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Ammonium uptake by seagrass epiphytes: Isolation of the effects of water velocity using an isotope label

Abstract—In a series of flume experiments, ^{15}N-labeled ammonium was used to isolate the effects of water velocity on ammonium uptake by epiphytes from that of an assemblage of organisms that included seagrass leaves, epiphytes, and phytoplankton. Rates of NH_4^+ uptake for epiphytes, seagrass leaves, and the total assemblage were dependent on water velocity. Ammonium uptake rates for epiphytes, normalized to chlorophyll *a*, increased by an order of magnitude (0.65 to 6.8 $\times 10^{-8}$ g N removed [mg Chl *a*]$^{-1}$ s^{-1}) over a range of velocity (0.02–0.20 m s^{-1}) and were correlated to uptake by the entire assemblage. The relationship between NH_4^+ uptake and velocity for the epiphytes was within the range expected for mass-transfer limited uptake, which suggests that water flow strongly influences NH_4^+ uptake by this important component of seagrass communities. Our results demonstrate that isotopically labeled nutrients can be used to isolate the effects of water velocity on rates of nutrient uptake by an individual component of a community and to evaluate how uptake rates for the component compare to those of the community as a whole.

Rates of nutrient uptake by benthic organisms can influence important ecological processes (i.e., photosynthesis and calcification) and can be strongly mediated by water flow and boundary layer characteristics adjacent to uptake surfaces (Patterson 1992). If uptake is controlled by rates of diffusion of nutrients through the diffusive boundary layer (physically limited), then enough uptake sites or metabolic enzymes are present to take up all of the nutrients that are delivered to an organism's surface. In this case, an increase in water velocity reduces the thickness of the diffusive boundary layer, leading to a higher rate of nutrient uptake. For individual organisms, water velocity is positively correlated to nutrient uptake by algae (e.g., Gerard 1982; Hurd et al. 1996) and nutrient-dependent processes, including photosynthesis in algae (e.g., Wheeler 1980; Koch 1993) and seagrasses (Koch 1994) and photosynthesis and calcification in corals (Dennison and Barnes 1988; Patterson et al. 1991). Rates of nutrient uptake have also been shown to be dependent on water velocity for assemblages of algae (Larned and Atkinson 1997) and coral (e.g., Bilger and Atkinson 1992; Thomas and Atkinson 1997) and for naturally occurring seagrass beds (Thomas et al. 2000).

In a recent study by Thomas et al. (2000), ammonium uptake by seagrass communities was shown to be dependent on water velocity and the morphology of the canopy. In their experimental approach, the seagrass community is viewed as a single entity whose rough surface influences the flow of water over the benthos, which in turn influences nutrient uptake by the whole community. However, seagrass communities are composed of a diverse assemblage of organisms that remove nutrients from the water column, including seagrass plants, epiphytes attached to the seagrass leaves, and phytoplankton. Although Thomas et al.'s data provide estimates of whole community uptake, they do not provide information about the kinetics of nutrient uptake for individual components of the community. These components vary in their morphology, physiology, and location relative to the canopy. Therefore, water flow may have variable effects on the different components of a seagrass community, which in turn may collectively contribute to the community scale response quantified in Thomas et al. (2000).

In the present study, we isolate the effects of water velocity on uptake of an important nutrient (NH_4^+) by a single component of seagrass communities (epiphytes) while it is situated within an assemblage of seagrass leaves. By use of an isotope-labeling approach, rates of NH_4^+ uptake for epiphytes are measured while they are attached to the host plant in order to obtain ecologically relevant estimates of NH_4^+ uptake kinetics for epiphytes. Epiphytes play an integral role in the ecology of seagrass communities, including food web dynamics (e.g., Fry and Parker 1979) and nutrient cycling (e.g., Harlin 1973; McRoy and Goering 1974). In addition, epiphytes are a major contributor to the overall productivity of seagrass meadows (e.g., Moncreiff et al. 1992) and are considered an important factor influencing the distribution and abundance of seagrasses (Kuo and McComb 1989). Although the important roles of epiphytes in seagrass communities are well documented, few data are available on the nutrient uptake kinetics of epiphytes and their contribution to total dissolved inorganic nitrogen (DIN) inputs to seagrass communities (Hemminga et al. 1991). Our study is intended to further our understanding of the factors that influence nutrient uptake by epiphytes by describing the effects of water velocity on NH_4^+ uptake and how uptake by epiphytes relates to the nutrient dynamics of the community as a whole.

Isotope labeling approach—To separate nutrient uptake by epiphytes from other components of the community (i.e., seagrass plants and phytoplankton), we used ^{15}N-labeled NH_4^+ to trace uptake from the water column into the epiphytes attached to seagrass leaves. Earlier studies have demonstrated the effectiveness of using isotope labels in understanding relationships between epiphytes and their host plant (e.g., Harlin 1973; McRoy and Goering 1974; Johnstone 1979). In addition, numerous studies have used isotope labels to partition nutrient uptake among community components and understand nutrient cycling in both freshwater (e.g., Pelton et al. 1998; Eriksson 2001; Hamilton et al. 2001) and marine systems (e.g., Winning et al. 1999; Koop et al. 2001). In our experiments, we expanded this application of isotope labels to isolate the effects of water velocity on NH_4^+ uptake by a single community component while it was situated in a complex assemblage of organisms.

To assess the effects of water velocity on rates of NH_4^+

uptake, ^{15}N accumulation in epiphyte tissues and the total uptake of NH_4^+ from the water column by all organisms combined was measured in 14 flume experiments conducted over a range of velocity (0.02–0.20 m s^{-1}). This range was chosen to best represent ambient water velocity observed at the site. The flume (volume 180 L) was of a racetrack design and was transported into the field and placed on a table along the shore for experiments. An electric trolling motor housed in a drop box at one end of the flume imposed controlled, unidirectional flow. Experiments were conducted at Emerson Point Park located at the mouth of the Manatee River in Southwest Tampa Bay on the west coast of Florida. Seagrass leaves (*Thalassia testudinum*), with epiphytes attached, were collected from one of two donor beds (sites) located close to shore and transplanted into the flume. The two sites were ~150 m apart on either side of Emerson Point. Six experiments using leaves from donor site 1 were conducted on 7–8 December 1999, 29 March 2000, and 4 April 2000, and eight experiments using transplants from donor site 2 were completed on 8–9 December 1999 and 3, 7, and 21 November 2000. On each day, experiments were completed within 1 h of each other and between 1100 and 1500 h. The specific velocity for each experiment and the order of low- and high-velocity experiments was randomized.

The flume size was minimized to allow for detection of nutrient uptake from the water column, and flume dimensions allowed for only the top 10–13 cm of individual leaves to be used in experiments. Because seagrass leaves in the donor beds were only 12–15 cm in length, the severed leaves included most of the blade and attached epiphytes. New and senescing leaves were not used in the experiment because of limited epiphyte growth on new leaves and the difficulty in cleanly removing epiphytes from senescing leaves. Leaves ($n = 150$) were transplanted to a removable Plexiglas floor (0.14 m^2) by affixing them into drilled holes with rubber stoppers.

Before each experiment the flume was filled with seawater from the study site and spiked with labeled ^{15}N-NH_4^+ (as 98 atom % $^{15}(NH_4)_2SO_4$ or $^{15}NH_4Cl$), to achieve a final water column concentration of ~6 μM. The beginning concentration fluctuated between 6 and 7 μM because of background NH_4^+ concentrations. Although this spike was higher than ambient NH_4^+ levels at the time of the experiments (0.5–1 μM), a 6 μM spike was used in order to allow accurate detection of NH_4^+ depletion in the water column over time and assess the effects of velocity on potential rates of NH_4^+ uptake. After mixing (~3 minutes), the Plexiglas floor with seagrass leaves attached was placed into the flume and held in place with flow straighteners. Bulk water velocity (U_b) was estimated by timing neutrally buoyant particles over a known distance ($n = 20$). Experiments were conducted for one hour so that a sufficient number of water and tissue samples for determining uptake rates could be collected.

Rates of NH_4^+ uptake by epiphytes were determined by measuring ^{15}N accumulation in epiphyte tissues over time during flume experiments. During each experiment, three leaves with epiphytes were randomly removed from the flume after 15-, 30-, 45-, and 60-min intervals. Three leaves were required for each sample to ensure adequate amounts of epiphyte tissue for analysis. At the end of each 15-min interval, epiphytes (all attached organisms) were removed from the seagrass leaves by gently scraping the leaves with a dull edge and were pooled to represent an epiphyte sample for the interval. Epiphyte samples were briefly rinsed with DI water over a 35-μM screen to remove salt (Winning et al. 1999) and placed on ice. Samples of epiphytes from each donor site were also collected and processed for determination of ambient ^{15}N in the epiphyte tissues. In addition to epiphytes, whole seagrass leaves ($n = 3$) were randomly selected from the flume at the end of each experiment, cleaned of epiphytes, pooled, and retained for ^{15}N analysis. All samples of epiphytes and seagrass were dried at 60°C for 24 h, weighed, homogenized, and stored in glass vials. Dry weights were used along with blade densities to estimate total biomass (g dry wt) of epiphytes and seagrass in the flume during each experiment.

Epiphyte and seagrass samples were analyzed by use of elemental analyzer isotope ratio mass spectrometry for determination of nitrogen content (% N) and atom % ^{15}N in the tissues. Specific uptake rates for epiphytes (V_{epi}) were calculated by use of the equation $V_{epi} = (da_s/dt)/(a_w - a_s)$, where a_s is the atom % ^{15}N in the epiphyte tissue, a_w is the atom % ^{15}N of the enriched substrate, and t is time (in s) (Dugdale and Goering 1967). The units for V_{epi} are g N removed (g N tissue)$^{-1}$ s^{-1} or simply s^{-1}. The numerator (da_s/dt) was calculated as the slope of the least-square regression of a_s versus time. The atom % ^{15}N of the enriched water (a_w) was based on the amount of 98 atom % $^{15}NH_4^+$ added and background NH_4^+ concentration (assumed to reflect ^{15}N concentration of atmospheric N ~0.37 atom % ^{15}N). To compare NH_4^+ uptake by epiphytes with uptake by their host plant, uptake rates for the seagrass leaves (V_{grass}) were also estimated for each of the experiments. Because seagrass leaves were retained only at the end of each experiment, V_{grass} was based on the final excess atom % ^{15}N in the tissue (Dugdale and Goering 1967). It is noted that the use of the above equation in calculating specific uptake rates for epiphytes and seagrass leaves assumes that the atom % ^{15}N of the source pool did not change during the course of the experiment. Dilution of ^{15}N in the source pool resulting from inputs of nonlabeled NH_4^+ into the water column (via regeneration or excretion) would expectedly result in underestimated uptake rates (Laws 1984). Although we acknowledge this potential source of error, the short duration of these experiments along with the high concentration and atom % ^{15}N of the spike likely minimized dilution.

Specific uptake rates (V_{epi} and V_{grass}) were normalized to nitrogen concentration (% N) of the epiphyte and seagrass tissues to calculate an uptake rate for epiphytes (ρ_{epi}) and seagrass leaves (ρ_{grass}) in units of g N removed (g dry wt)$^{-1}$ s^{-1} (Dugdale and Goering 1967). These values were multiplied by the total biomass of each component in the flume to estimate the contribution of epiphytes and seagrass to the total NH_4^+ removed from the water column during each experiment. Because of observed differences in the composition of epiphytes between donor sites (abundance of autotrophs vs. heterotrophs), uptake rates (ρ_{epi}) were normalized to chlorophyll *a* concentration in the tissues to obtain an uptake rate that was representative of the autotrophic fraction ($\rho_{Chl} = \rho_{epi} \times$ Chl a^{-1} in units of g N removed (mg Chl *a*)$^{-1}$

s^{-1}; Dickson and Wheeler 1995; Frankovich and Fourqurean 1997). Differences in the abundance of autotrophs that actively remove NH_4^+ from the water column and heterotrophs (i.e., bryozoans) that do not would predictably result in misleading rates of ^{15}N accumulation for the fraction of epiphytes that are actively removing NH_4^+ from the water column. Chl *a* concentrations (mg Chl *a* [g dry wt]$^{-1}$) were based on epiphyte samples collected at the end of each experiment and estimated by use of spectrophotometric methods as outlined in Strickland and Parsons (1968).

Rates of NH_4^+ uptake for the entire assemblage of organisms (seagrass leaves, phytoplankton, and epiphytes combined) were determined by measuring the rate of decline in NH_4^+ concentration in the water column over the duration (~1 h) of flume experiments. Methods of sample collection and analysis are outlined in detail in Thomas et al. (2000). In all but one experiment, uptake rates were based on a set of seven water samples collected over time. These samples were analyzed for NH_4^+ concentration by use of an autoanalyzer to an accuracy of 0.05 μM. Only the beginning and end bottle were used for one experiment because of loss of water samples. Ammonium concentrations for these samples were determined by use of the indophenol blue method (Solorzano 1969).

A first-order rate constant (k) describes the decline in NH_4^+ concentration in the flume over time (rate of decline, $-dC/dt = k[C]$), where C is the concentration of NH_4^+, t is time, and k is the first-order rate constant (s^{-1}). This constant (k) was estimated for each experiment as the slope of the least-square regression of the natural log of concentration versus time (see Bilger and Atkinson 1992; Thomas and Atkinson 1997; Thomas et al. 2000 for discussion). Each first-order rate constant was then normalized for water volume (Vol) in the flume (180 L) and the planar surface area (A) of the bottom covered by the seagrass leaves (0.14 m^2) to calculate an uptake rate constant (S) in units m s^{-1} ($S = k \times \text{Vol } A^{-1}$). Although Vol and A did not change during the experiments, this conversion was done to provide data in a form that is consistent (and therefore comparable) with studies elsewhere on nutrient uptake kinetics in benthic communities (Thomas and Atkinson 1997; Thomas et al. 2000). In addition to S, the total NH_4^+ removed over time (in g N removed s^{-1}) was calculated from the regression of k versus time for each experiment to compare uptake rates for epiphytes (ρ_{Chl}) with total NH_4^+ uptake by the assemblage in similar units.

Uptake by epiphytes—Coefficients of determination (r^2) for linear regressions of the atom % ^{15}N in epiphyte tissues versus time (da_s/dt) used in calculating specific uptake rates for epiphytes (V_{epi}) had a mean value of 0.85 (range, 0.69–0.99; SD, 0.11; $n = 14$). Multiplying V_{epi} by nitrogen content in epiphyte tissues provided NH_4^+ uptake rates (ρ_{epi}) that ranged from 0.45 to 3.3×10^{-8} and 0.66 to 6.8×10^{-8} g N removed (g dry wt)$^{-1}$ s^{-1} for sites 1 and 2, respectively. Water velocity had a significant effect on ρ_{epi} for epiphytes at site 1 ($\rho_{epi} = [11.6 \times 10^{-8}]U_b^{0.89}$, $r^2 = 0.80$, $P < 0.05$) and site 2 ($\rho_{epi} = [20.7 \times 10^{-8}]U_b^{0.73}$, $r^2 = 0.64$, $P < 0.05$). Although velocity had a similar effect on uptake by epiphytes at both sites (homogeneity of slopes, $P > 0.05$), estimates of ρ_{epi} were significantly lower for site 1 than site 2 (ANCOVA, ln ρ_{epi} vs. ln U_b, $P < 0.01$).

The disparity in ρ_{epi} between sites can be explained by differences in epiphyte composition. Epiphyte samples at site 1 included encrusting corallines, diatoms, attached macroalgae and a large portion (~30%) of epifauna (bryazoans), whereas those at site 2 were primarily composed of autotrophic organisms (corallines and macroalgae). Significantly lower Chl *a* concentrations at site 1 (~0.57 mg Chl *a* [g dry wt]$^{-1}$; SD, 0.20; $n = 12$) versus site 2 (~1.01 mg Chl *a* [g dry wt]$^{-1}$; SD, 0.25; $n = 15$) reflected these differences in composition (t test, $P < 0.001$, df = 25). Because all organisms attached to the leaves were included in the analysis the abundance of epifauna composed of inactive nitrogen at site 1 would expectedly result in the lower estimates of ρ_{epi} observed for these samples. This was confirmed by normalizing uptake rates (ρ_{epi}) to Chl *a* concentrations (ρ_{Chl}). Estimates of ρ_{Chl} were similar for both sites and ranged from 0.79×10^{-8} to 5.8×10^{-8} and 0.65×10^{-8} to 6.8×10^{-8} g N removed (mg Chl *a*)$^{-1}s^{-1}$ for sites 1 and 2, respectively (Fig. 1A). Contrary to estimates of ρ_{epi}, there was no significant difference between sites for ρ_{Chl} over the range of velocity (ANCOVA ln ρ_{chl} vs. ln U_b, $P = 0.14$) and the dependence of ρ_{Chl} on velocity for the pooled samples was on the order of 0.80 ($\rho_{chl} = [18.9 \times 10^{-8}]U_b^{0.77}$, $r^2 = 0.64$, $P < 0.001$). Although rates of uptake (ρ_{Chl}) and epiphyte biomass were similar between sites, the epiphytes at site 2 contained a larger fraction of autotrophic epiphytes and therefore removed a greater portion of NH_4^+ from the water column than epiphytes from site 1 (Fig. 2A; ANCOVA, ln uptake [epiphytes] vs. ln U_b, $P < 0.05$). Ammonium uptake by seagrass leaves was also dependent on water velocity (Fig. 2B). In general, uptake rates for the seagrass leaves (ρ_{grass}) were lower than those for epiphytes (ρ_{epi}) and ranged from 0.3 to 2.0×10^{-8} g N removed (g dry wt)$^{-1}s^{-1}$. It is noted that total uptake of NH_4^+ by seagrass leaves at site 1 was significantly higher than uptake by seagrass leaves at site 2 (Fig. 2B; ANCOVA, ln uptake [grass] vs. ln U_b, $P < 0.001$).

Uptake by the entire assemblage—Linear regressions of the natural log of NH_4^+ concentration versus time used to determine first-order rate constants (k) were significant for 11 of the 14 flume experiments and had a mean r^2 value of 0.93 (range, 0.75–0.99; SD, 0.09; $n = 11$). Regressions from two experiments conducted at low velocities (0.02 and 0.04 m s^{-1}) were not significant and showed no NH_4^+ uptake from the water column. This is likely due to sampling periods being set too short to detect uptake at this low velocity. No regression statistics were available for the experiment conducted at 0.10 m s^{-1}, because uptake was based on samples collected at the beginning and end of the experiment (see Isotope labeling approach section). The uptake rate constant was a function of velocity to the 0.59 power for experiments that use donor leaves from site 1 ($S = [25.3 \times 10^{-5}]U_b^{0.59}$, $r^2 = 0.92$, $n = 5$, $P < 0.01$) and to the 0.35 power for experiments that use donor leaves from site 2 ($S = [19.7 \times 10^{-5}]U_b^{0.35}$, $r^2 = 0.62$, $n = 7$, $P < 0.05$, see Fig. 1B).

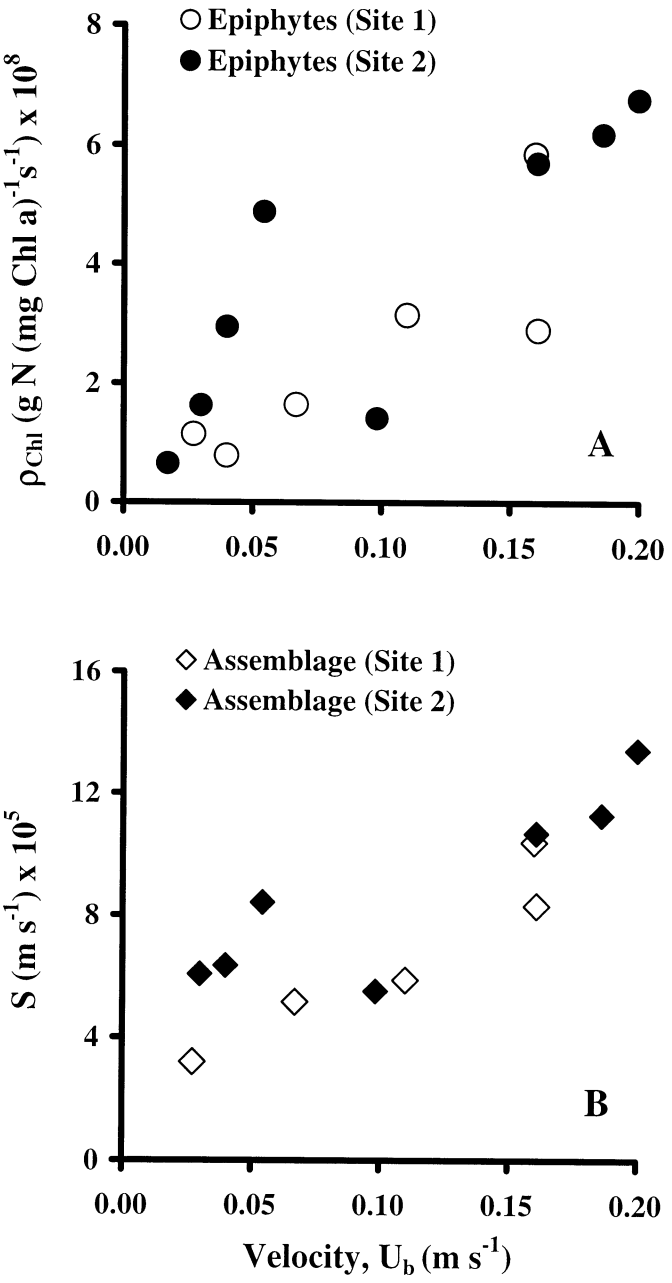

Fig. 1. (A) Rates of NH_4^+ uptake by epiphytes (ρ_{Chl}) and (B) uptake rate constants (S) for the assemblage as a function of water velocity (U_b). Estimates of (ρ_{Chl}) were similar between sites and equally affected by water velocity (ANCOVA ln ρ_{chl} vs. ln U_b, $P = 0.14$). The dependence of ρ_{Chl} on velocity for the pooled samples was on the order of 0.80 ($\rho_{chl} = [18.9]U_b^{0.77}$, $r^2 = 0.64$, $P < 0.001$). Uptake rate constants (S) were a function of velocity to the 0.59 power for site 1 ($S = [25.3]U_b^{0.59}$, $r^2 = 0.92$, $n = 5$, $P < 0.01$) and to the 0.35 power for site 2 ($S = [19.7]U_b^{0.35}$, $r^2 = 0.62$, $n = 7$, $P < 0.05$).

Ammonium uptake by epiphytes versus assemblage—The total NH_4^+ removed by the assemblage ranged between 4.8 and 12.9 $\times$ 10^{-7} g N s^{-1}. Uptake rates for epiphytes (ρ_{Chl}) were a function of the total NH_4^+ removed by the assemblage and increased as total uptake by the assemblage increased

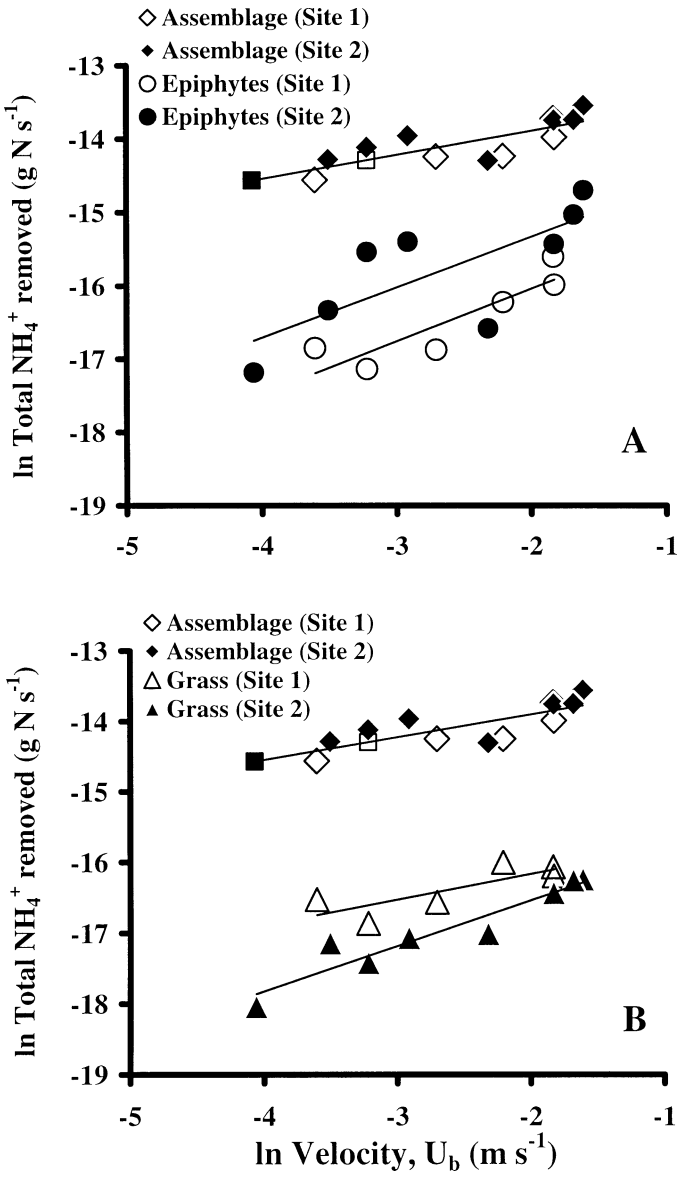

Fig. 2. Total ammonium removed during the experiments by epiphytes (A) and seagrass leaves (B) versus water velocity (U_b). The total ammonium removed by the entire assemblage (calculated from S) is presented in both graphs. Regression statistics are as follows: site 1 epiphytes (ln uptake = 0.72 ln U_b − 14.6; $r^2 = 0.79$, $P < 0.05$), site 2 epiphytes (ln uptake = 0.69 ln U_b − 14.0; $r^2 = 0.56$, $P < 0.05$), site 1 seagrass (ln uptake = 0.36 ln U_b − 15.4; $r^2 = 0.64$, $P = 0.05$), and site 2 seagrass (ln uptake = 0.64 ln U_b − 15.2, $r^2 = 0.90$, $P < 0.001$). For the entire assemblage, there was no significant difference between sites (ANCOVA, $P = 0.08$) and a common regression line for pooled data is shown (ln uptake = 0.32 ln U_b − 13.3; $r^2 = 0.61$, $P < 0.01$). Two data points based on this regression (squares on the line) are included to represent uptake rates for two experiments for which S could not be determined. Note that NH_4^+ uptake by epiphytes at site 1 was significantly lower than uptake by epiphytes at site 2 (ANCOVA, $P < 0.05$). The reverse was true for the seagrass leaves (ANCOVA, $P < 0.001$).

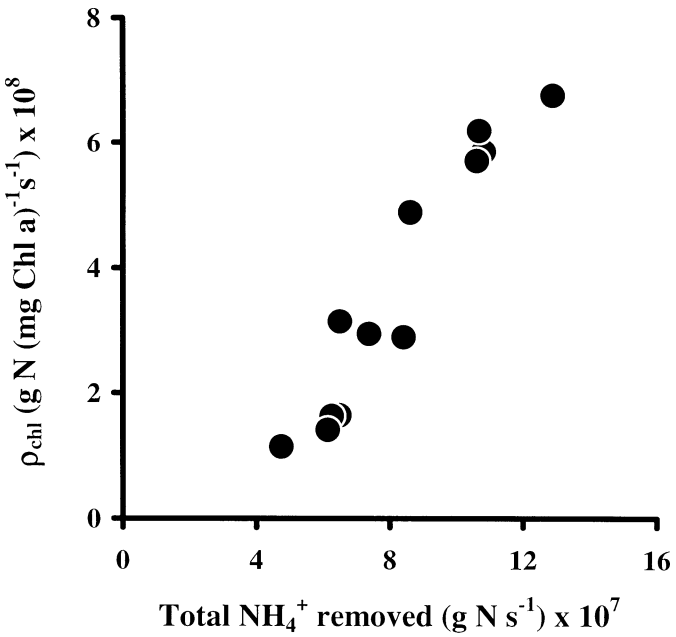

Fig. 3. Uptake rates normalized to Chl *a* for epiphytes (ρ_{Chl}) as a function of total ammonium uptake by the assemblage (model II geometric mean regression [Sokal and Rohlf 1981], $\rho_{Chl} = [0.84 \times 10^{-8}]x - 3.3$, $r = 0.96$, $P < 0.001$, where x is the total NH_4^+ removed by the assemblage in g N s^{-1}). There was no significant difference (ANCOVA, $P = 0.97$) in the relationship of ρ_{Chl} versus total uptake between sites and therefore the data were pooled.

(Fig. 3; Model II geometric mean regression [Sokal and Rohlf 1981], $\rho_{Chl} = [0.84 \times 10^{-8}]x - 3.3$, 95% confidence limits on slope are 0.66 and 1.01, $r = 0.96$, $P < 0.001$, where x is the total NH_4^+ removed by the assemblage in g N s^{-1}). Unlike epiphytes, rates of uptake by seagrass leaves (ρ_{grass}) were not dependent on the total NH_4^+ uptake by the assemblage ($P = 0.18$). The total NH_4^+ removed by individual components (epiphytes and seagrass leaves) and by the assemblage was dependent on velocity (Fig. 2).

Uptake by epiphytes accounted for ~17% (range, 7%–32%; SD, 8; $n = 12$) of the total NH_4^+ removed from the water column by the assemblage. The relative contribution of epiphytes increased as a linear function of total NH_4^+ uptake by the assemblage ($r^2 = 0.57$, $P < 0.01$). Approximately 9% (range, 4%–17%; SD, 4; $n = 12$) of the total uptake during experiments was attributed to leaf uptake. The remaining ~74% (range, 61%–83%; SD, 7; $n = 12$) of the total NH_4^+ removed could be attributed to several potential mechanisms including phytoplankton uptake, loss of small cells during rinsing of epiphyte samples, and adsorption of NH_4^+ ions to flume walls. Although pH was constant (range, 7.8–8.1) and nitrate concentrations remained low (<0.2 μM) and did not change during experiments, some loss of ^{15}N because of volatilization of ammonia and nitrification may also have occurred (Laws 1984; Dugdale and Wilkerson 1986). A comprehensive mass balance of the removed $^{15}NH_4^+$ was beyond the objectives of this study, and future experiments that use a mass balance approach will be required to quantify the contributions of all potential mechanisms of NH_4^+ removal.

Discussion—The use of ^{15}N-labeled ammonium in flume experiments has enabled us to isolate the effects of water velocity on NH_4^+ uptake by epiphytes while measuring the effects of velocity on NH_4^+ uptake by an assemblage of organisms that included epiphytes, seagrass leaves, and phytoplankton. The outcome of these experiments indicates that (1) rates of NH_4^+ uptake for epiphytes are dependent on water velocity (Figs. 1A, 3A), and (2) estimates of NH_4^+ uptake rates normalized to Chl *a* (ρ_{Chl}) are correlated to the total NH_4^+ removed from the water column by the assemblage (Fig. 3). These results demonstrate the utility of using isotope labels in flume studies to investigate the effects of water flow on nutrient dynamics of individual components of a community without separating the component from other community members.

Rates of NH_4^+ uptake for epiphytes increased by an order of magnitude over a range of water velocity (0.02–0.20 m s^{-1}) commonly observed in the field (Fig. 1A; Thomas and Cornelisen unpubl. data). This result emphasizes the importance of water flow in regulating NH_4^+ uptake by this integral component of seagrass systems and is consistent with studies elsewhere that have demonstrated flow-dependent nutrient uptake by individual organisms (e.g., Gerard 1982; Hurd et al. 1996) and communities (e.g., Thomas and Atkinson 1997; Thomas et al. 2000). The dependence of uptake rates on velocity ($\rho_{epi} \sim U_b^{0.8}$) was within the range expected for mass-transfer–limited uptake (Fig. 1A; Kays and Crawford 1993; Thomas et al. 2000). Furthermore, despite differences epiphyte composition between donor sites, rates of NH_4^+ uptake normalized to Chl *a* (ρ_{Chl}) were comparable between sites and were similarly affected by water velocity (Figs. 1A, 2). Therefore, uptake rates for these epiphytes were not only dependent on water velocity but also on their autotrophic fraction. We recognize that organisms that do not contain Chl *a* (i.e., heterotrophic bacteria) can remove ammonium from the water column (Hoch and Kirchman 1995); however, normalizing to Chl *a* in our experiments accounted for the differences in uptake rates (ρ_{epi}) between sites and allowed for comparison of epiphyte samples that differed in relative abundance of autotrophs and heterotrophs.

Ammonium uptake by the seagrass leaves was also dependent on water velocity and was higher for leaves at site 1 than for those at site 2 (Fig. 2B). Differences in NH_4^+ uptake rates between sites may have resulted from variations in long-term nutrient availability and uptake affinity of the leaves (Lee and Dunton 1999). However, close proximity of the donor beds and similar N tissue concentrations suggest a comparable nutrient history between sites and indicates that differences in uptake were likely due to interactions between epiphytes and their host plant. If this is the case, greater spatial coverage of epiphytes and/or higher NH_4^+ uptake rates for epiphytes at site 2 relative to site 1 (Fig. 2A) may have reduced uptake by the seagrass leaves at site 2. Studies elsewhere have provided evidence of these potential effects of epiphytes on nutrient uptake by seagrass leaves (Johnstone 1979; Sand-Jensen et al. 1985).

Rates of NH_4^+ uptake for epiphytes (ρ_{chl}) were a linear function (over the range of our data) of the total NH_4^+ removed by the entire assemblage, which indicates that NH_4^+ uptake by epiphytes was tightly coupled to uptake by the

entire assemblage (Fig. 3). It is possible that this relationship is nonlinear; however, we do not have data in the lowest range of NH_4^+ uptake. A nonlinear relationship is suggested if uptake by epiphytes occurs over all ranges of uptake by the assemblage and the intercept is zero (no uptake by epiphytes when there is no uptake by the assemblage and vice versa). Our results suggest that a nonlinear relationship may exist, because the relative contribution of epiphytes increased with total NH_4^+ removed by the assemblage (Fig. 2A). Such a relationship between uptake by epiphytes and uptake by the community would indicate that the epiphytes become an increasingly important contributor to NH_4^+ uptake as total NH_4^+ removed from the water column increases with velocity.

As implied by the close relationship between uptake by epiphytes and the community (Fig. 3) and the high dependence of NH_4^+ uptake by epiphytes on water velocity, epiphytes contributed a significant portion of the velocity-dependent NH_4^+ uptake by the entire assemblage (Fig. 2A). Seagrass leaves were also positively affected by water velocity, yet contributed less than epiphytes to total uptake by the assemblage (Fig. 2B). Seagrass plants remove a significant portion of their required nitrogen from the water column (e.g., Iizumi and Hattori 1982; Lee and Dunton 1999) and it has been suggested that leaf uptake contributes largely to the overall input of DIN to seagrass communities (Hemminga et al. 1991). The ability to estimate the relative contribution of epiphytes to total DIN inputs is confounded by the fact that seagrass plants use both water column and sediment nutrients (Iizumi and Hattori 1982; Lee and Dunton 1999). Nonetheless, our data reveal that epiphytes are an important pathway for DIN inputs and identify water velocity as a major factor influencing DIN inputs via both epiphytes and seagrass leaves.

The application of ^{15}N-labeled ammonium in flume experiments allowed us to isolate the effects of water velocity on NH_4^+ uptake by epiphytes while they were situated within a seagrass assemblage. As a result, we have demonstrated the importance of water velocity in controlling rates of NH_4^+ uptake by this important component of seagrass communities. Furthermore, our data indicate that epiphytes are tightly coupled to uptake by the entire community and along with seagrass leaves contribute to DIN inputs to seagrass communities as well as the uptake response of the assemblage to water velocity. Future studies conducted in situ through the use of field flumes and labeled nutrients will provide valuable information on the role of the physical environment on nutrient cycling processes at the scale of individual organisms (i.e., seagrass plants) and groups of organisms (i.e., epiphytes and phytoplankton) and how these processes are reflected in the response of the entire community.

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Life history bottlenecks in *Diaptomus clavipes* induced by phosphorus-limited algae

Abstract—Considerable information has accumulated on food quality effects on certain cladocerans; meanwhile, other zooplankton have received comparatively little nutritional study. In particular, little is still known about nutrition of copepods with more complex ontogenetic changes. Recent studies have identified strong ontogenetic differences in phosphorus content of copepods, which have been found to be highest for late naupliar stages. Laboratory experiments were set up to examine the life cycle of a calanoid copepod relative to mineral P limitation on a pure algae diet. We hypothesized that P imbalance would cause nauplii particularly to be affected by algal nutrient status. Developmental rates, growth rates, and fecundity of *Diaptomus clavipes* under excess (1.0 mg C L^{-1}) P-deficient and P-sufficient *Scenedesmus obliquus* were determined. We found strong differences between diets in survival to maturity: nauplii developed into copepodites and adults that successfully reproduced when fed the P-sufficient algae but invariably died after molting into copepodite CII when reared on the P-deficient algae. Differences in developmental rates were small for most life stages of nauplii for both food types but were substantial both for copepodite CI and particularly for CII that lived without molting up to 22 d on P-deficient food before dying. Surprisingly, copepodites that fed on P-deficient algae had similar or higher specific somatic growth rates than animals on P-sufficient algae. Although these experiments demonstrate a strong ontogenetic component to copepod nutrition, and a heretofore undocumented dependence of copepod success on algal P content, the life history bottleneck occurs at a later stage than we hypothesized.

Probably among the most important and at the same time the most difficult task of the zooplankton ecologist is to assess nutritional status. To date, we are still far from a unified view of zooplankton nutrition, especially for copepods. Experiments of food limitation on copepods are scarce, perhaps because of the complex life history of copepods and their relatively long development times. However, studying the ontogeny of an organism, with focus on nutrition, raises the intriguing question of stage-dependent performance. Population bottlenecks may mean that success of one stage is a poor predictor of the success of other stages.

When food levels allow for maximum population per capita growth, food quality becomes particularly relevant for