

## The effect of dietary nitrogen content on trophic level $^{15}\text{N}$ enrichment

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### Abstract

Given the great potential value of stable isotopes in a variety of scientific investigations, surprisingly little attention has been paid to the underlying physiological and biochemical mechanisms that account for trophic increases in  $\delta^{15}\text{N}$  values. This has led to a general call for controlled studies investigating the relationship between organismal diet and corresponding isotopic composition. We conducted a series of laboratory studies varying dietary nitrogen content and measuring corresponding variations in organismal  $\delta^{15}\text{N}$  values. Specifically, we investigated the relationship between the  $\delta^{15}\text{N}$  values of the anomopod crustacean, *Daphnia magna*, and the C:N ratio of its food, the green algae, *Scenedesmus acutus*. Daphnids were raised to a standard life stage on three types of *S. acutus* as food, which ranged in C:N (atomic) from 7.3 to 24.8. The average C:N of the daphnids was 6.0.  $^{15}\text{N}$  enrichment was found to be strongly linearly related to the C:N of the algae, ranging from nearly zero to approximately 6‰, which would normally be considered a span of almost two trophic levels. The  $\delta^{15}\text{N}$  values of the daphnids and the diet-tissue isotope fractionation factor, ( $\Delta_{dt} = D. magna \delta^{15}\text{N} - S. acutus \delta^{15}\text{N}$ ), were inversely related to the nitrogen content of the algae ( $R^2 = 0.82$  and  $0.99$  in two separate runs). To our knowledge, this is the first controlled study of nitrogen balance and  $\delta^{15}\text{N}$  values in animals.

Use of stable isotopes in the investigation of biological, ecological, and conservation issues has become increasingly popular in the past two decades. Such isotopic investigations have centered on two main themes: tracer experiments (source–sink information) and the elucidation of trophic structure (process information) (Peterson and Fry 1987; Lajtha and Michener 1994). In the biological sciences, tracer experiments involving  $^{15}\text{N}$  either use naturally occurring isotopic concentration gradients or monitor additions of  $^{15}\text{N}$  enriched or depleted substrates to investigate nutrient cycling and other biogeochemical processes (Mariotti et al. 1984; Estep and Vigg 1985). Recently, the analysis of  $^{15}\text{N}$  has been found also to have applications in conservation biology. Increases in  $\delta^{15}\text{N}$  values are significantly correlated with the bioaccumulation of organochlorines and other anthropogenic biochemical inputs, and therefore  $\delta^{15}\text{N}$  values are useful tools in predicting the magnitude of bioaccumulation (Cabana and Rasmussen 1994; Kidd et al. 1995; Kiriluk et al. 1995).

The main use of  $\delta^{15}\text{N}$  values for process information has been to assign individual organisms to particular trophic levels. Analysis of trophic structure has been founded on an observation that  $\delta^{15}\text{N}$  values increase in a consistent fashion with trophic level increases. Comparison of consumers and their resources indicates that  $\delta^{15}\text{N}$  values increase approximately 3.4‰ with each trophic level (Minawaga and Wada 1984). Therefore, by analyzing the  $\delta^{15}\text{N}$  values of various organisms within an ecosystem, it may be possible to elucidate part of the trophic structure of the ecosystem (Kling et al. 1992; Hecky and Hesslein 1995). However, the value 3.4‰ is the mean of a statistical distribution of  $\delta^{15}\text{N}$  value differences across trophic levels. In their original report, Minawaga and Wada showed a range of 1.3‰ to 5.3‰ in

trophic level enrichment. The standard deviation of this distribution was 1.1‰. The extent to which this variation consists of analytical or sampling errors versus a true biological difference in  $^{15}\text{N}$  enrichment across trophic levels has not yet been determined.

Despite the great potential value of stable isotopes in a variety of scientific investigations, surprisingly little attention has been paid to the underlying physiological and biochemical mechanisms that account for the trophic increase in  $\delta^{15}\text{N}$  values. Thus, there is still considerable uncertainty about the cause of this enrichment (Rau et al. 1990), potentially complicating its use in elucidating trophic positioning. This has led to a general call for controlled studies investigating the relationship between organismal diet and corresponding organismal isotopic composition (Owens 1987; Hobson et al. 1996; Gannes et al. 1997, 1998).

It is believed that the fractionation (isotopic discrimination) of  $^{15}\text{N}$  from  $^{14}\text{N}$  in animals occurs during amino acid synthesis (Gaebler et al. 1966), which results in the retention of isotopically heavier  $^{15}\text{N}$  and the excretion of the isotopically lighter  $^{14}\text{N}$  (DeNiro and Epstein 1981; Minawaga and Wada 1984; Gu et al. 1994). The chemical basis for this fractionation involves the lower vibrational frequency of the chemical bonds of  $^{15}\text{N}$  compared with  $^{14}\text{N}$ . Heavier isotopes therefore form bonds of greater energy than their isotopically lighter counterparts and thus are less likely to undergo chemical reactions. Physiologically, this means that amine groups containing  $^{14}\text{N}$  are favored during transamination and deamination, which results in isotopically light excreted nitrogen and the enrichment of certain amino acids such as glutamate (Gannes et al. 1998).

In spite of the known variation in animals' nitrogen content across trophic levels, few studies have addressed the variation in  $^{15}\text{N}$  increases across trophic levels. In fact, to our knowledge, this is the first controlled study of nitrogen balance and  $\delta^{15}\text{N}$  values in animals. The purpose of this study was to examine the increase in  $\delta^{15}\text{N}$  values across tro-

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phic levels in a controlled laboratory setting by investigating the relationship between organismal  $\delta^{15}\text{N}$  values and nitrogen availability. We measured the  $^{15}\text{N}$  content of the omnivorous herbivore, *Daphnia magna*, raised on the chlorophyte, *Scenedesmus acutus*, manipulated to vary in nitrogen content (C:N).

## Methods

Algal stocks consisted of *S. acutus* from long-term, laboratory-reared cultures (Sterner et al. 1993; Sterner and Smith 1993). Algae were cultured in basal COMBO medium (Kilham et al. 1998) in continuous flow chemostats at 20°C with constant (24 h) light at  $9.0 \times 10^{15}$  Quanta  $\text{s}^{-1} \text{cm}^{-2}$ . Different flow rates were used to obtain food stocks of varying nitrogen concentrations. Inflowing medium had a N:P ratio of 5 (all ratios are molar). The high nitrogen content (HIN) food stock had a chemostat dilution ( $D$ ) of  $0.5 \text{ d}^{-1}$ , which results in an algal carbon to nitrogen ratio (C:N) of 9.6 (run 1) and 7.3 (run 2). The low nitrogen content (LON) food stock had  $D = 0.1 \text{ d}^{-1}$  resulting in a C:N of 24.8 (run 1) and 21.3 (run 2). An algal food stock of intermediate C:N was created by mixing equal amounts of low and high nitrogen foods (based on carbon content). This moderate nitrogen content (MON) mixed food stock had a C:N of 15.8 (run 1) and 10.8 (run 2). The algal carbon content was estimated from standard curves of *S. acutus* culture absorbance at 800 nm, versus C concentration of the culture for each food type. The algal food stocks were sampled for both CHN and  $^{15}\text{N}$  analysis by filtering 5 and 25 ml, respectively, of each algal food stock (in triplicate) onto precombusted 25-mm Whatman GF/F glass-fiber filters. The filters were then dried at 60°C for at least 24 h, rolled in tin disks, and analyzed at either the University of Minnesota, St. Paul Campus (run 1) or Arizona State University, Tempe (run 2) (see sample analysis). For  $^{15}\text{N}$  analysis, three replicate algal samples per food stock were taken at the beginning and the end of each experiment.

*Daphnia magna* were reared entirely on each of the above algal food stocks from neonates to a standard life stage, i.e., primiparous females bearing their first clutch and with eggs in stage one of development (Threlkeld 1979). This was accomplished by placing stem mothers with eggs at developmental stage five (embryo with single median black eye), in basal COMBO medium with  $500 \mu\text{g C L}^{-1}$  of the appropriate algae for food. In this way, the neonates were released from the stem mothers directly into the appropriate experimental algal food stock. Food quantities were above the incipient limiting level (Lampert 1977). The day following the isolation of gravid females, neonate ( $\leq 24$  h of age) length was measured and 12 individuals were placed into 2-liter polycarbonate roller bottles (three replicate bottles per algal food) containing 2 liters of basal COMBO and  $500 \mu\text{g C L}^{-1}$  of the appropriate algal food. The roller bottles were then placed horizontally on roller racks and rotated at 1 rotation per minute. Temperature and light were constant (24 h) at 20°C and  $0.35 \times 10^{15}$  Quanta  $\text{s}^{-1} \text{cm}^{-2}$ , respectively. Each day, the daphnids were rinsed with basal COMBO and transferred into fresh basal COMBO with  $500 \mu\text{g C L}^{-1}$  of

fresh algal food. Animals were harvested and measured on the first day eggs were visible in their brood chambers. Harvest occurred after approximately 8 d of growth for daphnids raised on HIN, 9 d for daphnids raised on MON, and 13 d for daphnids raised on LON. Organismal mass and growth rates were based on conversion from length to dry weight ( $W = 1.89 \times 10^{-6} \times \text{length}^{2.25}$ ) using the regression equation for *D. magna* given in Dumont et al. (1975), and calculations were done in accordance with Bird and Prairie (1985). The harvested *Daphnia* were placed in tin capsules and dried for at least 24 h at 60°C before CHN and  $^{15}\text{N}$  analysis. Approximately two *Daphnia* were used per CHN analysis and approximately 10 *Daphnia* were used per  $^{15}\text{N}$  analysis.

*Daphnid starvation*—Another experiment was conducted to see what changes in N isotopic signature occurred in *D. magna* under starvation conditions. *D. magna* were raised from neonates to age 5 d on HIN food as described above. On the sixth day, 40 individuals were measured (length in mm) and placed into tin capsules for  $^{15}\text{N}$  analysis. The remaining daphnids were rinsed with basal COMBO and placed in fresh basal COMBO without the addition of any algal food. This process of harvesting 40 daphnids each day for  $^{15}\text{N}$  analysis and placing the remaining individuals in fresh basal COMBO without algal food was continued until no living animals were left to sample (on the fifth day, when the daphnids were 10 d old).

*Sample analysis*—Stable isotope data were analyzed in two runs at two independent laboratories. The  $^{15}\text{N}$  analyses of run 1 were performed using a Fisons Optima Continuous Flow Model Mass Spectrometer at St. Paul, Minnesota and the  $^{15}\text{N}$  analyses of run 2 were performed using an Europa Scientific 20/20 Stable Isotope Analyzer at Tempe, Arizona. CHN analyses for run 1 were performed using a Perkin Elmer elemental analyzer 2400 CHN. CHN analyses for run 2 were performed in conjunction with the  $^{15}\text{N}$  analyses using the Europa scientific 20/20 stable isotope analyzer.

For the analysis of the  $^{15}\text{N}$  samples, atmospheric nitrogen was used as the standard for the Fisons optima continuous flow model mass spectrometer and an aquatic solution of sucrose and ammonium sulfate (4.24% N and 33.69% C) was used as the standard for the Europa Scientific 20/20 stable isotope analyzer. For both machines, a maximum of 16 samples were run in between running sets of two to four standards.  $^{15}\text{N}$  abundance was expressed as a per mil (‰) deviation from the standards (Mariotti 1983):

$$\delta^{15}\text{N}\text{‰} = \left[ \frac{(^{15}\text{N}/^{14}\text{N}) \text{ sample}}{(^{15}\text{N}/^{14}\text{N}) \text{ standard}} - 1 \right] \times 1,000.$$

Standards carried through this procedure at both analyzing laboratories usually differed by less than 0.2‰. However, the standards from the end of run 1 did show a high degree of variability (standard error = 3.67) for unknown reasons. Replicate samples from run 1 did not show this high variability though (standard errors ranged from 0.03 to 0.26).

## Results

Body mass increases and growth rate,  $G = (\ln M_t - \ln M_0)/t$ , were greatest when daphnids were raised on HIN (Ta-

Table 1. Variations in *D. magna* size, age, growth, reproductive condition, and survivorship when raised on the three *S. acutus* food stocks and with no algal food (starvation experiment).

Food stock	Length (mm)	Mass ( $\mu\text{g}$ )	$\Delta$ mass ( $\mu\text{g}$ )	Age (d)	G ( $\text{d}^{-1}$ )	Clutch size	% survivorship ( $\text{d}^{-1}$ )
Low nitrogen	2.2 $\pm$ 0.02	63 $\pm$ 11	57	13.3 $\pm$ 1.5	0.16	3.7 $\pm$ 1.4	99
Moderate nitrogen	2.3 $\pm$ 0.03	71 $\pm$ 12	64	8.8 $\pm$ 1.2	0.24	6.0 $\pm$ 2.4	97
High nitrogen	2.4 $\pm$ 0.02	74 $\pm$ 10	67	7.8 $\pm$ 0.4	0.27	7.7 $\pm$ 2.9	99
None—starvation experiment	1.4 $\pm$ 0.01	22 $\pm$ 5	-0.3	6-10	-0.003	NA	NA

ble 1), were smallest when daphnids were raised on LON, and were intermediate when daphnids were raised on MON. In general, daphnid mass at the time of harvest was approximately eight times greater than the mass of the neonates at the start of the experiments. Therefore, the increase in daphnid  $\delta^{15}\text{N}$  values seen in this study should be almost entirely a result of production within the experimental time. Because we harvested *D. magna* at a standard life stage and growth rates differed with the different food stocks, individuals raised on different algal foods had significantly different ages at the time of harvest (ANOVA,  $df = 248$ ,  $p < 0.001$ ). Daphnids raised on HIN algal food were younger than daphnids raised on LON at the time of harvest, with daphnids raised on MON being intermediate (Table 1). Daphnids raised on HIN also tended to be larger and have more eggs in their brood chambers at the time of harvest than daphnids raised on LON, with daphnids raised on MON being intermediate (Table 1). Both animal mass and the number of eggs in the brood chambers at the time of harvest were significantly different between individuals raised on different algal food stocks (ANOVA,  $df = 248$ ,  $p < 0.001$ ).

The C:N of *S. acutus* in run 1 ranged from 9.6 (HIN) to 24.8 (LON), with MON being intermediate (15.8). The C:N of *S. acutus* in run 2 ranged from 7.3 (HIN) to 21.3 (LON), with MON being intermediate (10.8). The C:N of *D. magna* was strongly correlated with algal C:N in both run 1 and

run 2 ( $y = 0.126x - 4.20$ ,  $R^2 = 0.70$ ) (Fig. 1). There was not a significant run effect in the relationship between algal C:N and zooplankton C:N (partial F test,  $df = (2,2)$ ,  $p = 0.30$ ). Although *D. magna* C:N did increase (4.6 to 7.5) with increasing *S. acutus* C:N, the increase is comparatively small given the algal C:N increase.

The  $\delta^{15}\text{N}$  value for  $(\text{NH}_4)_2\text{SO}_4$ , the nitrogen source for the algae, was  $-0.68 \pm 0.18\text{‰}$ . We could detect no differences in the N isotopic composition of the algae in any of the treatments. The  $\delta^{15}\text{N}$  values of the food stocks from the two runs ranged from  $-1.61 \pm 1.19\text{‰}$  to  $0.40 \pm 3.55\text{‰}$  (Fig. 2). Although in both runs the three algal food stocks had significantly different C:N, their corresponding  $\delta^{15}\text{N}$  values were not significantly different either between or within runs (run 1 ANOVA,  $df = 29$ ,  $p = 0.05$ ; run 2 ANOVA,  $df = 22$ ,  $p = 0.88$ ; combined run 1 and run 2 ANOVA,  $df = 52$ ,  $p = 0.50$ ). The  $R^2$  of the regression between algal C:N and algal  $\delta^{15}\text{N}$  values was small (Fig. 2). Additionally, the  $\delta^{15}\text{N}$  values of the algal food stocks were not significantly different from the  $\delta^{15}\text{N}$  value of the  $(\text{NH}_4)_2\text{SO}_4$  (ANOVA,  $df = 56$ ,  $p = 0.92$ ). The overall algal isotope fractionation factor, *S. acutus*  $\delta^{15}\text{N} - [(\text{NH}_4)_2\text{SO}_4] \delta^{15}\text{N}$ , was  $-0.11\text{‰}$ .

*D. magna*  $\delta^{15}\text{N}$  values were strongly correlated with *S. acutus* C:N in both runs individually (run 1  $R^2 = 0.99$ , run 2  $R^2 = 0.98$ ), and combined ( $R^2 = 0.96$ ) (Fig. 3). Therefore, as the nitrogen content of *S. acutus* decreased (increasing

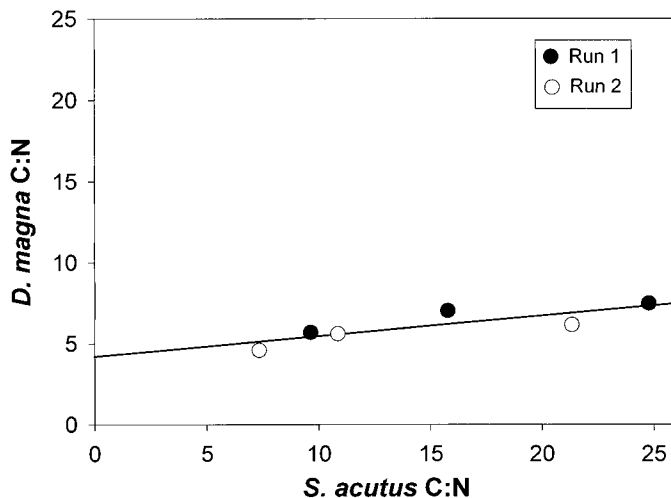


Fig. 1. Relationship between *D. magna* C:N and *S. acutus* C:N ( $y = 0.13x - 4.20$ ,  $R^2 = 0.70$ ,  $p = 0.04$ ).

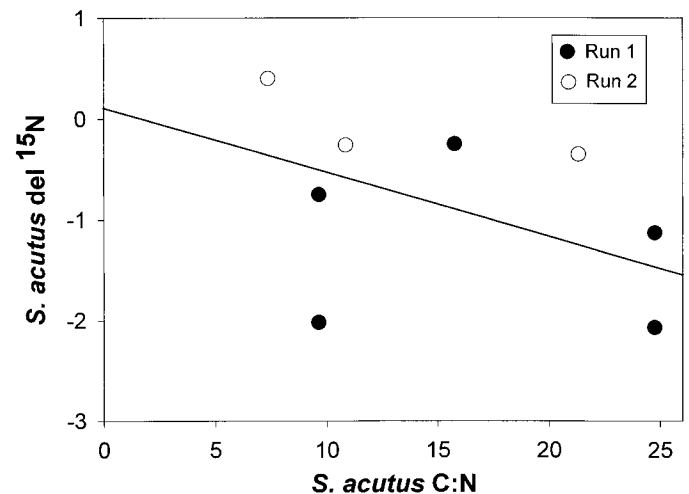


Fig. 2. Relationship between *S. acutus* C:N and *S. acutus*  $\delta^{15}\text{N}$  values ( $y = -0.06x + 0.11$ ,  $R^2 = 0.26$ ,  $p = 0.16$ ).

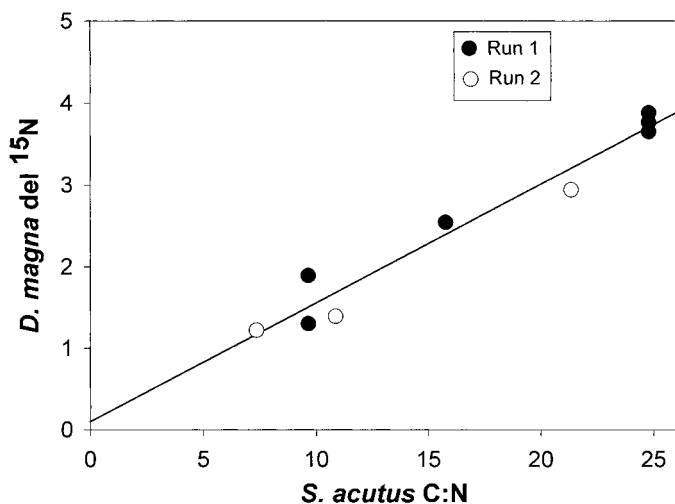


Fig. 3. Relationship between *S. acutus* C:N and *D. magna*  $\delta^{15}\text{N}$  values ( $y = 0.15x - 0.10$ ,  $R^2 = 0.96$ ,  $p < 0.001$ ).

C:N) the corresponding  $\delta^{15}\text{N}$  values of the daphnids feeding on this algae increased.

The difference in  $\delta^{15}\text{N}$  values between *D. magna* and *S. acutus* of differing C:N, the diet-tissue isotope fractionation factor ( $\Delta_{dt}$ ), was calculated using distinct algal  $\delta^{15}\text{N}$  values from each run, as well as a single pooled algal  $\delta^{15}\text{N}$  value (because algal  $\delta^{15}\text{N}$  values were not significantly different between runs). Again, the trend of increased  $\Delta_{dt}$ , *D. magna*  $\delta^{15}\text{N} - S. acutus$   $\delta^{15}\text{N}$ , with decreased algal nitrogen content was observed regardless of whether the algal  $\delta^{15}\text{N}$  value used was the pooled value or not.  $\Delta_{dt}$  was strongly correlated with *S. acutus* C:N (run 1  $R^2 = 0.82$ , run 2  $R^2 = 0.99$ , combined run 1 and run 2  $R^2$  with distinct algal  $\delta^{15}\text{N}$  value = 0.78, combined run 1 and run 2  $R^2$  with pooled algal  $\delta^{15}\text{N}$  value = 0.97) (Fig. 4).

*D. magna*  $\delta^{15}\text{N}$  values increased from  $-0.09$  to  $0.36\%$  during 5 d of starvation (Fig. 5). The mean growth rate of the daphnids during this period of starvation was  $-0.003$  (Table 1). However, since this starvation experiment was not replicated, we could not test if these observed differences were statistically significant.

## Discussion

Evidence is accumulating that isotopic fractionation across trophic levels may depend on N availability. Fractionation theory suggests that the  $\delta^{15}\text{N}$  value of a substrate will increase as the reaction continues due to the preferential conversion of the substrate  $^{14}\text{N}$  to product (Owens 1987). However, under highly nitrogen-limited conditions, when all of the substrate is converted to product, mass balance dictates that the  $\delta^{15}\text{N}$  value of the product will be identical to that of the original substrate. Studies concerning the relationship between nitrogen limitation and  $\delta^{15}\text{N}$  values have to date mainly addressed biotic uptake of nitrogen from inorganic pools.

$^{15}\text{N}$  enrichment is already known to vary across trophic levels within the range of 1.3 to 5.3‰ (Minawaga and Wada 1984), though this range represents a collection of different

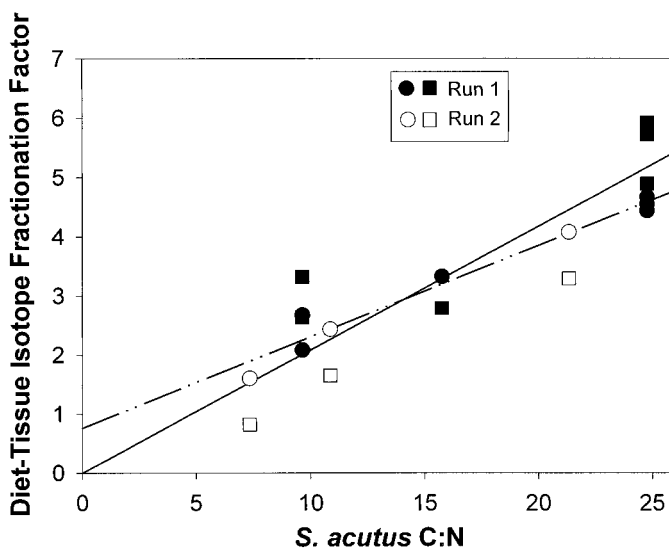


Fig. 4. Relationship between the diet-tissue isotope fractionation factor, ( $\Delta_{dt} = D. magna$   $\delta^{15}\text{N} - S. acutus$   $\delta^{15}\text{N}$ ) and *S. acutus* C:N. Squares are calculated with distinct *S. acutus*  $\delta^{15}\text{N}$  values (solid line  $y = 0.21x - 0.002$ ,  $R^2 = 0.78$ ,  $p = 0.002$ ) and circles are calculated with a pooled *S. acutus*  $\delta^{15}\text{N}$  values (dashed line  $y = 0.15x - 0.76$ ,  $R^2 = 0.97$ ,  $p < 0.001$ ).

consumers feeding on different kinds of foods. To our knowledge, ours is the first study to examine isotopic enrichment in an individual species of metazoan consumer as a function of N availability. In spite of the known variation in  $\Delta_{dt}$ , most studies have adopted a figure of about 3.4‰ as indicative of a single trophic step. Unfortunately, there is little known about the causes of the variation in  $\Delta_{dt}$  across trophic levels, and therefore use of  $^{15}\text{N}$  signatures to assign organisms to trophic positions may be more difficult than is often acknowledged. We would expect some of this variation to be related to the species involved, but our study suggests that even within a single consumer-resource species pair, variation in isotopic enrichment of  $^{15}\text{N}$  can be large.

In animals, increases in  $\Delta_{dt}$  occur due to preferential loss

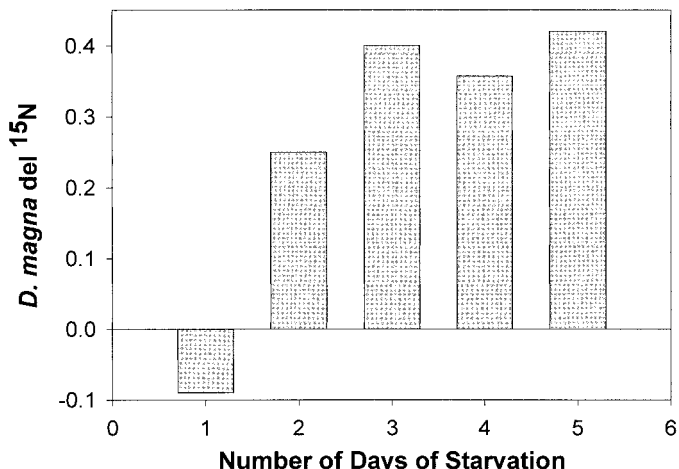


Fig. 5. Relationship between the  $\delta^{15}\text{N}$  value of *D. magna* and the number of days of starvation.

of  $^{14}\text{N}$ .  $^{14}\text{N}$  loss has been shown to increase during periods of both water and nutritional stress (Hobson et al. 1993; Ambrose and DeNiro 1986). Under conditions of water stress, the concentration of excreted urea in mammals increases (Ambrose and DeNiro 1986). Steele and Daniel (1978) found that for cattle, the  $\delta^{15}\text{N}$  value of excreted urea was less than that of the cattle's diet. Therefore, water-stressed mammals may have elevated  $\Delta_{di}$  resulting from increased excretion rates of relatively isotopically light urea. During starvation, N uptake is near zero, but N loss remains, even if at a low rate. Unfed animals have been found to show increasing  $\delta^{15}\text{N}$  values due to the recycling of existing nitrogen as lean body mass is lost without replacement of excreted  $^{14}\text{N}$  (Hobson et al. 1993). Data from our study, although not replicated, support this trend (Fig. 5).

An additional way to eliminate or dramatically reduce N intake is through forcing the animal to consume food very deficient in N, a situation very common in natural ecosystems (White 1993). The range of C:N of the *S. acutus* food stocks used in this study straddles the nitrogen threshold for planktonic cladocerans (i.e., 13) presented by Urabe and Watanabe (1992), and therefore should provide a good experimental range from consumer N deficiency to N sufficiency. Our study between algal food of varying C:N and the planktonic herbivore *D. magna* did generate considerable variation in trophic enrichment of  $^{15}\text{N}$ . The  $\Delta_{di}$  we observed, in fact, would be considered diagnostic of a range in trophic position of this single consumer of almost two full trophic levels.

There was a strong increase in organismal  $\delta^{15}\text{N}$  values with decreased N content (i.e., increased algal C:N) (Figs. 3 and 4), a trend that was highly reproducible between the two independent analytical runs. Greatest isotopic enrichment occurred for animals feeding on the most N-deficient foods. The mechanism for this trend is not clear. However, there are number of plausible hypotheses.

We designed our study to harvest animals at a standard stage, namely, the time of first reproduction immediately following the majority of somatic growth. However, it is impossible simultaneously to standardize all potentially relevant variables. There were differences in body size, clutch size, age, as well as C:N ratios among the animals from the different food types. Individuals raised on LON were smaller and had fewer eggs at the time of harvest than those raised on HIN, with individuals raised on MON being intermediate (Table 1). Increased  $\delta^{15}\text{N}$  values with increased size has been reported for Dover sole (Rau et al. 1981; Spies et al. 1989) though it was not observed in another study on Ontario Lake trout (Kiriluk et al. 1995). Harvested daphnids sampled for our starvation experiment were smaller (Table 1) and had much lower  $\delta^{15}\text{N}$  values than the larger adults harvested from our other experiments (Figs. 3 and 5). Conversely, larger daphnids raised on HIN had lower  $\delta^{15}\text{N}$  values than the smaller daphnids raised on LON. From this study, we cannot determine whether size per se is the explanation for the trends that we observed. Mictic eggs, such as those observed in our study, are generally lower in N content than whole *Daphnia* (Sterner and Schulz 1998). Reproductive *Daphnia* growing on low C:N algae and producing large clutches therefore would be expected to have instantaneous lower N demands than *Daphnia* still putting all of their production

into somatic growth. Thus, differential clutch sizes should not explain the observed trend, but without egg  $\delta^{15}\text{N}$  values it remains a possible explanation.

*D. magna* raised on LON took longer to develop stage 1 eggs and were therefore older at the time of harvest than individuals raised on HIN, and individuals raised on MON were intermediate in age at the time of harvest. Animals with higher  $\delta^{15}\text{N}$  values therefore were older. The question of whether organismal  $^{15}\text{N}$  generally increases with age is debated in the literature (Owens 1987). Spies et al. (1989) and Rau et al. (1981) found a significant increase in  $\delta^{15}\text{N}$  values with increased age of Dover Sole, and Gaebler et al. (1966) observed  $^{15}\text{N}$  enrichment of liver amino acids with age in rats. However, Kiriluk et al. (1995) found no correlation between  $\delta^{15}\text{N}$  values and age of lake trout, and Minawaga and Wada (1984) found no correlation between  $\delta^{15}\text{N}$  values and age in two species of marine mussels. Daphnid  $\delta^{15}\text{N}$  values did increase with increasing daphnid age in both our starvation experiment and our other experiments in which algal C:N was varied (Figs. 3 and 5). We cannot say why the daphnids that were the same age had lower  $\delta^{15}\text{N}$  values in the starvation experiment as compared with daphnids from the other experiments. Our study does not clarify the debate of increasing organismal  $\delta^{15}\text{N}$  values with age. Clearly, the relationship between organismal  $^{15}\text{N}$  and age needs further study.

The algal food fed to *D. magna* should more correctly be considered seston rather than pure *S. acutus* since it inevitably consisted of a bacterial component as well as a detrital component originating from *D. magna* excretion between daily algal and basal COMBO replacement. Macko and Estep (1984) showed an inverse relationship between amino acid C:N and  $\Delta$ , the difference in amino acid  $\delta^{15}\text{N}$  values and bacteria  $\delta^{15}\text{N}$  values. Bacteria grown on aspartic and glutamic acids were enriched in  $^{15}\text{N}$  relative to the substrate. They attribute this relationship to the use, in biosynthetic reactions, of ammonia released after deamination of the substrate leaving an isotopically heavy excreted ammonia pool. Therefore, if the bacteria, which made up an unknown portion of the diet of *D. magna* in this study, varied in isotopic signature among the food types, this could have affected the overall *D. magna* isotopic signature. However, *Daphnia* are relatively unselective feeders (DeMott 1982, 1985). Therefore, our filtered seston samples are most likely a good indication of the daphnids' diet. Additionally, an attempt was made to ascertain the  $\delta^{15}\text{N}$  value of the bacterial component by filtering six 50-ml aliquates of algal food through 3- $\mu\text{m}$  cellulose nitrate filter and then filtering 250 ml of the filtrate through a GF/F filter. The quantity of bacterial nitrogen on the final filter was below the detection limits, which implies that the bacterial component was either not prevalent or was associated with the larger particles.

Finally, the observed trend in our study may result from isotopic enrichment associated with nutritional stress. As available nitrogen decreases, organisms are forced to rely more heavily on internal nitrogenous resources. The preferential excretion of  $^{14}\text{N}$  from this internal nitrogen reserve may result in increased organismal  $\delta^{15}\text{N}$  values with decreased nitrogen availability (increased C:N). This isotopic enrichment associated with the nutritional stress of limited

nitrogen availability may overshadow any increased isotopic enrichment associated with increased nutrient assimilation in the presence of excess nitrogen, thereby causing increased organismal  $\delta^{15}\text{N}$  values with decreased nitrogen availability.

Regardless of the exact mechanism responsible for the observed trend in this study, we have clearly demonstrated that there is a high degree of variability in organismal  $\delta^{15}\text{N}$  values even in studies involving a single consumer-resource species pair. In fact, the results from this study point to a far more complicated process of trophic enrichment in  $\delta^{15}\text{N}$  values than seems to have been previously recognized and may provide some insights into the mechanisms of isotopic fractionation. There is still a great need for further study into the relationship between organismal  $\delta^{15}\text{N}$  values and dietary nutritional content, particularly concerning the relationship between organismal  $\delta^{15}\text{N}$  values and organismal size and age, but this study sheds some light into the importance this relationship has in the future of stable isotopic investigation.

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*Received: 22 March 1999*  
*Accepted: 15 December 1999*  
*Amended: 6 January 2000*