

Ammonium excretion by a symbiotic sponge supplies the nitrogen requirements of its rhodophyte partner

Simon K. Davy^{1,*}, Donelle A. Trautman^{1,2}, Michael A. Borowitzka² and Rosalind Hinde¹

¹*School of Biological Sciences, A08, University of Sydney, New South Wales 2006, Australia and* ²*School of Biological Sciences and Biotechnology, Murdoch University, Murdoch, Western Australia 6150, Australia*

*Author for correspondence at present address: Institute of Marine Studies, University of Plymouth, Plymouth PL4 8AA, UK
(e-mail: sdavy@plymouth.ac.uk)

Accepted 13 August 2002

Summary

Symbioses between sponges and algae are abundant in the nutrient-poor waters of tropical reefs, yet very little is known of the nutritional interactions that may promote this abundance. We measured nitrogen flux between the sponge *Haliclona cymiformis* and its symbiotic partner, the rhodophyte *Ceratodictyon spongiosum*, and assessed the potential importance of this flux to the symbiosis. While the association can take up dissolved inorganic nitrogen (DIN) as ammonium and nitrate from the surrounding sea water, enrichment of the water with nitrate did not affect its rates of photosynthesis and respiration. Much of the DIN normally assimilated by the alga is waste ammonium excreted by the sponge. A nitrogen budget for the symbiosis shows that the nitrogen required for algal

growth can potentially be provided by sponge catabolism alone, but that only a small amount of nitrogen is available for translocation back to the sponge in organic compounds. The stable isotope composition ($\delta^{15}\text{N}$) was consistent with our interpretation of the sponge supplying excretory DIN to its algal partner, while the results also suggested that this DIN limits nitrogen deficiency in the alga. If our observations are typical of sponge–alga symbioses, then the supply of excretory nitrogen could be a major reason why so many algae form symbioses with sponges on coral reefs.

Key words: symbiosis, sponge, rhodophyte, *Haliclona cymiformis*, *Ceratodictyon spongiosum*, nitrogen flux.

Introduction

Coral reefs are of major ecological and economic importance (Hinrichsen, 1997), and the symbiosis between corals and endosymbiotic dinoflagellates ('zooxanthellae') has long been cited as the predominant reason for their success (Davies, 1992). This is because of the phototrophic nature of these symbioses (Muscatine, 1990) and their ability to conserve and recycle essential elements such as nitrogen (Cook, 1983; Rahav et al., 1989). However, corals are not the only ecologically important organisms on reefs, and sponges, in particular, make significant contributions to both reef biomass and function (Wilkinson, 1983; Hinrichsen, 1997). As with corals, many reef sponges harbour micro-algal symbionts, with about 50% of the sponge species that inhabit middle- and outer-shelf reefs of the Great Barrier Reef containing cyanobacteria, diatoms, unicellular chlorophytes or dinoflagellates (Wilkinson, 1987; Rützler, 1990). Some sponges also form intercellular symbioses with macroalgal rhodophytes or chlorophytes (Bergquist and Tizard, 1967; Price et al., 1984; Trautman et al., 2000). Notably, these sponge–alga symbioses frequently flourish in areas where corals are scarce (Trautman et al., 2000). However, while it is known symbiotic sponges have the potential to be phototrophic (Wilkinson, 1983, 1987; Borowitzka et al.,

1989) and symbiotic algae enhance the growth of their sponge partners (Wilkinson and Vacelet, 1979; Frost and Williamson, 1980; Hill, 1996), the nutritional interactions in sponge–alga symbioses are still poorly defined. In particular, unlike the situation in coral–zooxanthella symbioses (Cook, 1983; Wang and Douglas, 1998), nitrogen fluxes between sponge and algal partners have never been explored. We used the haplosclerid sponge *Haliclona cymiformis* (Esper) and its rhodophyte partner *Ceratodictyon spongiosum* (Zanardini) to determine the potential importance of nitrogen excreted by the sponge to the growth and survival of the algal partner.

The *Haliclona*–*Ceratodictyon* symbiosis is locally common in shallow (<4 m) tropical reef waters of the Indo-Pacific region (Trautman et al., 2000); neither partner is known to occur alone in the field. The profusely branching macroalga forms a dense network, with the intercellular encrusting sponge spreading around and between the algal branchlets and covering most of the alga; only branchlets at the apex of the thallus are free of sponge tissue. The whole association is heavily branched, dark green in colour and may form clumps of up to 1 m across (Fromont, 1993). The close proximity of the autotrophic and heterotrophic partners in this symbiosis would certainly

facilitate nutritional exchanges but, until now, this has never been investigated.

Materials and methods

Experimental organisms

Branches of the *Haliclona*–*Ceratodictyon* symbiosis were collected from <2 m depth in the lagoon of One Tree Reef at the southern end of the Great Barrier Reef, Australia. The branches were kept submerged constantly and were maintained in vigorously aerated, running sea water for no more than 1 h prior to use. All branches were collected during summer, with the exception of those used for measuring ammonium uptake, which were collected during winter. Cultures of *Ceratodictyon spongiosum*, initiated >1 year previously by Dr A. Grant (University of Sydney), were maintained in Grund medium (Price et al., 1984) at an irradiance of 5–10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and a temperature of 21°C on a 12 h:12 h L:D cycle.

Nitrogen flux

To determine whether the association can take up exogenous ammonium and nitrate, sponge–alga branches ($N=3$ or 5), each from a different clump, were cleaned of debris and placed in glass beakers containing glass microfibre (Whatman GF/C) filtered sea water (FSW; 200 ml and 600 ml for ammonium and nitrate experiments, respectively). Controls consisted of FSW alone ($N=3$). Ammonium levels were increased to 20 $\mu\text{mol l}^{-1}$ by spiking with NH_4Cl ; existing nitrate levels (2–5 $\mu\text{mol l}^{-1}$) were sufficient for the experiments. All beakers were aerated regularly and kept at an irradiance of 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (using quartz halogen lamps) and at ambient temperature (mean 23.5°C and 27.5°C in winter and summer, respectively). Known volumes (5–10 ml) of water were removed from each beaker at regular intervals, sterilised using a 0.2 μm filter and stored at –20°C in acid-washed tubes. Samples were analysed within one week of storage, using the phenol–hypochlorite method for ammonium and the cadmium-reduction method for nitrate; nitrate samples were corrected for nitrite content (Parsons et al., 1984). All values were normalised to branch dry mass, which was determined after drying to constant mass at 60°C. One-way analysis of variance (ANOVA) was used to determine if ammonium and nitrate concentrations changed significantly over time.

To determine the rate of ammonium excretion by the sponge and how excretion is affected by algal photosynthesis, the release of ammonium in the dark by branches maintained previously in darkness for 4 h, 8 h or 24 h was measured. This was carried out as above, except that 1 l of FSW was used, experiments were performed in darkness, and 40 ml samples were collected after 0 h, 2 h, 4 h and 6 h ($N=5$ for each time point).

Enrichment with nitrate is known to enhance photosynthesis and respiration in nitrogen-deficient macroalgae (Littler et al., 1988; Littler and Littler, 1992). To test the physiological effects of enrichment with nitrate in the laboratory, clumps of

the association were collected and placed in aquaria containing sea water (controls) or sea water plus 10 $\mu\text{mol l}^{-1}$ NaNO_3 for 24 h ($N=10$ for each treatment). A branch (5–10 g wet mass) was then cut from each clump, and dark respiration and maximal rate of photosynthesis (P_{max}) were measured with a Radiometer oxygen electrode (Copenhagen, Denmark; $N=3$ for each branch). Field experiments were performed the same way, except that the clumps were incubated in sea water or 10 $\mu\text{mol l}^{-1}$ NaNO_3 /sea water at the site of collection in sealed 3 l plastic bags. P_{max} and respiratory rate were then measured in the laboratory. Photosynthesis and respiration by freshly collected branches, which had not been enclosed in plastic bags, were also measured.

Biomass ratios

The ratio of sponge to algal biomass was estimated by cutting sections (approximately 1 cm long) from the tips of branches and from 8–10 cm below the tip ($N=20$ for each branch position, with each branch coming from a different clump). The sections were blotted dry and cut in half longitudinally, and each piece was wet weighed. One piece from each section was then stripped of sponge material by teasing apart, shaking in a 1:5 sodium hypochlorite:water solution and rinsing in water. The whole and ‘cleaned’ pieces were dried to constant mass at 60°C. The proportion of the association consisting of each partner was calculated from the dried masses of the whole and ‘cleaned’ pieces, adjusted for their original wet masses.

Stable isotope analysis ($\delta^{15}\text{N}$)

For stable isotope analysis, branches from four different clumps were squeezed in FSW to liberate sponge cells. The resultant cell suspension was centrifuged (1000 g for 15 min), the supernatant was decanted, and the cells were resuspended in 20% FSW and pelleted again. Algal samples were isolated from 10 clumps by teasing apart and shaking in several changes of 10% FSW (which lysed and removed the sponge cells). Sponge and algal samples were dried to constant mass at 60°C, ground to a fine powder and analysed by mass spectrometry (analysis performed by CSIRO Division of Land and Water, Adelaide, Australia). This gave values for percentage nitrogen and $\delta^{15}\text{N}$ (in ‰).

Nitrogen sufficiency

The nitrogen status of the alga was assessed by measuring the extent to which ammonium enhanced the rate of dark carbon fixation (Cook et al., 1992, 1994). Within 24 h of collection, branches ($N=3$) were teased apart to obtain clean samples of alga. Pieces of alga were then blotted dry, and 5–6 mg of each algal sample were weighed into a 5 ml vial containing either 0.6 ml FSW or 0.6 ml 20 $\mu\text{mol l}^{-1}$ NH_4Cl in FSW ($N=2$ for each condition). Three ‘background’ vials contained alga and 0.6 ml 10% formalin in FSW. $\text{NaH}^{14}\text{CO}_3$ stock (30 μl containing 18.5 kBq) was then added to each vial in a dark room. The formalin vials were immediately sampled (50 μl) for added radioactivity, and all the vials were then

sealed and left in darkness at 22°C. After 2 h, the vials were acidified with 1 mol l⁻¹ HCl and dried using a heating block. Distilled water (0.6 ml) and scintillation fluid (5 ml) were then added, and radioactivity was measured by liquid scintillation counting. The ammonium enhancement ratio was calculated (Cook et al., 1992) following correction for background activity and normalization of dark carbon-fixation rates to algal weight.

The nitrogen status was also measured after the supply of nitrogen had been varied. This could only be done with the cultured alga, as the whole association could not be kept alive long enough for use in such experiments. Cultured alga was maintained for 3 weeks in medium containing 100 µmol l⁻¹ NH₄Cl; the medium was replaced every 2 days. Cultures were then switched to nitrogen-free medium for up to 6 weeks, and their ammonium enhancement ratio was measured ($N=2$ for each of three separate cultures) at a series of time points. Three further cultures were kept continuously in 100 µmol l⁻¹ NH₄Cl and analysed, as above, at the conclusion of the experiment.

Results

Uptake of dissolved inorganic nitrogen (DIN) from sea water

Nitrogen, an element essential for growth, can potentially be taken up as dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON) and particulate organic nitrogen (PON). In the present study, we determined that the *Haliclona–Ceratodictyon* symbiosis in One Tree Lagoon can remove inorganic nitrogen from the surrounding sea water as ammonium (NH₄⁺) and nitrate (NO₃⁻) (one-way ANOVA, $P<0.0001$, $N=3-5$ for both nutrients; Fig. 1); levels of these nutrients changed little in control experiments where sea water was incubated without the association (one-way ANOVA, $P>0.2$, $N=3$ for both nutrients). As occurs in many macroalgae (Lobban and Harrison, 1997), ammonium was taken up more rapidly than nitrate, probably because nitrate needs to be reduced to ammonium prior to assimilation (Syrett, 1981). However, in One Tree Lagoon, DIN availability may be limited, as shown by nutrient concentrations at the collecting sites [ammonium, 1.3–2.5 µmol l⁻¹ in winter and 0.8–2.2 µmol l⁻¹ in summer; nitrate, 2–5 µmol l⁻¹ in summer; nitrite, <0.5 µmol l⁻¹ (negligible) in summer; winter concentrations of nitrate and nitrite were not determined].

While the *Haliclona–Ceratodictyon* symbiosis could take up DIN from sea water, elevated levels of nitrate did not significantly enhance P_{\max} or respiratory rates (one-way ANOVA, $P>0.1$, $N=10$ in all experiments; Fig. 2).

Excretion of DIN by the sponge and its potential importance to the alga

Given the relatively low levels of DIN in the sea water and the failure of added DIN to enhance algal metabolism in the intact association significantly, waste material from the feeding activity of the sponge (i.e. excretory ammonium) may be an important nitrogen source for the algal partner. When we prevented algal photosynthesis by pre-incubating the

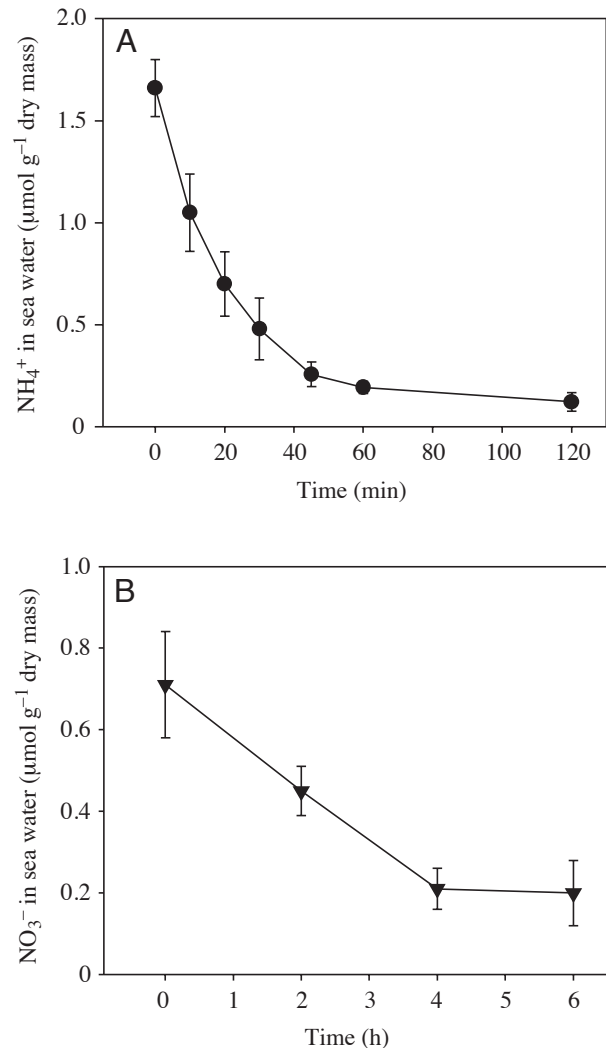


Fig. 1. Uptake of dissolved inorganic nitrogen from sea water by the *Haliclona–Ceratodictyon* symbiosis. (A) Uptake of ammonium from sea water spiked with 20 µmol l⁻¹ NH₄Cl, when branches were incubated in the light ($N=3$). (B) Uptake of nitrate from 'normal', unspiked sea water, when branches were incubated in the light ($N=5$). Values are means \pm S.D. Ammonium and nitrate concentrations in sea water without the association did not change significantly over time (one-way analysis of variance, $P>0.2$, $N=3$ for both nutrients), with ammonium concentrations being 20.84 \pm 0.83 µmol l⁻¹ and 18.84 \pm 2.89 µmol l⁻¹ and nitrate concentrations being 2.10 \pm 0.27 µmol l⁻¹ and 1.36 \pm 0.43 µmol l⁻¹ at the start and end of the experiments, respectively.

association in darkness for 24 h, the sponge excreted a significant amount of ammonium to the external medium during a further 6 h in the dark (one-way ANOVA, $P<0.0001$, $N=5$; Fig. 3). This release translated to approximately 4.6 µg ammonium-N g⁻¹ dry mass h⁻¹ (0.110 mg ammonium-N g⁻¹ dry mass day⁻¹). By contrast, branches that had been pre-incubated for ≤ 8 h in the dark released little ammonium (one-way ANOVA, $P>0.05$, $N=5$), suggesting that ammonium assimilation can, potentially, continue throughout the night.

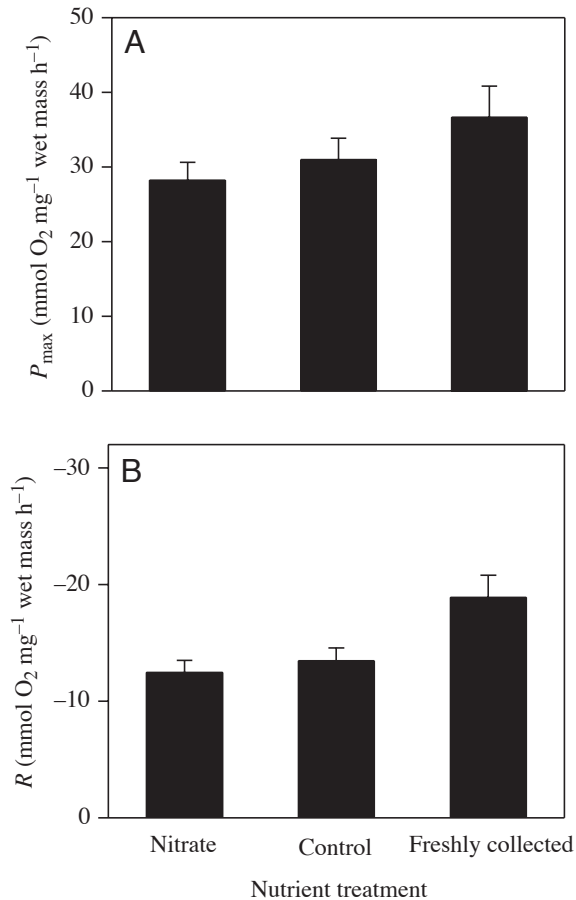


Fig. 2. The influence of nutrient enrichment in the field on the physiology of the *Haliclona-Ceratodictyon* symbiosis. (A) The maximum rate of gross photosynthesis (P_{\max}). (B) The dark respiration rate (R). Treated branches ($N=10$) were placed in either sea water plus $10 \mu\text{mol l}^{-1}$ NaNO_3 ('Nitrate') or sea water only ('Control') for 24 h. Treated branches were compared with branches harvested directly from the field ('Freshly collected'). Values are means \pm S.D. Measurements obtained in laboratory-based experiments were similar to those obtained in the field experiments and are therefore not shown.

To assess the potential importance of waste ammonium to the alga, it is necessary to consider the nitrogen required for algal growth. The whole sponge-alga association grows at a rate of $0.83\% \text{ day}^{-1}$ in the field (Trautman et al., 2000) and, on a dry mass basis, the alga comprises $70 \pm 7.5\%$ of the symbiosis and contains $1.9 \pm 0.1\%$ nitrogen (compared with $6.7 \pm 0.2\%$ nitrogen for the sponge). Therefore, assuming that the growth rate of the whole association is representative of the growth rates of the symbiotic partners, we estimate that $0.108 \text{ mg N g}^{-1} \text{ association dry mass day}^{-1}$ is used to support algal growth. Interestingly, this rate almost exactly matches the maximum measured rate of ammonium-N release from the sponge (Fig. 4).

The supply of DIN by the sponge raises the question of how this influences the nitrogen status of the alga. The enhancement of dark carbon fixation in the presence of

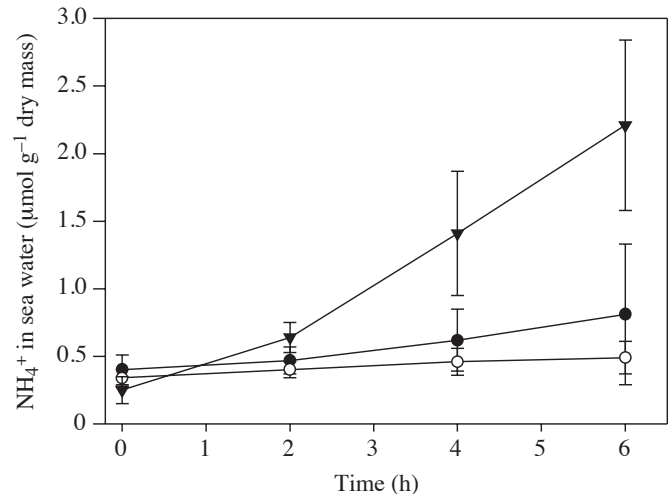


Fig. 3. Ammonium flux from the *Haliclona-Ceratodictyon* symbiosis in darkness. Branches had been pre-incubated in darkness for 4 h (filled circles), 8 h (open circles) or 24 h (filled triangles) ($N=5$ for each time point). Values are means \pm S.D.

ammonium is inversely related to nitrogen limitation (Cook et al., 1992). In the case of freshly harvested *C. spongiosum*, the enhancement ratio (the ratio of dark carbon fixation in sea water plus ammonium to dark carbon fixation in sea water alone) was approximately 1.4. Similar values were obtained when cultured *C. spongiosum* was grown for up to 9 weeks in medium containing $100 \mu\text{mol l}^{-1}$ ammonium (one-way ANOVA, $P>0.05$, $N=3$). However, enhancement ratios as high as 2.6 were observed when cultured *C. spongiosum* was grown in nitrogen-free medium over several weeks (Fig. 5).

Supply of nitrogen to the sponge

From Fig. 4, it can be seen that $<2\%$ of waste nitrogen is surplus to algal growth requirements. This surplus may be recycled back to the sponge as amino acids (A. Grant, personal communication), although the possible amount recycled is very small. We therefore propose that almost all of the nitrogen demands of the association must be met from external sources. Even though the sponge contributes only $30 \pm 7.5\%$ of the biomass of the association, its higher nitrogen content ($6.7 \pm 0.2\%$ versus $1.9 \pm 0.1\%$ on a dry mass basis) means that it uses more nitrogen for growth ($0.167 \text{ mg N g}^{-1} \text{ association dry mass day}^{-1}$) than the alga ($0.108 \text{ mg N g}^{-1} \text{ association dry mass day}^{-1}$). Therefore, to meet the nitrogen demands of both the alga and the sponge, at least $0.275 \text{ mg N g}^{-1} \text{ dry mass}$ must be supplied to the symbiosis from external sources each day (Fig. 4). If, as in cnidaria-alga symbioses (Crossland et al., 1980), DON is secreted into the water as mucus, more nitrogen will be required.

Additional evidence for the source of nitrogen comes from the stable isotope composition ($\delta^{15}\text{N}$), which has been used to elucidate nutritional sources in a variety of marine symbioses (e.g. Muscatine and Kaplan, 1994; Kline and Lewin, 1999).

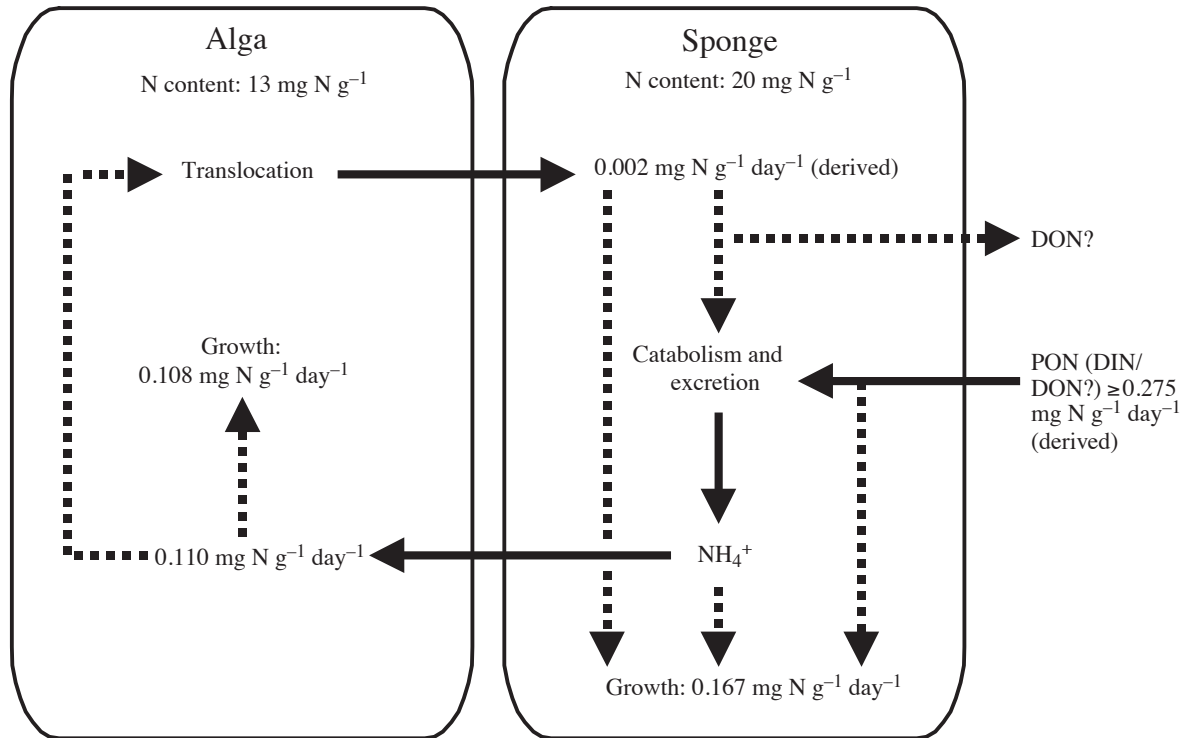


Fig. 4. Nitrogen budget for the *Haliclona*–*Ceratodictyon* symbiosis. The budget incorporates the maximum ammonium release rate in darkness ($0.110 \text{ mg N g}^{-1} \text{ dry mass day}^{-1}$), the growth rate of the association ($0.83\% \text{ day}^{-1}$, as reported by Trautman et al., 2000), the alga:sponge biomass ratio (70%:30% alga:sponge) and the nitrogen content of the two partners (alga, 1.9%; sponge, 6.7%; both on a dry mass basis). Nitrogen that is surplus to the growth requirements of the alga is assumed to be translocated back to the sponge, and the budget is balanced by the presumed acquisition of nitrogen from the ambient environment. Abbreviations: dissolved inorganic nitrogen (DIN); dissolved organic nitrogen (DON); particulate organic nitrogen (PON). Biomass units are $\text{mg nitrogen g}^{-1}$ dry mass of the whole association. Flux units are $\text{mg nitrogen g}^{-1}$ dry mass of the whole association per day. Solid arrows are used for proven fluxes (quantified directly except where stated as ‘derived’), while broken arrows are used for possible fluxes.

$\delta^{15}\text{N}$ was $+2.23 \pm 0.18\text{‰}$ in freshly collected *C. spongiosum* and $+4.88 \pm 0.28\text{‰}$ in its sponge partner, *H. cymiformis*.

Discussion

Our data suggest that the sponge *Haliclona cymiformis* releases waste ammonium that may potentially provide all of the nitrogen required by its rhodophyte partner, *Ceratodictyon spongiosum*, and help maintain the nutrient status of the alga. However, it is unlikely that much nitrogen is translocated back to the sponge, which must be largely reliant upon heterotrophic feeding to meet its nitrogen demands. We therefore propose a largely unidirectional flow of nitrogen in the *Haliclona*–*Ceratodictyon* symbiosis, with this nitrogen benefiting the rhodophyte partner in the nutrient-poor waters of the Great Barrier Reef. In addition, the sponge may protect the alga against grazing by herbivores, although this has yet to be investigated. The benefits for the sponge are not clear at present but may be related to the stable structure offered by the alga, as the association is commonly found on loose rubble (Trautman et al., 2000). Alternatively, the compounds translocated to the sponge may be important qualitatively, if not quantitatively. Such interactions may explain the local

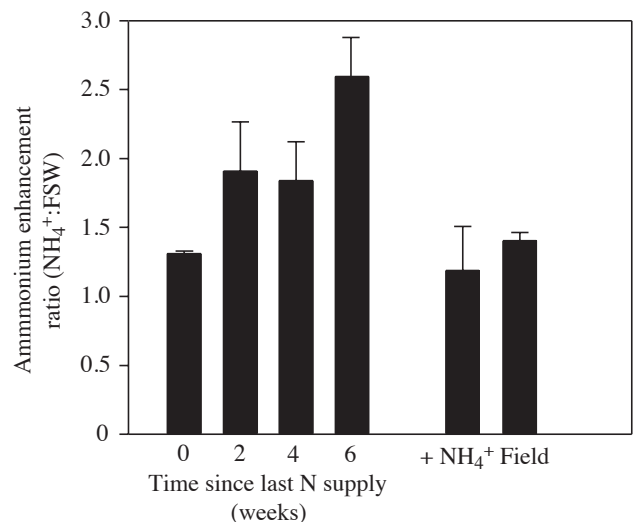


Fig. 5. Nitrogen status of *Ceratodictyon spongiosum* in the field and in culture, as measured by the enhancement of dark carbon fixation in the presence of ammonium ($20 \mu\text{mol l}^{-1} \text{ NH}_4\text{Cl}$). *C. spongiosum* was first cultured in $100 \mu\text{mol l}^{-1} \text{ NH}_4\text{Cl}$ for 3 weeks and then either deprived of nitrogen for up to 6 weeks or provided with $100 \mu\text{mol l}^{-1} \text{ NH}_4\text{Cl}$ for a further 6 weeks (+ NH_4^+). Freshly collected *C. spongiosum* (‘Field’) was analysed within 24 h. $N=3$ for each time point/condition. Values are means \pm s.d. FSW, filtered sea water

abundance of the *Haliclona*–*Ceratodictyon* symbiosis, which has a high biomass per unit area (up to 270 g wet mass m⁻²) compared with most other shallow-water sponges and red algae on coral reefs (Trautman et al., 2000).

Sponges are highly efficient filter-feeders (Reiswig, 1971; Pile et al., 1996), and *H. cymiformis* is known to filter bacteria and picoplankton from the water column in One Tree Lagoon (A. Grant, personal communication). Indeed, feeding upon particulate organic matter, which tends to be enriched in ¹⁵N, no doubt contributes to the relatively high δ¹⁵N value of the sponge. At present, the δ¹⁵N value of the sponge's prey is unknown, as is the δ¹⁵N value of any substrates that may be translocated from the algal partner, so our data must be interpreted with caution. However, the sponge value of +4.88‰ is very similar to the value of +4.74‰ reported for the non-symbiotic coral *Tubastrea coccinea* and is higher than the values of +2.79–4.11‰ reported for a range of shallow-water symbiotic corals (Muscatine and Kaplan, 1994). These corals are known to receive small quantities of nitrogenous compounds from their zooxanthellae (Muscatine and Cernichiari, 1969; Markell and Trench, 1993).

Clearly, the feeding efficiency of sponges means that the acquisition of PON is not a problem, even in relatively unproductive reef waters, and that a sponge is an ideal partner for an alga in a nutrient-poor habitat. The δ¹⁵N value measured for *C. spongiosum* (+2.23‰) was within the range of +0.95–3.54‰ reported for zooxanthellae from a variety of shallow-water coral species (Muscatine and Kaplan, 1994). It was also similar to values of +2.2–3.1‰ reported for the non-symbiotic, tropical rhodophyte *Halymenia dilatata* (Kline and Lewin, 1999). Therefore, our data are consistent with the alga acquiring its nitrogen as DIN from sea water and/or the sponge.

The uptake of excretory nitrogen presumably enhances the nutritional status of *C. spongiosum*. Indeed, this uptake may explain why exogenous nitrate did not increase the P_{\max} and the respiratory rate of the association (Fig. 2) and why dark carbon fixation by the alga only increased slightly in the presence of ammonium (Fig. 5). In comparison, when nitrate is supplied to free-living macroalgae on coral reefs, it generally elevates P_{\max} and, sometimes, the respiratory rate (Littler et al., 1988; Littler and Littler, 1992). For example, when nitrate was supplied to various species of the green algal genus *Halimeda*, P_{\max} was elevated by up to 1.7 times (Littler et al., 1988). Ammonium enhancement experiments have demonstrated a clear link between animal feeding and nitrogen status of the algal partner in zooxanthellate sea anemones and corals (Cook et al., 1992, 1994). For example, ammonium enhancement ratios for zooxanthellae from the reef coral *Madracis mirabilis* were approximately 1.2 in well-fed corals, as opposed to ≥1.7 in starved corals (Cook et al., 1994).

It should be recognised, however, that, as in cnidaria–alga symbioses (Rees, 1986; Wang and Douglas, 1998), the provision of photosynthetically fixed carbon to a symbiotic sponge may stimulate a degree of ammonium assimilation by

the sponge itself. This would no doubt reduce the availability of waste ammonium to the alga, although evidence to date suggests that this flux of photosynthate in sponge–alga symbioses may be relatively small. For example, cyanobacterial symbionts in a range of reef sponges translocated only 1–5% of their photosynthate (Wilkinson, 1983), and preliminary evidence from the *Haliclona*–*Ceratodictyon* symbiosis suggests a similarly low translocation rate (A. Grant, personal communication). In any case, it seems that the *Haliclona*–*Ceratodictyon* symbiosis forms a coherent unit, where carbon skeletons generated in the alga during photosynthesis enable the uptake of excretory ammonium from the sponge during both day and night. This interaction, in turn, may enhance the nutrient status of the macroalgal partner in an otherwise nutrient-poor environment.

By contrast, while it is known that the amino acids alanine, leucine, glutamate, threonine and aspartate are translocated by *C. spongiosum* (A. Grant, personal communication), our results suggest that there is a limited potential for nitrogen recycling in the *Haliclona*–*Ceratodictyon* symbiosis. Whether nitrogen recycling is important in cnidaria–alga symbioses is debatable. In their nitrogen budget for the reef coral *Stylophora pistillata*, Rahav et al. (1989) estimated that zooxanthellae need only 10% of the excretory nitrogen from the host for their growth and, potentially, recycle 90% back to the host. However, while zooxanthellae may release amino acids, such as alanine (Muscatine and Cernichiari, 1969), and nitrogen-containing glycoconjugates (Markell and Trench, 1993), the material they translocate consists predominantly of nitrogen-poor compounds, such as glycerol and glucose (Muscatine, 1967; Trench, 1971). In addition, as demonstrated in the zooxanthellate sea anemone *Aiptasia pulchella*, cnidarian hosts can assimilate ammonium (Wang and Douglas, 1998). Hence, nitrogen recycling in cnidaria–zooxanthella symbioses may not be as significant as initially thought.

Our findings therefore reveal that sponge–alga symbioses may not only be autotrophic (Wilkinson, 1983) but may also benefit from the transfer of nitrogen between the symbiotic partners. Similarly, in associations between massive root-fouling sponges (*Tedania ignis* and *Haliclona implexiformis*) and the red mangrove (*Rhizophora mangle*), the sponge obtains organic carbon from the roots of the mangrove, and the growth of the mangrove is enhanced by the uptake of excretory nitrogen from the sponge (Ellison et al., 1996). However, study of a wider range of sponge–alga symbioses is still required to determine how general such interrelationships are, while the direct demonstration of nitrogen transfer between the sponge and alga requires the use of a tracer, such as ¹⁵N. More information is also required concerning the sites and mechanisms involved in ammonium assimilation, the quantities and identities of metabolites passing between the symbiotic partners, and the temporal and spatial variability of the various nutritional fluxes. The roles of symbiotic bacteria, which are harboured by numerous marine sponges (Wilkinson, 1984), also await clarification. For example, nitrifying bacteria may enhance local concentrations of nitrate (Corredor et al.,

1988). Clearly though, it is evident that to understand the growth and success of coral reefs fully, we need to understand the biology and ecology not just of corals but of all symbiotic organisms and, in particular, sponges.

This work was funded by a Royal Society Postdoctoral Fellowship to S.K.D. and an Australian Research Council grant to R.H. and M.A.B. We thank Dr Adrienne Grant for assistance in the field and Matthew Bulbert for maintenance of algal cultures.

References

- Bergquist, P. R. and Tizard, C. A.** (1967). Australian intertidal sponges from the Darwin area. *Micronesica* **3**, 175-202.
- Borowitzka, M. A., Hinde, R. and Pironet, F.** (1989). Carbon fixation by the sponge *Dysidea herbacea* and its endosymbiont *Oscillatoria spongelliae*. *Proc. 6th Int. Coral Reef Symp.* **3**, 151-156.
- Cook, C. B.** (1983). Metabolic interchange in algae–invertebrate symbiosis. *Int. Rev. Cytol.* (suppl.) **14**, 177-210.
- Cook, C. B., Muller-Parker, G. and D'Elia, C. F.** (1992). Ammonium enhancement of dark carbon fixation and nitrogen limitation in symbiotic zooxanthellae: effects of feeding and starvation of the sea anemone *Aiptasia pallida*. *Limnol. Oceanogr.* **37**, 131-139.
- Cook, C. B., Muller-Parker, G. and Orlandini, C. D.** (1994). Ammonium enhancement of dark carbon fixation and nitrogen limitation in zooxanthellae symbiotic with the reef corals *Madracis mirabilis* and *Montastrea annularis*. *Mar. Biol.* **118**, 157-165.
- Corredor, J. E., Wilkinson, C. R., Vicente, V. P., Morell, J. M. and Otero, E.** (1988). Nitrate release by Caribbean reef sponges. *Limnol. Oceanogr.* **33**, 114-120.
- Crossland, C. J., Barnes, D. J., Cox, T. and Devereaux, M.** (1980). Compartmentation and turnover of organic carbon in the staghorn coral *Acropora formosa*. *Mar. Biol.* **59**, 181-187.
- Davies, P. S.** (1992). Endosymbiosis in marine cnidarians. In *Plant–Animal Interactions in the Marine Benthos* (ed. D. M. John, S. J. Hawkins and J. H. Price), pp. 511-540. Oxford: Clarendon Press.
- Ellison, A. M., Farnsworth, E. J. and Twilley, R. R.** (1996). Facultative mutualism between red mangroves and root-fouling sponges in Belizean mangal. *Ecology* **77**, 2431-2444.
- Fromont, J.** (1993). Descriptions of species of the Haplosclerida (Porifera: Demospongiae) occurring in tropical waters of the Great Barrier Reef. *Beagle* **10**, 7-40.
- Frost, T. M. and Williamson, C. E.** (1980). *In situ* determination of the effect of symbiotic algae on the growth of the freshwater sponge *Spongilla lacustris*. *Ecology* **61**, 1361-1370.
- Hill, M. S.** (1996). Symbiotic zooxanthellae enhance boring and growth rates of the tropical sponge *Anthosigmella varians* forma *varians*. *Mar. Biol.* **125**, 649-654.
- Hinrichsen, D.** (1997). Coral reefs in crisis. *BioScience* **47**, 554-558.
- Kline, T. C. and Lewin, R. A.** (1999). Natural $^{15}\text{N}/^{14}\text{N}$ abundance as evidence for N_2 fixation by *Prochloron* (Prochlorophyta) endosymbiotic with didemnid ascidians. *Symbiosis* **26**, 193-198.
- Littler, M. M. and Littler, D. S.** (1992). Photosynthesis versus irradiance curves for six species of macroalgae from the Seychelles Islands under four levels of nutrient enrichment. *Atoll Res. Bull.* **374**, 1-14.
- Littler, M. M., Littler, D. S. and Lapointe, B. E.** (1988). A comparison of nutrient- and light-limited photosynthesis in psammophytic versus epilithic forms of *Halimeda* (Caulerpales, Halimedaceae) from the Bahamas. *Coral Reefs* **6**, 219-225.
- Lobban, C. S. and Harrison, P. J.** (1997). *Seaweed Ecology and Physiology*. Cambridge: Cambridge University Press.
- Markell, D. A. and Trench, R. K.** (1993). Macromolecules exuded by symbiotic dinoflagellates in culture: amino acid and sugar composition. *J. Phycol.* **29**, 64-68.
- Muscantine, L.** (1967). Glycerol excretion by symbiotic algae from corals and *Tridacna* and its control by the host. *Science* **156**, 516-519.
- Muscantine, L.** (1990). The role of symbiotic algae in carbon and energy flux in reef corals. In *Coral Reefs* (ed. Z. Dubinsky), pp. 75-87. Amsterdam: Elsevier.
- Musatine, L. and Cernichiari, E.** (1969). Assimilation of photosynthetic products of zooxanthellae by a reef coral. *Biol. Bull.* **137**, 506-523.
- Muscantine, L. and Kaplan, I. R.** (1994). Resource partitioning by reef corals as determined from stable isotope composition II. $\delta^{15}\text{N}$ of zooxanthellae and animal tissue versus depth. *Pacif. Sci.* **48**, 304-312.
- Parsons, T. R., Maita, Y. and Lalli, C. M.** (1984). *A Manual of Chemical and Biological Methods for Sea Water Analysis*. Oxford: Pergamon Press.
- Pile, A. J., Patterson, M. R. and Witman, J. D.** (1996). *In situ* grazing on plankton <10 μm by the boreal sponge *Mycale lingua*. *Mar. Ecol. Prog. Ser.* **141**, 95-102.
- Price, I. R., Fricker, R. and Wilkinson, C. R.** (1984). *Ceratodictyon spongiosum* (Rhodophyta), the macroalgal partner in an alga–sponge symbiosis grown in unialgal culture. *J. Phycol.* **20**, 156-158.
- Rahav, O., Dubinsky, Z., Achituv, Y. and Falkowski, P. G.** (1989). Ammonium metabolism in the zooxanthellate coral *Stylophora pistillata*. *Proc. R. Soc. Lond. B* **236**, 325-337.
- Rees, T. A. V.** (1986). The green hydra symbiosis and ammonium. I. The role of the host in ammonium assimilation and its possible regulatory significance. *Proc. R. Soc. Lond. B* **229**, 299-314.
- Reiswig, H. M.** (1971). Particle feeding in natural populations of three marine demosponges. *Biol. Bull.* **141**, 568-591.
- Rützler, K.** (1990). Associations between Caribbean reef sponges and photosynthetic organisms. In *New Perspectives in Sponge Biology* (ed. K. Rützler), pp. 455-466. Washington DC: Smithsonian Institution Press.
- Syrett, P. J.** (1981). Nitrogen metabolism of microalgae. *Can. Bull. Fish. Aquat. Sci.* **210**, 182-210.
- Trautman, D. A., Hinde, R. and Borowitzka, M. A.** (2000). Population dynamics of an association between a coral reef sponge and a red macroalga. *J. Exp. Mar. Biol. Ecol.* **244**, 87-105.
- Trench, R. K.** (1971). The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. II. Liberation of fixed ^{14}C by zooxanthellae *in vitro*. *Proc. R. Soc. Lond. B* **177**, 237-250.
- Wang, J. T. and Douglas, A. E.** (1998). Nitrogen recycling or nitrogen conservation in an alga–invertebrate symbiosis? *J. Exp. Biol.* **201**, 2445-2453.
- Wilkinson, C. R.** (1983). Net primary productivity in coral reef sponges. *Science* **219**, 410-412.
- Wilkinson, C. R.** (1984). Immunological evidence for the Pre-cambrian origin of bacterial symbioses in marine sponges. *Proc. R. Soc. Lond. B* **220**, 509-517.
- Wilkinson, C. R.** (1987). Interocean differences in size and nutrition of coral reef sponge populations. *Science* **236**, 1654-1657.
- Wilkinson, C. R. and Vacelet, J.** (1979). Transplantation of marine sponges to different conditions of light and current. *J. Exp. Mar. Biol. Ecol.* **37**, 91-104.