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Uptake and assimilation of ^{15}N-ammonium by the symbiotic sea anemones *Bartholomea annulata* and *Aiptasia pallida*: conservation versus recycling of nitrogen

Abstract The uptake of ammonium and subsequent movement of nitrogen between symbiotic dinoflagellates (zooxanthellae) and host tissue in the sea anemones *Bartholomea annulata* (Le Sueur) and *Aiptasia pallida* (Verrill) were studied in time course experiments using $^{15}NH_4^+$ in 1994. Although there was net uptake of ammonium during exposure to the tracer, there was significant, concurrent efflux, and the anemones continued to lose ammonium over 11 days in low-nutrient seawater. There was no clear evidence of translocation of nitrogen from the zooxanthellae to the host in either species. Host tissue in both species was capable of ammonium assimilation based on simultaneous appearance of ^{15}N in host and zooxanthellae, as well as in aposymbiotic *A. pallida* and symbiont-free body column tissue of *B. annulata*. The amount of tracer in *B. annulata* tentacles from a single anemone was significant, with 66% of total ^{15}N atoms present in the host at all times during exposure to $^{15}NH_4^+$ and during the subsequent 11-day chase period. The degree of labeling in the tentacles of *B. annulata* and symbiotic *A. pallida* was much greater than host tissue elsewhere in *B. annulata* or in aposymbiotic *A. pallida*, demonstrating a strong influence of the symbionts on host metabolism. Our data are consistent with the conservation paradigm that photosynthate from the zooxanthellae reduces the host catabolism of nitrogenous compounds for respiration so that there is little ammonium to be recycled. Although there was little support for nitrogen recycling in our studies, there is likely a continuum between the recycling and conservation paradigms that depends on the availability of photosynthate and hence the coupling between carbon and nitrogen cycles of the partners.

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Introduction

It is well known that reef corals and other invertebrates containing symbiotic dinoflagellates (zooxanthellae) take up and assimilate inorganic nitrogen and phosphorus from seawater (D'Elia 1977; Muscatine 1980; Wilkerson and Trench 1986). The efficient uptake and retention of these scarce nutrients by recycling between the algae and host tissue is considered to be one of the major reasons for the success of reef corals in oligotrophic waters (Lewis and Smith 1971; Muscatine and Porter 1977).

There is good evidence that the algal endosymbionts are the primary site of assimilation of these nutrients, particularly nitrogen (Falkowski et al. 1993). First, zooxanthellae assimilate ammonium via the high affinity GS/GOGAT (glutamine synthetase/2-oxoaminoglutamate aminotransferase) pathway (Wilkerson and Muscatine 1984; Summons et al. 1986; Anderson and Burris 1987; Swanson and Hoegh-Guldberg 1998; Roberts et al. 1999). When this pathway is blocked with the inhibitor azaserine, corals lose the ability to assimilate ammonium and begin to release it (Rahav et al. 1989). Second, aposymbiotic (alga-free) corals and sea anemones do not effect net uptake of ammonium from seawater, rather they lose or excrete ammonium (Muscatine et al. 1979; Szmant-Froelich and Pilson 1984; Wilkerson and Muscatine 1984). Third, after prolonged darkness, ammonium uptake from seawater by symbiotic corals and sea anemones ceases and ammonium is excreted (Szmant-Froelich and Pilson 1977; Muscatine and D'Elia 1978; Szmant et al. 1990).

One role for the algae in maintaining this "nitrogen efficiency" of the symbiosis would be to recycle host excretory ammonium by fixing it into organic nitrogen

compounds that are then translocated back to the host (Muscatine and Porter 1977). Muscatine et al. (1984) and Rahav et al. (1989) estimated that 90% of ammonium assimilated by zooxanthellae in the coral *Stylophora pistillata* may be translocated to the host, with the remaining fraction retained for zooxanthella growth. Alanine derived from symbiont photosynthesis is released by isolated zooxanthellae in the presence of host tissue (Muscatine and Cernichiari 1969; Trench 1971) and may be translocated in intact symbioses (Swanson and Hoegh-Guldberg 1998; Wang and Douglas 1999). Export of essential amino acids by the zooxanthellae would be an important role for the algae, and algal production of most of the essential amino acids has been observed in anemones (Swanson and Hoegh-Guldberg 1998; Wang and Douglas 1998). However, in several coral species, host tissue synthesized nearly all of the essential amino acids (Fitzgerald and Szmant 1997). In addition to amino acids or other small molecules, nitrogenous macromolecules might also be translocated to the animal from the zooxanthellae (Markell and Trench 1993).

Another explanation is the conservation of host nitrogen through the catabolism of translocated symbiont carbon (Rees and Ellard 1989; Szmant et al. 1990; Wang and Douglas 1998). According to this view, translocated compounds are used as energy sources for respiration by the host rather than amino acids or other nitrogenous compounds, thus reducing nitrogen excretion. Wang and Douglas (1998) found that supplying exogenous carbon to the symbiotic sea anemone *Aiptasia pulchella* increased free amino acid pools and reduced ammonium concentrations in the host, consistent with this view. A second role for the algae would be to provide the host with reducing power for assimilation of ammonium (Rees 1991). Wang and Douglas (1998) also found that increased carbon supply enhanced host GS activity, and this host assimilation would increase the conservation of N in the system. Other evidence for the assimilation of ammonium by host tissue in zooxanthellar associations includes the high levels of NADPH-dependent glutamate dehydrogenase (GDH) (Catmull et al. 1987; Yellowlees et al. 1994) and GS (Yellowlees et al. 1994) that have been reported from coral host tissue. Thus, the primary algal role is not directly nitrogen assimilation, but to supply carbon-rich compounds for host respiration in lieu of nitrogen-containing compounds, which results in reduced amounts of ammonium to be recycled and increased host ability to utilize ammonium. Nitrogen limitation of the zooxanthellae (Cook and D'Elia 1987; Cook et al. 1997) would then result from algal translocation of carbon rather than an independent control mechanism.

One way to assess the relative roles of "recycling" versus "conservation" is the direct measurement of partitioning of nitrogen between host and symbiont. Patterns of tracer distribution during, and subsequent to, a pulse of ^{15}N-ammonium should be consistent with either the recycling or conservation hypotheses. If recycling dominates, a pulse of exogenous ^{15}N should initially appear in the zooxanthellae as small precursor molecules such as alanine and essential amino acids; this would be followed by increased ^{15}N content of the host pool of low molecular weight compounds as these molecules are translocated. The tracer would appear in host macromolecules only after a lag period while the tracer first filled the algal, and then the host precursor pools. Once the pulse ended, recycling would eventually cause the isotopic composition of the host and zooxanthellae to approach the same value as host-derived nitrogen was incorporated by the algae and vice versa.

In contrast, the conservation paradigm predicts that $^{15}NH_4^+$ would appear simultaneously in precursor molecules of host tissue and zooxanthellae as both partners assimilated ammonium. After incorporation of a ^{15}N pulse into macromolecules, the proportion of tracer in each partner would remain constant and not approach isotopic equilibrium, given minimal exchange of tracer and reduced production of ammonium due to the sparing effects of translocated photosynthate.

Earlier studies employing ^{15}N with symbiotic anthozoans (Muscatine and D'Elia 1978; Burris 1983; Muscatine et al. 1984) did not follow the tracer separately in the symbiotic partners. Recently, Roberts et al. (1999) conducted short-term, pulse-chase experiments using $^{15}NH_4^+$ with the anemone *Anemonia viridis* and found rapid assimilation into zooxanthellae, suggesting the algae were the primary site of assimilation. Wilkerson and Kremer (1992) measured the partitioning of $^{15}NH_4^+$ between scyphozoan medusae and their symbionts and found little evidence for nitrogen translocation.

We examined the fate of nitrogen in host tissue and zooxanthellae of the sea anemones *Bartholomea annulata* and *Aiptasia pallida* over short and long time periods after exposure to exogenously supplied $^{15}NH_4^+$. Low molecular weight compounds (LMWC) and macromolecules were analyzed to separate the dynamics of rapid turnover precursors from the long-lived components of both partners in the symbiosis. In addition, we analyzed the isotopic composition of ammonium in host tissues to determine if it was the precursor for the LMWC fraction. *B. annulata* was chosen as it is sufficiently large to permit repeated sampling of different regions, with varying amounts of zooxanthellae, from a single individual over an entire experiment. The use of symbiotic and aposymbiotic (alga-free) *A. pallida* enabled investigation of the role of host assimilation of ammonium.

Materials and methods

Study organisms

A single individual of the sea anemone *Bartholomea annulata* (Le Sueur) was collected from a reef off Bermuda in summer, 1994, 2 days before use and was maintained without feeding in 4 l of aerated, filtered seawater in an incubator (25°C, 80 μmol photons m^{-2} s^{-1}, 12 h light:12 h dark) as previously described (Cook et al. 1988). The oral disc was approximately 5 cm in diameter. This

species was chosen because its large size permitted repeated removal of single large tentacles during an experiment (a total of 14% were removed over 12 days) and repeated sampling of the body column. Sampling caused transient tentacle withdrawal, but the anemone quickly returned to normal appearance. In contrast to the tentacles, where host tissue contains numerous zooxanthellae, the body column was symbiont free, as verified by microscopic examination of a homogenized sample.

Clonal cultures of a second actinian, *Aiptasia pallida* (Verrill), originally collected from Walsingham Pond, Bermuda, were maintained under the same conditions as *B. annulata*. Aposymbiotic individuals of this clone (Muller-Parker et al. 1990) were maintained under the same regime as the symbiotic anemones except they were kept in a light-tight container. Daily feeding with brine shrimp ceased 2 days before the experiment to permit elimination of any unassimilated food in the gut. These experiments took place in summer, 1992.

Experimental protocols

The single individual of *B. annulata* was exposed to a 20 μM pulse of 99% $^{15}NH_4^+$ for 9 h at the beginning of the light portion of the incubator photoperiod. During the pulse period, the anemone was repeatedly sampled by removing two tentacles and a piece of the body column after 0.5, 1, 2, 4, and 9 h. The incubation solution was then decanted, the animal and beaker washed several times with 0.45 μm filtered, low nutrient, Sargasso seawater (FSSW), and the beaker refilled with 4 l unlabeled FSSW to begin a tracer reallocation period. Tentacles and body column were sampled as above at 10, 14, 19, and 24 h into the reallocation period: the anemone was rinsed and the seawater was replaced at 24 h. Sampling and rinsing was repeated daily until day 7, and final samples were taken 12 days from the beginning of the experiment. The anemone was not fed during the experiment. The protocol did not include a $^{14}NH_4^+$ "chase" or feeding so that changes in the distribution of tracer would result from internal reallocation and excretory losses rather than from external inputs. A sample of the incubation medium was removed at each time point for analysis of ammonium concentration and isotopic composition by forming an indophenol dye for determination of concentration and then isolating the dye by solid phase extraction for determination of the isotopic composition (McCarthy et al. 1996).

In the experiments with symbiotic *A. pallida*, approximately 40 individuals were placed in a 1 l beaker containing 500 ml of FSSW. The beaker was returned to the incubator for 24 h to allow the anemones to attach. A magnetic stirrer provided gentle stirring during this time and during experiments. Just prior to addition of $^{15}NH_4^+$, the anemones and beaker were vigorously rinsed with FSSW to remove mucus and other debris. To maximize the amount of label in the zooxanthellae compared to the host, the animals were exposed to 10 μM, 99% $^{15}NH_4^+$ in FSSW for 8 days. The labeling solution was changed every other day, and the anemones were fed daily with freshly hatched brine shrimp. A period of tracer reallocation began by washing the animals several times with FSSW to remove exogenous label and continued with daily changes of FSSW. Four anemones were removed after 0, 1, 2, 3, 4, 5, 6, 8, and 11 days during this period to detect ^{15}N movement between the host and symbiont. Before each sampling, the anemones were vigorously rinsed to remove adhering mucus and other debris. The anemones were not fed during the chase period.

Aposymbiotic *A. pallida* were exposed to a pulse of 10 μM, 99% $^{15}NH_4^+$ for 2 days to ensure sufficient ^{15}N was present in all fractions for accurate isotopic analysis. Three animals were sampled after 2, 4, 8, 24, and 47 h during the pulse, and the surrounding seawater was sampled for $^{15}NH_4^+$ after 0, 2, 4, 8, 24, and 47 h. The remaining animals were then washed several times with FSSW to remove the tracer, and three additional individuals were removed at 0.1, 1, 2, and 4 days during the reallocation period. The aposymbiotic anemones were treated identically to the symbiotic anemones.

Separation and fractionation protocols

The procedure for separation of zooxanthellae and host, and fractionation of LMWC and macromolecular compounds, is outlined in Fig. 1. Whole individuals of *A. pallida* (four symbiotic, three aposymbiotic), and tentacles or body column pieces of the single *B. annulata* were homogenized by ultrasonic disruption in 15 ml of cold 0.002% Triton X100 in FSSW. We found that 30% of full power for 20 s maximized host tissue disruption with minimal damage to the zooxanthellae; $<3\%$ of the total chlorophyll fluorescence remained in the supernatant after centrifugation. A 200 μl sample of the homogenate was spotted on a precombusted (450°C, 4 h) GF/A filter for isotopic analysis, and the remaining

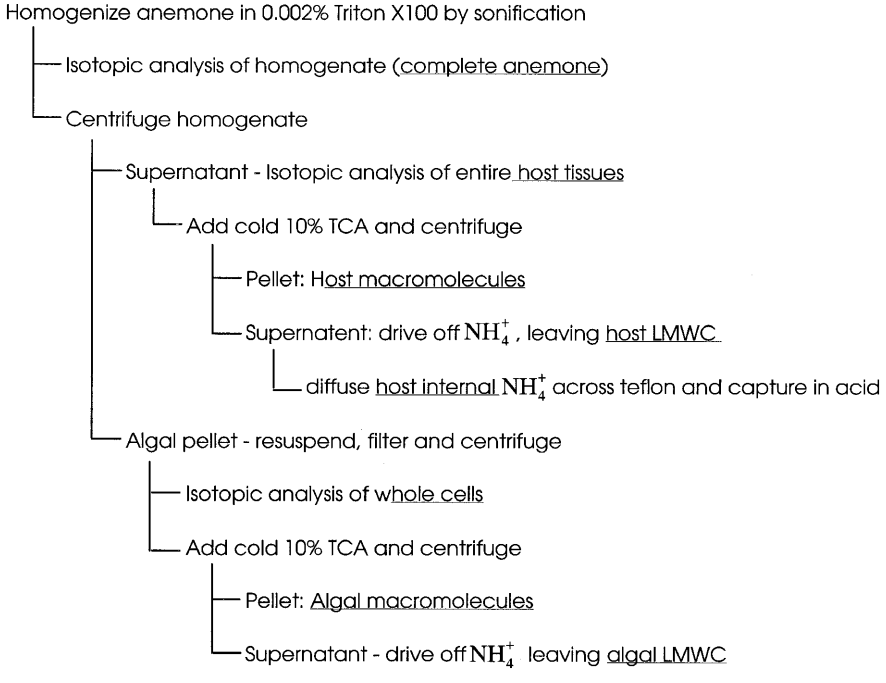

Fig. 1 *Bartholomea annulata*, *Aiptasia pallida*. Fractionation protocol for host tissue and zooxanthellae. *Underlined text* denotes measured fractions

homogenate was centrifuged at 1,000 *g* for 4 min to sediment the zooxanthellae. Two milliliters of the supernatant (host tissue) were added to 2 ml of 10% trichloroacetic acid (TCA) and maintained at 2°C for several days. Insoluble host macromolecules were then separated from the soluble, low molecular weight fraction (LMWC) by centrifugation at 16,000 *g* for 2 min. One milliliter of the resulting supernatant was removed, treated with 70 µl of 2.5 M NaOH to drive off residual NH_4^+ (hence our LMWC samples do not include NH_4^+ from internal pools), and 0.5 ml was dried onto a precombusted GF/A filter in successive 0.1 ml volumes. This treatment raises the potential for loss of some label by alkaline hydrolysis of the amido-N from glutamine which would alter the magnitude but not the kinetics of the LMWC pool. After discarding the remaining supernatant, the precipitated macromolecules were transferred to a precombusted GF/A filter and dried.

To recover $^{15}NH_4^+$ from host tissue samples, sufficient NaOH-citrate was added to the remaining 13 ml of host supernatant to raise the pH to 11, converting the cation to gaseous ammonia. This solution was slowly pumped through a 1 m length of Teflon tubing (2 mm outer diameter, 2 µm pore size, 50% porosity, W.L. Gore) encased in 3 mm inner diameter Tygon tubing. The space between the tubing walls was filled with 0.1% HCl which trapped ammonia diffusing from the supernatant through the inner tubing wall. The acidic solution containing the $^{15}NH_4^+$ was then dried onto a GF/A filter for isotopic analysis.

The algal pellet was resuspended in 5 ml of 0.002% Triton X100, centrifuged at 1,000 *g* for 1 min, and resuspended in FSSW. The suspension was then filtered through a 15 µm mesh Nitex screen; this mesh size was found by visual inspection to effectively remove remaining host tissue debris, resulting in a clean preparation of algal cells. The filtered suspension was again centrifuged and the zooxanthella pellet was suspended in 2 ml of FSSW. A 200 µl sample was pipetted onto a precombusted GF/A filter for analysis of the whole-cell isotopic content. The remaining algal suspension was added to an equal volume of 10% TCA and stored at 2°C for several days. The algal LMWC and macromolecules were then separated by centrifugation and prepared for isotopic analysis in the same manner as the host tissues.

Isotopic and mass analyses

The filters containing the various fractions were dried overnight at 75°C. They were then stored in a desiccator before being sealed under vacuum in quartz combustion tubes; the nitrogen on the filters was converted to N_2 gas by Dumas combustion (Fiedler and Proksch 1975; Lipschultz 1984). The isotopic ratio of the gas was determined by emission spectrometry using a diode array emission spectrometer (Lipschultz 1993). The results of all isotopic analyses are reported as atom % $^{15}N[^{15}N/(^{14}N+{}^{15}N)\times 100]$.

Turnover rate (k) was calculated by linear regression of $\ln A(t) = \ln A_0 + kt$, where $A(t)$ is the sample atom % ^{15}N at time t, and A_0 is the value at t_0. Uptake rates were calculated as Δatom % $^{15}N/(\Delta t \times R)$, where Δatom % ^{15}N is the change in sample isotopic composition over the time interval (Δt), and R is the exponential average atom % ^{15}N of the ^{15}N precursor over Δt.

The mass of nitrogen in *B. annulata* tentacle tissues was measured by elemental analysis (Control Equipment). In addition, the zooxanthella (N_z) and host nitrogen (N_h) mass fraction compared to the total (N_t) mass was calculated from the atom % excess (atom % ^{15}N–0.366) of the host (A_h), zooxanthella (A_z) and homogenate (A_t) tissues using the following equation: $(N_z \times A_z) + (N_h \times A_h) = N_t \times A_t$ and the assumption that $N_z + N_h = N_t = 1$. The proportion of ^{15}N atoms in the host tissue or zooxanthellae was calculated as $(N_i \times A_i)/A_t$ where N_i is either the host or zooxanthellae mass fraction and A_i is the atom % excess in that fraction.

Bacterial contamination

Bacterial activity could potentially affect the distribution of ^{15}N in our experiments. To assess this possibility, bacteria associated with *A. pallida* were counted using DAPI (Porter and Feig 1982). Few bacteria were observed on the tentacles, and only low numbers relative to the number of zooxanthellae were present in homogenates of whole anemones. In contrast, extremely dense assemblages of rod-shaped cells were seen on mucous "rings" that had been shed by the anemones and were floating in the growth chambers. Assimilation experiments with the heavily colonized, shed rings indicated that $^{15}NH_4^+$ was rapidly assimilated by the bacteria. However, mucous rings were easily removed from the anemones by a vigorous stream of FSSW; freshly removed rings had far fewer bacteria than old, naturally shed ones. Our protocol of washing the anemones prior to taking samples removed the rings and so reduced the bacterial biomass in our samples. In addition, the total mass of nitrogen on these rings was far smaller than that of the anemone itself, further limiting their influence on the isotopic composition of our samples. The body column of *B. annulata* was readily cleansed of surface bacteria by scraping the area before taking samples. Further evidence for limited bacterial influence comes from a piece of *A. pallida* host tissue that completely resisted sonic disruption. This piece had no bacterial component yet its atom % ^{15}N was similar to that of the other host tissue (data not shown). Thus, while we cannot rule out the influence of bacteria, we feel confident that bacteria were not a major source of contamination. Ferrier (1991) and Fitzgerald and Szmant (1997) concluded that bacteria did not significantly affect uptake of free amino acids by corals.

Results

^{15}N-partitioning in *Bartholomea annulata*

The concentration of exogenously supplied ammonium decreased linearly by 1.6 µM h^{-1} ($r^2 = 0.996$) during the 9 h pulse period (Fig. 2). The isotopic composition (atom % ^{15}N) of the ammonium also declined from the 99% initial value to just below 50% in 9 h. The incubation water was replaced with tracer-free FSSW immediately after the pulse, and at the end of each subsequent 24 h period. This reset the ammonium concentration to undetectable levels and the isotopic composition to natural abundance (0.366%); thereafter, the concentration of ammonium increased over each 24 h period to between 2.5 and 5 µM. The atom % ^{15}N of this external ammonium immediately after the pulse was nearly 8% and then gradually declined over each subsequent 24 h period, approaching natural abundance levels after 12 days.

During the pulse period, the label rapidly appeared in host internal ammonium and in the LMWC of both host and zooxanthellae in the tentacles (Fig. 3a). The atom % ^{15}N of internal ammonium reached a plateau by 4 h; following the pulse period, it rapidly declined (minimum half-life of about 10 h) due to input of isotopically lighter ammonium or loss of ^{15}N to the seawater. The atom % ^{15}N of symbiont and host LMWC increased at similar rates for the first 2 h of the pulse; thereafter, the zooxanthella LMWC increased more rapidly. Both LMWC compartments then either lost ^{15}N or gained ^{14}N, resulting in decreasing atom % ^{15}N until they equilibrated with the internal ammonium compartment by 48 h (Fig. 3b).

The zooxanthellae accumulated label continuously during the pulse period; during the first 10 h of the chase

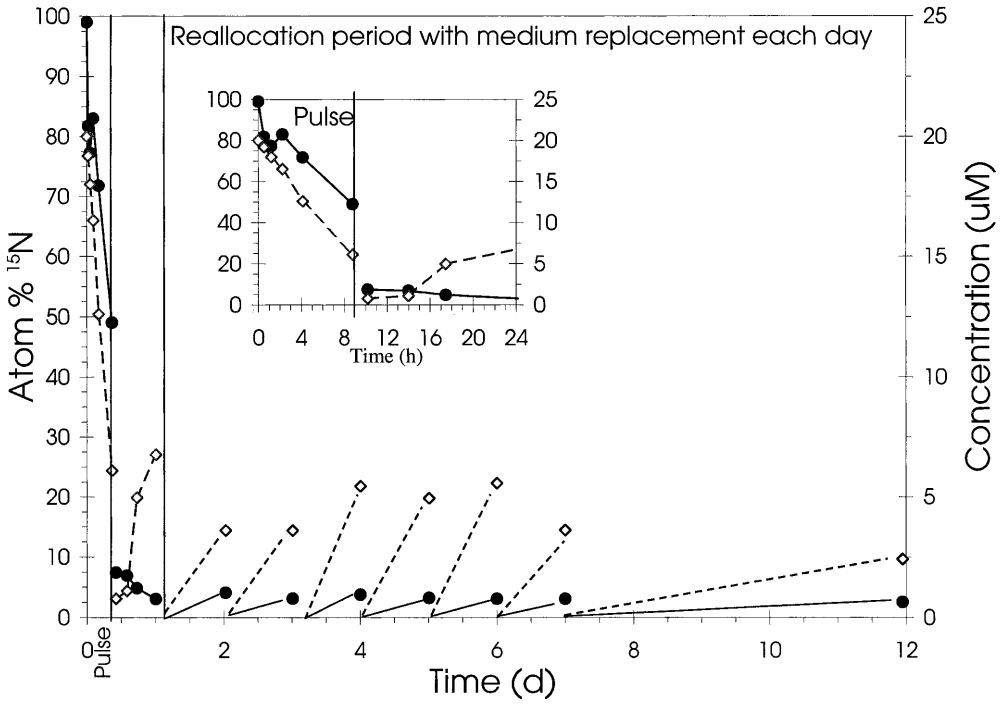

Fig. 2 *Bartholomea annulata*. Ammonium concentration (*diamonds*) and atom % ^{15}N (*circles*) in the medium during the 12 days after addition of tracer. *Lines* from the baseline to datapoints during the reallocation period reflect the unmeasured increase from undetectable concentration and 0.37% ^{15}N present after each sampling. *Inset* shows initial 24 h

period, the atom % ^{15}N of all the algal fractions continued to increase (Fig. 4a). The atom % ^{15}N of the algal LMWC fraction then declined, while the whole cell and macromolecular fractions exhibited little change during this period; after 24 h, the atom % ^{15}N of the LMWC was less than that of the macromolecules. The atom % ^{15}N of zooxanthella macromolecules was slightly lower than that of the whole cell until 24 h, after which the pattern reversed. Maximum atom % ^{15}N values of both the whole cell and macromolecular fractions were reached by 17 h. After 24 h, the isotopic content of whole cells and the macromolecular fraction remained constant until day 7, when there was a slight decline to a new plateau which continued to the end of the experiment (Fig. 4b).

The isotopic composition of the host tissue in the tentacles is shown in Fig. 5. The atom % ^{15}N of the LMWC increased rapidly, but at half the rate of the algal LMWC during the 9 h pulse period; the atom % ^{15}N of the macromolecules increased at a slower rate. Following removal of the label, the isotopic enrichment of the LMWC fraction declined for the first 7 h, then increased fractionally during the next 8 h, while the macromolecular fraction increased steadily during this period. Thereafter, the atom % ^{15}N of host LMWC steadily declined until it was just above natural abundance levels (Fig. 5b). In contrast, labeling of the macromolecules remained constant until day 6, when it decreased slightly, but remained higher than that of the LMWC until the end of the experiment.

Tissue from the alga-free body column (Fig. 6) had lower atom % ^{15}N values than the host tissue in the tentacles (Fig. 5), but otherwise displayed a similar pattern. (The last datum of the LMWC during the pulse appeared to be spuriously low). The atom % ^{15}N of the LMWC rapidly increased during the pulse and then declined during the reallocation period. The host supernatant and macromolecules were virtually identical in isotopic composition, reaching a maximum by the first 24 h of the experiment, and remaining constant over the entire reallocation period.

The percentage of nitrogen in zooxanthellae from the tentacles measured by CHN analysis and calculated from the isotopic measurements over the entire experiment was 24% and 23%, respectively. Using the independently measured proportions of nitrogen in each component (CHN analysis) and the isotopic composition of each component, the proportion of ^{15}N atoms in the host was fairly constant at all time points, averaging 66% (±2% SE) over the entire experiment (Fig. 7).

^{15}N-partitioning in symbiotic *Aiptasia pallida*

In contrast to *B. annulata*, symbiotic *A. pallida* were exposed to $^{15}NH_4^+$ continually for 8 days, resulting in zooxanthellae and host tissue highly enriched in ^{15}N (Fig. 8). One day after removal from the tracer, the LMWC of zooxanthellae still retained an atom % ^{15}N approaching 25%, while that of the host was $<2\%$. At that time, the macromolecules of the algae had values of nearly 15%, three times greater than the host macromolecules (Fig. 8a). In contrast to *B. annulata*, the isotopic composition of all zooxanthella fractions declined continuously during the reallocation period, with the LMWC isotopic compositions declining faster than the other two compartments for 11 days, by which time all three were in equilibrium. The atom % ^{15}N of host tissue

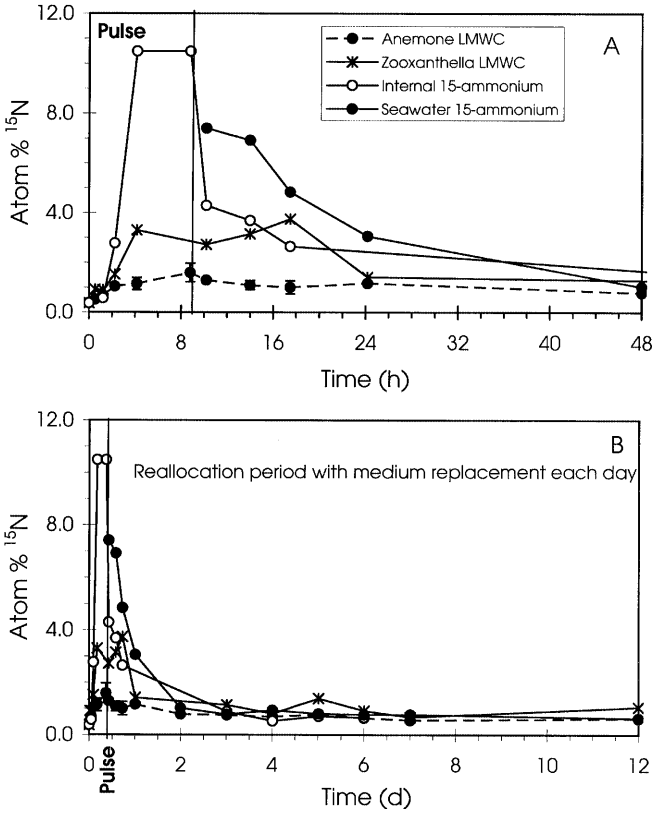

Fig. 3a, b *Bartholomea annulata*. Appearance of ^{15}N in internal pools of ammonium and low molecular weight compounds (*LMWC*) in host tissue and of LMWC in zooxanthellae during exposure to $^{15}NH_4^+$ and subsequent 11.6 day incubation in FSSW: **a** first 48 h of the experiment and **b** entire time course. *Errors bars*: sample standard deviations ($n=2$ tentacles). Atom % ^{15}N of the medium during reallocation period repeated from Fig. 2 for comparison

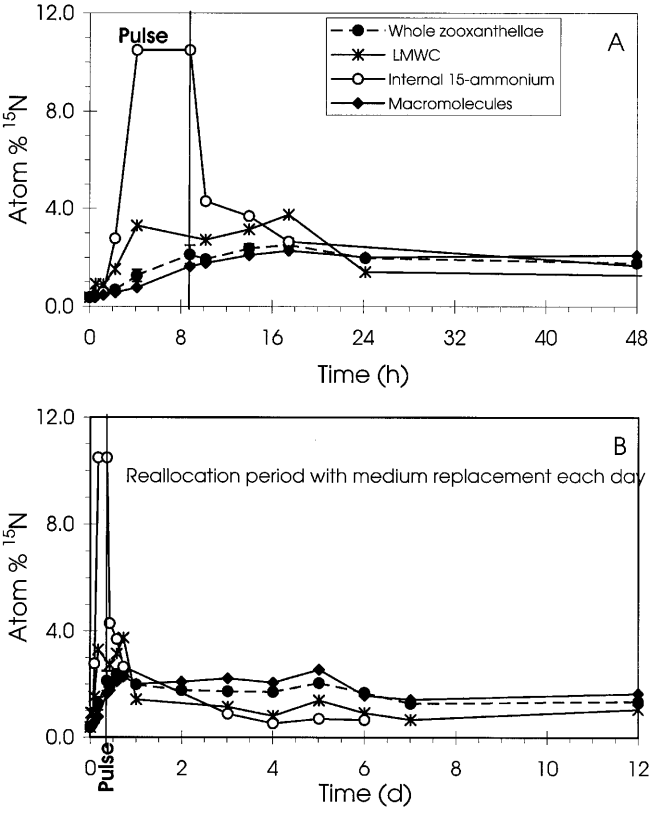

Fig. 4a, b *Bartholomea annulata*. ^{15}N isotopic composition of internal pools of ammonium compared to zooxanthellae and their LMWC and macromolecular components during exposure to $^{15}NH_4^+$ and subsequent 11.6 day incubation in FSSW: **a** first 48 h of the experiment and **b** entire time course. *Errors bars*: sample standard deviations ($n=2$ tentacles)

and its LMWC and macromolecule pools all remained constant during the entire 11 day period (Fig. 8b), but these values were always lower than those of the algal compartments. Efflux of labeled ammonium into the seawater slowly diminished until the atom % ^{15}N of the external ammonium approached natural levels at the end of the experiment.

^{15}N-partitioning in aposymbiotic *A. pallida*

The atom % ^{15}N of ammonium in the medium containing aposymbiotic anemones rapidly declined from 100% to $<10\%$ during the 2 day pulse period (Fig. 9). Since none of the tissue fractions exceeded 0.5% during this period, this decline was due to the efflux of unlabeled ammonium. Label rapidly accumulated in the host LMWC fraction for the first 8 h, after which the diminishing isotopic composition of the medium caused the atom % ^{15}N of all host fractions to reach a plateau. After the tracer was removed, the atom % ^{15}N of all fractions remained constant for the remainder of the experiment.

Discussion

Interaction between seawater and internal ammonium pools

Traditional uptake experiments using unlabeled compounds measure net fluxes rather than discrete, unidirectional influx or efflux between the medium and experimental organisms. In our experiment with *Bartholomea annulata*, we simultaneously monitored the concentration and isotopic composition of external ammonium (Fig. 2), permitting calculation of both influx and efflux (Glibert et al. 1982). The ammonium concentration declined during the pulse addition, indicating net uptake. The concurrent decrease in the proportion of $^{15}NH_4^+$ in the external medium could only have resulted from the simultaneous efflux of $^{14}NH_4^+$. We calculate that efflux from *B. annulata* was 32% of the gross uptake rate during the pulse period. Since the calculation assumes that only unlabeled ammonium is lost, the continued loss of tracer during reallocation (Fig. 2) strongly suggests that this is an underestimate. In addition, labeled ammonium acquired during the pulse was lost from the anemone during the entire chase

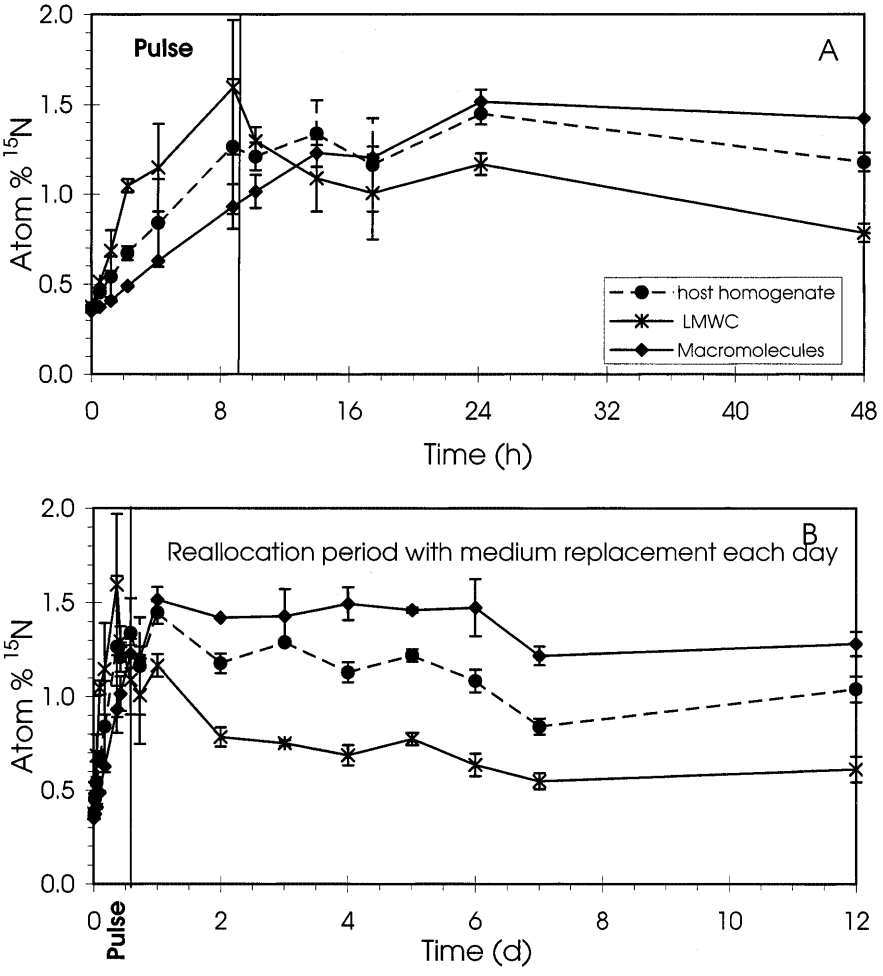

Fig. 5a, b *Bartholomea annulata*. ^{15}N isotopic composition of the host fraction, LMWC, and macromolecular components of tentacles during exposure to $^{15}NH_4^+$ and subsequent 11.6 day incubation in FSSW: **a** first 48 h of the experiment and **b** entire time course. *Error bars*: sample standard deviations ($n = 2$ tentacles)

period, suggesting that a threshold concentration exists which controls the balance of efflux and influx and hence the direction of the net flux.

A similar bi-directional flux has been observed for phosphate in corals. D'Elia (1977) used $^{32}PO_4^{3-}$ and observed both net uptake and the concomitant decrease in the specific activity of phosphate in the seawater. Applying the equations of Glibert et al. (1982) to his data indicates that the efflux of phosphate was ~50% of gross uptake, which is similar to our value for ammonium with anemones. Recent work by Kelty (2000) found a similar degree of phosphate efflux in *Aiptasia pallida*. Thus, our measurements for ammonium fluxes and those of D'Elia (1977) and Kelty (2000) for phosphorus indicate that previous measurements of net uptake of inorganic nutrients by corals and anemones (e.g. Muscatine and D'Elia 1978; Wilkerson and Muscatine 1984) have probably underestimated gross uptake rates.

We found rapid transport and assimilation of ammonium within *B. annulata*, with ^{15}N appearing in both host tissue and zooxanthellae within 30 min. Rapid assimilation of ^{15}N was also observed in several species of coral within 5 min (Burris 1983), and in the host and zooxanthellae of the anemone *Anemonia viridis* in 10 min (Roberts et al. 1999). Rapid uptake of the ammonium analogue, ^{14}C-methylamine, was reported for both *Aiptasia pallida* and the coral *Madracis mirabilis* (D'Elia and Cook 1988). Streamer et al. (1986) and Swanson and Hoegh-Guldberg (1998) reported that $^{14}CO_2$ appeared in zooxanthellae in *Acropora* and *Aiptasia*, respectively, within 1–2 min, again indicating rapid transport of inorganic compounds from the external media to the zooxanthellae. The speed of the label reaching the zooxanthellae is striking since they are separated from the seawater source of the tracer by at least two animal membranes, host cytoplasm, host vacuolar space, and their own cell membrane.

We also found rapid turnover of the internal pool of host ammonium in the tentacles of *B. annulata* (Fig. 3). Assuming ammonium from the seawater was the sole source of ammonium for the host internal pool, turnover of internal ammonium due to uptake was approximately 2.5% h^{-1}. However, since the maximum atom % ^{15}N of the internal ammonium did not reach isotopic equilibrium with the medium (~75%: Fig. 2), there must have been a significant input of unlabeled NH_4^+ from internal catabolic processes, so that the true turnover rate must have been considerably faster. Further evidence for internal production of ammonium by host tissue is the rapid decline of the atom % ^{15}N of internal ammonium

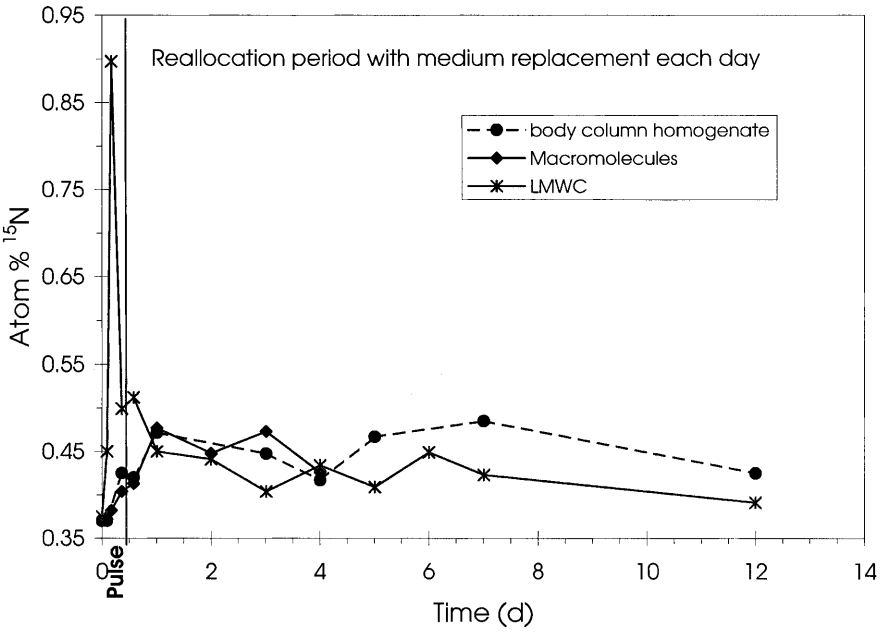

Fig. 6 *Bartholomea annulata*. ^{15}N isotopic composition of host homogenate, LMWC, and macromolecular fraction from the body column during exposure to $^{15}NH_4^+$ and subsequent 11.6 day incubation in FSSW

during the tracer reallocation period (Fig. 3). Assuming no recycling of ^{15}N, the turnover rate over the first 3 days was ~3% h^{-1}.

Several sources of ammonium could be responsible for the observed tracer kinetics in the tentacles. Local catabolism of host amino acids in the tentacle could be one source, since host LMWC had a lower isotopic content than the internal ammonium (Fig. 3). Another possibility is that ammonium generated by host body regions with few or no zooxanthellae is present in the coelenteron and tentacle lumen, and is taken up by host tissue. Experiments with *A. pallida* fed ^{15}N labeled zooplankton found rapid appearance of the tracer in zooxanthellae (Piniak and Lipschultz, in press), and Cook (1971) reported a similar result using ^{35}S-labeled food with *Aiptasia* sp., supporting the potential for transport within the coelenteron from digestive mesenteries to distal regions such as the tentacles. Regardless of the source, it is clear that the ammonium pool in the tentacles is very dynamic, responding rapidly to external influences and strongly influenced by host catabolism.

There are several potential complications to the measurements of ammonium within the tentacle tissues. Ultrasonic disruption of the anemone clearly integrates ammonium from all sites within the host tissue including the vacuolar space that is in direct contact with the zooxanthellae. There is little that can be done to overcome the homogenization of spatial structure, but it is important to keep in mind. During sample processing, deamination of proteins via enzymatic processes could produce ammonium, altering the isotopic ratio of the internal ammonium pool. However, preliminary experiments indicated that there was no change in ammonium concentration over 1 h if the disrupted anemones were kept on ice. Sample processing was completed in 0.5 h so this source of error should have been minimal. Finally, dynamics of $^{15}NH_4^+$ observed in anemones might not extend to corals, since it has been suggested that anemones have higher internal ammonium concentrations than corals (Wilkerson and Trench 1986).

Translocation of nitrogen between zooxanthellae and host

The paradigm that zooxanthellae recycle host ammonium by assimilation and translocation of compounds such as alanine (Muscatine et al. 1972) back to the host implies rapid turnover of zooxanthella nitrogen. Rahav et al. (1989) calculated that assimilation of host excretory nitrogen by zooxanthellae from *Stylophora pistillata* would result in a turnover time of 7.7 days for the total nitrogen pool of the zooxanthellae. Szmant et al. (1990) used a similar method and calculated turnover times for nitrogen of 13 days for zooxanthellae from *Acropora cervicornis* and 22 days for those from *Montastrea annularis*. Rahav et al. (1989) estimated that only 10% of the nitrogen was retained for zooxanthella growth, with the remainder going to the host whose nitrogen had a turnover time of 77 days. In contrast, Szmant et al. (1990) calculated that algal nitrogen turnover due to host excretory nitrogen balanced algal growth, implying no translocation to the host. Both reports assumed complete mixing of host excretory nitrogen with algal nitrogen. If a similar assumption is made for our experiments, then the whole-cell atom % ^{15}N of the zooxanthellae should have approached isotopic equilibrium with host nitrogen. Assuming a 12 day turnover time of zooxanthella nitrogen, the two compartments should have reached 63% of equilibrium during the 12 day reallocation period. However, in *B. annulata*, the atom % ^{15}N of the host homogenate and whole zooxanthellae never converged with that of the macromolecules (Figs. 4b, 5b), implying much longer turnover times.

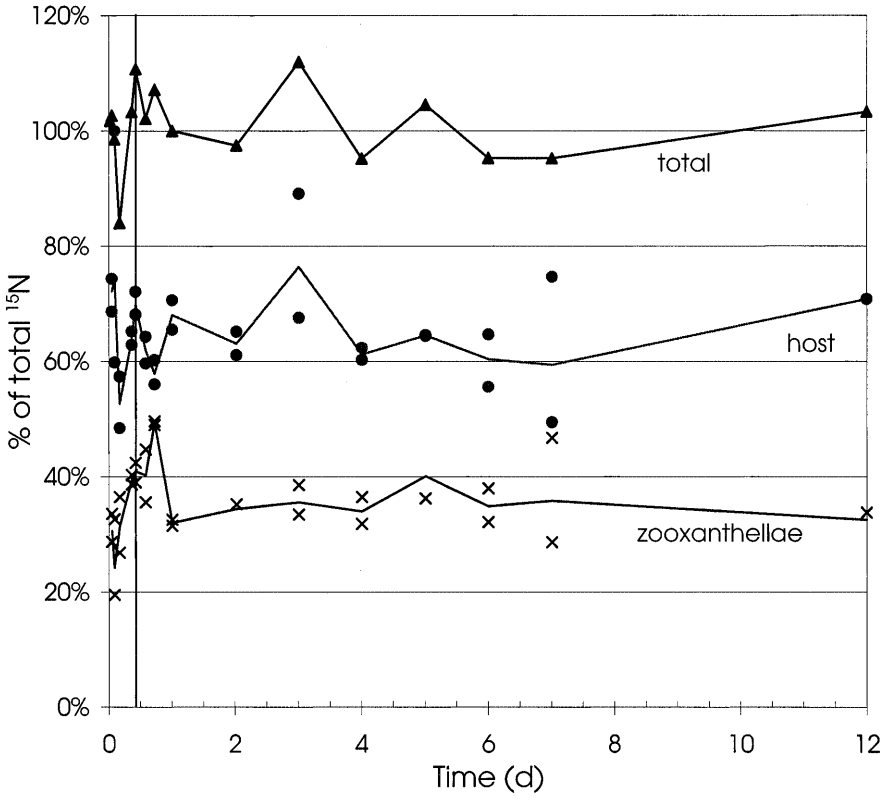

Fig. 7 *Bartholomea annulata*. Percentage of ^{15}N in host tissue and zooxanthellae of individual tentacles over entire experiment compared to the ^{15}N in the homogenate (*total*). *Each data point* represents one tentacle; the *line* is the average value

Rather than complete mixing of host excretory nitrogen with zooxanthella nitrogen, a more likely scenario is rapid turnover of precursor molecules in the LMWC, with much slower turnover of macromolecular nitrogen. Algal assimilation of host ammonium would cause rapid turnover of algal LMWC which, if translocated, would cause rapid turnover of host LMWC. Such cycling of host excretory nitrogen through the small LMWC pool in the zooxanthellae would result in a turnover time much shorter than the 7.7 days calculated by Rahav et al. (1989). Similarly, the host LMWC compartment would have a turnover time < 77 days.

The turnover of both compartments can be estimated during the reallocation period based on isotope dilution with ^{14}N from internal metabolism. Turnover estimated from isotope dilution was indeed faster in *B. annulata*: 59% day^{-1} for the zooxanthellae from 14 to 96 h (Fig. 4) and 55% day^{-1} for the host LMWC from 10 to 96 h (Fig. 5). Roberts et al. (1999) calculated turnover rates for host and zooxanthella LMWC from *Anemonia viridis* of 4.3% and 72% day^{-1}, respectively, but used data from the ^{15}N pulse period and assumed that the precursor for both pools was the external $^{15}NH_4^+$. However, a better choice of precursor is the internal ammonium pool, which has a far lower, and rapidly changing, isotopic composition (Fig. 3), at least in *B. annulata*. If these kinetic assumptions are applied to the *Anemonia* data, the rates would be much higher. Roberts et al. (1999) also measured both pools during the chase period, which included exposure to external $^{14}NH_4^+$, in contrast to our reallocation period. Although exposure to external $^{14}NH_4^+$ should have lowered the atom % ^{15}N of both LMWC pools, no dilution was detected for the zooxanthellae, and only slight dilution was observed in the host over the first hour of the 2 h chase, suggesting host assimilation of the unlabeled $^{14}NH_4^+$ and none by the zooxanthellae. Roberts et al. (1999) did find rapid declines in the atom % ^{15}N of the glutamate, aspartate, alanine, glycine, and valine pools of the zooxanthellae, which is possible if these constituents are minor components of the entire pool of LMWC.

The rapid turnover of both LMWC pools in *B. annulata* could be attributed to translocation; however, several lines of evidence suggest that the LMWC pools of the host and zooxanthellae interacted primarily with host ammonium and were relatively independent of each other. Zilversmit's rule of tracer kinetics for compartments linked in series requires a time lag between the appearance of label in a precursor compartment and one receiving the tracer (Jacquez 1985). ^{15}N moving from the host internal ammonium compartment through zooxanthellae LMWC to host LMWC should result in a lag while sufficient ^{15}N accumulates in the zooxanthella LMWC. This transfer should be seen in the much larger pool of host LMWC. In contrast, significant host assimilation of $^{15}NH_4^+$ should produce no lag, and the atom % ^{15}N of the host and zooxanthella LMWC would increase in tandem. We found no lag in the appearance of tracer in the host LMWC, and the host and algal LMWC compartments responded in concert to the tracer during the pulse period and to its removal during the reallocation period (Fig. 3). The atom % ^{15}N of

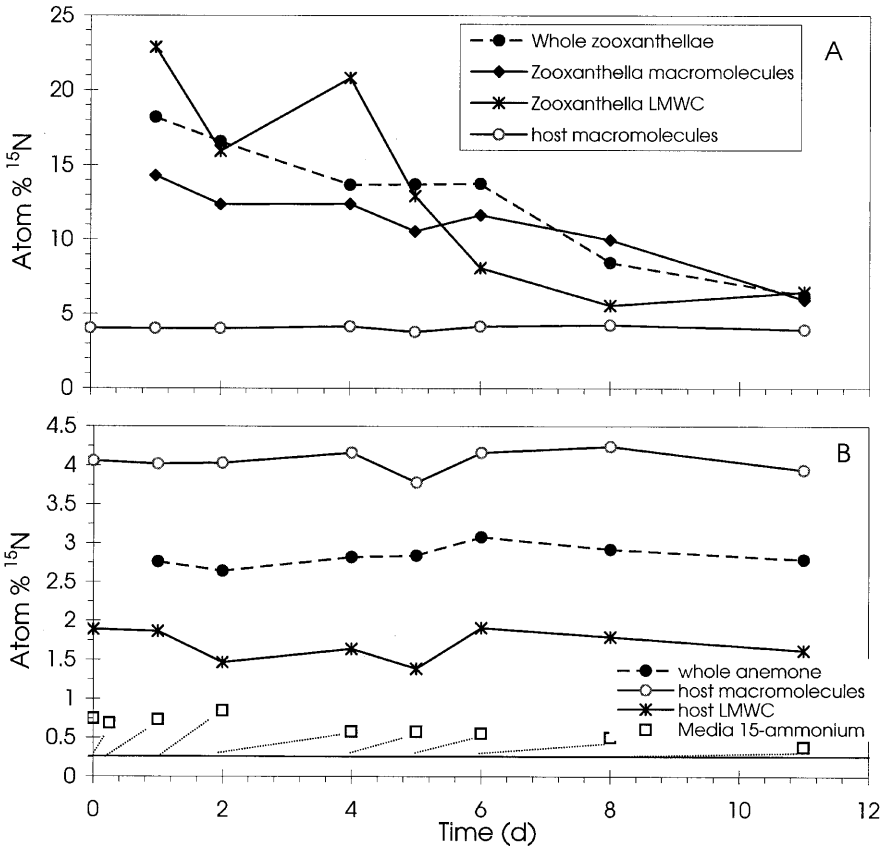

Fig. 8a, b *Aiptasia pallida*. ^{15}N isotopic composition of symbiotic anemones after 8 days of exposure to $^{15}NH_4^+$ and 11 days in unlabeled FSSW: **a** zooxanthella fractions and **b** host fractions and medium $^{15}NH_4^+$. *Lines* from baseline to datapoints during reallocation period reflect the unmeasured increase from 0.37% ^{15}N present after each sampling. Data for host macromolecules are included in Fig. 8a for comparision with declining zooxanthella isotopic composition. *Each data point* represents the combined tissues of four anemones

zooxanthella LMWC continued to increase after the animal LMWC reached a maximum; this possibly resulted from retention of labeled ammonium in the perialgal space or in the endoderm as compared to the entire tentacle.

Zilversmit's rule also requires that the maximum atom % ^{15}N of the receiving compartment is reached at the intersection with the declining isotopic composition of its precursor compartment (Jacquez 1985). The rapidly diminishing atom % ^{15}N of the internal ammonium compartment in the host did intersect the broad maxima of both the animal and zooxanthella LMWC pools (Fig. 3), suggesting again that ammonium is the primary precursor for the host LMWC, rather than the zooxanthella LMWC. Neither kinetic analysis supports substantial translocation of low molecular weight compounds from the zooxanthellae to the host on the time scale of our experiments.

Another source of ^{15}N for both LMWC pools could be the catabolism of proteins to amino acids. Sustained inputs of such amino acids to LMWC pools during the reallocation period would cause isotopic equilibrium with macromolecular pools. However, the isotopic composition of macromolecules in both zooxanthellae and host remained substantially greater than the LMWC pools over a 12 day period (Figs. 3, 4, 5). The sustained isotopic dilution and subsequent equilibrium of the two LMWC pools with host ammonium again implies that the interaction with host ammonium dominates rather than the interaction with the macromolecules.

If the zooxanthellae play a primary role in assimilation, nitrogen would need to be transported from regions with high zooxanthellar density such as the tentacles, to tissues with reduced algal content such as the body column or basal disc. There should therefore be a lag before ^{15}N appears in those tissues, and the atom % ^{15}N should continue to increase during the reallocation period. However, tracer appeared immediately in the alga-free body column of *B. annulata* (Fig. 6), just as it did in host tissue from the tentacles (Fig. 5). This implies local host assimilation of ammonium during the pulse, rather than movement of nitrogen from tissues with more symbionts. In addition, there was no further atom % ^{15}N increase in the body column during the reallocation period (Fig. 6), indicating little transport of nitrogen from tissues with high zooxanthella content.

The relatively small isotopic difference between zooxanthellae and host tissue in the tentacles in the experiment with *B. annulata* during the short pulse may have obscured translocation. In any tracer experiment, the detection of movement between compartments is partly a function of the isotopic difference between them. At isotopic equilibrium, for instance, movement of tracer could occur between two compartments, but would not be observed. The experiment with *A. pallida* was designed to enhance the isotopic difference between zooxanthellae and host by pre-loading with ^{15}N. In this

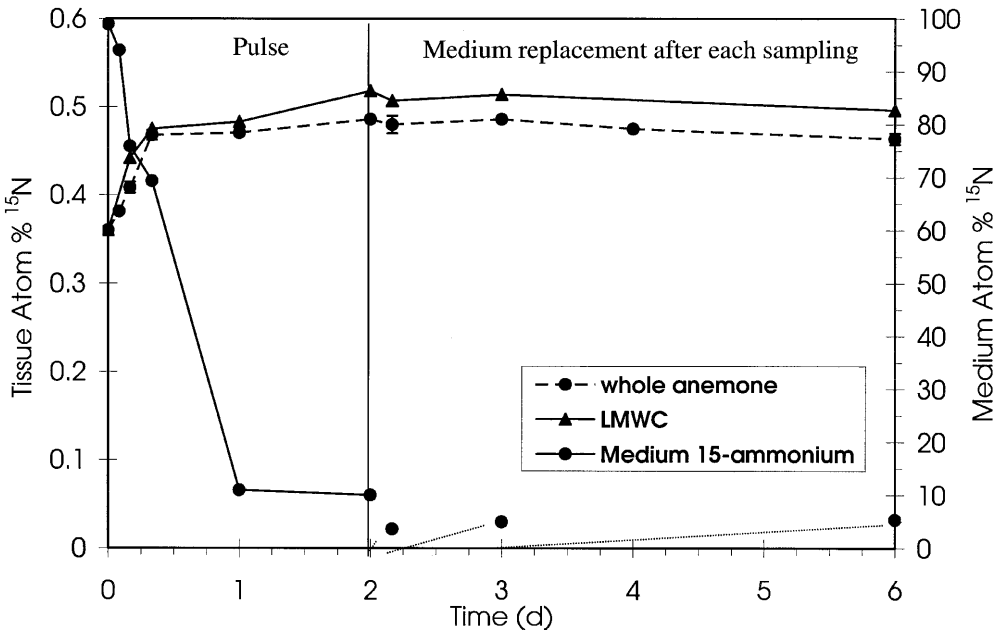

Fig. 9 *Aiptasia pallida*. ^{15}N isotopic composition of aposymbiotic anemones and medium $^{15}NH_4^+$ during 48 h of exposure to $^{15}NH_4^+$ followed by 6 days in unlabeled FSSW. *Lines connecting points* during reallocation period reflect unmeasured increase from 0.37% ^{15}N present after each sampling. *Each data point* represents combined tissues of three anemones

experiment, all fractions of zooxanthellae were highly labeled after 8 days of exposure to ^{15}N (Fig. 8a). Any translocation of algal ^{15}N should have increased the atom % ^{15}N of the host fractions during the reallocation period, but both the atom % ^{15}N of the host macromolecules (mean ± SE: 4.05 ± 0.05%) and LMWC (1.70 ± 0.08%) remained constant (Fig. 8b). The isotopic ratio of the host LMWC also was far lower than that of the algal LMWC, which again indicates that the dominant input of nitrogen to the host was not from the zooxanthellae. As with *B. annulata*, these data do not support substantial translocation from the zooxanthellae to the host on the time scale of these experiments.

The steady decline in the atom % ^{15}N of zooxanthella fractions from *A. pallida* was not observed in the experiments with *B. annulata*, and indicates incorporation of isotopically lighter nitrogen that was only available from the host. A likely explanation is that the continued exposure of *A. pallida* to high concentrations of ammonium for 8 days during exposure to the tracer resulted in an increased growth rate of the zooxanthellae during the subsequent reallocation period (e.g. Cook et al. 1988; Muller-Parker et al. 1994b). Increased growth by the algae implies that the host will receive less translocated photosynthate, and so would rely on stored reserves, including amino acids, to supply energy. Catabolic ^{14}N ammonium from the host could produce the observed decline in zooxanthella atom % ^{15}N.

Nitrogen enrichment stimulates the growth of zooxanthellae in anemones (Cook et al. 1988; Muller-Parker et al. 1989) and corals (Hoegh-Guldberg and Smith 1989; Hoegh-Guldberg 1994; Muller-Parker et al. 1994a). Given a 12 day doubling time for zooxanthellae from well-fed *A. pallida* (Cook et al. 1988), the number of zooxanthellae should have doubled and so the percentage of zooxanthella nitrogen to total nitrogen should have increased during the reallocation period. However, the ratio of zooxanthella nitrogen to total nitrogen between the first day of reallocation and during the last 6–11 days remained constant ($P > 0.10$, *t*-test) at 0.15 ± 0.2 (mean ± SE), based on mass balance of the atom % ^{15}N of the zooxanthellae and the homogenate of the entire anemone. This discrepancy is best explained by the loss of labeled cells by expulsion (Steele 1977; Hoegh-Guldberg et al. 1987; Smith and Muscatine 1999). (Consumption of zooxanthellae by the host would enrich with the isotopically heavier zooxanthella nitrogen.) Small pellets of expelled zooxanthellae were commonly observed in the incubation chamber, and their atom % ^{15}N was identical to that of freshly isolated zooxanthellae. Thus, pre-loading *A. pallida* with $^{15}NH_4^+$ probably increased algal growth, which was subsequently balanced by expulsion of highly labeled zooxanthellae. Growth of the remaining zooxanthellae likely required isotopically light nitrogen from the host, so that the ensemble of cells became progressively less enriched.

We find little direct evidence for the recycling of nitrogen between host and symbiont in our study. Szmant et al. (1990) found low rates of ammonium efflux by host corals; their calculations of turnover times of zooxanthella nitrogen based on growth rates suggested no recycling at all. Calculations of the extent of recycling generally assume that production of large amounts of ammonium by the host after a prolonged period of darkness occurs as part of the normal metabolism of the symbiosis (e.g. Rahav et al. 1989). Ammonium efflux rates of symbiotic corals following 48 h of darkness have been used to calculate zooxanthella nitrogen turnover times (Rahav et al. 1989; Szmant et al. 1990). However, Szmant et al. (1990) showed that ammonium influx rates in symbiotic corals in darkness diminished to zero by 12 h; uptake and efflux remained in balance until 36 h, when net loss

rates began increasing. Since corals never are exposed to such prolonged periods of darkness in the field, extrapolating such arbitrary efflux rates to a normal light regime will lead to erroneous conclusions. Little difference has been observed between uptake of ammonium by corals at night and during the day (Muscatine and D'Elia 1978; Shyka and Lipschultz, unpublished data), suggesting that energy reserves are generally sufficient over 12 h periods. Since prolonged darkness reduces translocated photosynthate, the conservation paradigm suggests that unfed hosts in the dark should consume nitrogen-containing compounds to support respiration with resultant production of ammonium. Rather than measuring processes normally operating within the symbiosis, the result of prolonged darkness is conversion of the symbiosis into a more heterotrophic organism with typical excretion patterns.

Role of zooxanthellae and host in the assimilation of NH_4^+

Symbiotic cnidarians typically take up ammonium in the light (Szmant-Froelich and Pilson 1977; Muscatine and D'Elia 1978; Wilkerson and Muscatine 1984), while aposymbiotic ones do not (Muscatine et al. 1979; Szmant-Froelich and Pilson 1984; Wilkerson and Muscatine 1984). These observations support the dominant role of zooxanthellae in ammonium assimilation in these associations. However, evidence is accumulating that host assimilation of ammonium is also important (Miller and Yellowlees 1989; Szmant et al. 1990; Rees 1991; Wang and Douglas 1998). Glutamine dehydrogenase (GDH) activity in coral host tissue exceeds glutamine synthase (GS) activity in the zooxanthellae (Yellowlees et al. 1994). GS has also been reported in coral tissues (Yellowlees et al. 1994). Wang and Douglas (1998) found that GS and host ammonium concentrations in *A. pulchella* were inversely correlated, with low GS activity and high ammonium present in darkness. Addition of organic carbon reversed the effect of darkness, presumably by supplying exogenous carbon for catabolism in place of host carbon reserves. This underscores the algal role in the conservation paradigm as providing an energy source to conserve nitrogen. The host assimilation of inorganic nitrogen via GDH and GS pathways permits the host to meter the flow of nitrogen to the zooxanthellae, thereby controlling their density (Miller and Yellowlees 1989; Rees 1991).

Our experiments with *B. annulata* and *A. pallida* indicate a significant role for host assimilation of ammonium. First, the rapid, parallel appearance of tracer in host and zooxanthella LMWC (Fig. 3) is consistent with direct assimilation of ammonium by the host. The application of Zilversmit's rule for precursor and product indicated that host internal ammonium was the precursor for host LMWC as well as zooxanthella LMWC. Third, tracer appeared in the body column LMWC of *B. annulata* (Fig. 6) even though no zooxanthellae were present in this region. Fourth, the lack of atom % ^{15}N increase in host macromolecules during the reallocation period (Figs. 5, 8) implied local assimilation by the host, rather than translocation from zooxanthellae or other areas of the animal. Finally, the amount of tracer present in the host tentacle tissue compared to the entire tentacle of *B. annulata* was substantial (66%) over the entire experiment (Fig. 7). It appears that host tissue from both anemones was able to intercept a large proportion of the external ammonium and retain it for the duration of the experiment.

In their ^{15}N study, Roberts et al. (1999) concluded that zooxanthellae were the primary site for assimilation, not the sea anemone host. The primary evidence for this was that the zooxanthellae had between 5.6 and 16.9 times the labeling as did the host. Our results with different species of anemones are similar, but our interpretation differs. The preponderance of nitrogen in host tissue balances a lower degree of labeling so that significant amounts of ^{15}N is found in the host at all time points (e.g. Fig. 7). If the zooxanthellae contained 20% of the total nitrogen and had 5.6 times higher atom % ^{15}N than host tissue, then the host actually had 42% of the total $^{15}N(0.8\times1)/[(0.2\times5.6+0.8\times1)]$ acquired by the anemone. It is interesting that the calculations for "fractional synthetic rate" used by Roberts et al. (1999) were based on external ammonium as the precursor pool for both zooxanthellae and the host, implying direct host assimilation of ammonium rather than translocation.

Swanson and Hoegh-Guldberg (1998) examined the incorporation of ammonium into amino acids derived from short-term photosynthetic products in *A. pulchella*. They found that photosynthetic ^{14}C rapidly appeared in amino acids in the zooxanthellae, while only small amounts of labeled amino acids were found in the host, suggesting that zooxanthellae were the primary site of ammonium assimilation. In particular, glutamine, the product of GS activity, had 15 times more label in the zooxanthellae than in host tissue. One problem with this approach is that the specific activity of ^{14}C compounds would be higher in the algae due to photosynthesis, while that of the host would be diluted by unlabeled host free amino acid pools. Swanson and Hoegh-Guldberg (1998) considered this unlikely, since rapid translocation of recently fixed carbon would mitigate the differential as the compounds would be used for carbon "backbones" for ammonium assimilation. However, this depends on the extent of translocation and the rate at which translocated carbon is respired by the host. The rate of translocation in *A. pallida*, for instance, is relatively low ($<20\%$ of total fixed carbon; Davy and Cook 2001). Although the amount of ^{14}C in some amino acids from the zooxanthellae was large, amino acids in the host actually had 0.1–2% of the ^{14}C as a fraction of the total ^{14}C, as compared to 0.1–3% in the zooxanthellae over all time periods from 1 to 240 min (Swanson and Hoegh-Guldberg 1998). This indicates that equal amounts of ammonium were needed to synthesize amino acids from carbon skeletons in both host and zooxan-

thellae, similar to our findings and those of Roberts et al. (1999).

Zooxanthellae may play an important role in ammonium assimilation by producing essential amino acids for the host. It is unclear, however, how many or which amino acids are actually essential for cnidarians. Fitzgerald and Szmant (1997) found that scleractinian corals could produce all essential amino acids from ^{14}C-glucose except threonine. Anemones, however, are not capable of de novo production of all essential amino acids (Swanson and Hoegh-Guldberg 1998; Wang and Douglas 1999). Thus, although some amino acids may require an algal source, evidence suggests that the host can utilize significant amounts of ammonium for production of the other amino acids (e.g. taurine) which can be a significant proportion of the total free amino acid pool (Swanson and Hoegh-Guldberg 1998; Wang and Douglas 1998).

The amount of tracer in the body column of *B. annulata* was always far less than in macromolecules from the tentacles, and was surprisingly similar to that in tissues of aposymbiotic *A. pallida* (Fig. 9). One explanation for the lower assimilation in these tissues is the lack of translocated photosynthate compared to the tentacles with greater zooxanthellar densities. When aposymbiotic *A. pulchella* were supplemented with various carbon sources, GS activity and other metabolic indicators shifted towards values comparable to those of symbiotic anemones (Wang and Douglas 1998). This result is consistent with the role of translocated carbon in conserving host nitrogen.

The low degree of ammonium assimilation in the body column of *B. annulata* and aposymbiotic *A. pallida* also suggests a requirement for some form of organic nitrogen to replace that lost via efflux and growth. Given that ^{15}N was not translocated from the tentacles, host feeding or use of exogenous dissolved organic nitrogen should be more important in these tissues. Ammonium is produced via respiration and excreted in regions distant from the tentacles, while both host and zooxanthellae in the tentacles act as a strong net sink. However, experimental protocols rarely accommodate the obvious fact that different regions of the animal function differently, especially in symbiosis.

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