IMPORTANCE OF PREY DERIVED AND ABSORBED NITROGEN TO NEW GROWTH; PREFERENTIAL UPTAKE OF AMMONIA OR NITRATE FOR THREE SPECIES OF UTRICULARIA

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ABSTRACT

The rootless, aquatic, submerged, carnivorous plants in the genus *Utricularia* are useful subjects in determining the relative importance of organic and dissolved inorganic nitrogen (DIN) to new growth, measured in length, number of bladders, and number of side branches, using enriched ^{15}N as a label. *Utricularia gibba*, *Utricularia geminiscapa*, and *Utricularia vulgaris* were used as subjects, but none of these three species grew differently while treatment varied. Nonetheless, each species displayed new growth differently from each other. *U. gibba* grew 23.8% longer than *U. vulgaris*. *U. geminiscapa* grew 38.2% more side branches than *U. gibba*, and 30.9% more side branches than *U. vulgaris*. *U. vulgaris* grew 7.2% more side branches than *U. gibba*. In the DI^{15}N treatment, *U. geminiscapa* incorporated the most ^{15}N; in the organic ^{15}N, treatment *U. vulgaris* incorporated the most ^{15}N. Rates of uptake were compared for NH_4^+ and NO_3^-. *U. geminiscapa* had the most negative slope for NH_4^+ uptake, and the least negative slope for NO_3^- uptake, while *U. vulgaris* had the least negative slope for NH_4^+ uptake and the most negative slope for NO_3^- uptake. In general, uptake of DIN may be preferential to NH_4^+ rather than NO_3^- for *U. gibba* and *U. vulgaris*, while equal preference is given by *U. vulgaris*, however statistical analysis on preferences shows that widespread variability casts doubt as to the believability of these data.

Keywords: *Utricularia*, nitrogen uptake, growth, ^{15}N, isotopic labeling, carnivorous plants

INTRODUCTION

Carnivorous plants absorb nutrients from dead animals adjacent to their surfaces and thus obtain increased fitness and furthermore must have some morphological, physiological, or behavioral feature to attract, capture, and/or digest prey (Givnish *et al*. 1984; Richards, 2001). Aquatic carnivorous plants in the genus *Utricularia* encompass more than 250 species, and are distributed throughout tropical and temperate climates (Meyers and Strickler, 1979). They grow in sunny, moist, nutrient-poor environments, as nutritional benefits of carnivory would decrease in photosynthetically limited environments (Givnish *et al*, 1984). *Utricularia* are rootless hydrophytes, living suspended in the water column, growing along a main axis, and dying away behind as new growth appears at the tips. *U. geminiscapa* and *U. vulgaris* grow side branches, while *U. gibba* does not. Each axis of *U. vulgaris* produces alternate, finely divided, four-lobed leaves in a linear age sequence (Friday and Quarmby, 1994).

Carnivory

Aspects of plant carnivory have been widely studied. *Utricularia*, as a carnivorous plant, has been classified as a true predator, utilizing a sit-and-wait approach to predation (Harms, 1999). Filamentous structures, originally labeled antennae and bristles by Charles Darwin, are used to help guide prey to the bladders, as they resemble the filamentous algae that the prey eat (Meyers and Strickler, 1979; Friday, 1991).

Bladders maintain a negative hydrostatic pressure, by a watertight seal, which is released by contact with trigger hair (Friday,1991). The trap door rapidly opens, the bladder expands, and water and prey flow into the bladder, and the doors shuts. The trap is reset, returning to negative hydrostatic pressure by withdrawing ions and water from the lumen. (Meyers and Strickler, 1979; Friday,1991; Knight and Frost, 1991). For *U. vulgaris*, traps are able to be reset in 15-40 minutes multiple times, but are only able to trap for the first 10-19 days of their 30 day life cycle, losing effectiveness after just 4-6 days. (Friday, 1989, 1991).

While bladders do pose a cost to the overall production of the plant, since bladders do not photosynthesize as well as leaves (Knight, 1991; 1992), carnivory helps overcome nitrogen deficiency at low substrate concentrations of nitrogen and help overcome deficiencies of other nutrients at higher nitrogen levels (Jobson *et al*, 2000). Prey, including rotifers, chydorid cladocerans, copepods and insect larvae (Friday and Quarmby, 1994) are assimilated quickly into new growth. Roughly 30% of 15N labeled mosquito larvae has been shown to appear in immature plants within 2 days (Friday and Quarmby, 1994).

Isotopic labeling with ^{15}N provides a method to distinguish nutrients derived from different sources. It can also be used to measure foliar uptake and use in *Utricularia,* as known quantities of nutrients can be supplied precisely to individual leaves or bladders. (Friday and Quarmby, 1994). ^{15}N has been used previously to study nitrogen uptake in non-carnivorous hydrophytes such as *Myriophyllum spicatum* and to follow the fate of prey-derived nitrogen in terrestrial carnivorous plants such as *Drosera erythrorhiza* (Friday and Quarmby, 1994).

Dissolved nutrients

Dissolved nutrients may also be taken up through absorption over the entire shoot surface, including bladders, but *Utricularia* do not have access to the sediment nutrient pool due to the rootless, free-floating nature of the plants (Friday, 1992, 1994). The relative importance of absorption of nutrients to new growth, compared to carnivory has not been as well studied. Isotopic labeling of DIN may prove to be useful in comparing each relative importance to growth.

Given these observations, the following questions are pertinent:

1) Does source of nutrient, either through carnivory or through DIN, have an effect on new growth in *Utricularia*, in terms of length, number of new bladders, or number of new side branches, thus demonstrating a reinvestment in carnivory or surface area for absorption? If so, to what extent?
2) Are there varying degrees of this effect on new growth between different species of *Utricularia*, based upon species preference to or investment in carnivory or absorption of dissolved nutrients?

In this paper, new growth comparisons of three species of *Utricularia*: *U. gibba*, *U. geminiscapa*, and *U. vulgaris*, are made as the source of isotopically labeled nitrogen is varied between carnivory and absorption. It is important to note that in carnivory, organic nitrogen is found in amino acids, while dissolved inorganic nitrogen is provided in the form of KNO_3.

Uptake

While carnivory has been the focus of many studies on *Utricularia*, uptake of DIN has been less well studied in general. As mentioned, absorption of nutrients is possible over the entire living plant surface (Friday, 1992). Therefore it seems necessary that nutrient uptake would vary based along water chemistry of the habitat in which *Utricularia* grows and the concentrations of various forms of DIN which are found within.

Based upon this observation, two questions arise:

1) To what extent is there a preference by *Utricularia* for NH_4^+ or NO_3^-, both forms of DIN?
2) Does this preference vary between the species *U. gibba*, *U. geminiscapa*, and *U. vulgaris*?

One hypothesis would be that there is a strong preference to uptake NH_4^+ by all species of *Utricularia*, and that this preference does not vary significantly between species. This hypothesis is based on the observation of uses and energy yields of NH_4^+ and NO_3^- by organisms for biological processes such as respiration (Jorgensen, 1980).

MATERIALS AND METHODS

Three species of *Utricularia* were used in new growth and DIN uptake experiments: *U. gibba*, *U. geminiscapa*, and *U. vulgaris*. *U. gibba* was collected at Lake Bomoseen, VT on November 3, 2001; *U. geminiscapa* and *U. vulgaris* were collected at Johns Pond, MA on October 30, 2001. Both lakes are freshwater, oligotrophic systems. Because collection occurred during the fall, turions were observed on several of the plants, no flowers were present, and species identification of *U. geminiscapa* and *U. vulgaris* was difficult and not entirely certain. Upon collection, plants were separated by species, kept in water collected from Johns Pond, and stored at 18^{o}C in an environmental chamber set for 16:8 light dark cycle for 30 d.

Growth Experiment

Individual plants were chosen for the experiment based on the presence of a growing apical meristem, bladders, a simplistic architectural structure, and similar lengths. Morphological details including length, number of bladders, and number of side branches were recorded before and after the 8 d experiment. Individual plants were grouped according to size and then assigned to treatments according to stratified random arrangement, with the exception that each individual in the zooplankton treatment was checked to make sure bladders were present. Each individual was kept in a 140 ml container with 100 ml filtered Johns Pond water for the duration of the experiment (n=4 for treatment, all species). Plants were maintained under a Gro-lux light with a 12:12 light-dark cycle.

Enriched Organic Nitrogen Treatment

Daphnia, parthnogenic crustaceans, were chosen because they are among the natural prey of *Utricularia*, reproduce quickly and are easy to culture in the laboratory. Less than 3 d old individuals were used because it is believed they would incorporate more labeled nitrogen than adults. *Scenedesmus*, a four celled green algae, was cultured for 7 d (Stein, 1973; Kirsop and Doyle, 1991) in Carolina Freshwater Alga-Gro, pH 7.8, modified with 1.0 mg l^{-1} 10 at % enriched $^{15}NH_4Cl$ and 650 uM 10 at% $K^{15}NO3$. Culturing allowed *Scenedesmus* to take up labeled nitrogen before feeding to *Daphnia magna*. *Scenedesmus* was chosen because *Daphnia* prefer to eat this type of algae to others (Repka, 1997). Algae were replenished every second day for 8 d and *Daphnia* were allowed to reproduce. Less than 3 d old individuals were placed in 95% ETOH, then rinsed 6 times in fresh pond water. These were then fed to *U. gibba* and *U. geminiscapa* by bringing the individuals just under the surface of the water, being careful to keep plants, especially bladders, under water at all times. *Daphnia magna* were placed inside mature, pre-triggered traps using a dissecting microscope and fine forceps. The bladders on *U. vulgaris* were too small to be manipulated, so a paste of *Daphnia magna* was made from several individuals, pressed in 0.5 ml pond water after placing in 95% ETOH and rinsing 6 times in pond water. 1 cc of this paste was then dripped onto *U. vulgaris* individuals in 100 ml pond water using a 1 cc syringe.

DI^{15}N Treatment

Excess DI^{15}N was provided by creating a solution where 1.825 ml 14.2 mM 10 at % enriched $K^{15}NO3$ solution was added to 98.175 ml non-enriched 14.2 mM KNO3 solution to provide enrichment of 500 per mil. 1 ml of this solution was added to 100 ml of pond water. Nitrate was used because it is less volatile than NH_4^+ and will not disperse into nor is absorbed from the atmosphere. Therefore, the quantity of NO_3^- can be more precisely regulated. *Utricularia* do not compete with microbes in the pond water for access to NO_3^-, while NH_4^+ is utilized by microbes as electron acceptors for several metabolic processes, including respiration in the absence of oxygen (Jorgenson, 1980).

Control Samples

For each species, four individuals were each placed in 100 ml filtered pond water with no added enrichment, and held under the same conditions as described above. This tests the amount of new growth that occurs for each species due to photosynthesis with only background levels of NH_4^+ and NO_3^- available.

Preparation for 15N Analysis

Algae grown in enriched media were sampled and filtered onto ashed GF/F filters and washed with DI water. The filter was dried for 24 h and analyzed for $^{15}N/^{14}N$ abundance (d^{15}N). Five less than 3 d old *Daphnia magna*, cultured in *Scenedesmus*, were placed in 95% ETOH, and rinsed 6 times in tap water. *Daphnia* were placed on an ashed GF/F filter and dried for 48 h before being sent for 15N analysis. After the growth experiment ran for 8 d, morphological detail was recorded, including total length, number of bladders, and number of side branches. Within each treatment for all three species, the four replicas were combined onto one ashed GF/F filter and placed into scintillation vials to create one sample per treatment for ^{15}N analysis. Samples were then dried for 48 h and analyzed. Individuals under the *Daphnia* treatment were stripped of old bladders so that only light, bright green traps remained on the plant (Sheldon, personal communication). This was done to avoid measuring the d^{15}N value of the *Daphnia* in addition to, or instead of the value of the plant itself. All samples were analyzed for d^{15}N by Marshall Otter at the Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.

Uptake Experiment

For each of the three species, 0.12 g of plant material were placed in 100 ml of 17 uM NO_3^- and 17 uM NH_4^+ solution in 100 ml beakers, covered with transparent plastic wrap to prevent volatilization and uptake of NH_4^+ from the atmosphere, and then placed under Gro-lux lighting (n=3). Three controls were made, containing only the NH_4^+ and NO_3^- solution and no plants. Samples for initial concentrations of both NO_3^- and NH_4^+ were taken from the stock solution and used for all replicates of all species. A total of 15 ml were withdrawn from each beaker at 2 h, 4 h, 6 h, and 12 h. 5 ml were placed in a scintillation vial and frozen until analyzed for NO_3^-, while 10ml were placed in a separate scintillation vial, acidified with 10 ul 5N HCl and frozen to preserve NH_4^+ for analysis. NO_3^- was analyzed using a LACHAT Flow Injection Analyzer using methods adapted from Wood, *et al*. (Wood *et al*., 1967). 3 ml samples were analyzed for NH_4^+ (n=3) with a Shimadzu 1601 Spectrophotometer using methods modified from Strickland and Parsons (Strickland and Parsons, 1972) and Solarzano (Solarzano, 1969).

RESULTS

Growth

Morphological Details

Measurements were made for total length, number of bladders, and number of side branches before and after the 8 d growth experiment, where source of enriched nitrogen was varied. Increased length, numbers of bladders, or side branches was considered new growth. Plants grew 7.6 to 31.4% in length over the experiment, showed 37.8 to 60.9% new bladders, and 58.5 to 96.6% new side branches (Table 1).

Differences in growth patters were seen across species. *U. gibba* grew 23.8% longer than *U. vulgaris* (Tukey HSD, $p < 0.003$). *U. geminiscapa* grew 38.2% more side branches than *U. gibba* (Tukey HSD, $p < 0.001$), and 30.9% more side branches than *U. vulgaris* (Tukey HSD, $p < 0.009$). *U. vulgaris* grew 7.2% more side branches than *U. gibba* (Tukey HSD, $p < 0.003$). Meanwhile there was a trend for *U. vulgaris* to grow 6.9% more bladders than *U. gibba* (Tukey HSD, $p < 0.156$). Nonetheless, there was no significant difference of new growth across treatments within a species.

Table 1: Morphological Details showing initial, final and percent change in length, number of bladders and number of side branches.

	U. gibba	*U. geminiscapa*	*U. vulgaris*
Mean initial length (mm) ± SD	39.9 ± 10.8	42.7 ± 12.0	36.8 ± 16.4
Mean final length (mm) ± SD	61.5 ± 22.4	57.0 ± 20.3	39.3 ± 14.2
Mean percent new length (mm) ± SD	31.4 ± 16.5	22.3 ± 11.7	7.6 ± 14.2
Mean initial # bladders ± SD	4.8 ± 1.7	9.3 ± 4.5	11.6 ± 11.1
Mean final # bladders ± SD	8.3 ± 3.9	25.1 ± 9.3	22.7 ± 11.2
Mean percent new bladders ± SD	37.8 ± 16.3	60.9 ± 17.4	44.8 ± 45.0
Mean initial # side branches ± SD	1.0 ± 1.6	0.3 ± 0.5	2.0 ± 1.4
Mean final # side branches ± SD	2.8 ± 1.9	5.8 ± 2.0	5.8 ± 1.8
Mean percent new side branches ± SD	58.5 ± 42.9	96.6 ± 6.2	65.7 ± 18.7

^{15}N Incorporation

d^{15}N analysis was performed on samples of *Scenedesmus*, less than 3 day old *Daphnia magna*, and treated plant samples for each species of *Utricularia*, as mentioned above. Enriched *Scenedesmus* displayed a d^{15}N value of 2234 $^{o}/_{oo}$ vs. atmospheric N_2, while the enriched *Daphnia magna* sample showed a d^{15}N value of 731.7 $^{o}/_{oo}$ vs. atmospheric N_2. Comparing species of *Utricularia* for the DI^{15}N treatment, *U. geminiscapa* showed 33.9% higher d^{15}N value than *U. vulgaris* and 28.0% higher d^{15}N value than *U. gibba*, while *U. gibba* showed 8.2% more ^{15}N than *U. vulgaris* (Figure 1). The *Daphnia* treatment showed that *U. vulgaris* incorporated 96.8% more ^{15}N than *U. gibba*, and 45.7% more ^{15}N than *U. geminiscapa*, while *U. geminiscapa* exhibited 94.1% more ^{15}N than *U. gibba* (Figure 1). Statistical analysis was not performed on these data due to small sample size (n=1) for each species.

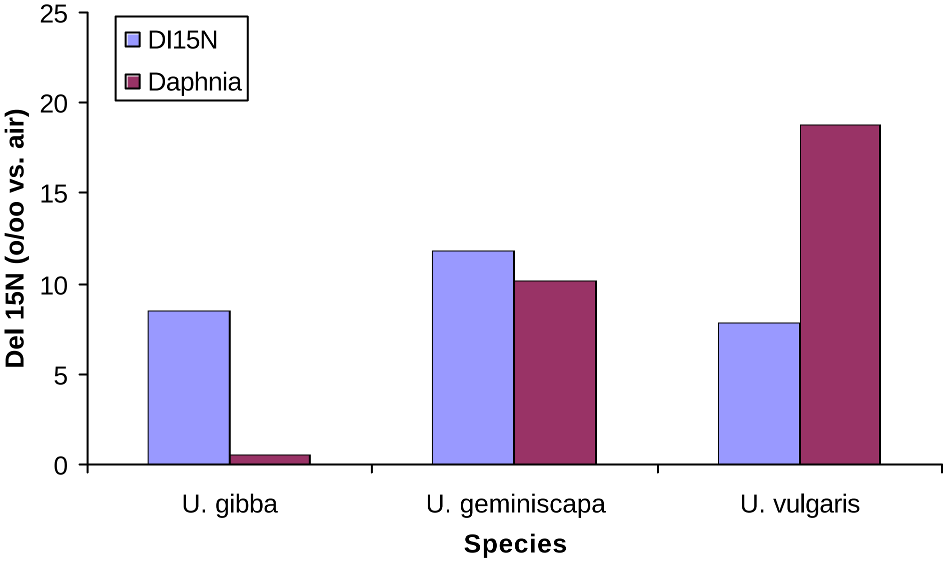
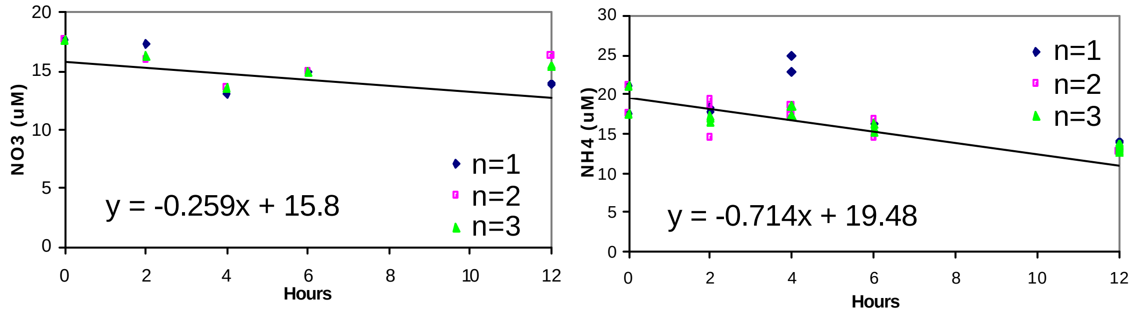


Figure 1: d^{15}N ($^{o}/_{oo}$ vs. atmospheric N_2) Incorporation of Utricularia across species and treatment.

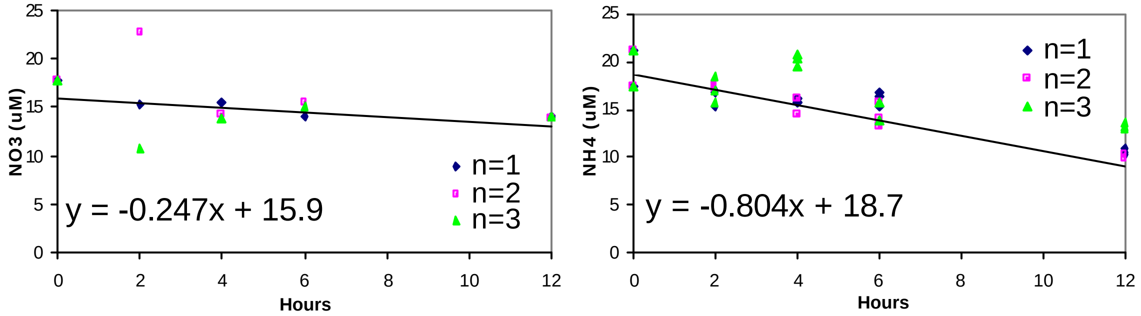
Uptake

Analyses of NO_3^- and NH_4^+ concentrations were performed, as described above, on the 15 ml samples taken from the solution of DIN used in the Uptake experiment. concentrations for both NO_3^- and NH_4^+ were plotted against time, and a resulting decreasing slope can be seen for both forms of DIN as they are taken up by all species of plants (Figure 2). A comparison of NH_4^+ and NO_3^- slopes within each species shows that NH_4^+ slopes were more negative than NO_3^- for *U. geminiscapa*, by 69.3%, and *U. gibba*, by 63.7%, but *U. vulgaris* showed that NO_3^- had a 1.3% more negative slope than NH_4^+. Comparing NH_4^+ slopes across species shows that *U. geminiscapa* ($p < 0.001$) shows a 44.0% more negative slope than *U. vulgaris* ($p < 0.005$), and an 11.2% more negative slope than *U. gibba* ($p < 0.001$), while *U. gibba* shows a 37.0% more negative slope than *U. vulgaris*. Comparing the slopes of NO_3^-, the slope of *U. vulgaris* is 45.8% more negative than *U. geminiscapa*, and 43.2% more negative than *U. gibba*, while *U. gibba* is 4.6% more negative than *U. geminiscapa*.



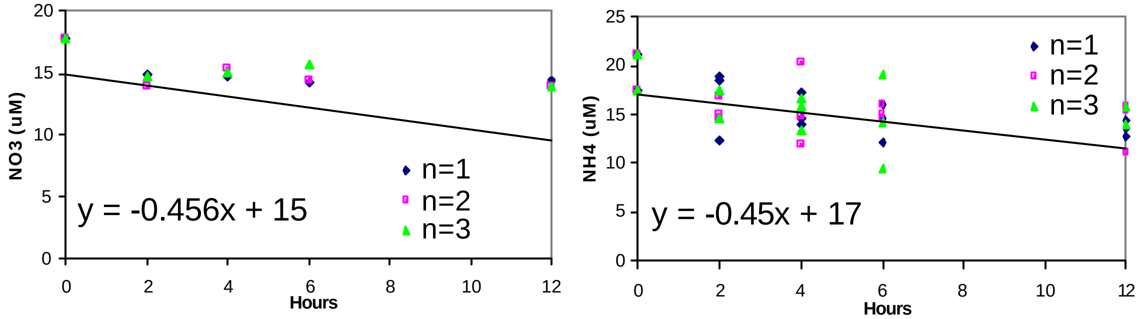
NO3 Concentration (uM) over 12 h while taken up by U. gibba

NH4 Concentration (uM) over 12 h while taken up by U. gibba



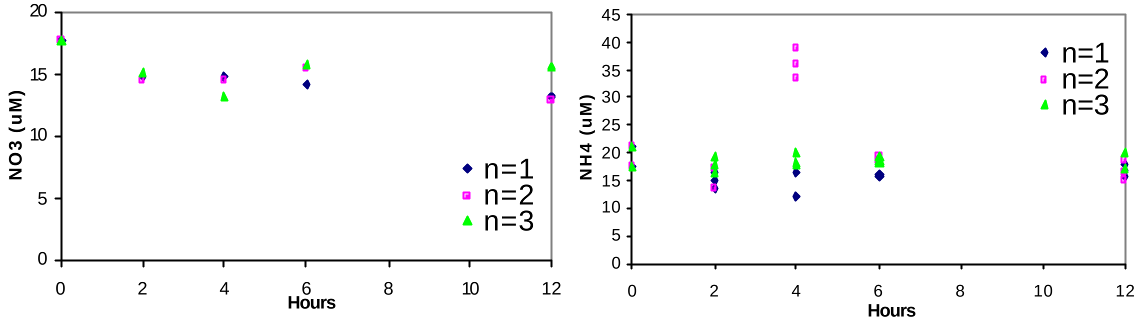
NO3 Concentration (uM) over 12 h while taken up by U. geminiscapa

NH4 Concentration (uM) over 12 h while taken up by U. geminiscapa



NO3 concentration (uM) over 12 h while taken up by U. vulgaris

NH4 Concentration (uM) over 12 h while taken up by U. vulgaris



Controlled NO3 concentration (uM) over 12 h, without plant material

Controlled NH4 concentration (uM) over 12 h, without plant material

Fig. 2: Concentrations of NO_3 and NH_4 (uM) over the 12 h Uptake experiment for each Utricularia species. The NH4 graphs do not include concentrations calculated from incomplete reactions due to analytical error.

Relative Preference Index

A relative preference index (RPI) can measure the degree of preferential selection between NH_4^+ and NO_3^- for *Utricularia*. Due to the widespread variability in the data collected, however, an index calculated as such may not be thoroughly accurate. Nonetheless, as an exercise, such an index is presented here to provide a rough estimate of relative preference. Such an index would be calculated as the ratio of the slopes describing the declining concentrations of NH_4^+ to NO_3^- according to the following formula (Deegan, personal communication). A RPI = 1 would indicate equal preference between NH_4^+ and NO_3^- while a RPI > 1 would indicate a preference for NH_4^+, and a RPI < 1 would indicate a preference for NO_3^-.

$$\frac{slope NH_4^+ uptake}{slope NO_3^- uptake}$$

Calculations based upon this equation show that *U. gibba* (RPI = 2.8) and *U. geminiscapa* (RPI = 3.3) clearly prefer NH_4^+ to NO_3^-, while *U. vulgaris* (RPI = 1.0) demonstrates an equal preference for both NH_4^+ and NO_3^-. (Figure 3) *U. geminiscapa* has a 15.2% greater RPI than *U. gibba* and a 69.7% greater NH_4^+ preference than *U. vulgaris*, while *U. gibba* has a 64.3% greater NH_4^+ preference than *U. vulgaris*. None of the three species was seen to prefer NO_3^- to NH_4^+.

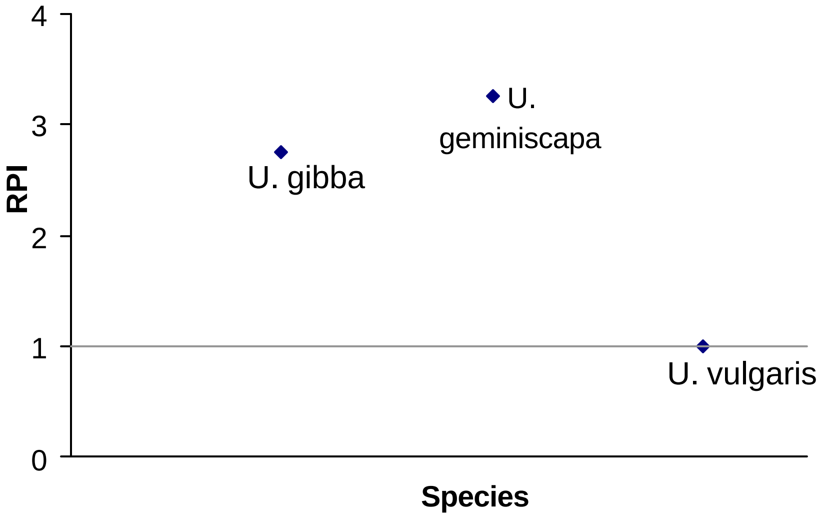


Figure 3: Relative Preference Index of NH_4^+ and NO_3^- for three species of *Utricularia*.A RPI = 1 would indicate equal preference between NH_4^+ and NO_3^- while a RPI > 1 would indicate a preference for NH4+, and a RPI < 1 would indicate a preference for NO3-.

DISCUSSION

Growth

While significant differences in growth patterns were seen across species, they were not significant across treatment within species. This suggests that while there may be physiological differences in growth patterns, new growth in terms of length, number of bladders and number of side branches is not significantly affected by the source of nitrogen, either through DIN or carnivory. Thus, it does not seem that either method of taking up nitrogen creates a specific reinvestment pattern of nitrogen for the plant any different from photosynthesis, measured by the control.

One might theorize that the different manifestations of new growth as exhibited by each species, may demonstrate a preferential allocation of nitrogen to specific types of new growth. For instance, *U. geminiscapa* may be showing a preference for growing side branches in early development rather than bladders or new growth along the main axis, as it grew significantly more side branches than either *U. gibba* or *U. vulgaris*. Furthermore, there was a trend where *U. vulgaris* grew more bladders than *U. gibba*. The rate of bladder growth for *U. vulgaris* is consistent with literature values for increase in the number of bladders at the beginning of the growing season (Friday, 1992), despite the different life histories of the plants.

Life histories of individual plants are important to development and results of new growth. All plants for this experiment were collected in the fall, and after incubation for 30 days, developing shoots were chosen as experimental units. Preferences for allocation of nutrients into new growth will change as the plant develops and will continue to vary seasonally as the plant continues to grow. (Friday, 1992) Potential nutrient allocation experiments through the development of *Utricularia* might be a worthwhile path of exploration in the future. Were this experiment to be redone, collection of plants during the summer growing season, with a larger sample size, would be important to provide for a greater number of larger bladders for each species, in addition to allow for greater maturity of plants. This maturity however, might produce data different from that obtained through this experiment. The larger sample size might decrease the variability somewhat.

d^{15}N incorporation became smaller as labeled nitrogen was transported from the *Scenedesmus* to the *Daphnia magana* to the *Utricularia*. This effect is a result of ecological efficiencies of nitrogen incorporation and the proportion of new growth in the prey that had taken up the labeled nitrogen. Additionally, some amount of labeled nitrogen was lost since smaller amounts of nitrogen are needed for amino acids than the concentration available. While incorporation from DI^{15}N did not seem to vary across species greatly, incorporation from carnivory varied dramatically (Figure 1). Furthermore, it appears that each species demonstrated different preferences in incorporation of ^{15}N: *U. gibba* preferring DI^{15}N, *U. geminiscapa* showing a slighter preference for DI^{15}N and *U. vulgaris* greatly preferring carnivory. d^{15}N values shown here were inconsistently larger than natural abundance levels of d^{15}N (8.2 $^{o}/_{oo}$, 8.3 $^{o}/_{oo}$, 8.9 $^{o}/_{oo}$ vs. atmospheric N_2) for *U. vulgaris* and for non-carnivorous plants (7.4 $^{o}/_{oo}$ vs. atmospheric N_2) located near collection sites of *Utricularia* (Sheldon, personal communication), casting shadow on the validity of these data. These inconsistencies may be due to plant history, stage of development, the small size of the traps, or species variability. Due to cost constraints, the four individual plants in each treatment were combined into one sample and so no statistical analyses can be performed on this data. In the future, with a larger budget, replicate samples may be analyzed and an understanding of the variances within and across species and treatments might be able to be understood. Comparisons between data from this experiment and plants with different life histories should be able to be made, as there is no significant variation of d^{15}N with age for *U. vulgaris* (Friday and Quarmby, 1994).

Uptake

Overall, concentrations of both NO_3^- and NH_4^+ decreased over time, while the rates of decline were higher for NH_4^+ than NO_3^-, consistent with the hypothesis presented, suggesting preferential uptake of DIN in the form of NH_4^+ by all three species of *Utricularia*. Contrary to the hypothesis, there were differences between species for NH_4^+ and NO_3^-. *U. geminiscapa* had the most negative slope for NH_4^+ and the least negative slope for NO_3^-, suggesting that it had the strongest preference for NH_4^+ of the three species. Meanwhile, *U. vulgaris* showed the most negative slope for NO_3^- and the least negative slope for NH_4^+, showing that it preferred NO_3^- the most for the three species. Note that while there is a decrease in DIN concentration over time, resulting in a negative slope, uptake is not negative over the length of the experiment.

Upon replication of this experiment, another calculation might prove to be useful. While RPI for this experiment was calculated from the rates of decreasing DIN concentrations, it can also be calculated from specific concentrations at any time, and plotted against the sum of each form of DIN to show preference of plants over time with varying concentrations, ranging from minimal to excess, of each form of DIN. McCarthy *et al*. (1977) use an equation that can be used to calculate an RPI from specific points of concentration. Their index is the ratio of the fraction of all the utilized (?) form of nitrogen to the fraction of all available nitrogen in that form (McCarthy *et al*, 1977). As with the RPI calculated in this experiment, a RPI = 1 would indicate that uptake is equal to availability and a RPI > 1 would indicate preference for that form of nitrogen.

$$\frac{\left(\dfrac{rNH_4^+}{rNH_4^+ + rNO_3^-}\right)}{\left(\dfrac{[NH_4^+]}{[NH_4^+]+[NO_3^-]}\right)}$$

This equation has the advantage of allowing a comparison of preferences while alleviating the necessity of beginning with equal concentrations of each form of DIN. Thus, preference can be determined in the field, where water chemistry across habitats does not hold to constant conditions.

Potential Sources of error

Despite careful procedures and measurements, there are several potential sources of error, which may have affected the results of the growth and uptake experiments. Because plants were collected in the fall, daylight hours were noticeably decreasing, and plant metabolism and growth was generally slowing down. Turion growth was quite noticeable on some plants. As a result, plants were maintained in a growth chamber for 30 d with 16:8 light dark cycle until taken for experimentation. Plants in both experiments may have received too much light to predict normal or generalized results for *Utricularia*. Individual plants for both experiments were placed roughly 0.5 m away from a Gro-Lux light on a 12:12 light cycle. In the natural environment, *Utricularia* suspend near the bottom of the water column, and receive much less light, as light decays exponentially according to Beer's Law (Friday, 1992, 1994). Because of these unnatural conditions, growth may have been encouraged more than in the natural environment. In addition to lighting concerns, the addition of excess DIN (both enriched and natural ^{15}N abundance) and prey may show results that do not reflect natural occurrences in the environment, but rather how these plants may behave under these specific conditions.

Note that *U. vulgaris* bladders for the Growth experiment were especially small, despite the time spent in the environmental chamber, and so the paste of *Daphnia magna* was used as the source of prey-derived labeled nitrogen. However, the growth and isotope data may not reflect direct carnivory, but rather the absorption of dissolved organic nitrogen in the form of amino acids.

Normally, during the process of NH_4^+ analysis, addition of reagents results in blue coloration (Wood *et al.*, 1967), but two reagents ran out during uptake NH_4^+ analysis, and air bubbles prevented the reaction from going to completion for several samples. Incomplete reactions result in a greenish color. Data from these incomplete reactions has been thrown out, as resulting data are consistently lower than expected for a compete reaction. Additional testing, with increased number of repetitions and sample measurements may help to decrease variability for both NH_4^+ and NO_3^-.

Unfortunately, the control samples for both NH_4^+ and NO_3^- in the Uptake experiment have a decreasing slope. This puts the validity of the experiment at risk, suggesting that there is loss of DIN through some means besides uptake, as no plant material was placed in control beakers. Volatilization of NH_4^+ and NO_3^- may account for a slope in these control samples. Furthermore, anomalous data, twice as high as expected, were found at t = 4h for the control NH_4^+ samples. These data may be due to analytical error, exposing samples to twice the required amount of reagents in an attempt to compensate for air bubbles or other malfunctions. Container contamination may also be a factor, as scintillation vials were last acid washed September, 2000 (Tholke, personal communication). Replication of the experiment with additional replications and measurements of samples might help to decrease the amount of variability seen in the data.

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