‰). Values for amino acids are means ± 1 SE from three analyses of each sample by GCIRMS. Aspartic acid includes asparagine. Glutamic acid includes glutamine.

† Amino acids commonly required in animal diets (essential amino acids).

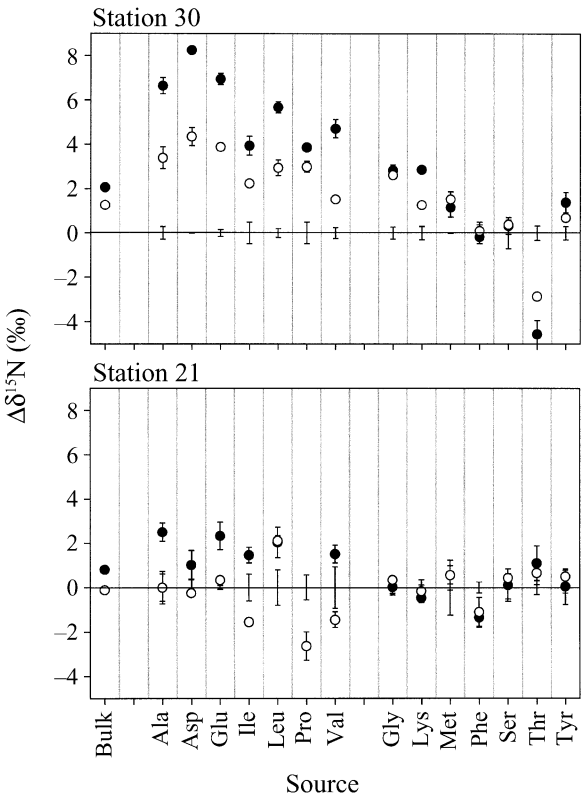


FIG. 2. Differences in stable N isotope values ($\Delta\delta^{15}$N) of bulk material and individual amino acids among size fractions of zooplankton from two locations in the tropical North Atlantic (stations are described in Table 2). Open symbols are 500–1000 μm minus 250–500 μm size fraction. Closed symbols are 1000–2000 μm minus 250–500 μm size fraction. All values are means from triplicate analyses. Standard errors for 250–500 μm size fraction are represented on the zero line, while standard errors for the 500–1000 and 1000–2000 μm size fractions are represented on the open and closed symbols respectively. Amino acid names are as in Fig. 1.

the amino acids showing the greatest increases between size fractions at both stations (Fig. 2). Methionine, phenylalanine, serine, and tyrosine showed little or no change at both stations (Fig. 2).

The isotope values of bulk material and individual amino acids at station 30 were generally lower than their counterparts at station 21 (Table 2). This difference between stations was greatest for the 250–500 μm size fraction and became less pronounced in the larger size fractions.

## DISCUSSION

Stable N isotope ratios of whole organisms and individual tissues have become routinely used for understanding trophic relations (Lajtha and Michener 1994, Gannes et al. 1998) and yet the underlying physiological and biochemical mechanisms that lead to increased $\delta^{15}$N from food source to consumer are still not completely understood (Rau et al. 1990, Gannes et al. 1997, Adams and Sterner 2000). Our examination of amino acid $\delta^{15}$N revealed how the major building blocks that make up bulk N-isotope ratios change from food source to consumer. Lab cultivation of *B. plicatilis* reared on *T. suecica* provided an unambiguous pairing between a consumer and its food. Comparison of increasing size fractions of zooplankton from the field provided a natural proxy for increasing trophic position since larger size fractions contain a greater proportion of omnivores and carnivores (Fry and Quiñones 1994).

Our results indicate that moderate increases in bulk $\delta^{15}$N from food source to consumer are underlain by large increases in the $\delta^{15}$N of some amino acids, and little or no change in others. Hence, amino acids from a single sample provide information both about trophic level and N sources at the base of the food web. Two lines of evidence suggest that this finding is generalizable. First, the ~2‰ shift in bulk $\delta^{15}$N observed between *T. suecica* and *B. plicatilis* falls within the range of 1.3–5.3‰ trophic enrichment reported by Minagawa and Wada (1984) for a variety of different consumer–food source combinations. Second, patterns of change in amino acid $\delta^{15}$N from food source to consumer in the lab (Fig. 1) were very similar to the patterns observed between mixed assemblages of zooplankton from different size fractions of field samples (Fig. 2). In both the lab and field comparisons, alanine and glutamic acid showed the largest changes, while phenylalanine, serine, and tyrosine showed almost no change. The larger differences in amino acid $\delta^{15}$N among size fractions at station 30 relative to station 21 (Fig. 2) suggest that samples from station 30 included zooplankton from a broader range of trophic levels, though the samples collected at these two stations were not obviously different in gross taxonomic composition.

Trophic-level information can be further defined by the difference in $\delta^{15}$N between amino acids such as phenylalanine, which does not change from food source to consumer, and amino acids like glutamic acid, which shows a large trophic change. In addition to representing very different patterns of trophic alteration, phenylalanine, and glutamic acid are among the easiest amino acids to analyze with high precision. Our analytical conditions produce particularly well defined and isolated peaks for these amino acids. We therefore used the difference between these two amino acids ($\Delta\delta^{15}N_{glu\text{-}phe} = \delta^{15}N_{glu} - \delta^{15}N_{phe}$) as an internal index to trophic position. This treatment of the data normalizes for differences in baseline $\delta^{15}$N : mean $\delta^{15}$N values of phenylalanine in *B. plicatilis* and 250–500 μm zooplankton from stations 21 and 30 range from −1.4 to −5.7‰ (Tables 1 and 2), while values for glutamic acid minus phenylalanine are all very near 11‰ (Fig. 3). The similarity in phenylalanine-normalized values indicates that the 250–500 μm size fractions from both field stations are dominated by herbivores. Increasing phenylalanine-normalized values in larger size fractions of zooplankton (Fig. 3) reflect increasing carnivore contributions that shift the assemblages to trophic

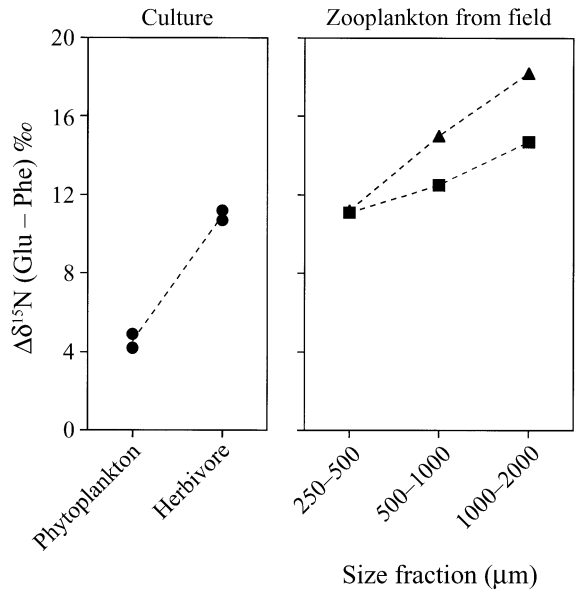


FIG. 3. Values of $\Delta\delta^{15}N$ of glutamic acid minus phenylalanine in *T. suecica* (Phytoplankton), *B. plicatilis* (Herbivore), and three size fractions of zooplankton collected from tropical North Atlantic waters at stations 21 (squares) and 30 (triangles) of cruise SJ9603. Differences were calculated from means listed in Tables 1 and 2.

positions between levels 1 and 2, assuming that the 7‰ difference between normalized values for phytoplankton and herbivores (Fig. 3) represents a one trophic level shift.

Examples in the literature comparing amino acid $\delta^{15}N$ of consumers to food sources are scarce, making it difficult to say how broadly the patterns we observed in planktonic organisms extend to other food web relationships. Hare et al. (1991) do, however, provide at least one example comparing the $\delta^{15}N$ of amino acids isolated by column chromatography from pigs (collagen) and their food. For the eight amino acids common to our study and theirs, the results are very similar: glutamic acid showed the largest increase; alanine, aspartic acid, proline, and valine showed moderate increases; glycine increased the least; and threonine decreased between food and consumer. Serine provides the one exception, having increased substantially between food and animal tissues in the Hare et al. 1991 study, and having changed very little in our study. This may reflect general differences in metabolic pathways between terrestrial vertebrates and the various invertebrates in our samples, though it is worth noting that serine is the immediate precursor of glycine, an amino acid that is particularly abundant in collagen. The large increase in $\delta^{15}N$ of serine in pig collagen relative to pig diet may therefore reflect the extensive throughput of amino acid skeletons to collagen glycine via the serine pool.

Within the 250–500 μm size fraction, the lower $\delta^{15}N$ values of zooplankton found at station 30 as compared to station 21 (Table 2) are likely coupled to $N_2$-fixation. The $N_2$-fixing cyanobacterium *Trichodesmium* was much more abundant at station 30 than at station 21 (E. Carpenter, *personal communication*), and particulate organic matter in regions where $N_2$ fixation is important has lower $\delta^{15}N$ values than in regions where $N_2$ fixation is negligible (Wada and Hattori 1976, Minagawa and Wada 1986, Carpenter et al. 1997). Direct consumption of *Trichodesmium* is probably a minor pathway of newly fixed N to the metazoan food web (Hawser et al. 1992). Instead, it is more likely that nitrogen fixed by *Trichodesmium* reaches zooplankton through the microbial loop (Capone et al. 1997), though the low $\delta^{15}N$ values we found for all size fractions of zooplankton at station 30 clearly imply that recently fixed N makes a substantial contribution to the entire pelagic ecosystem.

The $\delta^{15}N$ of different nitrogen pools within an organism reflect the $\delta^{15}N$ of nitrogen sources as well as the internal processing of N that causes fractionation (Fogel et al. 1997, Gannes et al. 1998). In our culture of the alga *T. suecica*, a single inorganic nitrogen source was added. Hence, variation in $\delta^{15}N$ among amino acids of *T. suecica* (Table 1) can be attributed to internal processing alone: each amino acid has a unique biosynthetic pathway and set of uses. For *B. plicatilis* (Table 1) and zooplankton from the field (Table 2), variations in $\delta^{15}N$ among amino acids reflect variations acquired from food sources as well as further internal processing by the animal. Fantle et al. (1999) found that the $\delta^{13}C$ of essential and nonessential amino acids in blue crabs (*Callinectes sapidus*) were very different. In contrast, the classic ''essential'' and ''nonessential'' groupings of amino acids did not show consistent patterns of variation in $\delta^{15}N$ with trophic position in our study. This may be because the amino acid requirements of planktonic consumers are different from those of rats, the model organism originally used in defining essential and nonessential amino acids. A variety of organisms have been shown to have amino acid requirements that differ from those of rats (Morris 1991). Alternatively, variation within the essential and nonessential categories may simply reflect varying degrees of conservation of different amino acids within each group.

Increases in the $\delta^{15}N$ values of proteins from food source to consumer have been attributed to fractionation during transamination and deamination (Gaebler et al. 1966, Gannes et al. 1998) that results in preferential retention of $^{15}N$ and excretion of $^{14}N$ (DeNiro and Epstein 1981, Gu et al. 1994). We should therefore expect to see increases in the $\delta^{15}N$ values of amino acids, whether acquired from food sources or synthesized, to the degree that they are subsequently broken down or transformed within the consumer. Since glutamic acid plays a central role in both synthesis of other amino acids (Lehninger 1975) and excretion of ammonia in crustaceans (Claybrook 1983), it is not sur-

prising that the $\delta^{15}$N of glutamic acid showed the largest increase between alga and rotifer in our feeding experiments (Fig. 1). Less expected was the relatively small change in the $\delta^{15}$N of serine between trophic levels (Fig. 1), as it is also considered a major contributor to excretion in crustaceans (Claybrook 1983). Differences in $\delta^{15}$N values among amino acids in humans have also been linked to their roles in bodily N cycling (Fogel et al. 1997). In contrast to crustaceans, fractionation during human excretion seems to be linked to losses of isotopically light nitrogen from the amide region of arginine.

Clearly, we still have much to learn about the mechanisms underlying increases in bulk $\delta^{15}$N with increasing trophic level. There is a growing body of evidence that $\delta^{15}$N can change in response to other factors such as starvation and water stress in terrestrial organisms (Gannes et al. 1997). These confounding factors make it difficult to use the $\delta^{15}$N of bulk material as an unambiguous trophic level indicator. Stable N isotope analysis of individual amino acids may help solve this problem, particularly if the $\delta^{15}$N values of particular amino acids are influenced by specific processes or conditions. Already it is apparent from our data that the very large increases in $\delta^{15}$N values of amino acids such as glutamic acid from food source to consumer allow greater definition of trophic position than is the case when using bulk material. At the same time, amino acids such as phenylalanine that do not show changes in $\delta^{15}$N across trophic levels provide information about nitrogen sources at the base of the food web. The new ability to acquire information both about trophic level and N sources at the base of the food web from single samples of consumer tissues offers a unique opportunity for understanding pathways of N transfer through food webs.

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