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Availability of two forms of dissolved nitrogen to the coral *Pocillopora damicornis* and its symbiotic zooxanthellae

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Abstract The relative contribution of dissolved nitrogen (ammonium and dissolved free amino acids DFAAs) to the nitrogen budget of the reef-building coral *Pocillopora damicornis* was assessed for colonies growing on control and ammonium-enriched reefs at One Tree Island (southern Great Barrier Reef) during the ENCORE (Enrichment of Nutrient on Coral Reef; 1993 to 1996) project. *P. damicornis* acquired ammonium at rates of between 5.1 and 91.8 nmol N cm⁻² h⁻¹ which were not affected by nutrient treatment except in the case of one morph. In this case, uptake rates decreased from 80.5 to 42.8 nmol cm⁻² h⁻¹ ($P < 0.05$) on exposure to elevated ammonium over 12 mo. The presence or absence of light during measurement did not influence the uptake of ammonium ions. Nitrogen budgets revealed that the uptake of ammonium from concentrations of 0.11 to 0.13 μM could completely satisfy the demand of growing *P. damicornis* for new nitrogen. *P. damicornis* also took up DFAAs at rates ranging from 4.9 to 9.8 nmol N cm⁻² h⁻¹. These rates were higher in the dark than in the light (9.0 vs 5.1 nmol m⁻² h⁻¹, $P < 0.001$). Uptake rates were highest for the amino acids serine, arginine and alanine, and lowest for tyrosine. DFAA concentrations within the ENCORE microatolls that received ammonium were undetectable, whereas they ranged up to 100 nM within the control microatolls. The contribution of DFAAs to the nitrogen budget of *P. damicornis* constituted only a small fraction

of the nitrogen potentially contributed by ammonium under field conditions. Even at the highest field concentrations measured during this study, DFAAs could contribute only $\approx 11.3\%$ of the nitrogen demand of *P. damicornis*. This contribution, however, may be an important source of nitrogen when other sources such as ammonium are scarce or during periods when high concentrations of DFAAs become sporadically available (e.g. cell breakage during fish-grazing).

Introduction

Coral reef ecosystems are characterized by high rates of productivity, yet are found in tropical waters that are generally deficient of essential nutrients such as inorganic nitrogen and phosphorus (Odum and Odum 1955). Coral reefs retain a high productivity in the otherwise nutrient-poor tropical oceans by recycling of nutrients between reef biota. Recycling by the biota of coral reef communities leads to a greater retention of nutrients and hence a reduced rate of loss of nutrients back to the dilute tropical water column (Muscatine and Porter 1977). Symbiotic dinoflagellates and reef-building corals recycle inorganic nitrogen and phosphorus in addition to carbon dioxide and photosynthetic products (Trench 1979). Nitrogen fluxes back and forth as organic (free or protein-bound amino acids) and inorganic forms (ammonium) between host and symbiont (Muscatine 1967; Markell and Trench 1993). Recycled nitrogen represents a significant component of the nitrogen budget of the symbiotic dinoflagellates within reef-building corals, with up to 90–98% of the nitrogen used originating from internal sources (Rahav et al. 1989; Atkinson et al. 1994).

Despite recycling, new nitrogen must be acquired from the environment for the growth of symbiotic associations such as those of reef-building corals and dinoflagellates. There are two major ways that new nitrogen enters these associations: (a) Nitrogen may originate from the direct absorption of inorganic nitro-

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gen such as ammonium (D'Elia et al. 1983; Wilkerson and Muscatine 1984) and nitrate (Marubini and Davies 1996), or dissolved organic nitrogen from the water surrounding the coral (Schlichter 1982; Ferrier 1991); (b) reef-building corals may acquire nitrogen via the catabolism of food captured by the animal host (Lewis and Price 1975; Clayton and Lasker 1984). While studies have shown that corals do capture food particles (e.g. Porter 1977; Sebens et al. 1996), the relative contribution of nitrogen captured during feeding to overall nitrogen budgets remains unclear. Recent evidence from the study of the stable isotopes in corals has suggested that nitrogen may originate from dissolved sources as opposed to those acquired during the capture and catabolism of prey or particulates (Yamamuro et al. 1995; Hoegh-Guldberg et al. 1999). The relative role of the two different nitrogen sources will depend on species and feeding mode. It is important to note, however, that few if any studies have shown how much of the daily nitrogen needs of reef-building corals and their symbiotic dinoflagellates can be supplied by either source. In the latter case, estimates of the rate of growth of the nitrogen budget of coral-algal associations are required.

The present study compares the uptake of two forms of dissolved nitrogen with the nitrogen demand of the coral *Pocillopora damicornis* and its symbiotic dinoflagellates. Ammonium is reputedly taken up by the symbiotic dinoflagellates after passing by diffusion across the host cytoplasm (but see discussion of alternative model in Swanson and Hoegh-Guldberg 1999). Dissolved free amino acids are taken up by sodium-dependent co-transporters located on the outer epithelium of the coral. The results of this study show that while ammonium is taken up at rates that are sufficient to supply the nitrogen requirements of *P. damicornis* at ambient concentrations, dissolved free amino acids (DFAAs) are not. The transport of DFAAs supplies merely a maximum of 11.3% of the nitrogen requirements of *P. damicornis* when (infrequently seen) maximum field concentrations of DFAAs are present.

Materials and methods

This study constituted part of the ENCORE (Enrichment of Nutrient on CORal REef) project conducted at One Tree Reef (23° 30' S; 152° 06' E) on the eastern edge of the Capricorn Group of reefs on the southern Great Barrier Reef, Australia. The ENCORE project was sponsored by the Great Barrier Reef Marine Park Authority (GBRMPA), Australian Research Council (ARC) and the University of Sydney, and ran from February 1993 to February 1996. The ENCORE project involved 12 replicate patch reefs or microatolls (16 to 25 m diam), some of which received inorganic nitrogen (NH_4Cl) and phosphorus (NaH_2PO_4) from telemetrically controlled nutrient-dispersal units over a 2 yr period (Larkum and Steven 1994). Three microatolls were randomly assigned to four treatments: (1) control (no nutrient addition), (2) nitrogen (NH_4Cl added), (3) phosphorus (NaH_2PO_4 added), and (4) nitrogen and phosphorus (both NH_4Cl and NaH_2PO_4 added). The ENCORE project began with a pre-enrichment phase (February 1993 to August 1993). The period in which nutrients were added was divided into two phases. During the first phase (September 1993 to December 1994), nutrients were added to microatolls at every low

tide to reach initial concentrations calculated at 10 μM ammonium and 2 μM phosphate. Nutrient-monitoring studies revealed that nutrients rapidly decreased in the microatolls after addition (Koop et al. 1995). Consequently, nutrients were added hourly (three times over the 4 h periods in which the microatolls were ponded) using twice the original amount of nutrients per addition during a second phase of the project (January 1995 to February 1996). Concentrations during the second phase were 10 to 25 times those of control microatolls for ammonium and 10 to 100 times background for phosphate at each low tide (Koop et al. 1995).

Collection and maintenance of *Pocillopora damicornis*

Nutrient uptake was studied for the brown and pink morphs of the reef-building coral *Pocillopora damicornis* (Takabayashi and Hoegh-Guldberg 1995). The two morphs are primarily distinguished by having a low (brown morph) or high (pink morph) concentration of the coral pigment, pocilloporin (Dove et al. 1995). Sub-colonies or nubbins were constructed from colonies (20 to 30 cm diam) collected from One Tree Island lagoon. Branch tips (3 to 5 cm) were attached to numbered Perspex plates (30 mm \times 30 mm \times 3 mm) with marine epoxy (Vepox, Vessey Chemicals). Nubbins were then attached to weighted plastic racks (IG051 mat, Nally) that were placed in the ENCORE microatolls. Nubbins grew normally and looked in every way like corals growing in adjacent reef areas.

Ammonium-uptake measurements

Long-term changes in the rate of uptake of ammonium by colonies of *Pocillopora damicornis* were examined using colonies of brown and pink morphs that had been growing in the microatolls since the beginning of the first phase of nutrient addition in the ENCORE project. These colonies had been exposed to both the first and second phase of the ENCORE project (September 1993 to February 1996) and were used for this part of the study in December 1995. All nubbins used for this part of the study were derived from different parent colonies, and hence were likely to be genetically distinct individuals.

All experiments were carried out in the middle of the day when photosynthetically active radiation (PAR) was at least 190 μmol (photon) $\text{m}^{-2}\text{s}^{-1}$. Replicate corals were collected from the control and nitrogen microatolls just prior to each experiment, and were brought back to University of Sydney's research station on One Tree Island. Each colony was immersed in an outdoor Perspex chamber (15 cm diam, 10 cm tall; continuously stirred using a battery-powered propeller) containing 1 litre of 0.45 μm -filtered seawater with ammonium added to give an initial concentration of either 2 μM in the first experiment or 10 μM in the second experiment. In each experiment, three colonies of each morph from each microatoll were placed in a clear Perspex chamber to measure uptake under natural light conditions and three were placed in blackened chambers for dark uptake. During the experiments, the chambers were held at the same temperature as the temperature in the lagoon. Corals were returned to their microatolls within 1 h of completion of each experiment.

The rate of uptake of ammonium ions was measured for each replicate coral, with measurements (5 ml) being taken immediately after the start of the experiment, and then every 15 min until the end of 1 h. Controls consisted of identical chambers without coral colonies. The amount of ammonium in each sample was measured using a modification of the phenol-hypochlorite method (Solórzano 1969). Aliquots (0.2 ml) of 40 mM trisodium citrate, followed by 1 ml each of phenol nitroprusside and alkaline hypochlorite (both reagents from Sigma Chemical Company) were added to each sample. Samples were left for 1 h in the dark for the colour to develop (Gravitz and Gleye 1975), and the absorbance was then measured on a spectrophotometer at 630 nm against a seawater blank (made from the same filtered seawater used in the experiment). The concentration of ammonium in each sample was determined from standard curves made from the same seawater during each experiment.

Uptake studies with dissolved free amino acids (DFAAs)

Replicate colonies of the brown morph of *Pocillopora damicornis* ($n = 3$) were incubated in filtered seawater in either light (12 VDC, mercury iodide) or dark. Light was provided by metal halide lights during the DFAA incubations and ranged between 800 and 1000 $\mu\text{mol (photon) m}^{-2} \text{s}^{-1}$. Colonies were habituated to experimental conditions for 1 h prior to the beginning of an experiment. At the beginning of each experiment, a mixture containing the following amino acids were added in equal amounts (400 nM) so that the final concentration of all amino acids was 5.6 μM : aspartate, glutamate, serine, histidine, arginine, glycine, threonine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine and lysine. Samples were removed at 0, 15, 30, 60 and 120 min after the addition of the amino acid mixture and stored for HPLC analysis as described in the following paragraph.

To avoid contamination of the samples by bacteria or "human-derived" DFAAs, surgical gloves and sterile plastic syringes were used during sample collection. Immediately after collection, samples were filtered using 0.2 μm polycarbonate syringe filters (Nalgene) and were stored in residue-free Eppendorf tubes at -20°C at One Tree Island Research Station. Later, samples were transported (on dry ice) to the University of Sydney, where they were stored at -20°C until HPLC (usually within 2 mo). The concentration of amino acids in each sample was measured using HPLC following pre-column OPA-derivatization (Mopper and Lindroth 1982). Unknowns were identified and quantified using co-chromatography with known standards (Fig. 1), and HPLC analysis software (Maxima 820, Millipore-Waters) was used to determine peak areas. Changes in the concentration of amino acids were used to calculate the uptake rates of experimental corals for individual amino acids and total combined amino acids. Between 3 and 6 replicate colonies or individuals were used together with control incubations (no corals).

The concentration of DFAA species was measured within and just outside the control and nitrogen ENCORE microatolls in July 1994. Using sterile syringes, the microatolls were sampled at low tide at three sites (two replicates within site): (1) 10 cm from the bottom of each microatoll, (2) 10 cm below the surface of each microatoll, and (3) 10 cm below the surface immediately outside each microatoll. Samples were filtered and stored as described in the foregoing paragraph.

Measurement of biomass parameters

Uptake rates were standardised to a number of coral biomass parameters. At the end of each uptake experiment, experimental corals were fixed in 4% formalin in seawater for 12 h. The surface

area of *Pocillopora damicornis* was measured using the foil method of Marsh (1970). Colonies of *P. damicornis* were decalcified using 10% (v/v) HCl, and decalcified tissues were blotted dry and weighed (wet weight). A subsample of each mass of tissues was homogenised, and the number of symbiotic dinoflagellates was counted using a hemocytometer.

Nitrogen-budget calculations

Nitrogen budgets were constructed for *Pocillopora damicornis* to estimate the contribution made by the transport of ammonium and DFAAs at ambient concentrations. These calculations assumed minimal losses of nitrogen from *P. damicornis* per unit time. This assumption is consistent with observations that symbiotic corals such as *P. damicornis* are net sinks for nitrogen (Muscatine and D'Elia 1978; Rahav et al. 1989; Atkinson et al. 1994). Losses back to the water column are minimal. Consequently, a lower-range estimate of the nitrogen demand of *P. damicornis* and its symbiotic dinoflagellates can be estimated from: (a) the growth rates of *P. damicornis* in control and ammonium-enriched treatments (Hoegh-Guldberg 1999) and (b) the average mass of N atoms per cm^2 for *P. damicornis* (0.206 to 0.287 mg N cm^{-2} ; Muller-Parker et al. 1994). Although these measurements are undeniably simple estimates of nitrogen demand, they do allow assessment of whether the rate of acquisition of dissolved nitrogen sources is of the same magnitude as the rate at which nitrogen is needed by the association.

Statistical analysis

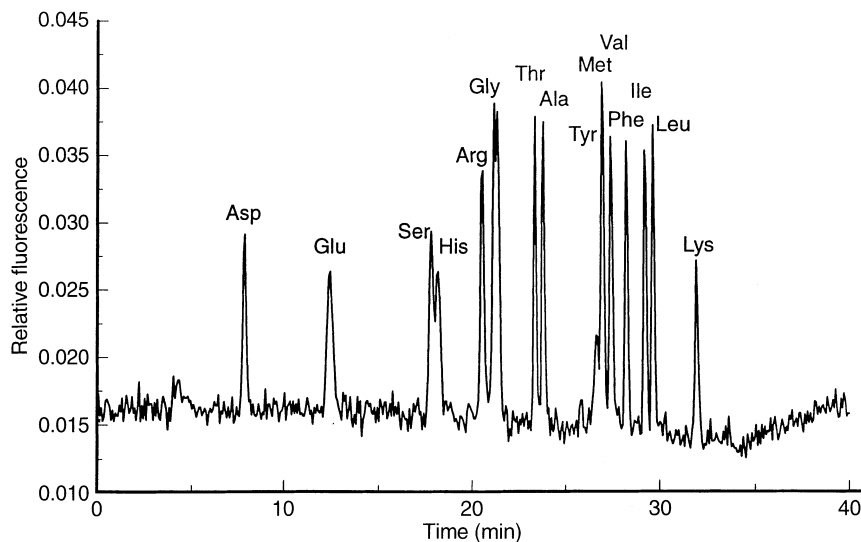
Data were analysed using ANOVA software, GMAV5 (AJ Underwood and MG Chapman, University of Sydney). The homogeneity of variances was verified prior to analysis using Cochran's test (Underwood 1981), and was found to be homogeneous in all data sets.

Results

Uptake of ammonium

Pocillopora damicornis rapidly depleted ammonium from the surrounding water. Rates of ammonium acquisition ranged between 5.1 and 91.8 $\text{nmol N cm}^{-2} \text{h}^{-1}$ (on a per area of coral tissue basis) or 5 and 60 $\text{nmol } 10^6 \text{ cell}^{-1} \text{h}^{-1}$ (on a per symbiotic dinoflagellate basis). Rates were unaffected by whether measurements were made in

Fig. 1 HPLC chromatograms of standards used to identify dissolved free amino acid (DFAA) species. Each amino acid was present at 400 nM (See third subsection of "Materials and methods" for full DFAA names)



the light or dark ($P > 0.05$), but were significantly higher when measured at $10 \mu\text{M}$ than at $2 \mu\text{M}$ ($P < 0.05$; Fig. 2). There was no effect of the long-term exposure to ammonium, except for colonies of the pink morph at substrate concentrations of $10 \mu\text{M}$ (Fig. 2). In this case, uptake rates were lower in those colonies that been exposed to higher ammonium concentrations in the preceding 2 yr, decreasing from 80.5 to $42.8 \text{ nmol cm}^{-2} \text{ h}^{-1}$ ($P < 0.05$).

Dissolved free amino acids (DFAAs)

DFAAs within undisturbed waters in One Tree Island lagoon ranged between 0 and $0.122 \mu\text{M}$ and mostly comprised serine and glycine. The total concentration of DFAAs in the water column was significantly lower (undetectable) in microatolls that received $20 \mu\text{M}$ ammonium ions as opposed to the control microatolls (Fig. 3; ANOVA, $F_{1,4} = 46.35$, $P < 0.0024$).

Pocillopora damicornis rapidly took up total DFAAs (Fig. 4) at rates ranging between 4.9 and $9.8 \text{ nmol N cm}^{-2} \text{ h}^{-1}$ (Table 1). The exception was a single colony of *P. damicornis* measured in the light in which there was an efflux of amino acids. Rates were

also calculated per colony (range: 1.44 to $2.16 \mu\text{mol N colony}^{-1} \text{ h}^{-1}$, ignoring the single outward flux) and as a function of wet tissue weight (range: 0.15 to $0.66 \mu\text{mol N g}^{-1} \text{ h}^{-1}$). Rates of DFAA uptake were higher in the dark ($P < 0.05$). Rates of uptake for individual amino acids ranged between 0.14 (tyrosine, light) and $0.98 \text{ nmol cm}^{-2} \text{ h}^{-1}$ (histidine, dark), and were generally similar between different amino acids (Fig. 5). The exception was tyrosine, which was taken up at rates that were consistently one-third to one-half that of the rates measured for the other amino acids (Fig. 5). There was no clear difference between the uptake of amino acids belonging to acidic, neutral or basic classes.

Nitrogen-budget calculations

Nitrogen budgets were constructed to compare the relative importance of ammonium- and DFAA-uptake systems in providing nitrogen for *Pocillopora damicornis* (Table 2). The nitrogen demand of the brown morph of *P. damicornis* was 23.80 and $21.09 \text{ nmol N cm}^{-2} \text{ d}^{-1}$ in the control and N microatolls at One Tree Island, respectively. The rate at which N atoms could be supplied by the inward transport of amino acids was 2.7 and $1.8 \text{ nmol N cm}^{-2} \text{ d}^{-1}$ (control and N corals respectively, Table 2) at the highest ambient concentration of DFAAs (100 nM). This represented 11.3 and 8.5% of the total nitrogen demand of *P. damicornis* in control and N microatolls. The concentration of DFAAs that would be able to satisfy the nitrogen demand of *P. damicornis* ranged between 0.89 and $1.18 \mu\text{M}$ (Table 2). The concentration at which the uptake of ammonium would satisfy the nitrogen needs of brown *P. damicornis* was 0.13 and $0.11 \mu\text{M}$ for control and N corals, respectively (Table 2). *P. damicornis* was ≈ 7 to 10 times more effective at acquiring N via inward transport of ammonium ions as opposed to DFAAs

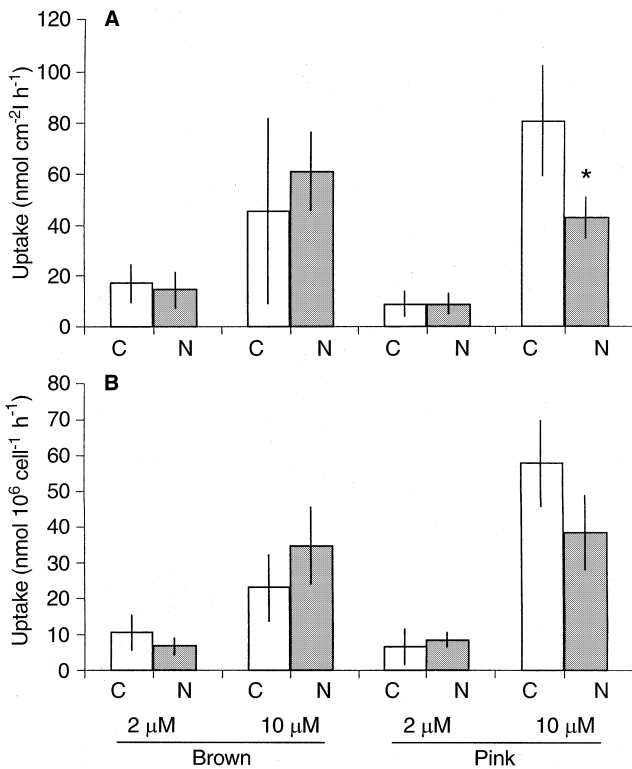


Fig. 2 *Pocillopora damicornis*. Rate of ammonium uptake as a function of substrate concentration (2 or $10 \mu\text{M}$) and nutrient treatment (C control; N ammonium-treated ENCORE microatolls) for two colour morphs (brown and pink; pink morph contains higher concentrations of coral pigment pocilloporin). Data expressed per surface area of coral skeleton (A) and per symbiotic dinoflagellate cell (B)

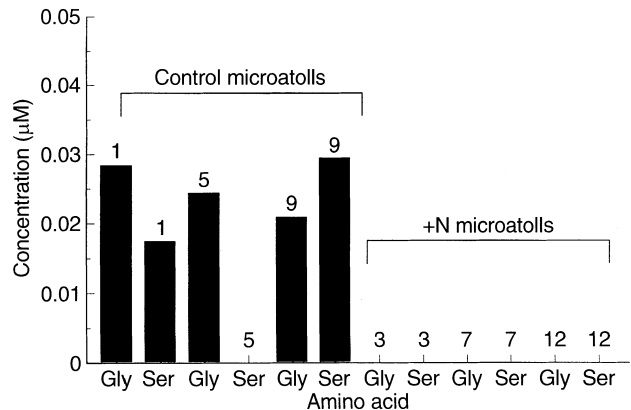


Fig. 3 Concentration of dissolved free amino acids in control and N-treated microatolls in ENCORE experiment 8 mo after beginning of fertilization. Data are means of two replicate samples taken from two sites within water column of each atoll. Glycine (Gly) and serine (Ser) were only amino acids detected (numbers above bars designated numbers of microatolls atolls within ENCORE project)

Fig. 4 *Pocillopora damicornis*. Typical concentration profiles of dissolved free amino acids in uptake experiments with small colonies. **A** Control incubation with no colony present (light = 800 to 1000 $\mu\text{E m}^{-2} \text{s}^{-1}$); **B** control incubation with no colony present in darkness; **C** colony present in light; **D** colony present in darkness (lines indicate regression fits used to calculate rates of uptake)

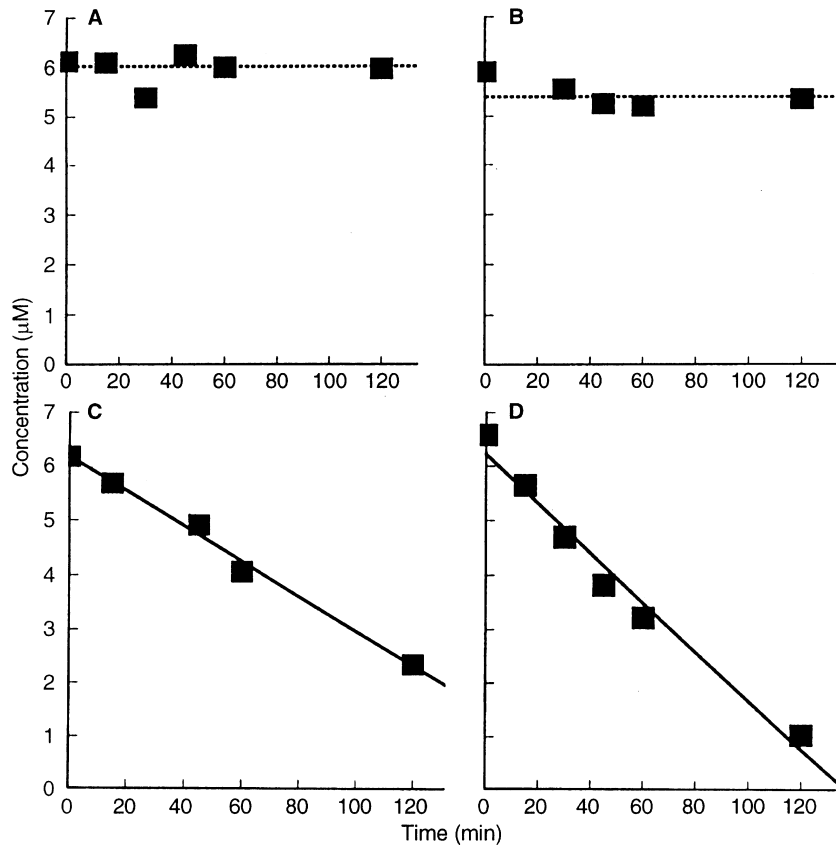


Table 1 *Pocillopora damicornis*. Total uptake rates of dissolved free amino acids by colonies incubated in light (800 to 1000 $\mu\text{E m}^{-2} \text{s}^{-1}$; Colonies L1–L3) and dark (D1–D3). Rates are standardised to colony, colony surface-area, or wet weight of decalcified tissue

Colony	Rate of uptake as:		
	$\mu\text{mol colony}^{-1} \text{h}^{-1}$	$\text{nmol cm}^{-2} \text{h}^{-1}$	$\mu\text{mol g}^{-1} \text{h}^{-1}$
L1	1.52	5.37	0.19
L2	1.44	4.90	0.15
L3	-1.52	-5.33	-0.21
D1	2.16	9.77	0.66
D2	1.44	6.52	0.25
D3	1.76	8.50	0.33

(calculated by dividing the ratio of the “break-even points” for ammonium by that for DFAAs in Table 2).

Discussion

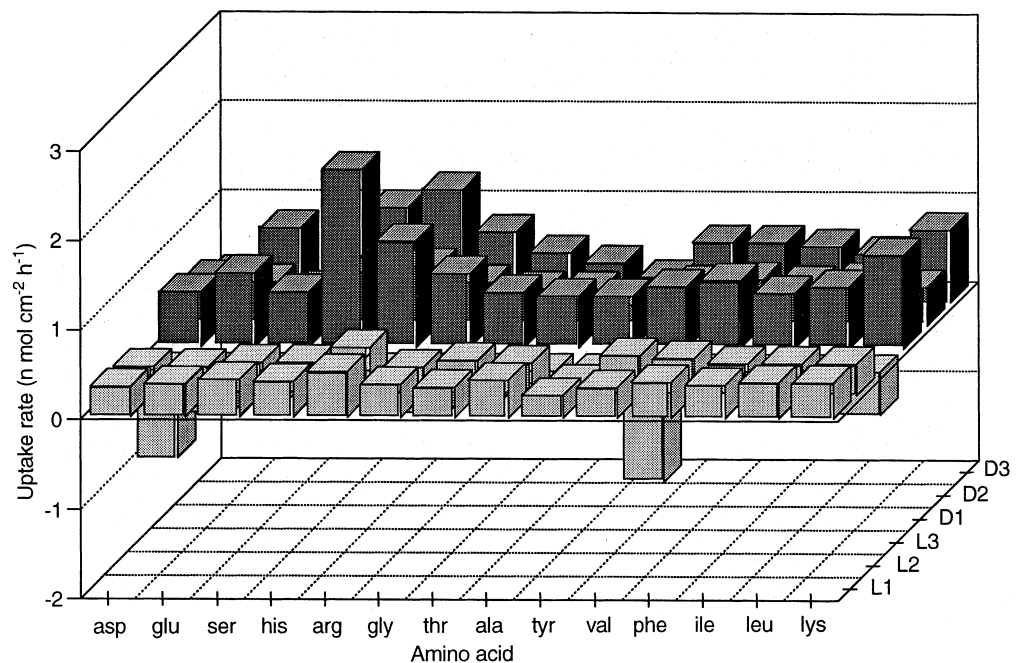
The results of the present study demonstrate that ambient concentrations of dissolved nitrogen can supply the nitrogen requirements of the symbiotic coral *Pocillopora damicornis*. Of the two potential sources investigated, ammonium was the only resource that appeared to be able to completely satisfy the nitrogen demand of *P. damicornis* at field concentrations, with the inward flux of ammonium readily supplying the nitrogen

requirements of *P. damicornis* at ammonium concentrations as low as 100 nM. This is well within the bounds of the concentrations normally found in the water surrounding *P. damicornis*. The potential of DFAAs to supply significant amounts of nitrogen, however, was an order of magnitude smaller. Even at the rare maximum ambient concentrations of DFAAs measured in the field (100 nM), only 8.5 to 11.3% of the nitrogen requirements of *P. damicornis* and its symbiotic dinoflagellates could be supplied.

Availability of dissolved nitrogen to *Pocillopora damicornis*

Koop et al. (1995) monitored the ammonium concentrations in the ENCORE atolls during several sampling periods during the ENCORE project. Their measurements revealed that the concentration of ammonium in the control microatolls (in One Tree Island lagoon) rarely decreased below 0.5 μM for most of the year and experienced small increases of up to 1–2 μM during summer. Their results confirm those of Hatcher and Frith (1985), who reported values of $3.68 \pm 0.92 \mu\text{M}$ and $4.32 \pm 1.93 \mu\text{M}$ (mean \pm SE, regular samples, $n > 30$, period December 1979 to January 1981) for two sites in One Tree Island lagoon within the area used for the ENCORE experiment. Conditions within the ammonium-treated microatolls in the ENCORE

Fig. 5 *Pocillopora damicornis*. Rate of uptake of 14 amino acids by colonies incubated in light (L1–3) and dark (D1–3). Each amino acid present at 400 nM, total amino acid concentration presented to colonies was 5.6 μ M



project were identical, except for the periods when the microatolls were ponded and ammonium ions were added. The latter conditions led to transient increases in ammonium concentrations that remained $\geq 10 \mu$ M for ≈ 3 h every low-tide period. At the end of low tide, new water flowing into the microatolls flushed the re-

maining concentrations of ammonium down to background levels similar to those in the control microatolls. These dynamics are typical for tropical waters, especially those associated with offshore coral reefs (Andrews and Muller 1983; Hatcher 1988; Charpy-Roubaud et al. 1990).

Table 2 *Pocillopora damicornis*. Nitrogen budget for brown morph exposed for 12 mo to control or ammonium (NH_4^+)-treated microatolls within ENCORE experiment at One Tree Island. Fraction of new area added per day (average growth rate, Line 1) divided by 100 was multiplied by mass of N atoms per control and NH_4^+ -treated corals (Line 2). Resulting demand for N atoms (Line 3) was then converted into units of $\text{nmol cm}^{-2} \text{d}^{-1}$

(Line 4) Mean daily uptake of ammonium and dissolved free amino acids (DFAAs) was calculated for various concentrations (Lines 5 to 9). Percent contributions (Lines 10 and 11) were calculated by dividing uptake rates (Lines 7 and 9) by demand for N atoms (Line 4). Break-even points (Lines 12 and 15) were calculated as shown and represent concentrations at which uptake equals demand

	Control	NH_4^+ -treated microatolls
Demand for N atoms by <i>P. damicornis</i>		
(1) Average growth rate (% per day; Hoegh-Guldberg 1999)	0.162	0.103
(2) Mass N (mg) per cm^2 (Muller-Parker et al. 1994)	0.206	0.287
(3) Demand for N atoms ($\mu\text{g cm}^{-2} \text{d}^{-1}$)	0.33	0.30
(4) Demand for N atoms ($\text{nmol cm}^{-2} \text{d}^{-1}$)	23.80	21.09
Supply of nitrogen from DFAA uptake		
(5) Mean DFAA uptake at 6 μ M ($\text{nmol cm}^{-2} \text{h}^{-1}$; this study)	7.01	7.01
(6) Mean DFAA uptake at 6 μ M ($\text{nmol cm}^{-2} \text{d}^{-1}$) = (5) \times 24 h	160.8	107.2 ^a
(7) Mean DFAA uptake at 100 nM ($\text{nmol cm}^{-2} \text{d}^{-1}$) = (6) \times 0.1 μ M \div 6 μ M	2.7	1.8
Supply of nitrogen from uptake of NH_4^+		
(8) Mean uptake at 2 μ M ($\text{nmol cm}^{-2} \text{d}^{-1}$; this study)	379.9	379.9
(9) Mean uptake at 10 μ M ($\text{nmol cm}^{-2} \text{d}^{-1}$; this study)	1277.6	1277.6
Percent contribution of DFAAs to nitrogen needs of <i>P. damicornis</i>		
(10) Contribution at 100 nM (%) = (7) \div (4) \times 100 \div (1)	11.3	8.5
(11) Contribution at 6 μ M (%) = (6) \div (4) \times 100 \div (1)	675.6	508.2
(12) Break-even point (μ M) = 6 μ M \times (4) \div (6)	0.89	1.18
Percent contribution of NH_4^+ to nitrogen needs of <i>P. damicornis</i>		
(13) Contribution at 2 μ M (%) = (8) \div (4) \times 100 \div (1)	1596.4	1801.1
(14) Contribution at 10 μ M (%) = (9) \div (4) \times 100 \div (1)	5368.7	6057.2
(15) Break-even point (μ M) = 2 μ M \times (4) \div (8)	0.13	0.11

^aTakes into account that DFAA levels decreased to zero in ammonium microatolls for ≈ 8 of every 24 h

Measurement of the concentrations of DFAAs in the water column at One Tree Island revealed that concentrations are very low and similar to those found earlier for polar (Welborn and Manahan 1990), temperate (Poulet et al. 1991; Coffin 1989) and tropical (Ferrier 1991; Schlichter and Leibezeit 1991) seas. The principal amino acids making up this fraction were serine and glycine, which were also found to be common in other studies of tropical water columns (Ferrier 1991; Schlichter and Leibezeit 1991; Hoegh-Guldberg et al. 1997). Interestingly, the concentration of DFAAs appeared to be sensitive to the long-term addition of ammonium. The addition of ammonium to the microatolls of the ENCORE experiment led to a reduction in the concentration of the DFAAs pool in the water column. The reason for this decrease was not explored in the current study, but may be related to changes in the abundance and composition of the microbial community induced by the long-term addition of ammonium.

Acquisition of ammonium by *Pocillopora damicornis*

Pocillopora damicornis and its symbiotic dinoflagellates acquired ammonium from 2 to 10 μM solutions at rates (range: 5.1 to 91.8 $\text{nmol N cm}^{-2} \text{h}^{-1}$) that were similar to those reported for symbiotic dinoflagellates of reef-building corals in previous studies (Muscatine and D'Elia 1978; Muscatine et al. 1979; Wafar et al. 1993). For *P. damicornis* from Hawaii, Muscatine and D'Elia, measured maximum uptake rates of ammonium that ranged between 3.57 and 9.21 $\mu\text{mol N chlorophyll } a^{-2} \text{h}^{-1}$. Assuming a mean chlorophyll *a* concentration for Hawaiian *P. damicornis* of 4 $\mu\text{g cm}^{-2}$ (actual range = 2.0 to 6.5 $\mu\text{g cm}^{-2}$; Muller-Parker et al. 1994), these rates convert to maximum uptake values ranging between 14.3 and 36.8 $\text{nmol N cm}^{-2} \text{h}^{-1}$. These values compare well with the rates of ammonium uptake at 10 μM (range: 9.8 to 45.4 $\text{nmol N cm}^{-2} \text{h}^{-1}$). The ammonium uptake by *P. damicornis* was not influenced by the whether measurements were made in light or dark. Muscatine and D'Elia reported that the extent to which corals (*P. damicornis*) will take up ammonium in the dark was strongly influenced by the length of the preceding period of photosynthetic activity. When the energy reserves from preceding periods of photosynthetic activity were deliberately run down in lengthy dark periods (>19 h), *P. damicornis* tended to show a net efflux of ammonium. As the corals in the current study were collected directly from the experimental microatolls (1 m depth) in the early afternoon on a summer day, it appears that exposure of the corals to darkness during the current experiment was probably too small to affect the energy available for the transport of ammonium.

The incubation of corals in ammonium did not affect the rate of ammonium uptake except in the case of the pink (= pocilloporin) morph of *Pocillopora damicornis* measured at 10 μM ammonium. In this case, uptake was

lower ($P < 0.05$) in corals exposed to elevated ammonium over 12 mo. The lack of a broad general response in this case ran counter to expectations from previous studies, which found a reduced ammonium uptake in symbiotic dinoflagellates isolated from *P. damicornis* grown for 6 wk in the presence of 20 μM ammonium (Yellowlees et al. 1994). Yellowlees and co-workers traced declines in ammonium uptake to reduced glutamine synthetase activities in the corals, which were exposed to increased ammonium. These decreases were interpreted as evidence of regulatory controls to reduce nitrogen influx as excess inorganic nitrogen becomes available. The lack of a strong decreasing trend in the present experiment may be a result of the overall low loading of ammonium on the nutrient-treated microatolls. The addition of ammonium during the ENCORE experiment occurred only during the period in which the microatolls were ponded (3 to 4 h each low tide). The rapid uptake of ammonium in the microatolls during this period resulted in high concentrations of ammonium that only persisted for a couple of hours during each low tide (Koop et al. 1995). Counter to this explanation is the observation that *P. damicornis* growing in microatolls that received ammonium had low growth rates and higher rates of mortality than those growing in control microatolls (Hoegh-Guldberg 1999). This may indicate that the ammonium concentrations of the ENCORE project were higher than might be considered "healthy" for *P. damicornis*, and hence might be expected to induce biochemical controls on the influx of nitrogen. These effects on growth and mortality, however, may also be related to secondary effects (e.g. great rates of algal overgrowth or disease infestation) as opposed to primary effects on coral/algal metabolism.

Co-transport of dissolved free amino acids

Dissolved free amino acids (DFAAs) have been proposed as a source of nitrogen for both reef-building corals (Ferrier 1991) and symbiotic clams (*Tridacna gigas*; Hawkins and Klumpp 1995; but see Ambariyanto and Hoegh-Guldberg 1999). In the present study, *Pocillopora damicornis* readily took up amino acids from the surrounding water when amino acids were offered to them at a total concentration of 6 μM . The concentration of all amino acids was reduced during the incubation periods, and uptake rates ranged from 0.080 to 0.660 $\mu\text{mol g}^{-1} \text{h}^{-1}$ over this period. The highest uptake rates were for the amino acids serine, arginine and alanine, and the lowest rates for tyrosine. Uptake was non-specific, with all classes of amino acids being taken up rapidly. This is similar to the results of Ferrier (1991), who also saw a similar non-specific uptake of classes of DFAAs by four Caribbean corals (*Montastrea annularis*, *Madracis mirabilis*, *Agaricia fragilis*, and *Favia fragum*). The non-specific nature of amino acid uptake by reef-building corals appears to be different to that seen by invertebrate larvae. In the latter, a clear preference is

shown by epithelial transport systems for neutral amino acids (Manahan 1990; Hoegh-Guldberg 1994).

It is important to realize that the observation of uptake by coral colonies (as with invertebrate larvae) does not rule out the possibility that bacteria inhabiting the coral surface are involved in the uptake process. The component of the complex community represented by a coral colony responsible for the uptake of DFAAs by corals remains to be determined. Manahan et al. (1982) has shown, however, that the uptake of DFAAs by echinoid larvae is due to the animal and not to contaminating or constituent bacteria. In their study, axenic larvae raised in sterile conditions were found to have high rates of amino acid transport. This is technically difficult to achieve with corals, given they have diffuse gonad tissues and complexities introduced by the symbiotic association of reef-building corals and symbiotic dinoflagellates. A less sophisticated test may depend on using antibacterial compounds to sterilise the surface of corals prior to uptake experiments.

Rates of DFAA uptake were lower in the light than in the dark. The influence of darkness on DFAA uptake may be related to changes in the concentration of amino acids in the cytoplasm of the symbiotic cnidarians when the symbiosis becomes photosynthetically active. A higher concentration internally (induced by photosynthetic activity) would form a steeper gradient (from outside to inside the cell) and hence mean a greater resistance to the inward transport of free amino acids. These results are in contrast to those of Schlichter and Leibezeit (1991), who reported that amino acid were released from colonies of the symbiotic soft coral *Heteroxenia fuscescens* when incubated in the light. The difference between these two studies is hard to resolve, except to note that one of the colonies of *Pocillopora damicornis* incubated in the light in the present study was also a net exporter of amino acids. Perhaps there is an effect of light such that, at some light levels, the concentration of amino acids builds up to the point where symbiotic cnidarians become net exporters of amino acids. This remains an intriguing yet exciting possible pathway for the flow of nitrogen within coral reef communities.

Relative importance of ammonium vs dissolved free amino acids in meeting nitrogen demand of growing *Pocillopora damicornis*

The tissue of *Pocillopora damicornis* colonies growing in the ENCORE microatolls expanded at rates between 0.103 and 0.162% d⁻¹ (Hoegh-Guldberg 1999). *P. damicornis* (brown morph) growing in the control microatolls required nitrogen at the rate of 23.8 nmol N cm⁻² d⁻¹; colonies growing in the N microatolls needed 21.1 nmol N cm⁻² d⁻¹. It is important to note that this estimate depends on the fact that corals are net sinks for nitrogen (Muscatine and D'Elia 1978; Rahav et al. 1989; Atkinson et al. 1994), and hence that the

export of nitrogen is relatively small. This discrepancy aside, the estimates generated here can be used as relative estimates of the extent to which the two forms of nitrogen uptake can supply the nitrogen required for tissue expansion. These estimates also provide a common ground for comparing the relative abilities of the inward transport of ammonium versus dissolved free amino acids to meet the nitrogen requirements of *P. damicornis*.

When the requirement of *Pocillopora damicornis* for nitrogen is compared to the rate at which it can be supplied, it becomes clear that the inward flux of ammonium and DFAAs differ in the extent to which they can satisfy this demand (Table 2). The inward flux of ammonium can meet the nitrogen requirements of *P. damicornis* at concentrations between 0.11 and 0.13 μM (Line 15: "Break-even point": Table 2). As concentrations of ammonium were rarely ever < 0.1 μM in the ENCORE microatolls and surrounding waters (Hatcher and Frith 1985; Koop et al. 1995), this suggests the important conclusion that ammonium is not limiting for *P. damicornis* even under control conditions within patch reefs ("microatolls") in One Tree Island lagoon. This has additional importance for interpreting the responses of organisms within the ENCORE experiment. If background levels of ammonium more than meet the nitrogen demand of corals such as *P. damicornis*, then the additional ammonium supplied during the ENCORE experiment might not be expected to stimulate coral growth. Data collected during the ENCORE experiment appears to support this interpretation. Both morphs of *P. damicornis* and *Acropora longicyathus*, for example, did not exhibit any increased growth rate in the ammonium-supplemented microatolls (Hoegh-Guldberg 1999).

Measurement of DFAAs in the water column at One Tree Island revealed low concentrations similar to those in tropical seas (Ferrier 1991; Schlichter and Leibezeit 1991), including a range of habitats in the central Great Barrier Reef (Hoegh-Guldberg et al. 1997). The absolute amounts of DFAAs imported were calculated for *Pocillopora damicornis* growing in the DFAA concentrations recorded in the microatolls. These calculations revealed that only 8.5 to 14.7% of the total nitrogen demand of *P. damicornis* could be supplied by the transport of DFAAs at the highest concentrations reported within this study (100 nM: Table 2). This conclusion is similar to that reached for giant clams growing in the ENCORE microatolls (Ambariyanto and Hoegh-Guldberg 1999). In the latter case, the giant clam *Tridacna maxima* also took up DFAAs from the surrounding seawater. However, the supply of either energy or nitrogen atoms from the transport of DFAAs, was trivial compared to the energy or total nitrogen demands of growing *T. maxima*. Despite the apparently small role of DFAAs in supplying N atoms for coral-dinoflagellate growth, the transport of DFAAs might perform a number of other functions, including (1) the recapture of amino acids which might leak across their highly

exposed and permeable membrane surfaces, (2) the acquisition of essential amino acids that are unavailable from dinoflagellate photosynthate (but see Swanson and Hoegh-Guldberg 1998) or coral feeding, and (3) the acquisition of small proportions of amino acids as part of routine osmoregulatory function (Hoegh-Guldberg et al. 1997). Whatever its function, however, the transport of DFAAs does not appear to play a significant role in supplying either the energy or nitrogen requirements of tropical organisms such as corals, clams or asteroid larvae (Hoegh-Guldberg et al. 1997; Ambariyanto and Hoegh-Guldberg 1999).

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