The Journal of Experimental Biology 211, 860-865
Published by The Company of Biologists 2008
doi:10.1242/jeb.012807

Uptake of dissolved free amino acids by the scleractinian coral *Stylophora pistillata*

Renaud Grover[1,*], Jean-François Maguer[2], Denis Allemand[1] and Christine Ferrier-Pagès[1]

[1]Centre Scientifique de Monaco, Avenue Saint Martin, MC-98000, Monaco and [2]Laboratoire de Chimie Marine, Institut Universitaire Européen de la Mer, Place Nicolas Copernic, F-29280 Plouzane, France

*Author for correspondence (e-mail: rgrover@centrescientifique.mc)

Accepted 19 January 2008

SUMMARY

This study was designed to assess the importance of dissolved free amino acids (DFAA) as a nitrogen source for the scleractinian coral *Stylophora pistillata*. For this purpose, experiments were performed using ^{15}N-enriched DFAAs, and %^{15}N enrichment was measured both in animal tissue and zooxanthellae at different DFAA concentrations, incubation time and light levels. As previously observed for urea, which is another source of organic nitrogen, DFAA uptake exhibited a biphasic mode consisting of an active carrier-mediated transport for concentrations below 3 μmol l^{-1} and a linear uptake for higher concentrations. The value of the carrier affinity (K_m=1.23 μmol l^{-1} DFAA) indicated good adaptation of the corals to the low levels of DFAA concentrations measured in most oligotrophic waters. DFAA uptake was also correlated with light. The DFAA contribution to the nitrogen requirements for tissue growth was compared to the contribution of ammonia, nitrate and urea, for which uptake was also measured in *S. pistillata*. Inorganic sources (NH_4^+ and NO_3^-) contributed 75% of the daily nitrogen needs against 24% for organic sources. Taken altogether, dissolved organic and inorganic nitrogen can supply almost 100% of the nitrogen needs for tissue growth.

Key words: coral, isotopic enrichment, uptake, kinetics, dissolved free amino acid.

INTRODUCTION

Coral reefs constitute a paradoxical ecosystem: they display a high gross primary productivity (Sorokin, 1993), whereas they thrive in oligotrophic environments containing sub-micromolar nutrient concentrations (Bythell, 1990; Furnas, 1991; Szmant, 2002). This high productivity results from an efficient multiscale strategy, combining high flow rates above the reef (Thomas and Atkinson, 1997), high nutrient recycling by the reef community (Szmant-Froelich, 1983; Uthicke, 2001) and coral mixotrophy (Titlyanov et al., 2000). At the coral level, mixotrophy multiplies the nutrient sources, by combining prey capture (Ferrier-Pagès et al., 2003), uptake of dissolved molecules (Bythell, 1990) and utilization of photosynthates produced by the symbiotic zooxanthellae living in the endodermal cells of the animal (Muscatine and Cernichiari, 1969). Previous studies have shown that corals are opportunistic with respect to nitrogen and retain both inorganic sources, such as ammonia and nitrate (Falkowski et al., 1993; Grover et al., 2002; Grover et al., 2003; Hoegh-Guldberg and Williamson, 1999; Marubini and Davies, 1996; Wilkerson and Trench, 1986), and organic sources such as urea (Grover et al., 2006) and dissolved free amino acids (DFAA) (Al-Moghrabi et al., 1993; Ferrier, 1991; Hoegh-Guldberg and Williamson, 1999).

DFAA is part of the dissolved organic nitrogen (DON), which is a heterogeneous mixture of urea, dissolved combined amino acids (DCAA), nucleic acids and unidentified species (Bronk, 2002). As DFAA are excreted and consumed by a large variety of marine organisms, they are subject to rapid turnover that leads to several orders of magnitude variability in seawater concentrations. DFAA therefore represent approximately 10% of the DON pool with concentrations ranging between 0.001 and 0.7 μmol l^{-1} (Bronk, 2002).

DFAA uptake by corals has not been thoroughly investigated, and previous works are all based on depletion measurements of a DFAA-enriched medium using, *de facto*, elevated concentrations. HPLC techniques have been used to monitor the specific amino acid uptake by different coral species (Ferrier, 1991; Hoegh-Guldberg and Williamson, 1999). The concentrations used, however, ranged from 4 to 5.6 μmol l^{-1}, and were several times higher than those *in situ*. Finally, Al-Moghrabi et al. (Al-Moghrabi et al., 1993) performed physiological approaches by using a unique radiolabelled DFAA (valine) in order to figure out uptake regulation by light.

The present work investigates the uptake of a natural mixture of DFAA, at *in situ* concentrations, in the scleractinian coral *Stylophora pistillata*, using ^{15}N-labeled products. DFAA uptake was measured with different incubation times, DFAA concentrations and light levels. To highlight a possible discrimination between various DFAA, depletion measurements were also performed, whereby corals were incubated with single amino acids.

MATERIALS AND METHODS

Biological material

Experiments were performed in the laboratory using colonies of the scleractinian coral *Stylophora pistillata* (Esper 1797), which were collected in the Red Sea and maintained for several months in aquaria. Microcolonies of about the same size (4 cm long, 2 cm wide) were obtained by cutting terminal portions of branches of parent colonies and were used only when the animal tissue entirely covered the skeleton. They were maintained in two 40 l aquaria, supplied with oligotrophic Mediterranean seawater pumped at 50 m depth, and containing low levels of nutrients (Grover et al., 2002). Temperature was maintained at 26±0.2°C using heaters connected to temperature controllers. Submersible pumps were used for water agitation and metal halide lamps (Philips, HPIT, Guildford, Surrey, UK) provided light with a 12 h:12 h light:dark photoperiod. Salinity and irradiance were measured using a conductivity meter (Meter

LF196, WTW, Weiheim, Germany), and a 4π quantum sensor (Li-Cor, LI-193SA, Lincoln, NE, USA), respectively. Corals were fed twice a week with *Artemia salina* nauplii during the healing period. They remained unfed 3 weeks before and during the experiments, however, to avoid external nitrogen input that could interfere with DFAA uptake (Grover et al., 2002; Muller-Parker et al., 1988).

Measurements of dissolved free amino acids (DFAA) in seawater by spectrofluorimetry

The contribution of planktonic bacteria to DFAA uptake was checked by measuring DFAA concentrations in 3 tanks filled with natural seawater, after 0, 12 and 24 h of incubation. Natural DFAA concentrations in the incubation medium were also measured every day and before each experiment. They were taken into account in the calculations of the total DFAA concentrations during the experiments. This DFAA quantification was performed using a spectrofluorometer (Xenius SAFAS, Monaco, Monaco) according to the method of Parsons et al. (Parsons et al., 1984). Briefly, o-phthalaldehyde reacts with DFAA in the presence of β-mercaptoethanol to produce fluorescent compounds, the fluorescence intensity being proportional to DFAA concentration. Seawater samples were first filtered through a 0.22 μm syringe filter (Acrodisc, PALL, East Hills, NY, USA) to remove any particle that could interfere with the fluorescence measurements. 5 ml of each sample was added to 5 ml of reactive solution (o-phthalaldehyde + β-mercaptoethanol) and mixed for 2 min. Samples were then transferred into a 4 ml quartz SUPRASIL cell and excited at a 342 nm wavelength. Emission wavelengths between 400 nm and 500 nm were recorded in order to quantify the maximal fluorescence intensity. Standard solutions of glycine from 0.2 to 1.0 μmol l^{-1} were prepared for internal calibration and to set up the photomultiplier voltage. DFAA concentration in the samples was obtained according to the following formula:

$$[\text{DFAA}] = (F_S - F_B) \times F \,, \quad (1)$$

where F_S=average fluorescence of triplicate seawater samples, F_B=average fluorescence of triplicate blanks, and F=conversion factor (μmol l^{-1}/relative fluorescence intensity), according to the calibration curve.

DFAA depletion experiments

In order to assess the relative uptake of the main DFAAs by *S. pistillata*, eleven amino acids (glycine, valine, alanine, glutamate, glutamine, aspartate, asparagine, histidine, leucine methionine, serine) (Aldrich, St Quentin, Falavier, France) were tested separately. Each DFAA was dissolved into 0.22 μm filtrated seawater at a final concentration of 3 μmol l^{-1}, and transferred into three 250 ml beakers, each containing a coral microcolony. Beakers were incubated during 6 h in the light (300 μmol photons m^{-2} s^{-1}), and at a constant temperature of 26.5°C, using a water bath. Seawater was continuously stirred using a magnetic stirrer. DFAA depletion was monitored in each beaker by sampling 5 ml of the medium every hour. This depletion was linear during the first 3 h, before decreasing asymptotically, due to the lowering of the DFAA concentration in the medium. Only the linear decrease was taken into account for the uptake rate calculations. Results were normalized to skeletal surface area and expressed as nmol DFAA h^{-1} cm^{-2}.

DFAA uptake kinetics

Experiments using ^{15}N-enriched DFAA (thereafter called ^{15}N-DFAA) were performed to measure DFAA uptake rates for different incubation times, DFAA concentrations and light intensities. ^{15}N-DFAA originated from an algal mix (98% ^{15}N enrichment, ISOTEC, Sigma-Aldrich, St Quentin, Falavier, France) whose composition was close to the natural seawater DFAA composition.

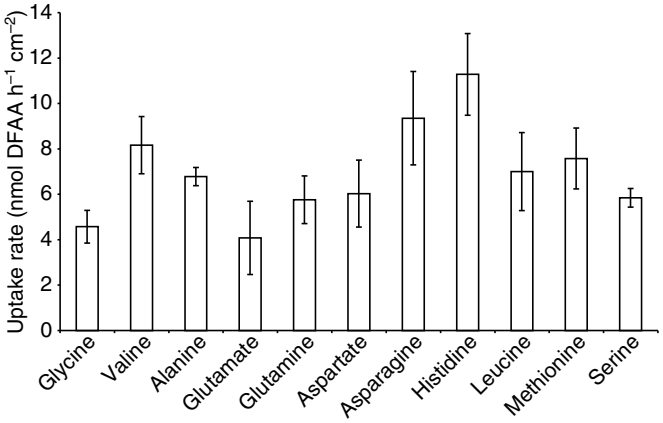


Fig. 1. Uptake rates (nmol DFAA h^{-1} cm^{-2}) by an entire microcolony of 11 amino acids tested separately at a final concentration of 3 μmol l^{-1}, during 6 h incubation. Each value represents the mean ± s.d. of three individuals.

For all experiments, microcolonies were incubated individually in 250 ml beakers immersed in a water bath maintaining a constant temperature of 26.5°C. To avoid DFAA depletion in the beakers during the incubation, ^{15}N-DFAA-enriched seawater was continuously pumped at a flow rate of 7 ml min^{-1} with a peristaltic pump from a batch solution to the beakers. At the end of the incubation, microcolonies were rinsed in a large volume of filtered seawater during 30 min to wash the cœlenteron and were then frozen until subsequent analysis (as described below). Each experimental condition was performed with triplicate samples.

To assess the effect of the incubation length on DFAA uptake rate, microcolonies were incubated either in 0.5 or 3 μmol l^{-1} ^{15}N-DFAA-enriched seawater during 2, 5, 7 and 21 h under a constant light intensity of 300 μmol photons m^{-2} s^{-1} (N=24 microcolonies). This experiment confirmed that the uptake rate was constant and linear during the whole incubation. The concentrations used (0.5 and 3 μmol l^{-1}) were considered as 'normal' and 'high', compared to that *in situ*. To assess the effect of light on the uptake rates, microcolonies were incubated either in 0.5 or 3 μmol l^{-1} ^{15}N-DFAA-enriched seawater for 7 h and under three light intensities: 0 (dark), 160 and 300 μmol photons m^{-2} s^{-1}. Finally, for the determination of DFAA uptake rates *versus* concentration, microcolonies were incubated in six different ^{15}N-DFAA concentrations equal to 0.2, 0.5, 1, 3, 8 and 13 μmol l^{-1} for 7 h and under a constant light intensity of 300 μmol photons m^{-2} s^{-1} (N=18).

DFAA uptake kinetics by freshly isolated zooxanthellae

In order to obtain freshly isolated zooxanthellae (FIZ), tissue was removed from the skeleton of three big colonies and zooxanthellae were isolated by centrifugation as described below. They were then re-suspended in 200 ml filtered seawater and divided into several beakers for a 5 h incubation with three different ^{15}N-DFAA concentrations equal to 0.5, 3 and 7 μmol l^{-1} (using triplicate samples for each concentration). Experiments were performed in the light (160 μmol photons m^{-2} s^{-1}) and under a constant temperature of 26.5°C. At the end of the incubation, each sample was filtered through a pre-combusted (450°C) GF/F filter and rinsed

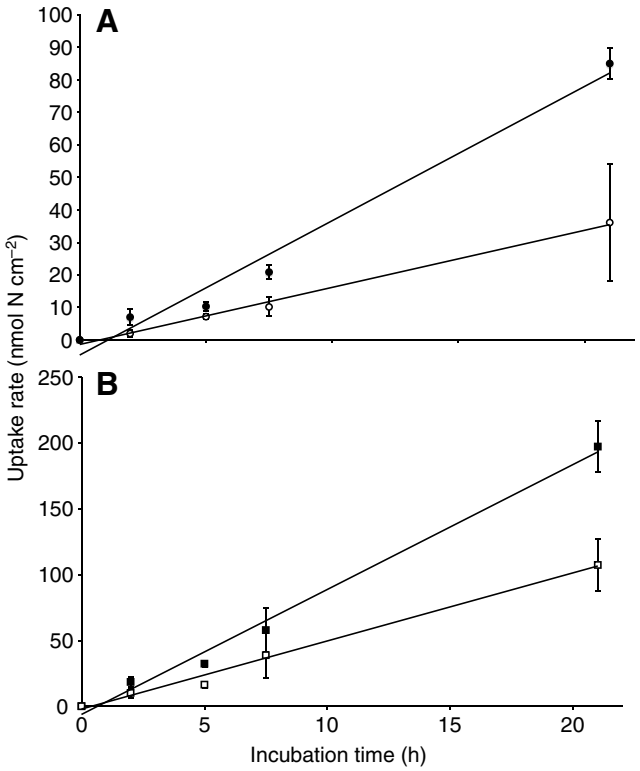


Fig. 2. (A) Calculated DFAA uptake rates (nmol N cm^{-2}) at 0.5 μmol l^{-1} in the zooxanthellae (open circles) and in the animal tissue (filled circles), during a 21 h incubation. Open circles, y=1.75x–1.38, r^2=0.99; filled circles, y=4.13x–4.67, r^2=0.98. (B) Calculated DFAA uptake rates (nmol N cm^{-2}) at 3 μmol l^{-1} in the zooxanthellae (open squares) and in the animal tissue (filled squares), during 21 h incubation. Open squares, y=5.18x–2.25, r^2=0.99; filled squares, y=9.49x–6.27, r^2=0.99. Each point represents the mean ± s.d. of three individuals.

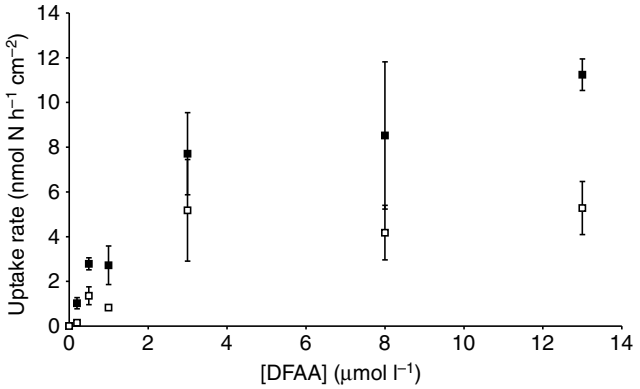


Fig. 3. Concentration-dependent uptake of DFAA (nmol N h^{-1} cm^{-2}) in the zooxanthellae (open squares) and the tissue (filled squares) compartments during 7 h incubation at 300 μmol photons m^{-2} s^{-1}. Each point represents the mean ± s.d. of three individuals.

with a small volume of filtered seawater. Filters were then dried at 60°C for 8 h and stored in a desiccator until analysis. Results will be expressed as $\%^{15}N_{enrichment}$ and will not be converted into uptake rate, because the exact number of zooxanthellae on the filter could not be determined.

Analysis

Tissue extraction

Tissue extraction was performed as described (Grover et al., 2006). Briefly, coral tissue was removed from the skeleton with a flow of argon under pressure in 5 ml filtered seawater and homogenized using a Potter tissue grinder. Animal and zooxanthellae fractions were then separated by centrifugation and zooxanthellae were rinsed three times in filtrated seawater to avoid animal contamination. Samples were freeze-dried for conservation until spectral analysis.

Isotopic enrichment quantification and determination of DFAA uptake rates

$^{15}N/^{14}N$ isotopic ratios in animal tissue and zooxanthellae were determined using an Isotope Ratio Mass Spectrometer (IRMS) and compared to natural $^{15}N/^{14}N$. The $\%^{15}N$ enrichment in the coral corresponds to the amount of nitrogen transferred from seawater into the animal and vegetal constituents of the coral. This flow can be converted into uptake rate (ρ) using the equation derived from Dugdale and Wilkerson (Dugdale and Wilkerson, 1986) and presented in Grover et al. (Grover et al., 2002), which takes into account the sample biomass and the skeletal surface area. In the present study, as in previous ones using the same technique (Grover et al., 2002; Grover et al., 2003; Grover et al., 2006), even after animal and algal separation, the results were normalized to skeletal surface area, in order to be comparable with other studies on nitrogen fluxes. Considering that animal and algal biomasses in a coral colony are not the same, however, normalization to animal tissue or zooxanthellae dry mass appeared to be more suitable for determining the contribution of each partner of the symbiosis for DFAA accumulation. Uptake rates in this study will therefore be expressed in ng N h^{-1} mg^{-1} animal tissue and mg^{-1} zooxanthellae, and in ng N h^{-1} cm^{-2}. Skeletal surface area of the microcolonies was measured according to the wax technique (Stimson and Kinzie, 1991).

RESULTS

Background DFAA concentration in seawater ranged from 0.11 to 0.37 μmol l^{-1} with an average value of 0.24±0.08 μmol l^{-1}. It remained within this range during the whole experiment. No change in DFAA concentration in control tanks filled with natural seawater was observed during a 24 h period, suggesting a negligible DFAA uptake by bacteria.

Uptake rates of the 11 amino acids incubated separately with microcolonies of *S. pistillata* are presented in Fig. 1. Mean rates varied between 4.08 and 11.28 nmol h^{-1} cm^{-2} and were minimal and maximal for glutamate and histidine, respectively. There was no significant correlation between uptake rates and DFAA characteristics (hydrophobicity, acidity heteroatom or chemical functions). Comparable results were obtained when data were normalized to protein concentration. In this case, uptake rates varied between 11.36 and 31.42 nmol DFAA h^{-1} mg^{-1} protein.

Kinetics experiments showed that ^{15}N-DFAA uptake was linear in animal tissue and in zooxanthellae for up to 21 h incubation at concentrations of both 0.5 μmol l^{-1} (Fig. 2A) and 3 μmol l^{-1} (Fig. 2B). At both concentrations, DFAA uptake rates, normalized to surface area, were twice as high in the animal tissue than in the zooxanthellae. Indeed, at 0.5 μmol l^{-1} DFAA, representing an *in situ* concentration, uptake rates were equal to 4.3 and 1.8 nmol N h^{-1} cm^{-2} for animal tissue and zooxanthellae, respectively. At 3 μmol l^{-1} DFAA, these rates were twice as high, with 9.8 and 5.3 nmol N h^{-1} cm^{-2} in animal tissue and zooxanthellae, respectively. Normalized to biomass, DFAA appeared to be 5–7 times more concentrated in the zooxanthellae than in the animal

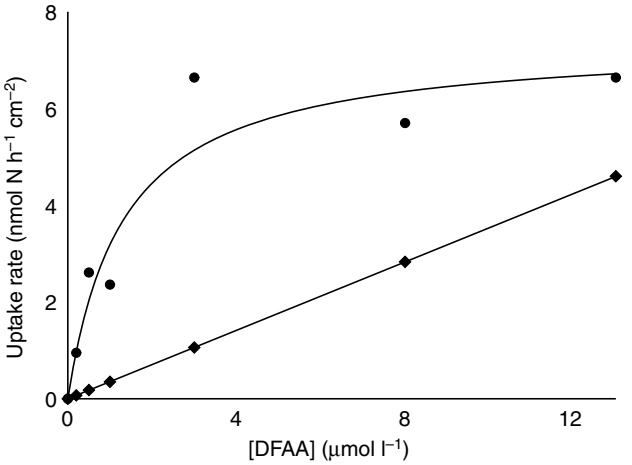


Fig. 4. Linear (diamonds) and saturable (circles) components of the biphasic mode of DFAA uptake by the animal tissue as a function of external concentration.

tissue. At 0.5 μmol l^{-1} DFAA, uptake rates ranged from 0.5 nmol N h^{-1} mg^{-1} tissue to 2.4 nmol N h^{-1} mg^{-1} zooxanthellae. At 3 μmol l^{-1} DFAA, uptake rates were equal to 1.0 nmol N h^{-1} mg^{-1} tissue and 7.0 nmol N h^{-1} mg^{-1} zooxanthellae.

The relationship between uptake rates and DFAA concentrations in seawater is presented in Fig. 3. DFAA transport through animal tissue followed a biphasic mode that combines carrier-mediated transport with passive diffusion through the animal membranes. Carrier-mediated DFAA transport through the membranes followed Michaelis–Menten kinetics:

$$\rho = \rho_{max}[DFAA] / (K_m+[DFAA]) , \quad (2)$$

where ρ_{max} is the maximal DFAA uptake rate, [DFAA] is the concentration, and K_m the solute concentration at which DFAA uptake is half-maximal.

For concentrations above 3 μmol l^{-1}, the carriers of the active transport were saturated, and a passive DFAA diffusion process through animal membranes became apparent, according to the equation:

$$\rho = K_d[DFAA] , \quad (3)$$

where K_d is the apparent diffusion permeability coefficient.

Therefore, over the entire range of concentrations tested, DFAA uptake through the animal tissue followed a combination of Michaelis–Menten and linear kinetics, which can be pooled in the following unique equation:

$$Flux = \{(\rho_{max}[DFAA]) / (K_m+[DFAA])\} + (K_d[DFAA]) . \quad (4)$$

In Fig. 4, Eqn 4 is split into its linear and Michaelis–Menten components, showing the two types of transport system for the animal tissue. It is obtained by subtracting the apparent diffusion (determined theoretically as a regression line calculated on uptake rates obtained for concentrations between 3 and 13 μmol N-DFAA l^{-1}) from the total uptake. This representation has already been used in previous uptake experiments (Al-Moghrabi et al., 1993). Note that the active transport is dominant up to approximately 16 μmol l^{-1}, the diffusive transport becoming dominant above this concentration (when the linear curve crosses the Michaelis–Menten curve). ρ_{max} and K_m were determined using the fitting software pro Fit 6.0.6 (Quantum Soft). The calculated values are

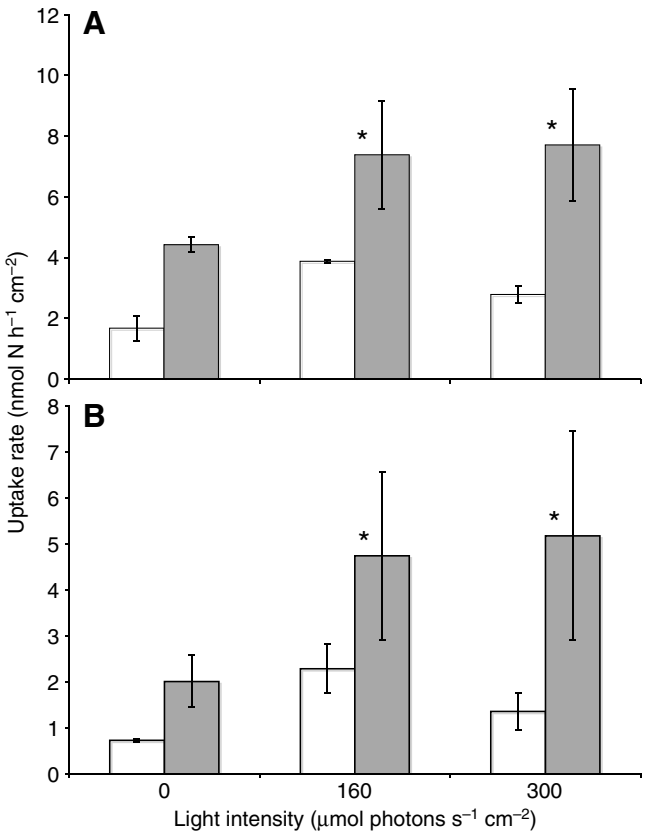


Fig. 5. Uptake rates at 0.5 μmol l^{-1} (white bars) and 3 μmol l^{-1} (grey bars) DFAA (nmol N h^{-1}cm^{-2}) during 7 h incubation under 0, 160 and 300 μmol photons m^{-2} s^{-1} in (A) coral tissue and (B) zooxanthellae. Each value represents the mean ± s.d. of three individuals. Asterisks indicate significant differences between uptake rates at 0.5 and 3 μmol l^{-1} for a given light intensity.

ρ_{max}=7.52 nmol N h^{-1} cm^{-2} and K_m=1.23 μmol l^{-1} DFAA. When normalized to animal tissue dry mass, these values are ρ_{max}=0.62 nmol N h^{-1} mg^{-1} tissue and K_m=0.63 μmol l^{-1} DFAA.

Fig. 5 shows the relationship between light intensity and DFAA uptake rate in both animal tissue (Fig. 5A) and zooxanthellae (Fig. 5B) at two concentrations (0.5 and 3 μmol l^{-1}). At 0.5 μmol l^{-1}, there was no significant effect of light on the uptake rate of animal tissue and zooxanthellae (P>0.05). Conversely, at 3 μmol l^{-1}, there was a significant difference between dark and light exposure, but not between the two light levels in both the animal compartment and the zooxanthellae. The observations are the same when data were normalized to biomass.

%^{15}N enrichment in freshly isolated zooxanthellae (FIZ) *versus* DFAA concentration in the incubation medium is presented in Fig. 6. %^{15}N in FIZ linearly increased with increasing DFAA concentration. Fig. 6 can be compared with the %^{15}N enrichment of zooxanthellae *in hospite* (Fig. 7), where it apparently reached a maximum at 3 μmol l^{-1} DFAA. Fig. 7 is derived from the data in Fig. 3 (same trend), but represents the raw data of %^{15}N enrichment in zooxanthellae *in hospite*.

DISCUSSION

This is the first study using the ^{15}N technique to measure DFAA uptake rates in scleractinian corals. An algal DFAA mixture was used, to mimic the *in situ* amino acid composition of the dissolved

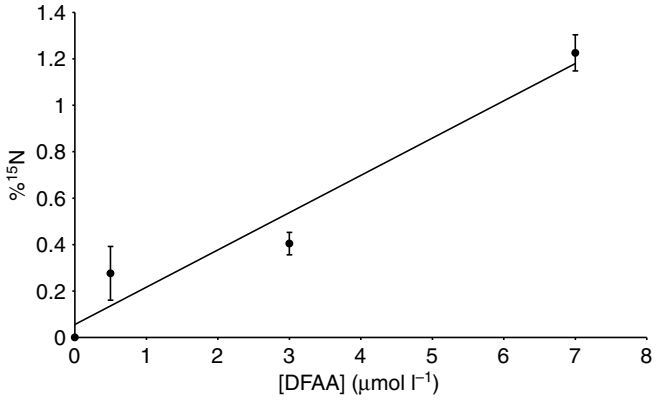


Fig. 6. Concentration-dependent %^{15}N enrichment in freshly isolated zooxanthellae after 5 h incubation in ^{15}N-DFAA under 160 μmol photons m^{-2} s^{-1}. Each value represents the mean ± s.d. of three individuals.

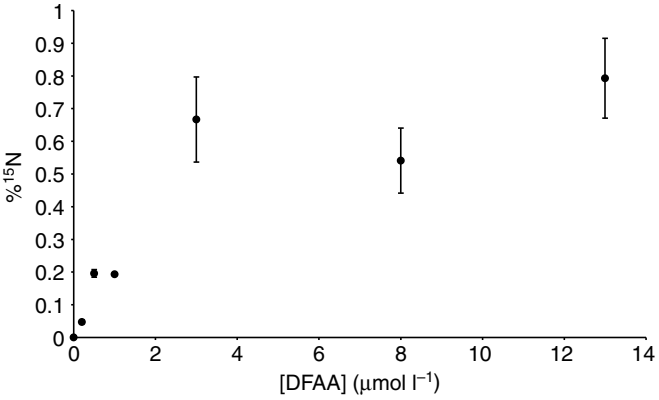
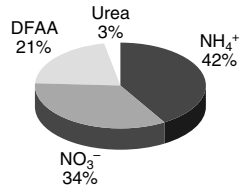


Fig. 7. Concentration dependent %^{15}N enrichment of zooxanthellae *in hospite*. Same conditions as in Fig. 3.



Daily nitrogen uptake rates (ng N cm^{-2} day^{-1}) at *in situ* nutrient concentrations				
[Nutrient] (μmol l^{-1})	NH_4^+ (0.2)	NO_3^- (0.3)	Urea (0.3)	DFAA (0.2)
Daily nitrogen uptake rate	789.6±331.2	495.7±104.8	69.1±17.8	393.5±107.52

Fig. 8. % Contribution of each nutrient for tissue growth.

organic matter as closely as possible. Compared to depletion experiments (Ferrier, 1991; Hoegh-Guldberg and Williamson, 1999), the ^{15}N technique exhibits some advantages, such as: (i) it allows the amount of DFAA taken up by the animal to be distinguished from that going into the zooxanthellae; (ii) kinetics can be performed at low DFAA concentrations; and (iii) more specific details can be obtained concerning both DFAA transport mechanisms through membranes and nitrogen allocation.

When data were normalized to skeletal surface area, animal tissue presented the highest uptake rate, as already observed for urea, which is another DON source for corals (Grover et al., 2006), in contrast to ammonium and nitrate, which were mainly taken up by the zooxanthellae. If biomass is taken into consideration, all nitrogen sources, including inorganic and organic, accumulated in the zooxanthellae, suggesting that they are the main nitrogen users. ^{15}N enrichment rapidly occurred in the zooxanthellae, since they were labeled in less than 2 h. If we consider that external DFAA has to migrate through the animal epithelial layers to reach the zooxanthellae, we can then suppose that DFAA uptake occurred, partly or entirely, from the seawater that fills the cœlenteric cavity. Considering that symbiotic zooxanthellae live within the endodermal cells that constitute the cœlenteron, this could explain the fast ^{15}N enrichment in the symbionts.

Usually, animals show preferences and a higher affinity for the eight essential DFAAs (valine, leucine, isoleucine, tyrosine, phenylalanine, histidine, methionine, lysine), since they are not capable of synthesizing these amino acids. However, corals are not pure animals, due to the presence of zooxanthellae in their tissue, and their capacity for DFAA synthesis remains controversial (Fitzgerald and Szmant, 1997; Wang and Douglas, 1999). In our depletion experiments, all amino acids were taken up, but with a significantly higher uptake rate for histidine compared to the other amino acids (except asparagine). Such preferential uptake for some amino acids was also evident in the work of Schlichter (Schlichter, 1978) and Ferrier (Ferrier, 1991) for the coral species *Montastrea annularis*. However, it was not observed for three other coral species [*Madracis Mirabilis*, *Agaricia fragilis* and *Favia fragum* (Ferrier, 1991; Hoegh-Guldberg and Williamson, 1999)]. These differences could either be due to reciprocal inhibitory effects between DFAAs, or to species-specific differences.

The results obtained also show good agreement between the depletion and the ^{15}N-techniques, since addition of 3 μmol l^{-1} DFAA (either as a mixture or as individual amino acids) in the incubation medium led to equivalent uptake rates (4–12 and 8–15 nmol DFAA cm^{-2} h^{-1} for the depletion and ^{15}N technique, respectively). These rates are in the same range as those previously measured (Ferrier, 1991) for several scleractinian species (3–19 nmol N cm^{-2} h^{-1}), and for *Pocillopora damicornis* (4.9–9.8 nmol N cm^{-2} h^{-1}) (Hoegh-Guldberg and Williamson, 1999). These two studies used equivalent DFAA concentrations in the external medium (4–5.6 μmol l^{-1}). Schlichter and colleagues (Schlichter, 1980; Schlichter et al., 1986) were the first to suggest that the absorption of amino acids through the membranes of the epidermal cells of anthozoans takes place against gradients of up to 1:10^6 and requires a carrier-mediated trans-epithelial transport. Our observations confirm this hypothesis, since DFAA uptake in *S. pistillata* followed a bimodal process with a major contribution of the carrier-mediated transport at DFAA concentrations lower than16 μmol l^{-1}. Such bimodal transport has been emphasized (Al-Moghrabi et al., 1993) for valine uptake by *Galaxea fascicularis*, in the light, which also showed an active transport at concentrations lower than 20 μmol l^{-1}. The value of the carrier affinity (K_m=1.23 μmol l^{-1}) found in this work for the DFAA mixture highlights how well corals are adapted to the low levels usually found in seawater. Uptake of amino acids by freshly isolated zooxanthellae (Fig. 6) followed a passive diffusion process, since no saturation in the uptake occurred even when amino acids concentrations were as high as 13 μmol l^{-1}. This result suggests that the saturation curve obtained with *in hospite* zooxanthellae (Fig. 3) is due to a limitation, by the animal tissue, of amino acid transport (carrier-mediated transport observed in Fig. 4).

The effect of light on DFAA uptake is complex. Indeed, light enhanced DFFA uptake, but only at concentrations above

0.5 μmol l^{-1}, suggesting that a minimal concentration is required to activate this process. Moreover, this enhancement was not proportional to light intensity since it was saturated at 160 μmol photons m^{-2} s^{-1}. Finally, the same phenomenon was observed both in the zooxanthellae and the animal tissue (Fig. 5). These observations strongly suggest that zooxanthellae are actively involved in DFAA uptake, but their contribution should necessarily be indirect and linked to their photosynthetic activity. It is indeed known that the transfer of photosynthates from the zooxanthellae to the animal is a source of energy, enhancing the animal metabolism and the incorporation of nitrogen into proteins. This mechanism has been called the 'light-enhanced amino acid assimilation' (Al-Moghrabi et al., 1993). In contrast to our observations and those of Al-Moghrabi et al. (Al-Moghrabi et al., 1993), a faster DFAA uptake in the dark was observed for the species *Pocillopora damicornis* (Hoegh-Guldberg and Williamson, 1999). This inconsistency could be due to species specificity, but remains to be further investigated.

Taking into account results obtained in previous studies concerning the uptake of dissolved inorganic and organic nitrogen by *S. pistillata* (Grover et al., 2002; Grover et al., 2003; Grover et al., 2006), a simple model can be designed to evaluate the importance of DFAA compared to the other nitrogen sources for animal tissue growth. For this purpose, we considered the daily uptake rates of each nitrogen source measured for the whole coral colony after 12 h incubation in the light (Fig. 8). These daily rates were compared to the daily nitrogen requirements for animal tissue growth. This latter value is based on the daily coral tissue growth and the mass of nitrogen per mg of coral tissue measured in our samples (55±9 ng N mg^{-1} tissue). Since no measurement of tissue growth was available for *S. pistillata*, we calculated it by multiplying the tissue mass by the specific growth rate u_a expressed as day^{-1}. This specific growth rate u_a was calculated according to the empirical formula given by Muscatine et al. (Muscatine et al., 1985), depending on the surface area of each sample. Finally, the % contribution of each nitrogen source to tissue growth was calculated by dividing the daily nitrogen uptake rate by the daily nitrogen requirement for tissue growth (Fig. 8). These calculations do not take into account nitrogen excretion by the coral, which is negligible for *Stylophora pistillata* (Rahav et al., 1989). The model suggests that total dissolved nitrogen provides 99% of the daily nitrogen necessary for tissue growth. Organic sources (urea+DFAA) only provide 24% of the tissue requirements, with a major contribution (21%) of DFAA. Inorganic nitrogen sources (NH_4^++NO_3^-) therefore account for 75% of the tissue needs. Since these inorganic sources are mainly taken up by the zooxanthellae (Grover et al., 2002; Grover et al., 2003), most of the dissolved nitrogen uptake is due to zooxanthellae activity.

In summary, our results show that DFAA can represent an important source of nitrogen for corals at *in situ* concentrations (200–500 nmol l^{-1}), with uptake rates as high as those measured for DIN at the same concentrations. DFAA uptake by *Stylophora pistillata* shows no discrimination, allowing the uptake of any available amino acid through the animal membranes, depending on the DFAA concentration in the surrounding water. A 'light-enhanced amino acid assimilation' process (Al-Moghrabi et al., 1993) has been confirmed, suggesting DFAA uptake is a diurnal event.

REFERENCES

Al-Moghrabi, S., Allemand, D. and Jaubert, J. (1993). Valine uptake by the scleractinian coral *Galaxea fascicularis*: characterization and effect of light and nutritional status. *J. Comp. Physiol. B* **163**, 355-362.

Bronk, D. A. (2002). Dynamics of dissolved organic nitrogen. In *Biogeochemistry of Marine Dissolved Organic Matter* (ed. D. A. Hansell and C. A. Carlson), pp. 153-247. London, San Diego: Academic Press.

Bythell, J. C. (1990). Nutrient uptake in the reef-building coral *Acropora palmata* at natural environmental concentrations. *Mar. Ecol. Prog. Ser.* **68**, 65-69.

Dugdale, R. C. and Wilkerson, F. P. (1986). The use of ^{15}N to measure nitrogen uptake in eutrophic oceans; experimental considerations. *Limnol. Oceanogr.* **31**, 673-689.

Falkowski, P. G., Dubinsky, Z., Muscatine, L. and McCloskey, L. (1993). Population control in symbiotic corals. Ammonium ions and organic materials maintain the density of zooxanthellae. *BioScience* **43**, 606-611.

Ferrier, M. D. (1991). Net uptake of dissolved free amino acids by four scleractinian corals. *Coral Reefs* **10**, 183-187.

Ferrier-Pagès, C., Witting, J., Tambutté, E. and Sebens, K. P. (2003). Effect of natural zooplankton feeding on the tissue and skeletal growth of the scleractinian coral *Stylophora pistillata*. *Coral Reefs* **22**, 229-240.

Fitzgerald, L. M. and Szmant, A. M. (1997). Biosynthesis of essential amino acids by scleractinian corals. *Biochem. J.* **322**, 213-221.

Furnas, M. J. (1991). Net in situ growth rates of phytoplankton in an oligotrophic, tropical shelf ecosystem. *Limnol. Oceanogr.* **36**, 13-29.

Grover, R., Maguer, J. F., Reynaud-Vaganay, S. and Ferrier-Pagès, C. (2002). Uptake of ammonium by the scleractinian coral *Stylophora pistillata*: effect of feeding, light, and ammonium concentrations. *Limnol. Oceanogr.* **47**, 782-790.

Grover, R., Maguer, J.-F., Allemand, D. and Ferrier-Pagès, C. (2003). Nitrate uptake in the scleractinian coral *Stylophora pistillata*. *Limnol. Oceanogr.* **48**, 2266-2274.

Grover, R., Maguer, J.-F., Allemand, D. and Ferrier-Pagès, C. (2006). Urea uptake by the scleractinian coral *Stylophora pistillata*. *J. Exp. Mar. Biol. Ecol.* **332**, 216-225.

Hoegh-Guldberg, O. and Williamson, J. (1999). Availability of two forms of dissolved nitrogen to the coral *Pocillopora damicornis* and its symbiotic zooxanthellae. *Mar. Biol.* **133**, 561-570.

Marubini, F. and Davies, P. S. (1996). Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. *Mar. Biol.* **127**, 319-328.

Muller-Parker, G., D'Elia, C. F. and Cook, C. B. (1988). Nutrient limitation in zooxanthellae: effects of feeding history on nutrient uptake by isolated algae. *Proc. 6th Int. Coral Reef Symp.* **3**, 15-19.

Muscatine, L. and Cernichiari, E. (1969). Assimilation of photosynthetic products of zooxanthellae by a reef coral. *Biol. Bull.* **137**, 506-523.

Muscatine, L., McCloskey, L. R. and Loya, Y. (1985). A comparison of the growth rates of zooxanthellae and animal tissue in the Red Sea coral *Stylophora pistillata*. *Proc. 5th Int. Coral Reef Symp.* **6**, 119-123.

Parsons, T. R., Maita, Y. and Lalli, C. M. (1984). *A Manual of Chemical and Biological Methods for Seawater Analysis*. Oxford: Pergamon Press.

Rahav, O., Dubinsky, Z., Achituv, Y. and Falkowsky, P. G. (1989). Ammonium metabolism in the zooxanthellae coral, *Stylophora pistillata*. *Proc. R. Soc. Lond. B Biol. Sci.* **236**, 325-337.

Schlichter, D. (1978). On the ability of *Anemonia sulcata* (coelenterata, anthozoa) to absorb charged and neutral aminoacids simultaneously. *Mar. Biol.* **45**, 97-104.

Schlichter, D. (1980). Adaptations of cnidarian for integumentary absorption of dissolved organic material. *Rev. Can. Biol.* **39**, 259-282.

Schlichter, D., Bajorat, K. H., Buck, M., Eckes, P., Gutknecht, D., Kraus, P., Krisch, H. and Schmitz, B. (1986). Epidermal nutrition of sea anemone by absorption of organic compounds dissolved in the oceans. *Zool. Beitr. N. F.* **30**, 29-47.

Sorokin, Y. I. (1993). *Coral Reef Ecology*. Berlin: Springer-Verlag.

Stimson, J. and Kinzie, R. A. (1991). The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. *J. Exp. Mar. Biol. Ecol.* **153**, 63-74.

Szmant, A. M. (2002). Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? *Estuaries* **25**, 743-766.

Szmant-Froelich, A. (1983). Functional aspects of nutrient cycling on coral reefs. *NOAA Symp. Ser. Undersea Res.* **1**, 133-139.

Thomas, F. I. M. and Atkinson, M. J. (1997). Ammonium uptake by coral reefs: effects of water velocity and surface roughness on mass transfer. *Limnol. Oceanogr.* **42**, 81-88.

Titlyanov, E., Bil, K., Fomina, Titlyanava, T., Leletkin, V., Eden, N., Malkin, A. and Dubinsky, Z. (2000). Effects of dissolved ammonium addition and host feeding with *Artemia salina* on photoacclimation of the hermatypic coral *Stylophora pistillata*. *Mar. Biol.* **137**, 463-472.

Uthicke, S. (2001). Nutrient regeneration by abundant coral reef holothurians. *J. Exp. Mar. Biol. Ecol.* **265**, 153-170.

Wang, J. T. and Douglas, A. E. (1999). Essential amino acid synthesis and nitrogen recycling in an alga-invertebrate symbiosis. *Mar. Biol.* **135**, 219-222.

Wilkerson, F. P. and Trench, R. K. (1986). Uptake of dissolved inorganic nitrogen by the symbiotic clam *Tridacna gigas* and the coral *Acropora* sp. *Mar. Biol.* **93**, 237-246.