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Symbiotic anemones can grow when starved: nitrogen budget for *Anemonia viridis* in ammonium-supplemented seawater

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Abstract The ability of endosymbioses between anthozoans and dinoflagellate algae (zooxanthellae) to retain excretory nitrogen and take up ammonium from seawater has been well documented. However, the quantitative importance of these processes to the nitrogen budget of such symbioses is poorly understood. When starved symbiotic *Anemonia viridis* were incubated in a flow-through system in seawater supplemented with 20 μ*M* ammonium for 91 d under a light regime of 12 h light at 150 μmol photons m^{-2} s^{-1} and 12 h darkness, they showed a mean net growth of 0.197% of their initial weight per day. Control anemones in unsupplemented seawater with an ammonium concentration of <1 μ*M* lost weight by a mean of 0.263% of their initial weight per day. Attempts to construct a nitrogen budget showed that, over a 14 d period, ≃40% of the ammonium taken up could be accounted for by growth of zooxanthellae. It was assumed that the remainder was translocated from zooxanthellae to host. However, since the budget does not balance, only 60% of the growth of host tissue was accounted for by this translocation. The value for host excretory nitrogen which was recycled to the symbionts equalled that taken in by ammonium uptake from the supplemented seawater, indicating the importance of nitrogen retention to the symbiotic association.

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Introduction

Many symbiotic cnidarians are able to deplete ammonium from seawater when exposed to light (e.g. Kawaguti 1953; Muscatine and D'Elia 1978; Wilkerson and Trench 1986) whereas aposymbiotic individuals excrete ammonium (e.g. Cates and McLaughlin 1976; Szmant-Froelich and Pilson 1977; Muscatine et al. 1979; Wilkerson and Muscatine 1984). Non-symbiotic ahermatypic corals also excrete ammonium (e.g. Muscatine and D'Elia 1978; Burris 1983). Some symbiotic corals show the capacity to take up nitrate (e.g. Franzisket 1973, 1974; D'Elia and Webb 1977; Webb and Wiebe 1978; Wilkerson and Trench 1986; Marubini and Davies 1996), but this has never been demonstrated for symbiotic anemones (Wilkerson and Muscatine 1984; Davies 1988; Roberts unpublished data).

It is now well established that zooxanthellae can translocate most of the carbon fixed by photosynthesis to the host (Davies 1984; Muscatine et al. 1984; Edmunds and Spencer Davies 1986; Tytler and Davies 1986; Davy et al. 1996). This organic carbon supply can meet, and sometimes exceed, the growth and respiratory demands of the host (Davies 1984; Muscatine et al. 1984; Edmunds and Spencer Davies 1986). Previous work on the temperate sea anemone *Anemonia viridis* (Forskål) has suggested that it could be autotrophic with respect to energy requirements when maintained without feeding under an illumination of 140 μmol photons m^{-2} s^{-1} with a 12 h light:12 h dark photoperiod (Tytler 1982; Tytler and Davies 1986). However, when anemones were maintained without feeding under this light regime for 84 d they lost weight, albeit at a lower rate than anemones maintained in darkness (Tytler and Davies 1986). These growth experiments were carried out in a circulating seawater

aquarium with no nutrient addition. Subsequently, Davies (1988) repeated these growth experiments, but incubated one group of anemones in seawater which was supplemented with ammonium. Under these conditions, there was only minimal weight loss over an 84 d period. Since any weight gain or weight loss is the result of processes of biosynthesis and catabolism, this observation was interpreted as demonstrating an enhancement of biosynthesis resulting from ammonium assimilation.

Previous studies on the effects of elevated ammonium on corals have shown striking increases in the population of algal symbionts (Hoegh-Guldberg and Smith 1989; Muscatine et al. 1989; Dubinsky et al. 1990; Stambler et al. 1991; Hoegh-Guldberg 1994; Muller-Parker et al. 1994) whilst effects on growth of the host have been ambiguous. Thus, exposure to 20 μ*M* ammonium in *Pocillopora damicornis* increased (Muller-Parker et al. 1994) or produced no effect (Achituv et al. 1994) on the protein content of the host. Host protein was also unchanged during similar experiments with *Styllophora pistillata* (Muscatine et al. 1989). However, these coral studies were usually of short duration (2 to 3 wk), which may have been too little time for changes in growth of host to be detectable.

The long-term (84 d) experiments of Davies (1988) utilized a protocol in which the anemones were held in tanks of seawater which were spiked to an initial concentration of 20 μ*M* ammonium once per day. As ammonium was taken up by the anemones, the concentration fell to an average of 5.6 μ*M* before being replenished. The average concentration to which the anemones were exposed was therefore considerably below the starting concentration of 20 μ*M*. It was therefore of interest to determine whether net growth could be achieved if the anemones were incubated at a constant higher level of ammonium by using a flow-through protocol. The second objective was to determine how the ammonium taken up was partitioned between zooxanthellae and host, and to assess the quantitative importance of any recycled excretory nitrogen.

Materials and methods

Specimens of the brown colour morph of *Anemonia viridis* were collected from between 1 and 2 m depth in Loch Sween, Argyll, on the west coast of Scotland, and maintained in recirculating seawater aquaria at the Division of Environmental and Evolutionary Biology (University of Glasgow). In these aquaria, the following conditions were standardized: temperature 15 °C (±1C°), 34‰ S; illumination 70 μmol photons m^{-2} s^{-1} provided by cool-white fluorescent strip lights and a 12 h light:12 h dark daily photoperiod. The anemones were fed weekly on chopped, frozen mussel tissue (*Mytilus edulis*). Since individual anemones may have experienced different environmental regimes in the field, they were acclimatised to these standard conditions for at least 4 wk before use in experiments. Aposymbiotic anemones were from stock which had been maintained in darkness for between 4 and 5 yr and fed twice weekly.

Effect of long-term exposure to ammonium on weight change of anemones

Twenty symbiotic anemones which had been maintained under standard laboratory conditions were buoyant-weighed (Tytler 1982) and divided randomly between two aquarium tanks. The anemones were maintained under an irradiance of 150 μmol photons m^{-2} s^{-1} which provided sufficient illumination to saturate photosynthesis (Tytler 1982) and was comparable to that used in the growth studies of Tytler and Davies (1986) and Davies (1988). The control tank was supplied with aquarium seawater at ambient ammonium concentrations (< 1 μ*M*), while the experimental tank was supplied with seawater containing ≃20 μ*M* ammonium [= 10 μ*M* $(NH_4)_2SO_4$]. This was produced by mixing 99 parts of seawater (at 3.4 litres h^{-1}) with one part of 1 m*M* ammonium sulphate (at 0.034 litre h^{-1}) in a glass chamber. The seawater and ammonium-supplemented seawater were delivered to their respective tanks at a constant rate by peristaltic pumps (Watson-Marlow). The ammonium concentrations were monitored in samples from both tanks using the method of Liddicoat et al. (1975), and the tanks were thoroughly cleaned twice a week to prevent algal growth. Each anemone was buoyant-weighed (Tytler 1982) at weekly intervals throughout the duration of the experiment using an electronic balance (Mettler AJ50). The weight of each anemone was then expressed as the percentage of the initial buoyant weight. The anemones were not fed during the experiment, and any anemones that divided were excluded from the analysis. It was intended to maintain these conditions for a period of at least 80 d. However, after 63 d, a failure in the seawater supply to the control group ended this treatment. Since the ammonium-treated group was supplied from a seawater reservoir, it was possible to continue their treatment for a total of 91 d.

Nitrogen budget

Rationale

The model on which the budget was based (Fig. 1) is that of Davies (1992). It was assumed that the ammonium taken up by starved anemones in 20 μ*M* ammonium-supplemented seawater was assimilated into the algal symbionts during daytime photosynthesis, and that any nitrogen which was not used in the growth of the symbionts was translocated as amino acids to the host. Here it would be incorporated into the host's intracellular amino acid pool and used in the biosynthesis of proteins for growth of the anemone. Concurrently, catabolism of host protein would yield excretory ammonium. Some of this ammonium would be used in resynthesis of protein in the host cytoplasm; the remainder would be recycled to the symbionts, since under the experimental conditions adopted there would be no net excretion of ammonium to the seawater during night or day (Davies 1988; Roberts unpublished data).

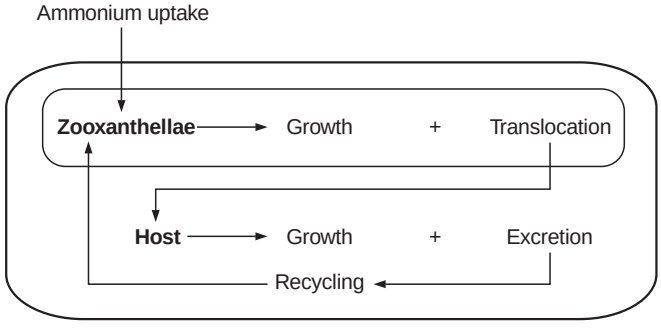

Fig. 1 *Anemonia viridis*. Simplified model of net nitrogen fluxes in an unfed anemone over 24 h period under 12 h light:12 h dark photoperiod (from Davies 1992)

The rate of nitrogen uptake was measured by the rate of depletion of ammonium from the incubating water; the rate of nitrogen utilisation in the growth of algae was determined from algal growth rate and measurement of their nitrogen content; translocation was estimated from the rate of assimilation minus the nitrogen used in algal growth; host growth was similarly calculated from the growth rate of host tissue and measurement of nitrogen content; the rate of catabolism was estimated from the rate of excretion of ammonium by aposymbiotic anemones. The budget was normalised to a 1 g dry weight individual and expressed on a 24 h basis.

Experimental procedure

Before the start of the experiment, 16 symbiotic anemones were maintained for 4 wk without feeding, under an illumination of 300 μmol photons m^{-2} s^{-1}. Eight anemones were buoyant-weighed and sacrificed in order to determine the dry weight and nitrogen content of both zooxanthellae and of host tissue (see below). These are referred to as "time zero" anemones. A further eight symbiotic anemones were buoyant weighed and transferred to individual 250 ml glass beakers containing autoclaved 0.3 μm-filtered seawater (AFSW). The beakers were fitted with glass spouts that overflowed when filled to ≃200 ml. After 1 h, the anemones had attached to the chambers and the seawater was removed and replaced with AFSW supplemented with 20 μ*M* ammonium. Each chamber was supplied with a glass inflow tube to deliver ammonium-supplemented seawater to the base of the chamber. This tube was connected to a ten-channel peristaltic pump (Autoclude VL) whose inlet came from a reservoir containing the chilled ammonium-supplemented AFSW. The flow rates to the chambers were ≃0.04 litres h^{-1}, but each flow rate was accurately recorded throughout the experiment in order to calculate the rate of ammonium uptake. Each chamber also contained a second glass tube connected to an aquarium air pump whose output was passed through a 2 *M* sulphuric acid trap to remove any atmospheric ammonia. Preliminary experiments showed that the air bubbled into the beakers provided good aeration and mixing of the water, and did cause the loss of ammonium within the chambers (data not shown). For the 14 d duration of the experiment, the experimental chambers were maintained at 15 °C in a water bath, at an illumination of 300 μmol photons m^{-2} s^{-1} provided by cool-white fluorescent lights within a light hood. The system was regularly cleaned to prevent algal growth. After 14 d, the anemones were removed, buoyant-weighed and treated in an identical way to the time zero anemones to determine dry weights and nitrogen contents.

Ammonium-uptake rate of symbiotic anemones

This was calculated by measuring the difference in ammonium concentration flowing into and out of each incubation chamber, using the following equation:

$$\begin{aligned}\text{Uptake rate} &= \Delta[NH_4^+]\,(\mu\text{mol l}^{-1}) \times \text{flow rate}\,(\text{l h}^{-1}) \\ &\quad \times (1/\text{g dry wt}) \\ &= \mu\text{mol}\,NH_4^+\,\text{g}^{-1}\,\text{h}^{-1}.\end{aligned}$$

Duplicate 250 μl seawater samples were taken during the light photoperiod and at the end of each 12 h dark photoperiod, and the ammonium concentration was measured using a micro-assay modification of the method of Parsons et al. (1984) in a 96-well microplate. Sodium dichloroisocyanurate was used instead of sodium hypochlorite (Solórzano 1969), and the absorbance of samples and standards was measured at 620 nm using an automated plate reader (Dynatech).

Ammonium-excretion rate of aposymbiotic anemones

The rate at which aposymbiotic anemones excreted ammonium was measured using the same protocol and under identical light and ammonium levels to those described in the foregoing subsection. The aposymbiotic anemones were unfed for a period of 3 wk before the experiment.

Growth

The incorporation of nitrogen into growth of zooxanthellae and host tissue was estimated from the difference between the nitrogen content at the start (predicted from the time zero anemones), and that measured at the end of the 14 d incubation period. At Day zero, after buoyant-weighing eight of the 16 light-adapted anemones, they were sacrificed and then cut longitudinally into two halves from oral disc to pedal disc. Both halves were blotted dry and weighed to provide the relative weights of the two pieces. One-half was then lyophilised and weighed. The dry weight to buoyant weight ratios obtained in this way were then used to predict the dry weights of the experimental anemones from their buoyant weights. The other halves were homogenised (Ultra-Turrax T25, Janke and Kunkel) in artificial seawater (w/v: 3.1% NaCl, 1.0% $MgSO_4$, 0.002% $NaHCO_3$) containing 0.05% (w/v) sodium dodecyl sulphate (SDS) (McAuley 1986). The SDS counteracted the foaming resulting from mucus production by the column and mesenteries. This tissue slurry was allowed to stand at room temperature for 20 min, after which it was centrifuged for 2 min at 350 ×*g*. The supernatant was removed, the zooxanthellae pellet resuspended in artificial seawater containing 0.05% SDS, and the centrifugation step was repeated several more times. The supernatants from the first two centrifugations were combined to produce the host fraction. The zooxanthellae and host fractions were then lyophilised and weighed.

The total nitrogen content of the two fractions was analysed using a micro-Kjeldahl procedure based on the method of Lang (1958). Following acid digestion of samples containing between 25 and 100 μg nitrogen, the ammonia produced was assayed using the Nessler reaction. The absorbance of samples and ammonium sulphate standards was measured at 490 nm (Phillips PU8720 spectrophotometer). The nitrogen content of zooxanthellae and host fractions was then used to predict the nitrogen content of the experimental anemones on Day zero. At the end of the 14 d experiment, the anemones were sacrificed, fractionated, and the dry weights and nitrogen contents of the fractions were determined as described above.

Results

Effect of ammonium on weight change of *Anemonia viridis*

During this experiment, four anemones divided (two from each treatment) and were excluded from the analysis, leaving eight individuals from both the control and ammonium-treated groups. The anemones treated with 20 μ*M* ammonium showed an overall increase in buoyant weight throughout the 91 d period of treatment. Over the 63 d of the control experiment, the anemones in unsupplemented seawater showed an overall decrease in buoyant weight. The rate of weight change for each treatment was calculated by fitting a regression line to the data (Fig. 2). The regression for ammonium-treated anemones produced a positive slope which was significantly different from the negative slope corresponding to control anemones (ANCOVA $F_{1,184} = 34.66$, $p < 0.001$) (Table 1). The control anemones decreased in weight by a mean of 0.263% of the initial buoyant weight per day, whilst the ammonium-

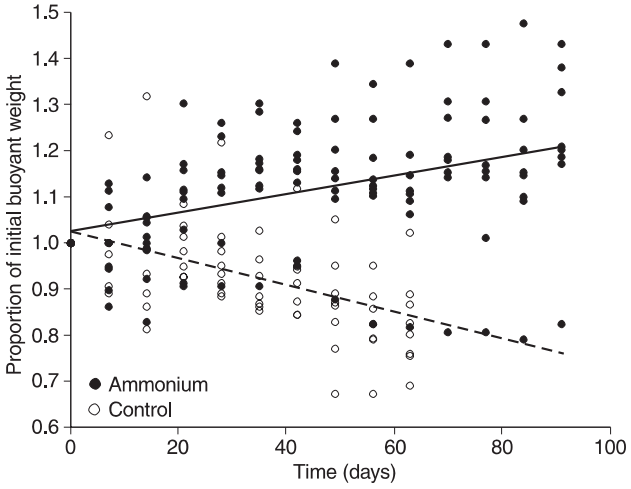

Fig. 2 *Anemonia viridis*. Effect of ammonium treatment on weight change of unfed anemones under 150 μmol photons m^{-2} s^{-1} illumination (12 h light:12 h dark photoperiod). One group of anemones ($n = 8$) was maintained for 91 d in seawater supplemented with 20 μ*M* ammonium; second (control) group ($n = 8$) was maintained in seawater at ambient ammonium concentration (< 1 μ*M*) for 63 d. Lines were fitted using linear regression for both ammonium (*continuous line*) and control (*dashed line*) treatments

treated anemones increased by a mean of 0.197% of their initial buoyant weight per day.

Nitrogen budget

Ammonium-uptake rate

Throughout the 14 d exposure, the anemones took up ammonium at a mean uptake rate over the 14 d of 2.20 μmol NH_4^+ g^{-1} h^{-1}, which is equivalent to 0.74 mg N g^{-1} d^{-1}. Since at the end of each 12 h dark period there was still detectable uptake by the anemones, there would be no net loss of ammonium from the symbiosis during the night.

Ammonium-excretion rate

The ammonium excretion rate recorded from aposymbiotic anemones under a constant inflow of 20 μ*M* ammonium was 2.23 μmol NH_4^+ g^{-1} h^{-1}, which is equivalent to 0.75 mg N g^{-1} d^{-1}. It was assumed that this corresponds to the ammonium that is recycled in symbiotic anemones, since they showed no net loss of ammonium in darkness.

Growth

During the course of the experiment, the mean anemone dry weight decreased by 5.4 ± 4.6%, and the relative biomass of the zooxanthellae showed a non-significant increase from 17.5 ± 9.0% to 18.2 ± 4.7% of the anemone dry weight (Table 2). The dry weight of the zooxanthellae decreased from 180 to 170 mg per (original) g dry weight of anemone, and the weight of host tissue decreased from 820 to 776 mg per (original) g dry weight of anemone. However, the total nitrogen content of both the zooxanthellae and host fractions increased significantly from 6.6% of dry weight to 9.5% in the zooxanthellae, and from 10.7 to 14.8% in the host tissue following exposure to 20 μ*M* ammonium for 14 d. This resulted in an increase in the nitrogen mass of both zooxanthellae and host tissues, from 11.9 to 16.2 mg N per g dry weight of tissue in the zooxanthellae and from 87.7 to 114.8 mg N in the host. For the 24 h nitrogen budget, this corresponds to a growth rate of 0.30 mg N g^{-1} h^{-1} for the zooxanthellae and 1.90 mg N g^{-1} d^{-1} for the host.

Translocation

This was calculated as the difference between nitrogen input to the zooxanthellae and that used in growth: i.e. $(0.74 + 0.75) - 0.30 = 1.19$ mg N g^{-1} d^{-1}.

The parameters measured to calculate the nitrogen fluxes in the budget model are given in Table 3, and the nitrogen budget thus derived is illustrated in Fig. 3.

Discussion and conclusions

Effect of ammonium on weight change of anemones

In previous experiments in which unfed *Anemonia viridis* were kept for 80 d in seawater which was spiked daily to a concentration of 20 μ*M* ammonium, only a minor weight loss was observed, whilst control anemones in seawater at ambient ammonium concentrations displayed a significant weight loss (Davies 1988). However,

Table 1 *Anemonia viridis*. Change in buoyant weight of anemones during incubation in 20 μ*M* ammonium for 91 d or in seawater at ambient ammonium concentration (< 1 μ*M*) for 63 d under 150 μmol photons m^{-2} s^{-1} illumination (12 light:12 h dark photoperiod) (*y* proportion of initial buoyant weight; *x* time in days; R^2-*adj* coefficient of determination adjusted for number of parameters in the model; *p* significance of regression) ($n = 8$)

Treatment	% Buoyant weight change d^{-1}	R^2-adj	*p*	Equation of line
Control	−0.263	24.6	< 0.001	$y = 1.03 - (0.00279x)$
Ammonium	0.197	12.4	< 0.001	$y = 1.03 + (0.00185x)$

Table 2 *Anemonia viridis*. Biomass and nitrogen (means ± SD) changes in anemones after 14 d incubation in 20 μ*M* ammonium ($n = 8$). Day 0 values derived from eight anemones sacrificed at start of experiment and normalised to 1 g dry wt individual. Day 14 values give corresponding predicted weights of 1 g dry wt individual at end of experiment. Significance of difference between control and experimental treatments was assessed using a two-sample *t*-test. Masses are expressed per gram anemone dry weight, where zooxanthellae account for 18% of total mass (i.e. zooxanthellae + host mass = 1 g) for both Day 0 and Day 14 groups

Parameter	Day 0	Day 14	Change	*p*
Biomass ratio (zooxanthellae as % anemone dry weight)	17.5 ± 9.0	18.2 ± 4.7	+0.7	>0.05
Mass zooxanthellae g^{-1} total (mg)	180	170	−10	
% N content of zooxanthellae	6.60 ± 1.10	9.50 ± 0.72	+2.9	<0.01
Mass zooxanthellae N g^{-1} total (mg)	12	16	+4	
Mass host g^{-1} total (mg)	820	776	−44	
% N content of host	10.73 ± 3.34	14.8 ± 3.4	+4.07	<0.05
Mass host N g^{-1} total (mg)	88	115	+27	

Table 3 *Anemonia viridis*. Parameters used to calculate nitrogen budget of a 1 g dry wt individual in 20 μ*M* ammonium. Nitrogen budget does not balance, since nitrogen translocated (1.19 mg N g^{-1} d^{-1}) only accounts for 63% of measured host growth (1.9 mg N g^{-1} d^{-1})

Parameter measured	Nitrogen flux in model	mg N g^{-1} d^{-1}
Rate of ammonium uptake	Nitrogen input to symbiosis	0.74
Rate of ammonium excretion	Nitrogen recycled	0.75
Change in zooxanthellae total N	Zooxanthellae growth	0.30
Change in host total N	Host growth	1.90
Input to zooxanthellae, less growth	Translocation	1.19

these experiments involved closed-chamber incubations, so that the actual ammonium concentration declined during the course of the day as it was taken up by the anemones. The present experiments were designed to overcome this shortcoming by using a flow-through incubation system in which the ammonium was continually replenished.

Over the first 14 d, there was no apparent change in the weight of either the control or ammonium-treated anemones. However, during the rest of the experiment the control anemones in seawater at ambient ammonium concentrations (< 1 μ*M*) lost weight, whereas anemones in seawater at 20 μ*M* ammonium gained weight. This has also been observed by Beaver (1996) who demonstrated that unfed *Anemonia viridis* increased in weight by 11.4% over 80 d in 20 μ*M* ammonium-supplemented seawater and that almost 60% of this weight change could be accounted for by increased host protein. In the present study, unfed symbiotic anemones increased in weight by 15.8% over the same time period in 20 μ*M* ammonium-supplemented seawater.

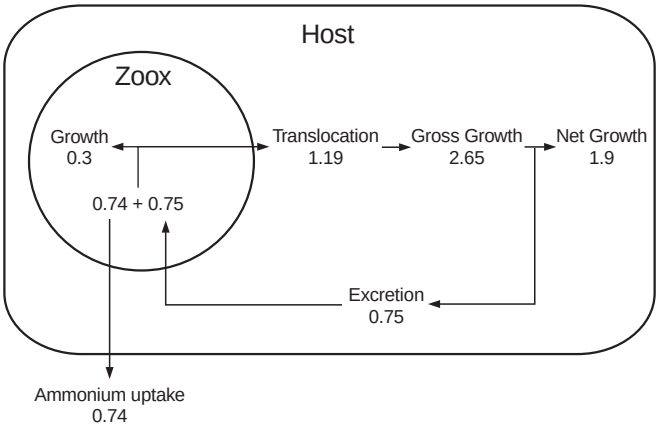

Fig. 3 *Anemonia viridis*. Diagram of 24 h nitrogen budget (mg N g^{-1} d^{-1}) of symbiotic anemone incubated under illumination of 300 μmol photons m^{-2} s^{-1} (12 h light:12 h dark photoperiod) in seawater containing 20 μ*M* ammonium. Since amount of nitrogen supplied by ammonium uptake does not account for observed growth, this budget cannot be balanced

These experiments have shown for the first time that net growth can be achieved with dissolved inorganic nitrogen as the only major source of nitrogen, under appropriate experimental conditions, which in this case were an illumination level of 150 μmol photons m^{-2} s^{-1} and a 20 μ*M* ammonium concentration. Direct comparisons with previous experiments are not possible, since these have used either lower illumination or lower concentrations of ammonium.

Weight change can be considered a result of metabolic processes, mainly protein synthesis producing weight gain, and catabolic processes resulting in weight loss. Previously it has been observed that starved symbiotic anemones exposed to daytime light in unsupplemented seawater lose weight more slowly than those kept in darkness (Tytler and Davies 1986). This could be explained by assuming that protein catabolism to ammonium occurs at a relatively constant rate, and this nitrogen is excreted during prolonged darkness. However, in those anemones exposed to daytime light, this nitrogen is retained within the symbiosis and is available for protein synthesis, allowing the rate of weight loss to be reduced.

Why then do starved symbiotic anemones lose weight at all, if exposed to photosynthetically saturating light

during the day? Under these conditions, excretion of nitrogen does not take place during darkness, since carbon skeletons are available within the zooxanthellae for its assimilation (Muscatine and D'Elia 1978; Davies 1988). With fixed carbon compounds being provided for energy metabolism by photosynthesis, and with excretory nitrogen being recycled, it is difficult to understand why weight loss is observed. This may point to another critical nutrient, perhaps phosphate, which is not being recycled during darkness, or to carbon loss by respiration.

Nitrogen budget

Whilst there is a large literature which demonstrates the uptake of ammonium by symbiotic anemones and corals, there is very little information on how the assimilated nitrogen is partitioned between algae and host. A budget derived by Davies (1992) from data of Rahav et al. (1989) for the coral *Stylophora pistillata* in unsupplemented seawater, used a predicted rather than a measured value for the rate of dissolved inorganic nitrogen (DIN) uptake, and the problem of an unbalanced budget therefore did not arise. The budget which we have calculated for *Anemonia viridis* under nitrogen-supplemented conditions fails to balance, since the nitrogen allocated to growth of zooxanthellae and host is 66% higher than the apparent nitrogen uptake. This could be due to either an overestimate of the rate of growth or to an underestimate of the rate of nitrogen intake.

Rate of growth is expressed in the nitrogen budget in terms of nitrogen increase. This was achieved in the course of a decrease in actual dry weight, by an increase in the relative proportion of nitrogen in the tissues of both algae and host. It appears unlikely that growth-measurement errors are a major contributor to this paradox. It is possible, however, that nitrogen uptake by ammonium assimilation is an underestimate. A similar conclusion was reached by Hawkins and Klumpp (1995), who found that only ~70% of the apparent nitrogen demand of the symbiotic giant clam *Tridacna gigas* could be met from ingested particles and dissolved inorganic nitrogen uptake. They concluded that additional sources of nitrogen could have been available to the clams, either as nitrate or as dissolved organic matter. It has been conclusively shown that *Anemonia viridis*, in common with other temperate anemones, is unable to assimilate nitrate (Wilkerson and Muscatine 1984; Davies 1988; Roberts unpublished data). However, the uptake of dissolved organic matter by both symbiotic and non-symbiotic cnidarians is well-documented (Stephens 1962; Shick 1975; Schlichter 1982; Ferrier 1991; Wilkerson and Kremer 1992). This source of nitrogen can make detectable contributions to the nitrogen demand of non-symbiotic cnidarians such as *Aurelia aurita* (Schick 1975) as well as symbiotic species such as *Linuche unguiculata* (Wilkerson and Kremer 1992). Furthermore, Ferrier (1991) demonstrated the uptake of free amino acids at environmentally realistic concentrations by four species of corals. However, in the nitrogen-budget experiments with *Anemonia viridis* described herein, the volume of seawater flowing over each anemone was extremely low in comparison with cnidarians in the sea, and so the total amount of dissolved organic nitrogen to which the anemones would be exposed would be low. The paradox of the unbalanced budget awaits further investigation.

Comparisons between the nitrogen budgets of *Stylophora pistillata* under unsupplemented conditions and *Anemonia viridis* incubated in 20 μM ammonium reveal two similarities. In both, the nitrogen allocated to growth of the zooxanthellae is approximately half of the net DIN uptake. This means that all the excretory nitrogen together with some of the assimilated DIN is recycled to the host. Secondly, both budgets emphasise the importance of the recycled excretory nitrogen. In *S. pistillata*, because of the low ambient DIN levels of the tropical seawater in which it lives, and correspondingly low uptake rates, the excretory nitrogen is about four times that taken up from the seawater. In *A. viridis*, in supplemented seawater, the excretory nitrogen equals that absorbed from the water. Whilst excretory nitrogen cannot, by definition, result in growth, if this were lost (as happens in non-symbiotic cnidarians), this relatively large part of the budget would have to be met from holozoic feeding.

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