

Uptake of ammonium by the scleractinian coral *Stylophora pistillata*: Effect of feeding, light, and ammonium concentrations

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Abstract

Experiments were designed to assess the uptake rates of 0.2, 1, and 5 μM ammonium by the scleractinian coral *Stylophora pistillata* maintained under different feeding regimes (highly fed, slightly fed, and starved) for 4–8 weeks. $^{15}\text{NH}_4$ was used to follow the incorporation of nitrogen in the zooxanthellae or in the animal tissue and to calculate uptake rates. After 4 or 8 weeks, fed corals contained significantly higher concentrations of protein, chlorophyll, and zooxanthellae than starved nubbins. They also contained significantly higher amounts of carbon and nitrogen per unit surface skeleton. Results obtained showed that the algal fraction was enriched with ^{15}N at up to 10 times the rate of the host, which suggests that the zooxanthellae are the primary site of assimilation. Uptake rates (measured in the algal fraction) varied according to the nitrogen concentration in seawater. They were ~ 20 times lower at 0.2 than at 1 or 5 μM $^{15}\text{NH}_4$ enrichment (2–30 vs. 120–510 $\text{ng N h}^{-1} \text{cm}^{-2}$) for both fed and starved nubbins. These rates were also affected by the feeding history of the host, because they were significantly lower for fed than for starved nubbins (analysis of variance, $P < 0.005$), at both high and low ammonium concentrations. On the basis of the nitrogen content of the zooxanthellae, we suggest that an external concentration of ammonium equal to 0.6 μM can sustain the growth of the zooxanthellae population.

Scleractinian corals thriving in nutrient-poor tropical waters have developed adaptations for conserving nitrogen. They live in symbiosis with dinoflagellates called zooxanthellae that can take up and retain dissolved inorganic nitrogen (ammonium and nitrate) from the surrounding seawater (Muscatine 1980; Wilkerson and Trench 1986; Falkowski et al. 1993; Marubini and Davies 1996). Zooxanthellae also use host metabolites as nutrient sources (Falkowski et al. 1984; Rahav et al. 1989), and holozoic feeding is therefore believed to provide zooxanthellae with nitrogen (Szmant-Froelich and Pilson 1984).

Different studies have investigated relationships between corals and nitrogen. It has been shown that assimilation of ammonium into glutamine is through the action of glutamine synthetase, found both in the host tissue and the algae (Yellowlees et al. 1994). Nitrate, in contrast, is converted into ammonium only in algae, through the action of nitrate and nitrite reductases (Miller and Yellowlees 1989). Most of the work (see Koop et al. 2001) has focused on the effect of

elevated nitrogen enrichment ($>10 \mu\text{M}$ ammonium or $>5 \mu\text{M}$ nitrate) on coral and zooxanthellae physiology. Such enrichment often induces an increase in zooxanthellae density (Hoegh-Guldberg and Smith 1989; Muscatine et al. 1989; Dubinsky et al. 1990; Stambler et al. 1991), a decrease in coral calcification or growth (Stambler et al. 1991; Marubini and Davies 1996), and a decrease in the rates of photosynthesis per algal cell (Dubinsky et al. 1990). Few studies, however, have measured the uptake rates of ammonium and nitrate under different environmental conditions. Most of the works were performed with cultured or freshly isolated zooxanthellae (D'Elia et al. 1983; Domotor and D'Elia 1984; McAuley and Smith 1995), and only one work determined the pathway of ammonium assimilation in the symbiotic sea anemone *Anemonia viridis* (Roberts et al. 1999). However, nitrogen uptake rates by the coral-zooxanthellae association have still been poorly investigated (Muscatine and D'Elia 1978; Burris 1983; Wilkerson and Trench 1986; Bythell 1990; Hoegh-Guldberg and Williamson 1999). Among the above studies, only one (Bythell 1990) measured the uptake rates under natural nitrogen concentrations ($<2 \mu\text{M}$ ammonium or nitrate), whereas others largely increased these concentrations far above normal seawater levels (2–10 μM ammonium).

The present experiments were therefore designed to assess the uptake rates of ammonium by the scleractinian coral *Stylophora pistillata* over a range of concentrations from 0.2 to 5 μM . For this purpose, $^{15}\text{NH}_4$ was used. Experiments were

Acknowledgments

The present study is part of the O.O.E. program supported by the Centre Scientifique de Monaco and the Council of Europe (Open Partial Agreement on Major Natural and Technological Disasters). Thanks are due to Dr. E. Tambutté and Marie-Dominique Pisay for their help and to Dr. N. Ounais and the staff of the public aquarium of the Oceanographic Museum for providing corals used in these experiments.

also conducted to evaluate whether ammonium uptake was affected by host nutrition, because it has been suggested that the uptake of dissolved nutrients depends on the feeding history of the host (Szmant-Froelich and Pilson 1984; Muller-Parker et al. 1988, 1990).

Material and Methods

Experimental set up—Experiments were performed in the laboratory by use of colonies of the scleractinian coral *S. pistillata* (Esper 1797) collected in the Gulf of Aqaba (Red Sea, Jordan). In a first set of experiments, 80 nubbins (4 cm long and 2 cm large) of about the same size were obtained by cutting terminal portions of branches of eight parent colonies and were used when the animal tissue entirely regenerated over the cut portion of the skeleton. They were then randomly divided into three aquaria supplied with oligotrophic Mediterranean seawater (pumped at 50 m depth and heated to 26°C). Metal halide lamps at 400 W (Philips, HPIT) provided a constant irradiance of $300 \pm 50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (photoperiod 12:12 h). Salinity and irradiance were measured by use of a conductivity meter (Meter LF196) and a 4π quantum sensor (Li-Cor, LI-193SA), respectively. Temperature (precision $\pm 0.05^\circ\text{C}$) was logged at 10-min intervals by use of a Seamon temperature recorder and varied between 26.5°C and 27.5°C. In the first tank, nubbins were fed three times a week with 5 g of *Artemia salina* nauplii (“highly fed nubbins”). In the second tank, nubbins were fed only once a week with the same amount of nauplii (“slightly fed nubbins”). In the last control tank, corals were not fed (“starved nubbins”). To ensure a good capture of the prey, seawater renewal was shut down for 2 h after introduction of the *Artemia*.

After 4 weeks under the above culture conditions, uptake rates of ammonium were measured for the three groups of nubbins. Each nubbin was incubated in a 250-ml beaker filled with a solution of 1 and 5 μM NH_4 . For all experiments, the tracer ^{15}N was added as $^{15}\text{NH}_4\text{Cl}$ (98% atom, CEA). Beakers contained a magnetic stirrer to homogenize the medium and were immersed in a waterbath to maintain a constant temperature of 26°C. The length of the incubation necessary to detect a satisfactory signal in the coral tissue was determined in preliminary experiments (8 h at 1 μM $^{15}\text{NH}_4$ and 5 h at 5 μM $^{15}\text{NH}_4$). For each concentration, uptake rates were measured under low and high irradiance (80 and 350 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Five nubbins were used for each condition. In order to avoid nutrient depletion in the beakers, solutions of $^{15}\text{NH}_4$ were continuously pumped with a peristaltic pump from a batch solution to the beakers. The flow rate was 7 ml min^{-1} and had also been determined during a set of preliminary experiments.

In a second set of experiments, 40 nubbins were prepared again and incubated for 8 weeks under the same culture conditions as above. Experiments were then performed to measure the uptake rates of 0.2 μM $^{15}\text{NH}_4$ by the highly fed and starved nubbins. This low ammonium concentration was comparable to the amounts measured in oligotrophic tropical seawater (Szmant 1997). Because ammonium concentrations used were very low, we preferred to maintain the corals 8

weeks instead of 4 under the different feeding regimes, to increase their difference in physiological state and therefore increase their response to nitrogen uptake. Each nubbin was incubated as described above with a solution of 0.2 μM $^{15}\text{NH}_4$, which was added to the seawater content of 0.4 μM to give a final concentration of 0.6 μM . Corals were incubated for either 12 h under high light (350 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$) or 24 h with 12 h high light (350 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$) and 12 h dark.

At the end of the incubation with $^{15}\text{NH}_4$, nubbins were rinsed in nutrient-depleted seawater (maintained in the dark for 1 month) for 30 min to wash the coelenteron. Tissues were then completely removed from skeleton with an “air pick” (air under pressure) and homogenized with a Potter tissue grinder. The homogenate was centrifuged (Biofuge 17RS-Heraeus) at $2000 \times g$ for 10 min at 4°C to pellet the zooxanthellae. The supernatant was centrifuged again at least two times to pellet residual zooxanthellae (Muscatine et al. 1989) and transferred into 25-ml polypropylene tubes. Pellets of zooxanthellae were resuspended and washed 3 times with filtered seawater to avoid tissue contamination. Tubes that contained tissues and zooxanthellae were then immersed in liquid nitrogen and freeze-dried by use of a Heto lyophilizer (CT 60).

Ammonium uptake rate determination—Carbon and nitrogen contents were measured for each sample by use of a carbon-hydrogen-nitrogen analyzer. The isotopic ratios $^{15}\text{N}/^{14}\text{N}$ of the animal tissues and zooxanthellae of the freeze-dried samples were determined by emission spectroscopy that used a GS1 optical spectrometer (SOPRA France) according to the method of Guiraud and Fardeau (1980). The enrichment of the samples with ^{15}N was recorded as “at. % excess”: $\text{atom \% excess } ^{15}\text{N} = (\text{at. \% } N_{\text{mes}}) - (\text{at. \% } N_{\text{natural}})$.

Ammonium uptake rates (ρ) in tissue and zooxanthellae fractions were calculated according to the equation of Dugdale and Wilkerson (1986). ρ is expressed in $\text{ng N h}^{-1} \text{ cm}^{-2}$:

$$\rho = \frac{N_{\text{mes}} - N_{\text{natural}}}{(N_{\text{enr}} - N_{\text{mes}}) t_{\text{inc}} S} \times M_{\text{sample}} \times M_{\text{N}} \times 10^6$$

where

- N_{mes} : % ^{15}N measured in the sample
- N_{natural} : natural abundance ^{15}N in control nubbins
- N_{enr} : ^{15}N enrichment of the incubation medium
- t_{inc} : incubation time of the nubbins (h)
- S : nubbin surface area (cm^2)
- M_{sample} : mass of the freeze-dried sample (mg)
- M_{N} : particulate nitrogen mass (mg) per mg of tissue or zooxanthellae

Measurements of protein, chlorophyll, and zooxanthellae concentrations—At the end of the 8 weeks of culture, three nubbins of starved and highly fed treatments were selected for chlorophyll and protein determination. Measurements were also performed after 4 weeks but gave similar results. Chlorophyll *a* and *c*₂ were extracted twice in 99% acetone (24 h at 4°C). The extracts were centrifuged (Heraeus centrifuge) at $11,000 \times g$ for 10 min at 4°C and the absorbances measured at 630, 663, and 750 nm. Chlorophyll concentra-

Table 1. Results of one-factor ANOVAs and *t* test, testing the effect of feeding on the carbon and nitrogen content of the zooxanthellae and tissue of coral colonies. *P*, probability; *, significant (5%); carbon and nitrogen concentration ($\mu\text{g cm}^{-2}$) in starved, slightly fed and highly fed corals; DW, dry weight. Data are presented as mean and standard error.

	<i>P</i>	Concentrations ($\mu\text{g cm}^{-2}$)		
		Highly fed	Slightly fed	Starved
After 4 wk incubation (ANOVA)				
Carbon content of the zooxanthellae fraction	0.76	473.5 \pm 161.0	576.3 \pm 56.5	399.6 \pm 22.6
Carbon content of the animal fraction	0.87	720.9 \pm 207.3	493.5 \pm 46.8	504.2 \pm 41.5
Nitrogen content of the zooxanthellae fraction	0.15	72.2 \pm 4.9	73.9 \pm 21.6	72.3 \pm 34.6
Nitrogen content of the animal fraction	0.05*	161.8 \pm 12.0	130.4 \pm 10.7	121.7 \pm 12.0
Mean C:N ratio of the zooxanthellae fraction		6.53 \pm 0.3	7.78 \pm 1.8	6.51 \pm 0.25
Mean C:N ratio of the animal fraction		4.48 \pm 0.5	4.10 \pm 0.7	4.16 \pm 0.20
		Concentrations ($\mu\text{g cm}^{-2}$)		
		Highly fed	Starved	
After 8 wk incubation (<i>t</i> test)				
Carbon content of the zooxanthellae fraction	0.05*	302.7 \pm 3.6	252.9 \pm 18.1	
Carbon content of the animal fraction	0.05*	688.1 \pm 102.1	388.3 \pm 51.4	
Nitrogen content of the zooxanthellae fraction	<0.001*	42.1 \pm 0.9	25.7 \pm 2.9	
Nitrogen content of the animal fraction	<0.001*	133.6 \pm 19.4	72.5 \pm 11.9	
Mean C:N ratio of the zooxanthellae fraction		7.15 \pm 0.3	9.69 \pm 0.25	
Mean C:N ratio of the animal fraction		4.73 \pm 0.8	5.38 \pm 0.50	

tions were computed according to the equations of Jeffrey and Humphrey (1975). The remaining tissue was then dissolved in 1 N NaOH (90°C for 30 min) and the protein content measured as described by Lowry et al. (1951) on three aliquots (1 ml) from each coral homogenate. The standard curve was established with bovine serum albumin, and absorbance was measured at 750 nm with a Multiscan bi-chromatic (Labsystem). Three nubbins were also taken for the determination of the zooxanthellae density. Zooxanthellae were removed with a Water-Pic and counted with a Neubaer cell. Surface area of the colonies was measured according to the aluminum foil technique (Marsh 1970).

Measurement of nutrient concentrations in the experimental tanks—Concentrations of ammonium, nitrite, and nitrate in the culture tanks were measured every week by use of a Technicon Autoanalyzer. All concentrations remained low and constant in the tanks during the whole experiment and equal to $0.35 \pm 0.10 \mu\text{M}$ for ammonium, $0.1 \pm 0.1 \mu\text{M}$ for nitrite, and $0.5 \pm 0.2 \mu\text{M}$ for nitrate.

Statistical treatments—The effect of light and feeding on the rates of ammonium uptake was assessed with an analysis of variance (ANOVA). When a significant effect was found, means were compared with a Bonferroni/Dunn post hoc test. Statistical analyses were performed with use of StatView 4.01 (Abacus Concepts). Data are reported as mean \pm standard error of the mean (SE).

Results

Physiological parameters—Fed and starved nubbins exhibited significant physiological differences after either 4 or 8 weeks. The amount of protein was significantly higher in highly fed than in starved nubbins (*t* test, $P < 0.001$). Con-

centrations varied from 421 ± 19 to $900 \pm 91 \mu\text{g cm}^{-2}$ for starved and highly fed nubbins, respectively. Chl *a* concentrations and zooxanthellae density were also significantly different (*t* test, $P < 0.001$). They varied from 3.8 ± 0.8 to $16.2 \pm 1.4 \mu\text{g Chl } a \text{ cm}^{-2}$ and from $(0.7 \pm 0.05) 10^6$ to $(2 \pm 0.1) 10^6$ zooxanthellae cm^{-2} for starved and highly fed nubbins, respectively.

Uptake rates—After 4 weeks of culture under the three feeding regimes, the average carbon content of the tissue and zooxanthellae was not significantly different between fed, slightly fed, and starved nubbins (Table 1). The average nitrogen content of the tissue of highly fed nubbins was, however, significantly higher than the content of the two other groups. The carbon and nitrogen content per zooxanthellae varied between 240 and 570 pg C cell^{-1} and between 36 and 120 pg N cell^{-1} for highly fed and starved nubbins, respectively. Zooxanthellae represented between 30% and 36% of the nitrogen in the symbiotic association. The average C:N ratios varied from 6.53 to 7.78 for the algae and from 4.10 to 4.48 for the tissue. After 8 weeks of culture, the average carbon and nitrogen contents of the tissue and zooxanthellae were significantly different between treatments and were higher in fed than in starved nubbins (Table 1). The carbon and nitrogen content per zooxanthellae varied between 150 and 360 pg C cell^{-1} and between 21 and 37 pg N cell^{-1} for highly fed and starved nubbins, respectively. Zooxanthellae represented in this case $\sim 25\%$ of the nitrogen contained in the symbiotic association. The C:N ratio varied between 7.15 and 9.59 for the algae and between 4.73 and 5.38 for the animal fraction.

The atom % excess measured for the first set of experiments (incubation with 1 and $5 \mu\text{M } ^{15}\text{NH}_4$) is presented in Fig. 1a,b. It is expressed as uptake rates in Fig. 2a,b. In the algal fraction, these rates varied from 120 to 510 ng N h^{-1}

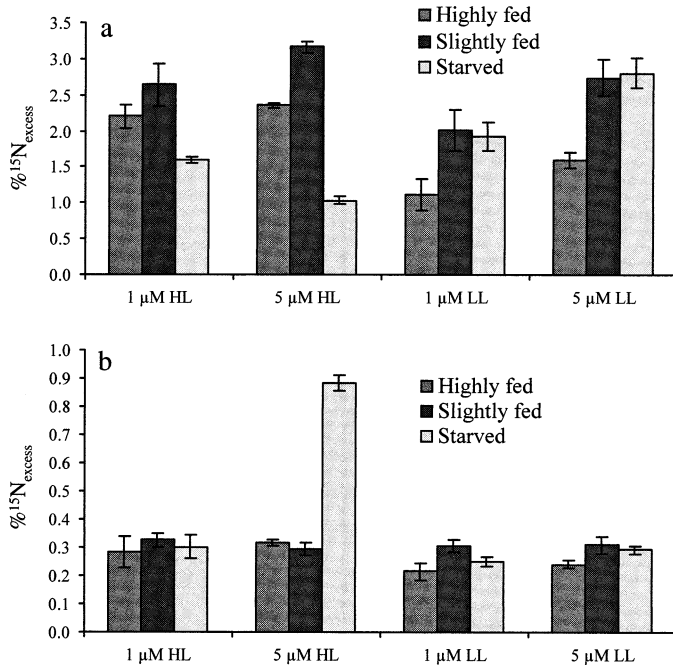


Fig. 1. Atom % ^{15}N excess after a 1 and 5 μM ^{15}N enrichment in (a) the zooxanthellae fraction and (b) the animal fraction. LL and HL: incubation under low (80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and high light (350 $\mu\text{mol m}^{-2} \text{s}^{-1}$), respectively. Grey, black, and white bars: highly fed, slightly fed, and starved nubbins, respectively. Mean and SE ($n = 5$ for each treatment). First set of experiments.

cm^{-2} depending on the light regime and nitrogen concentration. Uptake rates of the highly fed nubbins were significantly lower than the uptake rates of the two other groups of slightly fed and starved nubbins (Table 2). This trend was observed both at 5 and 1 μM $^{15}\text{NH}_4$ enrichment and under low or high light conditions, except for the group incubated under high light and 5 μM ammonium. In this case, a faster transfer of ^{15}N occurred between the zooxanthellae and the tissues (Fig. 2a,b). Light had an effect on the uptake rates except for a 5 μM NH_4 enrichment (Table 3). Uptake was

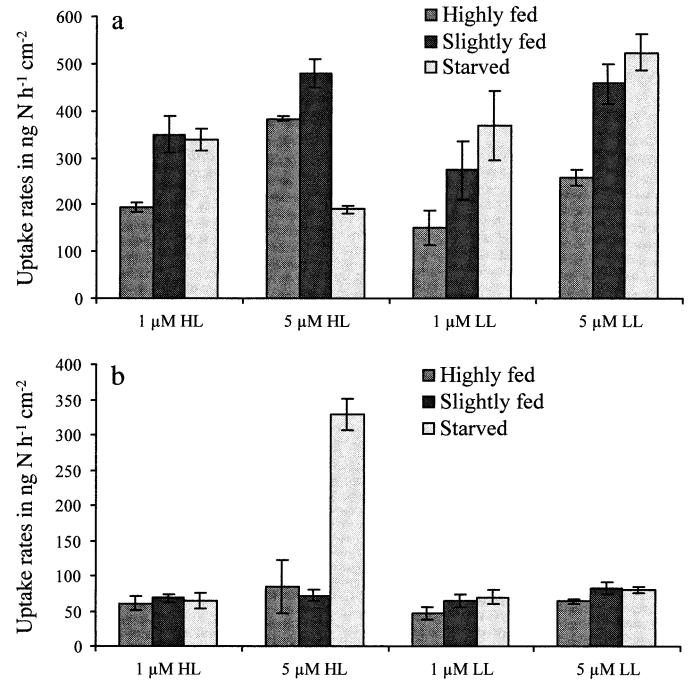


Fig. 2. Uptake rates ($\text{ng N cm}^{-2} \text{h}^{-1}$) of 1 and 5 μM $^{15}\text{NH}_4$ enrichment measured for (a) the zooxanthellae fraction and (b) the animal fraction. Mean and SE ($n = 5$ for each treatment). First set of experiments.

not dependent on the ammonium concentration in seawater (Table 3, Fig. 2a). Fig. 3a,b shows a significant difference in uptake rates expressed per chlorophyll content or algal cell density between highly fed and starved nubbins at both light levels and ammonium concentrations. Uptake rates in the animal fraction (Fig. 2b) were not significantly different between the three groups of corals, except for nubbins incubated at 5 μM $^{15}\text{NH}_4$ under high light for which a rapid transfer occurred. They ranged from 45 to 81 $\text{ng N h}^{-1} \text{cm}^{-2}$.

Uptake rates obtained during the second set of experiments with a 0.2 μM $^{15}\text{NH}_4$ enrichment are presented in Fig.

Table 2. First experiment. Results of one-factor ANOVAs testing the effect of feeding on the uptake of 1 and 5 μM ammonium by coral colonies incubated under two light levels (probabilities $P < 0.05$ are significant). When a significant effect was found, means were compared with a Bonferroni/Dunn post hoc test ($P < 0.008$ are significant); UF, SF, and HF: corals starved, slightly fed, and highly fed respectively. *, means significant at 5%.

	ANOVA <i>P</i>	Post hoc test		
		UF/HF <i>P</i>	UF/SF <i>P</i>	HF/SF <i>P</i>
Uptake of ammonium by the algal fraction				
Incubation at 5 μM $^{15}\text{NH}_4$ under high light	<0.001*	<0.001*	<0.001*	0.004*
Incubation at 5 μM $^{15}\text{NH}_4$ under low light	<0.001*	<0.001*	0.21	0.001*
Incubation at 1 μM $^{15}\text{NH}_4$ under high light	0.003*	0.003*	0.76	0.002*
Incubation at 1 μM $^{15}\text{NH}_4$ under low light	0.050*	0.002*	0.09	0.42
Uptake of ammonium by the animal fraction				
Incubation at 5 μM $^{15}\text{NH}_4$ under high light	<0.001*	<0.001*	<0.001*	0.52
Incubation at 5 μM $^{15}\text{NH}_4$ under low light	0.07	0.06	0.82	0.04
Incubation at 1 μM $^{15}\text{NH}_4$ under high light	0.29	0.13	0.72	0.23
Incubation at 1 μM $^{15}\text{NH}_4$ under low light	0.84	0.83	0.72	0.57

Table 3. First experiment. Results of one-factor ANOVAs testing the effect of light on the uptake of ammonium by coral colonies having three different feeding regimes ($P < 0.05$ significant). When a significant effect was found, means were compared with a Bonferroni/Dunn post hoc test. $P < 0.008$ significant); 1LL and 1HL: incubation under $1 \mu\text{M } ^{15}\text{NH}_4$ enrichment at low ($80 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and high ($350 \mu\text{mol m}^{-2} \text{ s}^{-1}$) light, respectively. 5LL and 5HL: incubation under $5 \mu\text{M } ^{15}\text{NH}_4$ enrichment at low and high light, respectively. *, means significant at 5%.

ANOVA	Uptake of ammonium by the algal fraction ($\text{nmol h}^{-1} \text{ cm}^{-2}$) (P)		
	Highly fed colonies <0.001*	Slightly fed colonies 0.017*	Starved colonies <0.001*
Post hoc tests			
1HL/1LL	0.11	0.25	0.64
5HL/5LL	<0.001*	0.74	<0.001*
1HL/5HL	<0.001*	0.06	0.02
1LL/5LL	0.01	0.01	0.01
1HL/5LL	0.02	0.11	0.007*
1LL/5HL	<0.001*	0.005*	0.009

4. They varied between 2 and 30 $\text{ng N h}^{-1} \text{ cm}^{-2}$. For all fractions, these rates were not significantly different between fed and starved nubbins, except the uptake of fed nubbins measured during 24 h (ANOVA, $P < 0.005$). However, uptake rates expressed per $\mu\text{g Chl } a$ or per algal cell (Fig. 5a,b) were significantly lower in fed than in starved nubbins (ANOVA, $P < 0.005$).

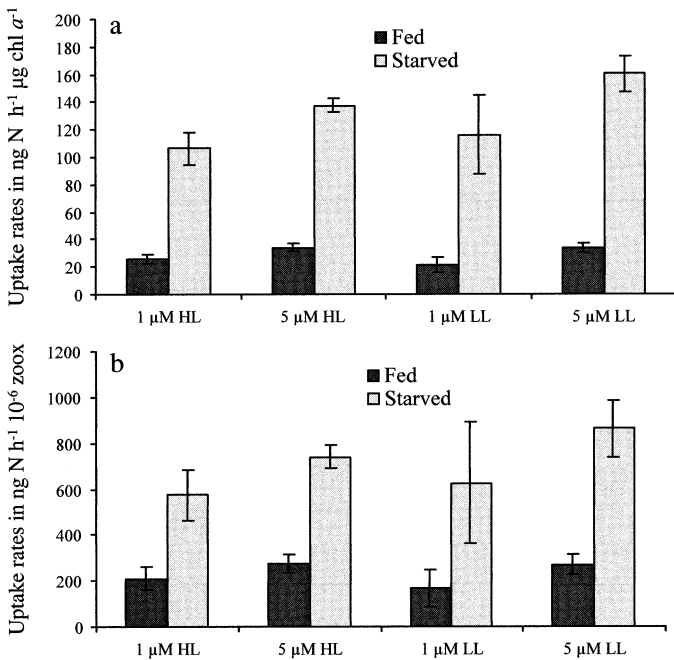


Fig. 3. Uptake rates of 1 and $5 \mu\text{M } ^{15}\text{NH}_4$ enrichment measured for the zooxanthellae fraction in (a) $\text{ng N h}^{-1} \text{ mg Chl } a^{-1}$ and (b) $\text{ng N h}^{-1} \text{ zooxanthellae}^{-1}$. Grey and black bars: fed and starved corals, respectively. Mean and SE ($n = 5$ for each treatment). First set of experiments.

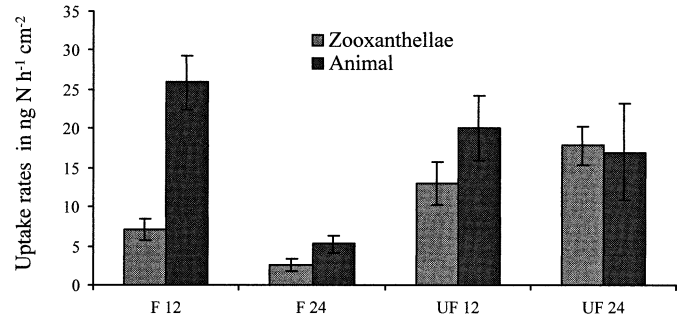


Fig. 4. Uptake rates ($\text{ng N h}^{-1} \text{ cm}^{-2}$) of $0.2 \mu\text{M } ^{15}\text{NH}_4$ enrichment measured for the zooxanthellae and animal fraction. F12 and F24: uptake rates measured for fed corals during 12 or 24 h incubation. UF 12 and UF 24: uptake rates measured for starved corals during 12 or 24 h incubation. Mean and SE ($n = 5$ for each treatment). Second set of experiments.

Discussion

In this study, uptake rates of ammonium by *S. pistillata* are compared for different ammonium concentrations in sea-water and for corals with a different feeding history. The ^{15}N technique has been intensively used in phytoplankton studies (see Dugdale and Wilkerson 1992) but can be found only in two experiments with sea anemones (Roberts et al. 1999; Lipschultz and Cook in press) and two with corals (Muscatine and D'Elia 1978; Burris 1983), in which only the percentage of enrichment ^{15}N in the tissue was measured. Most of the previous studies on ammonium uptake by sym-

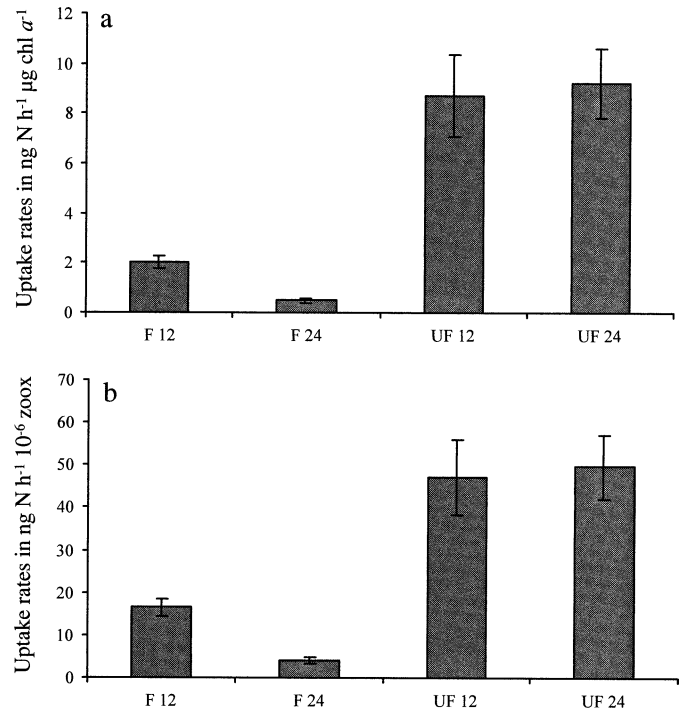


Fig. 5. Uptake rates of $0.2 \mu\text{M } ^{15}\text{NH}_4$ enrichment measured for the zooxanthellae fraction in (a) $\text{ng N h}^{-1} \text{ mg Chl } a^{-1}$ and (b) $\text{ng N h}^{-1} \text{ zooxanthellae}^{-1}$. Mean and SE ($n = 5$ for each treatment). Second set of experiments.

Table 4. Uptake rates of ammonium measured for symbiotic associations or isolated symbiotic dinoflagellates.

Reference	Species	Concentration (μM)	Uptake rates
Entire association			
Muscatine and D'Elia (1978)	<i>Pocillopora damicornis</i>	10	4.15 nmol ($\mu\text{g Chl } a$) ⁻¹ h ⁻¹
	<i>Pocillopora meandrina</i>	10	2.17 nmol ($\mu\text{g Chl } a$) ⁻¹ h ⁻¹
Wilkerson and Muscatine (1984)	<i>Aiptasia pulchella</i>	10	0.90 nmol ($\mu\text{g Chl } a$) ⁻¹ h ⁻¹
Rahav et al. (1989)	<i>Stylophora pistillata</i>	10	20.1 nmol cm ⁻² h ⁻¹ 6.70 nmol ($\mu\text{g Chl } a$) ⁻¹ h ⁻¹
Bythell (1990)	<i>Acropora palmata</i>	In situ	1.23 nmol cm ⁻² h ⁻¹
Hoegh-Guldberg and Williamson (1999)	<i>Pocillopora damicornis</i>	10	5.1–91.8 nmoles cm ⁻² h ⁻¹
Roberts et al. (1999)	<i>Anemonia viridis</i>	10 and 20	2200 nmol (g DW) ⁻¹ h ⁻¹
Zooxanthellae in vivo			
Szmant-Froelich and Pilson (1984)	<i>Astrangia danae</i>	In situ	1.14 nmol (10 ⁶ cells) ⁻¹ h ⁻¹ (starved corals) 1.3–2 nmol (10 ⁶ cells) ⁻¹ h ⁻¹ (fed corals)
Freshly isolated zooxanthellae			
D'Elia et al. (1983)	<i>Seriatopora hystrix</i>	10	5 nmol ($\mu\text{g Chl } a$) ⁻¹ h ⁻¹
	<i>Montastrea annularis</i>	10	7 nmol ($\mu\text{g Chl } a$) ⁻¹ h ⁻¹
Muller-Parker et al. (1988)	<i>Aiptasia pallida</i>	<10	14–43 nmol (10 ⁶ cells) ⁻¹ h ⁻¹
Yellowlees et al. (1994)	<i>Pocillopora damicornis</i>	20	42 nmol (10 ⁶ cells) ⁻¹ h ⁻¹
Wilkerson and Trench (1986)	<i>Tridacna gigas</i>	4–32	37 nmol (10 ⁶ cells) ⁻¹ h ⁻¹
Wilkerson and Muscatine (1984)	<i>Aiptasia pulchella</i>	10	13 nmol ($\mu\text{g Chl } a$) ⁻¹ h ⁻¹
Muscatine and Marian (1982)	<i>Mastigias</i> sp.	10	35 nmol ($\mu\text{g Chl } a$) ⁻¹ h ⁻¹
Zooxanthellae in culture			
McAuley and Smith (1995)	<i>Anemonia pallida</i>	100	20 nmol (10 ⁶ cells) ⁻¹ h ⁻¹

biotic associations have reported uptake from experiments in which ammonium depletion was monitored, usually from a high initial concentration (10 μM). We therefore present one of the first attempts to use the ¹⁵N tracer in corals to assess the uptake rates of near-natural ammonium concentrations. Corals indeed live in waters that contain very low levels of nutrients, with nitrogen concentrations being <2 μM (Szmant 1997). The main advantage of using the ¹⁵N technique is the avoidance of an important change in natural nitrogen concentrations because very low enrichments (0.1–0.2 μM) can be performed.

Direct comparisons of uptake rates measured in this work with previous studies are not possible, because they have used different coral species, isolated zooxanthellae, or different ammonium concentrations and flow rates. It has been demonstrated, for instance, that the uptake of ammonium can vary by two times during a 10-fold change in water velocity (Atkinson et al. 1994). Our data also mainly represent gross uptake rather than net uptake, as has usually been measured by concentration changes. However, we can say that our results are not completely different than uptake rates obtained in previous studies (Table 4). Uptake rates measured for the whole colony (1.5 nmol cm⁻² h⁻¹) are similar to those reported for reef-building corals in two previous studies (Table 4, Bythell 1990; Hoegh-Guldberg and Williamson 1999). When normalized per zooxanthellae, these rates (1–3.5 and 14–57 fmol zoox⁻¹ h⁻¹ for enrichments of 0.2 and 1–5 μM , respectively) are also in the same range of values than those found in several studies performed on freshly isolated or cultured zooxanthellae (Table 4).

Results obtained in this study showed that the uptake rates varied according to the nitrogen concentration in seawater.

They were ~20 times lower at 0.2 than at 1 or 5 μM ¹⁵NH₄ enrichment. However, they were not different between 1 and 5 μM ¹⁵NH₄, which indicates that incorporation of nitrogen in corals was already saturated at 1 μM ammonium. Concentration-dependent nutrient uptake dynamics have already been described for corals (Muscatine and D'Elia 1978; Wilkerson and Trench 1986). This has led to the development of a depletion-diffusion model of uptake in which the zooxanthellae deplete ammonium from the host cytoplasm after diffusion from seawater (D'Elia et al. 1983). In that study, the algal fraction was enriched with ¹⁵N at up to 10 times the rate of the host, which suggests that the zooxanthellae are the primary site of assimilation. This pattern has also been found in other zooxanthellar symbiosis when they have been investigated by use of a ¹⁵N tracer (Wilkerson and Kremer 1992; Hawkins and Klumpp 1995; Roberts et al. 1999). The presence of ¹⁵N in animal tissue after incubation in ¹⁵NH₄-spiked seawater is consistent with both the diffusion-depletion hypothesis (D'Elia et al. 1983) and the possibility that ¹⁵N is held in regulatory pools being maintained and released by animal enzyme activity (Wang and Douglas 1998). In other terms, ammonium could have been either recycled between zooxanthellae and their host or directly assimilated by the host (Ferrier 1996; Wang and Douglas 1998). Ferrier (1996) as well as Wang and Douglas (1998) indeed found glutamate synthetase activity in the animal tissues of sea anemones, which suggests that the animal is also able to take up some or all of the nitrogen.

Results obtained also showed that uptake rates were affected by the feeding history of the host, at both high and low ammonium concentrations. Uptake rates by highly fed nubbins were indeed significantly lower than the rates mea-

sured with starved corals. Previous studies that investigated the effect of feeding history on ammonium uptake by zooxanthellae also showed an inhibition in well fed animals (Cook et al. 1988; D'Elia and Cook 1988; Muller-Parker et al. 1988). This can be explained by a high recycling of nitrogen between the host and its symbionts (Trench 1974), organic nitrogen from the food being recycled by the host into inorganic nitrogen available for the zooxanthellae. McAuley and Cook (1994) and Smith and Muscatine (1999) also showed that the mitotic index, as well as the size of glutamate and glutamine pools (amino acids involved in ammonium assimilation), were higher in zooxanthellae freshly isolated from fed than from starved animals.

Fed nubbins, moreover, exhibited higher levels of proteins, Chl *a* and *c*₂ compared with the starved corals, in agreement with previous results (Stambler et al. 1991; Titlyanov et al. 1999). This is consistent with higher concentrations of total particulate carbon and nitrogen. The nitrogen content of the zooxanthellae fraction has been found to vary between 1.8 and 3 $\mu\text{mol N cm}^{-2}$ for starved and highly fed nubbins, respectively. These values are in agreement with the few existing data for *S. pistillata* (2.14 $\mu\text{mol N cm}^{-2}$, Muscatine et al. 1984; Falkowski et al. 1993) or for *Pocillopora damicornis* (Muller-Parker et al. 1994). Because fed corals contained three times more zooxanthellae per unit surface skeleton than starved corals, the amount of nitrogen per zooxanthellae was lower in fed than in starved corals. This suggests that an external supply of nitrogen is not stocked in internal pools in the cells but is used to increase the algal biomass (as well as the animal biomass, because proteins also doubled in fed corals). The C:N ratios of the zooxanthellae or the animal fraction are in agreement with the few values given in the literature (C:N = 6, Falkowski et al. 1993). They were lower in fed than in starved nubbins after 8 weeks' culture, which indicates that fed nubbins were retaining nitrogen. The same trend was observed after an enrichment with inorganic nitrogen (Falkowski et al. 1993) or particulate food (Muscatine et al. 1989). There was no change in the C:N ratio between fed and starved corals after 4 weeks' culture because of the high variability in the response of the nubbins to feeding.

Results obtained in the second set of experiments (daily cycle of nitrogen uptake, Figs. 4 and 5) suggest that the dark uptake of nitrogen by corals seems to be dependent on the nutritional status of the animal. This cycle indeed shows that, in starved corals, uptake rates measured over 12 h (daylight period) were comparable to those measured over 24 h (day and night incubation). This suggests that uptake by these animals and in our conditions was constant throughout the 24 h. Ammonium uptake in the dark has been demonstrated by a number of other studies (Muscatine and D'Elia 1978; Wilkerson and Trench 1986) and has been suggested to increase the rate at which carbon is fixed by the zooxanthellae in darkness (Cook et al. 1992). However, in fed corals, uptake rates were four times lower when measured for 24 than 12 h. Two hypotheses might explain such a difference: (1) zooxanthellae stopped taking up ammonium during the dark period, and/or (2) well-fed animals might have excreted nitrogen during the night. Both events might also have occurred simultaneously, because the difference between the

two uptake rates would have been lower if just one process was involved. On the other hand, the light intensity at which the corals were incubated (80 or 250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) apparently had no effect on the uptake rates. This trend has already been observed in previous studies on intact symbiosis (Muscatine and D'Elia 1978; D'Elia et al. 1983). Uptake of nitrogen seems to be inhibited only after a long incubation in darkness (Muscatine and D'Elia 1978), which suggests that zooxanthellae must be subjected to carbon depletion before nitrogen assimilation is reduced.

On the basis of the nitrogen content of zooxanthellae, it is possible to calculate whether an external concentration of ammonium equal to 0.6 μM can sustain the growth of the zooxanthellae population. In starved corals, ammonium uptake rate by the zooxanthellae fraction was estimated equal to 3.93 $\text{fmol N h}^{-1} \text{ zoox}^{-1}$ or 94 $\text{fmol N d}^{-1} \text{ zoox}^{-1}$. The nitrogen content of these zooxanthellae was found to be 2.5 pmol N zoox^{-1} . The external nitrogen supply would therefore support a generation time of 26 d for these algae. The growth rate of the zooxanthellae can also be estimated with the results obtained per unit surface skeleton of the corals. The nitrogen content of the zooxanthellae has been found to be 1.8 $\mu\text{mol N cm}^{-2}$ for starved corals. The daily nitrogen uptake with a 0.6 μM ammonium concentration was 0.065 $\mu\text{mol N cm}^{-2} \text{ d}^{-1}$. External ammonium would therefore sustain a maximal specific growth rate of 0.036 d^{-1} for the algae (or 27 d). The determination of the real growth rate of zooxanthellae remains a complex problem, because it is rather difficult to get direct determination of cytokinesis duration in the zooxanthellae of native corals. Nevertheless, growth rates of zooxanthellae isolated from *S. pistillata* have been estimated to vary between 0.013 d^{-1} (or 76 d; Muscatine et al. 1989) to 0.026 d^{-1} for fed corals (or 38 d; Rahav et al. 1989). Szmant et al. (1990) also gave a lower generation time of 20 d for zooxanthellae symbiotic with two other scleractinian coral species. These calculations suggest that the growth of the zooxanthellae in the host can be sustained by the small amounts of nitrogen taken up from the surrounding environment. This is in agreement with the observations of Hoegh-Guldberg and Williamson (1999), who also suggested that ammonium present in concentrations as low as 100 nM completely satisfy the nitrogen demand of *P. damicornis* at One Tree Island. In the nitrogen available for the symbiotic association, we must also take into account other inorganic forms such as nitrate (Marubini and Davies 1996), organic forms such as amino acids (Hoegh-Guldberg and Williamson 1999), and nitrogen recycled from the host catabolism. Falkowski et al. (1993) indeed calculated, for *S. pistillata*, that nitrogen recycled from the catabolism of the animal host would also sustain a maximal algal growth rate of 0.12 d^{-1} .

In nutrient-poor tropical seawater, symbiotic corals seem to be well adapted to cope with a paucity of environmental nitrogen, because zooxanthellae are able to retain all of the ammonium excreted by the animal (Muscatine et al. 1979) and to actively scavenge inorganic nitrogen dissolved in seawater, even at concentrations as low as 0.6 μM . Such concentrations are common in reef waters (Hoegh-Guldberg and Williamson 1999). Corals are also able to efficiently use nitrogen provided by food for increasing both the animal and

algal compartments. Both partners of the symbiosis therefore benefit from all sources of nitrogen available in the environment. This is important for these marine symbioses living in nutrient poor environments. Results obtained in this study support the general view that the uptake of inorganic nutrients in symbiotic associations depends on zooxanthellae, but the magnitude of the uptake is regulated by the supply of nutrients from the surrounding waters and from the host (via feeding). The next step of this work will be to assess how the assimilated ammonium (in small concentrations in seawater) is partitioned between algae and host. We suggest that by measuring the rates of transfer of ^{15}N from ammonium into the host fraction, the role of zooxanthellae and host fractions in ammonium uptake and assimilation can be addressed. The use of the ^{15}N technique will also make possible the comparison of the preferential uptake of the different sources of inorganic nitrogen in such symbiosis.

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Received: 2 August 2001

Accepted: 26 November 2001

Amended: 2 January 2002