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

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INTRODUCTION

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 from the surrounding seawater (Muscatine 1980; Marubini and Davies 1996). Few studies, 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 (McAuley and Smith 1995; D'Elia et al. 1983) and one work determined the pathway of ammonium assimilation in the sea anemone *Anemonia viridis* (Roberts et al. 1999). However, nitrogen uptake rates by the coral-zooxanthellae association are still poorly investigated (Muscatine and D'Elia 1978; 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 whereas others largely increased these concentrations far above normal seawater levels.

These 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, $^{15}NH_4$ was used. Experiments were also conducted to evaluate whether ammonium uptake was affected by host nutrition (Szmant-Froelich and Pilson 1984; Muller-Parker et al. 1990).

MATERIALS AND METHODS

Experiments were performed using colonies of the scleractinian coral *Stylophora pistillata* (Esper 1797), collected in the Gulf of Aqaba (Red Sea, Jordan).

In a first set of experiments, eighty nubbins were randomly divided into three aquaria supplied with oligotrophic Mediterranean seawater and placed under a 300 µmol photons m^{-2} s^{-1} irradiance (photoperiod 12:12 h). Highly fed nubbins were fed three times a week with 5 g of *Artemia salina* nauplii. Slightly fed nubbins were fed only once a week with the same amount of nauplii. Starved nubbins were not fed. After four weeks of treatment, uptake rates of ammonium were measured for the three groups of nubbins under different conditions of concentrations (1 or 5 µM $^{15}NH_4$) and irradiance (80 and 350 µmol photons m^{-2} s^{-1}) for several hours. For all experiments, the tracer ^{15}N was added as $^{15}NH_4Cl$ (98% atom, CEA, France).

In a second set of experiments, forty nubbins were raised during eight weeks under the same culture conditions as above. Uptake rates of ammonium were measured for highly fed and starved nubbins in seawater supplied with a 0.2 µM $^{15}NH_4$ concentration giving a final concentration of 0.6 µM. Corals were incubated either 12 h under high light (350 µmol photon m^{-2} s^{-1}) or 24 h with 12 h high light and 12 h dark.

At the end of the incubation with $^{15}NH_4$, nubbins were rinsed in seawater during 30 min to wash the coelenteron. Tissues were then removed from skeleton with an "air pick" and homogenized with a Potter tissue grinder. The homogenate was centrifuged 3 times at 2000 g for 10 min at 4°C to pellet the zooxanthellae. Tubes containing tissues and zooxanthellae were freeze-dried using a Heto lyophilizer.

Ammonium uptake rate determination

Carbon and nitrogen contents were measured for each sample using a CHN analyzer. The isotopic ratios ^{15}N / ^{14}N of the animal tissues and zooxanthellae of the freeze-dried samples were determined by emission spectroscopy using a GS1 optical spectrometer according to Guiraud and Fardeau (1980). The enrichment of the samples with ^{15}N was recorded as at. % excess: Atom % excess ^{15}N = (at. % N_{mes}) – (at. % $N_{natural}$).

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

$$\rho = \frac{N_{mes} - N_{natural}}{(N_{enr} - N_{mes}).t_{inc}.S} \cdot M_{sample} \cdot M_N \cdot 10^6$$

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

Measurement of nutrient concentrations in the experimental tanks

Concentrations of ammonium, nitrite and nitrate in the culture tanks were measured every week using a Technicon Autoanalyzer. All concentrations remained low and constant in the tanks during the whole experiment and equal to 0.35 ± 0.10 µM for ammonium, 0.1 ± 0.1 µM for nitrite and 0.5 ± 0.2 µM for nitrate.

RESULTS

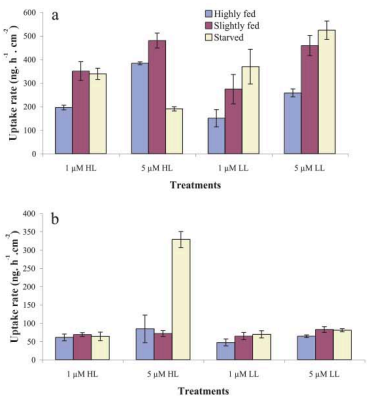
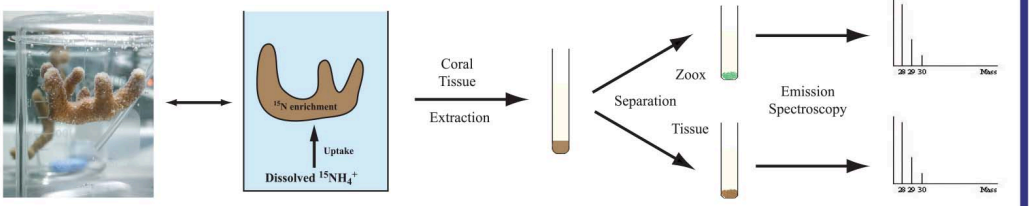


Figure 1. First set of experiments. Uptake rates (ng N cm^{-2} h^{-1}) of 1 and 5 µM $^{15}NH_4$ enrichment measured for a) the zooxanthellae fraction, b) the animal fraction. Mean and standard error of the mean (n = 5 for each treatment).



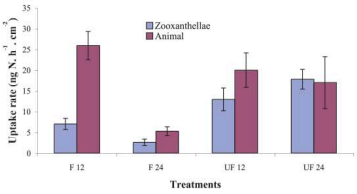


Figure 2. Uptake rates varied between 2 and 30 ng N h^{-1} cm^{-2}. For all fractions, these rates were not significantly different between fed and starved nubbins, except the uptake of fed nubbins measured during 24 h (ANOVA, $p < 0.005$).

DISCUSSION

Results obtained in this study showed that the uptake rates varied according to the nitrogen concentration in seawater. They were ca. 20 times lower at 0.2 than at 1 or 5 µM $^{15}NH4$ enrichment. However, they were not different between 1 and 5 µM $^{15}NH4$, indicating 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). In this study, the algal fraction was enriched with ^{15}N at up to 10 times the rate of the host, suggesting that the zooxanthellae are the primary site of assimilation. The presence of ^{15}N in animal tissue after incubation in $^{15}NH4$-spiked seawater is consistent with both the diffusion-depletion hypothesis (D'Elia et al. 1983) and the possibility that ^{15}N is held in regulatory pools being maintained and released by animal enzyme activity (Wang and Douglas 1998).

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 measured with starved corals. Previous studies investigating 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.

Results obtained in the second set of experiments 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 during 12 h (daylight period) were comparable to those measured during 24 h (day and night incubation). This suggests that uptake by these animals and in our conditions was constant throughout the 24 h. However, in fed corals, uptake rates were four times lower when measured during 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, since the difference between the two uptake rates would have been lower if just one process was involved.

In nutrient-poor tropical seawaters, 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).

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