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Interactions between zooplankton feeding, photosynthesis and skeletal growth in the scleractinian coral *Stylophora pistillata*

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Summary

We investigated the effect of zooplankton feeding on tissue and skeletal growth of the scleractinian coral *Stylophora pistillata*. Microcolonies were divided into two groups: starved corals (SC), which were not fed during the experiment, and fed corals (FC), which were abundantly fed with *Artemia salina* nauplii and freshly collected zooplankton. Changes in tissue growth, photosynthesis and calcification rates were measured after 3 and 8 weeks of incubation. Calcification is the deposition of both an organic matrix and a calcium carbonate layer, so we measured the effect of feeding on both these parameters, using incorporation of ^{14}C-aspartic acid and ^{45}Ca, respectively. Aspartic acid is one of the major components of the organic matrix in scleractinian corals. For both sampling times, protein concentrations were twice as high in FC than in SC (0.73 *vs* 0.42 mg P^{-1} cm^{-2} skeleton) and chlorophyll c_2 concentrations were 3–4 times higher in fed corals (2.1±0.3 μg cm^{-2}). Cell specific density (CSD), which corresponds to the number of algal cells inside a host cell, was also significantly higher in FC (1.416±0.028) than in SC (1.316±0.015). Fed corals therefore displayed a higher rate of photosynthesis per unit area (P^{g}_{max}= 570±60 nmol O_2 cm^{-2} h^{-1} and I_k=403±27 μmol photons m^{-2} s^{-1}). After 8 weeks, both light and dark calcification rates were twofold greater in FC (3323±508 and 416±58 nmol Ca^{2+} 2 h^{-1} g^{-1} dry skeletal mass) compared to SC (1560±217 and 225±35 nmol Ca^{2+} 2 h^{-1} g^{-1} dry skeletal mass, respectively, under light and dark conditions). Aspartic acid incorporation rates were also significantly higher in FC (10.44±0.69 and 1.36± 0.26%RAV 2 h^{-1} g^{-1} dry skeletal mass, where RAV is total radioactivity initially present in the external medium) than in SC (6.51±0.45 and 0.44±0.02%RAV 2 h^{-1} g^{-1} dry skeletal mass under dark and light conditions, respectively). Rates of dark aspartic acid incorporation were lower than the rates measured in the light. Our results suggest that the increase in the rates of calcification in fed corals might be induced by a feeding-stimulation of organic matrix synthesis.

Key words: coral, feeding, photosynthesis, calcification, organic matrix, ^{14}C-aspartate, ^{45}Ca.

Introduction

Scleractinian corals derive their nutritional needs, in part, from photosynthates translocated by their symbiotic zooxanthellae (Muscatine, 1990). The effects and mechanisms of autotrophy have been well documented (Muscatine, 1980). Corals nevertheless are true heterotrophs, ingesting a wide range of food such as dissolved and particulate organic matter (Anthony, 1999; Anthony and Fabricius, 2000), sediment (Rosenfeld et al., 1999), bacteria (Farrant et al., 1987; Sorokin, 1991) and zooplankton (Lasker et al., 1983; Lewis, 1992; Ferrier-Pagès et al., 1998; Sebens et al., 1996). The relative importance of phototrophy *vs.* heterotrophy is still partially understood. In shallow waters and high-light-adapted corals, photosynthesis alone may meet all the nutritional needs (Falkowski et al., 1984) while in deep living corals or in some species depending mostly on predation, photosynthesis may only supply a small fraction of this energy demand (Szmant-Froelich and Pilson, 1984; for a review, see Barnes and Chalker, 1990). Corals living in turbid environments are also more dependent on heterotrophy than those living in clear waters (Anthony and Fabricius, 2000).

The major role of feeding is to provide the symbiosis with essential nutrients such as nitrogen and phosphorus (Rahav et al., 1989; Cook et al., 1994). Both host and algal symbionts respond quickly to food availability (Fitt, 2000). At the algal level, Dubinsky et al. (1990) as well as Titlyanov et al., (2000a,b, 2001) showed an enhancement of the areal pigmentation and zooxanthellae density in fed corals, leading to an increase in the areal photosynthesis. At the animal level, heterotrophy tends to increase the amount of tissue synthesis (Jacques and Pilson, 1980; Sebens and Johnson, 1991; Kim and Lasker, 1998). An enhancement in skeletal growth has also been observed, suggesting that corals allocate a high proportion of the energy brought by food to calcification processes (Jacques and Pilson, 1980; Witting, 1999; Ferrier-

Pagès et al., 2003; Houlbrèque et al., 2003). Although it is well known that nutrients are continuously exchanged between the two partners (Muscatine, 1990), few studies have focused on the simultaneous effect of feeding on the algal and animal components (Witting, 1999; Ferrier-Pagès et al., 2003; Houlbrèque et al., 2003). Here, we build on this knowledge base by providing an experimental analysis of the interactions between heterotrophy, photosynthesis and calcification in corals.

Feeding has also been shown to enhance skeletal growth, suggesting that corals allocate a high proportion of the energy brought by food to calcification processes (Jacques and Pilson, 1980; Witting, 1999; Ferrier-Pagès et al., 2003; Houlbrèque et al., 2003). It is important to note that calcification is also a dual process, involving the secretion of an organic matrix and the deposition of a $CaCO_3$ fraction. The presence of an organic matrix in coral skeletons is widely documented (Goreau and Goreau, 1959; Wainwright, 1963; Young, 1971; Constantz and Weiner, 1988; Cuif and Gautret, 1995: Dauphin and Cuif, 1997) and is considered an essential prerequisite in the formation of a biomineral strucure (Goreau and Goreau, 1959; Cuif et al., 1997; Allemand et al., 1998). This matrix potentially plays key roles in various processes such as crystal nucleation and growth, crystal size and orientation and regulation of skeletal formation (Weiner and Addadi, 1991; Falini et al., 1996; Belcher et al., 1996). Cuif et al. (1999) demonstrated that the composition of the matrix was different between symbiotic and asymbiotic corals, and Allemand et al. (1998) suggested that heterotrophy is a source of aspartic acid, one of the major components of the coral matrix (Young, 1971; Cuif and Gautret, 1995; Dauphin and Cuif, 1997). We therefore investigated the effect of feeding on both organic matrix synthesis and calcification.

Materials and methods

Biological material

Experiments were conducted in the laboratory using microcolonies (1 cm long fragments) of the scleractinian coral *Stylophora pistillata* Esper, 1797. The advantages of using microcolonies in radioactive experiments are described in Tambutté et al. (1995). 140 terminal portions of branches were cut from ten different parent colonies placed and maintained on separate nylon nets. 14 nubbins were cut from each parent colony and kept on separated nylon nets during the healing period. After 3 weeks, coral fragments were entirely covered with new tissue and were ready to be used for the experiments. 35 microcolonies (3–4 microcolonies from each parent colonies) were assigned to each of the four aquaria. The growth of the microcolonies was monitored twice a week for 3 weeks, using the buoyant weight technique (Jokiel et al., 1978). An analysis of variance (ANOVA) performed on these growth rates showed no significant clone or aquaria effects ($P>0.05$).

Tanks were supplied with oligotrophic Mediterranean seawater, pumped from 50 m depth at a rate of 2 l h^{-1} and mixed using a submersible pump (Aquarium system, mini-jet MN 606, Mentor, OH, USA). The velocity of flow across the corals was approximately 0.6–1 cm s^{-1}, as measured by timing the passage of neutrally buoyant beads. Filtered seawater, maintained at 26°C, had low amounts of organic and inorganic nutrients (Ferrier-Pagès et al., 1998). Corals received a constant irradiance of 350 μmol photons m^{-2} s^{-1} (photoperiod was 12 h:12 h light:dark) using metal halide lamps (Philips, HPIT, 400 W, Eindhoven, The Netherlands). Tanks and nylon nets were cleaned several times per week in order to avoid algal growth on the nylon nets.

Experimental design

Microcolonies were then divided into two groups (two tanks per group) corresponding to two feeding levels: (1) starved corals (SC) were not fed during the whole experiment; (2) fed corals (FC) were fed 4 days per week (Monday, Wednesday, Thursday and Friday). On Monday and Friday corals were fed *Artemia salina* nauplii (2022±115 shrimps l^{-1}) and on Wednesday and Thursday they were fed freshly collected Mediterranean zooplankton (1005±164 organisms l^{-1}). Copepods represented 94% of the plankton, followed by lesser numbers of siphonophores, brachiopods, crustacean larvae and jellyfish. The ingestion of prey was controlled under a dissecting microscope during feeding (Ferrier-Pagès et al., 2003). The number of prey items ingested was proportional to prey density and capture rates varied from 0.06 to 1 prey items $polyp^{-1}$ day^{-1} (Ferrier-Pagès et al., 2003). These rates were in the same range as previous estimates for other coelenterates (Lasker et al., 1983; Sebens et al., 1996).

Plankton were collected using a WP2 net and immediately brought back to the laboratory. They were concentrated with a reverse filtration apparatus on a 10 μm filter, to remove small algae and detritus. They were then added to heated seawater, and the actively swimming portion of the sample was fed to the corals during a 1 h period. A 100 ml sample was collected from the aquaria at each feeding time to determine the nature and abundance of the planktonic prey using a binocular microscope (Wild M3, 40×) and a Dolfuss tank. After feeding, the aquaria were emptied entirely and refilled with fresh filtered seawater to avoid contamination by dissolved and organic nutrients coming from the degrading prey.

Microcolonies were maintained under these conditions for 8 weeks. Changes in photosynthesis, tissue composition (protein and chlorophyll contents), cell-specific density (CSD), rates of aspartic acid incorporation and calcification were measured after 3 and/or 8 weeks, depending on the assay.

Photosynthesis–irradiance (P/I) curves

Rates of photosynthesis and respiration were measured after 3 weeks on five microcolonies (replicates) taken from each tank (total=20 colonies, 10 fed and 10 starved corals, respectively). Each microcolony was placed in a respirometric glass chamber containing a ‘Strathkelvin 928®’ electrode (Glasgow, UK) and immersed in a water bath (26°C). The incubation medium was continuously stirred with a magnetic stirring bar. Photosynthesis *vs.* irradiance (P/I) curves were

constructed by measuring production rates across a range of irradiances. Samples were incubated for 10 min under different light levels (0, 50, 80, 120, 200, 300, 400, 500, 600, 800 μmol m^{-2} s^{-1}). Light was provided by a 400 W metal halide lamp (Philips, HPIT) attenuated to the desired intensity with screens placed between the light source and the aquaria. Before each experiment, the oxygen sensor was calibrated against air-saturated seawater (100% oxygen) and a saturated solution of sodium dithionite (zero oxygen). Oxygen was monitored every 10 s on an acquisition station. Rates of photosynthesis and respiration were estimated by regressing oxygen data against time.

The following function was fitted to the photosynthesis–irradiance data (Barnes and Chalker, 1990).

$$P_{net} = (P^{g}_{max}) \tanh(I/I_k) + R \,, \quad (1)$$

where P_{net} is the net photosynthetic rate, expressed in μmol O_2 cm^{-2} h^{-1} or in μmol O_2 (chlorophyll *a*)$^{-1}$ h^{-1}. P^{g}_{max} is the maximum gross photosynthetic rate (μmol O_2 cm^{-2} h^{-1}), tanh is the hyperbolic tangent, I is the irradiance (μmol photon m^{-2} s^{-1}), I_k is the irradiance at which the initial linear portion of the curve intersects P^{g}_{max} (Talling index, i.e. saturation constant) and R is the respiration rate in the dark (μmol O_2 cm^{-2} h^{-1}). From these P/I curves, two other parameters were determined: I_c, representing the light intensity at which the oxygen production equalled the oxygen consumption (compensation intensity), and α, the initial slope of the P/I curve (photosynthetic efficiency, $\alpha = P^{g}_{max}/I_k$).

From the same corals, chlorophylls *a* and c_2 were extracted twice in 100% acetone (24 h at 4°C). The extracts were centrifuged at 6000 ***g*** for 20 min and the absorbencies read at 630, 663 and 750 nm. Chlorophyll concentrations were computed according to the spectrometric equations of Jeffrey and Humphrey (1975) and were expressed per surface area (cm^2). Surface area was measured using the aluminium foil technique (Marsh, 1970).

Estimation of the cell-specific density (CSD)

After 3 weeks, CSD was determined for five colonies (replicates) taken from each tank (N=10 fed and 10 starved corals, respectively). Cells were extracted mechanically by shaking (using a wrist-action) crushed coral in a flask (Muscatine and Cernichiari, 1969). Host cells containing symbionts were observed under a Leica microscope (50×; Wetzlar, Germany), and the number of algae contained in each cell was counted for 300 host cells per sample. Data were expressed in terms of the frequency or percentage distribution of host cells (f_i) with a given number of algae per cell (r_i).

The average cell-specific density (CSD) was calculated as:

$$CSD = \sum(r_i \times f_i) / \sum f_i \,. \quad (2)$$

Measurements of skeletal calcium and aspartic acid incorporation

Measurements were performed both in the light and in the dark after 3 and 8 weeks of treatment. Corals were incubated in the morning to avoid variations due to endogenous circadian rhythms (Buddemeier and Kinzie, 1976; Tambutté et al., 1995). For each sampling period (3 and 8 weeks), three replicates microcolonies, randomly taken in each tank, were used for each measurement (a total of 48 colonies, N=6 for starved and fed corals and each measurement, respectively). Calcification rates were measured using ^{45}Ca according to the method of Tambutté et al. (1995). Organic matrix synthesis was measured as skeletal incorporation of ^{14}C-aspartic acid and protocols were adapted from Allemand et al. (1998) and Tambutté et al. (1995, 1996). ^{45}Ca- and ^{14}C-aspartic acid incorporations were studied in separate colonies.

Each microcolony was placed in a plastic holder and incubated for 2 h in an 8 ml beaker containing either 16 kBq ml^{-1} of $^{45}CaCl_2$ (NEN, LifeScience Products, France) or 833 Bq ml^{-1} of ^{14}C-aspartic acid (NEN) dissolved in seawater. Corals for dark incubations were sampled at the end of the night (1 h before the lights were switched on) and were maintained in the dark during the whole incubation. The results are expressed either as nmol Ca^{2+} g^{-1} dry skeletal mass or as a fraction of the total radioactivity initially present in the external medium (%RAV; ^{14}C-aspartic acid g^{-1} dry skeletal mass) (Allemand et al., 1998). Protein content of the radioactive tissue was measured using the BC Assay Kit (Interchim, Montluçon, France), based on the colorimetric determination of the amount of protein (Smith et al., 1985). The standard curve was established with bovine serum albumin.

In the Results, the amounts of protein and chlorophyll are normalized per unit surface area and the rates of photosynthesis per unit surface area or per amount of chlorophyll. The rates of calcification and aspartic acid incorporation are normalized per g skeletal dry mass in order to facilitate comparisons with the previous results of Allemand et al. (1998).

Statistical analyses

The effects of feeding on the physiological parameters were tested using a *t*-test (software Stat-View 4.01, Abacus concept, Inc, Berkeley, CA, USA). The effect of feeding on the CSD was analysed using a Pearson χ^2 test.

Results

Chlorophyll and protein content

After 3 and 8 weeks of treatments, protein concentrations were significantly higher in fed (FC) than in starved corals (SC) (Fig. 1A,B, Table 1). Concentrations of chlorophyll-*a* per unit surface skeleton were, however, comparable in fed and starved corals (Fig. 1C, Table 1). Conversely, feeding significantly increased chlorophyll c_2 concentrations (Fig. 1C, Table 1) roughly three- to fourfold.

Photosynthesis–irradiance (P/I) curves

Mean P/I curves obtained for each treatment are shown in Fig. 2. Table 2 represents the calculated mean values for P^{n}_{max}, I_k, I_c and α. When normalized per surface area, feeding significantly increased the rates of maximum net

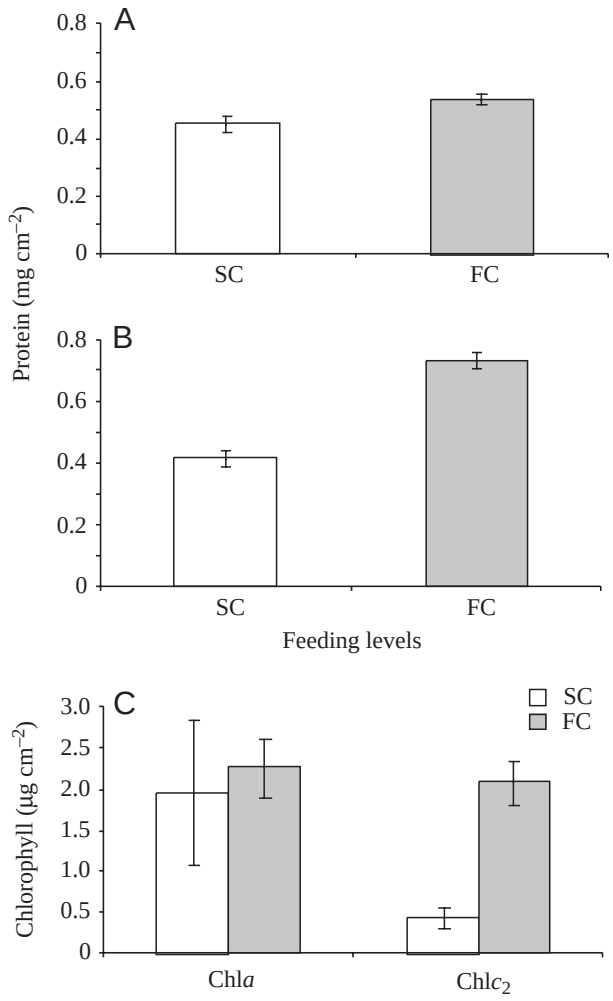

Fig. 1. Protein concentration after (A) 3 weeks of incubation (N=6) and (B) 8 weeks of incubation (N=6); (C) chlorophyll (Chl) concentrations after 3 weeks for starved and fed corals (N=10). Values are means ± S.D. White bars, starved corals (SC); grey bars, fed corals (FC).

Table 1. *The effect of feeding on the chlorophyll and protein contents, on the rates of light and dark calcification and on the light and dark uptake of aspartic acid for each sampling time*

		Effect of feeding	
	d.f.	F	P
Proteins			
T_0+3	1	16.17	**<0.01**
T_0+8	1	23.94	**<0.01**
Chlorophyll *a*	1	0.20	0.68
Chlorophyll c_2	1	116.26	**<0.01**
Photosynthesis parameters			
P^g_{max}	1	115.32	**<0.01**
I_k	1	46.02	**<0.01**
I_c	1	1.15×10^{-4}	0.99
Respiration	1	0.02	0.89
α	1	1.81	0.25
Calcification			
Light			
T_0+3	1	4.00	0.12
T_0+8	1	12.57	**0.02**
Dark			
T_0+3	1	38.01	**<0.01**
T_0+8	1	9.13	**0.03**
Uptake of aspartic acid			
Light			
T_0+3	1	0.02	0.89
T_0+8	1	22.80	**<0.01**
Dark			
T_0+3	1	0.20	0.68
T_0+8	1	12.13	**0.03**

P, probability; significant values (P<0.05) are indicated in bold. T_0, time at start of experiment; +3, +8 indicates +3 weeks and + 8 weeks feeding, respectively; α, initial slope; P^g_{max}, maximum gross photosynthetic rate; I_k, Talling index; I_c, compensation intensity (see Materials and methods for details).

photosynthesis (P^n_{max}) (570±60 *vs.* 200±20 nmol O_2 cm^{-2} h^{-1} for FC and SC, respectively) and the Talling index (I_k) (403±27 and 203±11 µmol photons m^{-2} s^{-1}, respectively, for FC and SC). Conversely, when data were normalised per chlorophyll *a* concentrations, feeding did not have any significant effect on P^n_{max} (*t*-test, d.f.=4, P=0.13) (Table 2). For both normalizations, there was no significant difference in the respiration rates between FC and SC (*t*-test, d.f.=0.6, P=0.50) (Table 2).

Cell-specific density

Fig. 3 shows the percentage distribution of the number of algae per host cell. Host cells containing a single dinoflagellate (singlet) predominate (62.3% and 70.4% of the total cells for FC and SC, respectively) followed in decreasing frequency by those containing two (doublet) (34.3% in FC and 28.3% in SC), three (triplet; 3.0% in FC and 0.7% in SC), and up to four cells (quadruplet; 0.4% in FC and 0.7% in SC). In FC, the number of doublets and triplets significantly increased during the incubation compared to the SC. Therefore, CSD was significantly higher in FC (1.416±0.028) than in SC (1.316±0.015) (Pearson χ^2 test, d.f.=1, P=0.04).

Calcification rates

After 3 weeks, there was no significant difference in the calcification rates measured in the light between FC and SC (Fig. 4A, Table 1). However, after 8 weeks, FC showed significantly higher light calcification rates than SC (Fig. 4B, Table 1). For the two sampling periods, feeding enhanced the dark calcification rates, which were twice as high in FC compared to starved corals (Fig. 4A,B, Table 1). For the two sampling periods, rates of dark calcification were 6–10 times lower than those of light calcification (Fig. 4A,B).

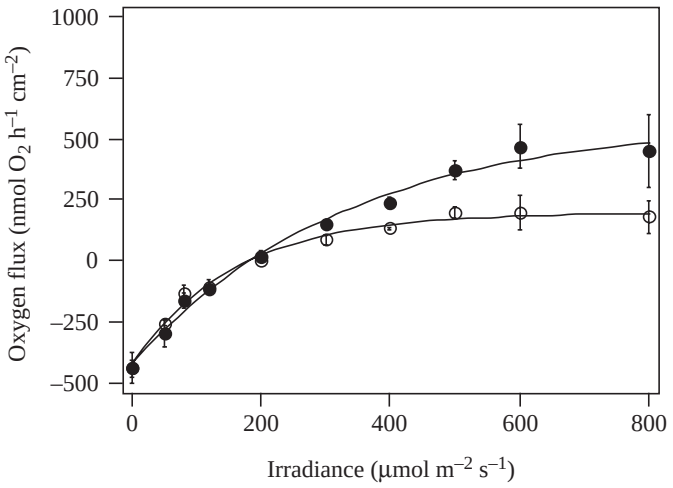

Fig. 2. Net photosynthesis (measured as oxygen flux) for starved (white circles) and fed (black circles) corals at various irradiance levels. Values are means ± S.D., *N*=10.

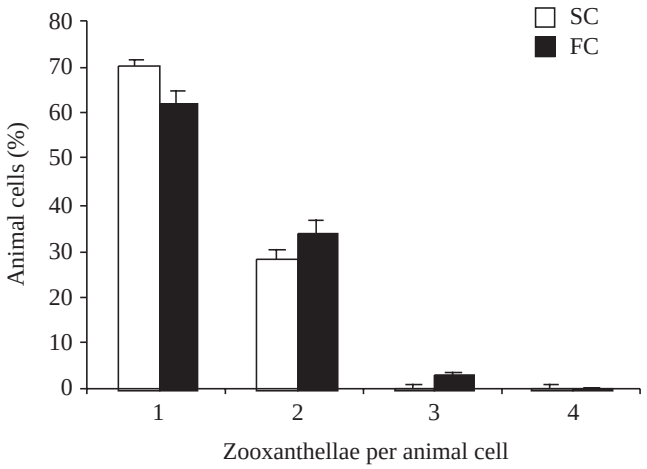

Fig. 3. Distribution percentage of zooxanthellae in starved and fed coral cells. Values are means ± S.D., *N*=10.

Effects of feeding on organic matrix synthesis

After 3 weeks, we found no significant effect of feeding on the light and dark incorporations (Fig. 5A, Table 1). After 8 weeks, however, feeding significantly changed the rate of incorporation in both the light and the dark (Fig. 5B, Table 1). Incorporation rates in the light were increased by two thirds in FC compared to SC. The uptake rates in the dark were three times higher in FC than in SC. In both cases, darkness markedly reduced the incorporation of ^{14}C aspartic acid into the skeleton (Fig. 5A,B). After 8 weeks, the rates of incorporation in the dark were, respectively, 14 and 8 times lower than the uptake rates in the light for starved and fed corals (Fig. 5B). Feeding did not affect the ratios of calcification rate/aspartic acid incorporation, measured in the light (Fig. 6). However this ratio is 1.82–2.64 higher in the dark for both fed and starved corals, respectively.

Table 2. *Parameter estimates for the photosynthesis curves*

Parameter estimates	SC	FC
r^2	0.98	0.99
P^n_{max} (μmol O_2 h^{-1} cm^{-2})	0.20±0.02	0.57±0.06
R (μmol O_2 h^{-1} cm^{-2})	–0.44±0.02	–0.43±0.04
α (nmol O_2 h^{-1} cm^{-2}) (μmol photons m^{-2} s^{-1})	1.04±0.28	1.21±0.18
P^n_{max} (μmol O_2) (mg Chl*a*)$^{-1}$ h^{-1})	106.83±24.9	190.900±33.8
R (μmol O_2) (mg Chl*a*)$^{-1}$ h^{-1})	–244.66±76.6	–167.55±29.45
I_k (μmol photons m^{-2} s^{-1})	203.04±10.99	403.81±27.48
I_c (μmol photons m^{-2} s^{-1})	182.98±6.90	182.88±5.83

r^2 is the statistical fit of the curve, P^n_{max} is the net maximal photosynthetic rate, R is the respiration rate in the dark and α is the initial slope (see Materials and methods for details). I_k, Talling index; I_c, compensation irradiance.

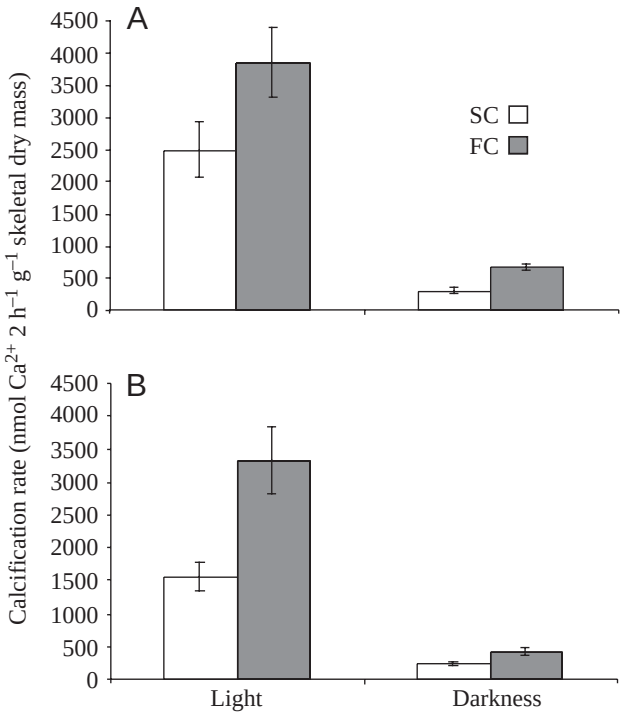

Fig. 4. Calcification rates measured after (A) 3 weeks and (B) 8 weeks in light and dark. Values are means ± S.D., *N*=6. Fed corals (black bars); starved corals (white bars).

Discussion

Feeding induced significant changes in most of the physiological parameters measured. The increase in tissue growth (significant after 3 weeks) was faster than the increase in skeletal growth (significant only after 8 weeks). This is in agreement with the findings of Anthony et al. (2002), who suggest that either tissue may react more rapidly than the skeleton to availability of resources, or that the energy content of the tissue may represent a major component of the total

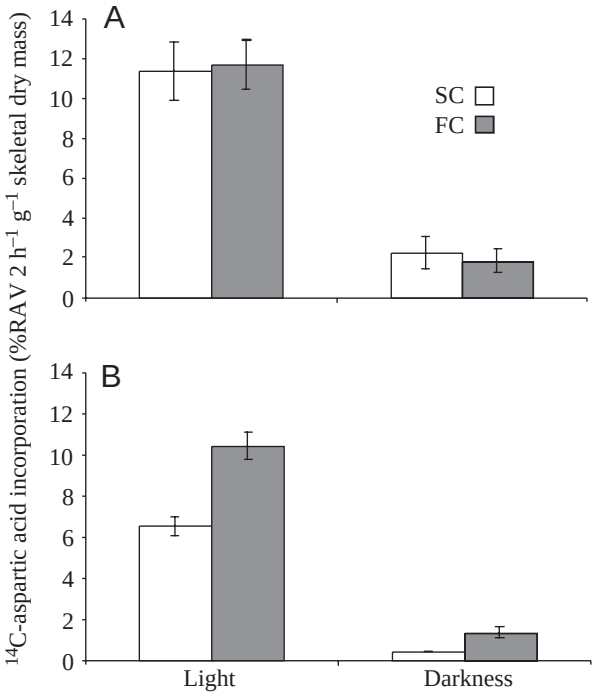

Fig. 5. Rates of ^{14}C-aspartic acid incorporation after (A) 3 weeks and (B) 8 weeks in light or dark conditions. Values are means ± S.D., *N*=6. Fed corals (black bars); starved corals (white bars).

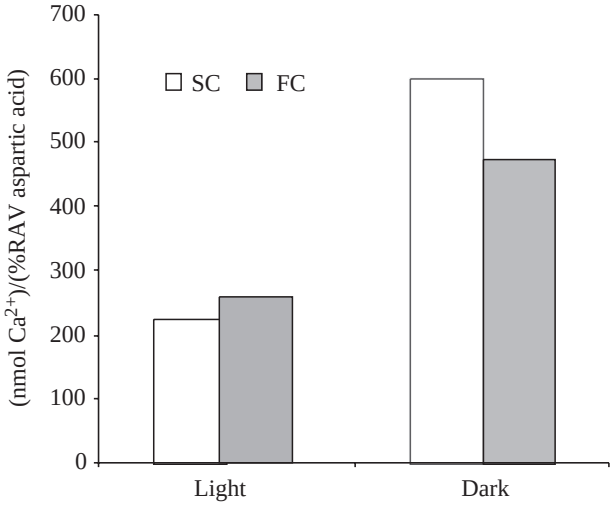

Fig. 6. Ratios of the calcification rates (nmol Ca^{2+} 2 h^{-1} g^{-1} skeletal mass) to aspartic acid uptake (%RAV 2 h^{-1} g^{-1} skeletal mass) for the microcolonies of *Stylophora pistillata* maintained for 8 weeks under the two feeding conditions.

energy investment in coral growth. In a previous experiment, Ferrier-Pagès et al. (2003) estimated that the amount of carbon and nitrogen ingested by *S. pistillata* fed with zooplankton over a 4 week period represented one-third of the carbon and nitrogen needed for the estimated tissue growth. In the present study, we also observed that feeding increased the number of zooxanthellae per host cell. Feeding therefore enhanced the growth of the algal component at a higher rate than the growth of the animal cells. A similar increase in CSD was observed in an environment enriched in inorganic nitrogen (Muscatine et al., 1998), suggesting that the algae are nitrogen-limited in the symbionts. When collected *in situ* in the same environment, different coral species do not display the same CSD, which ranged from ca. 50% of doublets in *Madracis mirabilis* to 20% in *Acropora palmata* (Muscatine et al., 1998). This may suggest different feeding capacities for different species, with 'effective' predators displaying a higher CSD than 'poor' predators. For example, previous studies have established that *M. mirabilis* (high CSD) is a 'voracious' feeder (Sebens et al., 1996) and capable of capturing more prey than more 'autotrophic' species with smaller CSDs. Our results are consistent with the study of Muscatine et al. (1998), since a difference in CSD was observed for microcolonies of *S. pistillata* benefiting from a food supply. However, the validity of this underlying relationship deserves further testing.

One of the aims in this work was to understand by which mechanism feeding enhances calcification. Both light and dark calcification rates were greatly enhanced by feeding; after 8 weeks of incubation, these rates were twice as high in fed than in starved corals. This stimulation had already been noticed in a previous experiment performed with the same species (Houlbrèque et al., 2003) and validates recent results showing a positive effect of heterotrophy on coral growth (Kim and Lasker, 1998; Anthony and Fabricius, 2000; Witting, 1999; Ferrier-Pagès et al., 2003). A light-enhanced (or dark-repressed) calcification (Goreau and Goreau, 1959; Barnes and Chalker, 1990; Gattuso et al., 1999; Houlbrèque et al., 2003) was also noticed with rates of dark calcification 6–10 times lower than rates of light calcification.

Calcification is carried out in two processes, i.e. the secretion of an organic matrix and the deposition of calcium carbonate. The presence of an organic matrix in corals has been a matter of much controversy (Constantz, 1986), but its existence is now well-demonstrated, both from studies performed on scleractinian corals (Goreau and Goreau, 1959; Cuif et al., 1997; Allemand et al., 1998) and on other calcifying organisms (Belcher et al., 1996; Falini et al., 1996). Cuif and Gautret (1999) showed that the amino acid composition of the organic matrix differs between zooxanthellate and azooxanthellate corals, suggesting that their nutritive source may affect the organic matrix synthesis. Aspartic acid, for example, is one of the major and most abundant amino acids in the coral matrix (Young, 1971; Cuif and Gautret, 1995; Dauphin and Cuif, 1997). Allemand et al. (1998) also showed that no aspartic acid pool was present inside the coral tissue, suggesting the need for a constant supply from an exogenous source. By using ^{14}C-aspartic acid as a precursor for organic matrix synthesis (Allemand et al., 1998), we measured a higher incorporation of this amino acid into the organic matrix of fed corals. Since feeding has increased the unlabelled amino acid pool present within the tissue, the rates of incorporation in fed animals are likely to be underestimated, suggesting that the

enhancement of the organic matrix synthesis by feeding is even higher.

Feeding might therefore have enhanced the construction of the organic matrix by (i) supplying additional input of energy, especially for the dark processes. Under high plankton concentrations, such as those provided for the fed corals in this study, uptake of organic carbon (and hence energy) may be significant and could provide some energy for calcium/proton exchange at night. Alternatively, the larger biomass of fed corals may have provided larger energy stores for dark processes (McConnaughey and Whelan, 1997; Anthony et al., 2002). Thus, feeding might have (ii) directly provided the necessary 'external' amino acids and/or (iii) indirectly increased photosynthesis and therefore the supply of 'autotrophic' amino acids. When normalized per amount of chlorophyll, the rates of photosynthesis were unchanged between fed and starved corals, suggesting that feeding does not increase the efficiency with which symbionts (or reaction centers) use light for photosynthesis. However, when normalized per surface area, the rates of photosynthesis were higher in fed corals, with a change in photosynthetic parameters, such as P^{n}_{max} and I_k. The increase in P^{n}_{max}, already observed by Titlyanov et al. (2001), generally corresponds to an increase in the number of photosynthetic units (Prezelin, 1987). Davy and Cook (2001) obtained similar results in fed and starved sea anemones and showed that the percentage translocation of photosynthates remained unchanged between the two treatments. This is presumably because the surplus carbon was stored by the algae rather than being translocated. However, several authors have demonstrated that even if the amount of translocated photosynthates is unchanged between starved and fed animals, their quality is completely different (Swanson and Hoegh-Guldberg, 1998; Wang and Douglas, 1999). Since the supply of nitrogen directly influences the zooxanthellar C:N ratio (Snidvongs and Kinzie III, 1994; Grover et al., 2002), feeding might have increased the amino acid synthesis compared to the production of non-nitrogenous compounds such as glycerol and glucose (Swanson and Hoegh-Guldberg, 1998; Wang and Douglas, 1999). This higher amount of translocated amino acids might have enhanced the synthesis of the organic matrix. However, this question can only be answered by investigating the quality of the photosynthates produced by fed and starved corals.

The second main conclusion that can be derived from this study is that the effect of feeding on aspartic acid incorporation is comparable to its effect on calcium incorporation. Feeding enhanced both light and dark processes, with a higher enhancement in the dark (threefold increase) than in the light (1.6-fold increase). To determine the link between the deposition of organic and mineral fractions, we compared the ratio of $CaCO_3$/aspartate incorporation. The dark ratio was 1.8–2.6 higher than the light ratio (for fed and starved corals, respectively), which suggests that the interactions between the organic and mineral fractions were affected by light/dark conditions. In the dark, there may have been a decrease in the organic matrix synthesis, an increase in the mineral fraction deposition or a combination of both events. From Figs 4B and 5B, it appears that the more important process should be a decrease of dark organic-matrix synthesis. This decreased fourteen-fold (Fig. 5B), while dark calcification decreased only sevenfold (Fig. 4B). This phenomenon, observed here for the first time, could be responsible for the diurnal bands observed in coral skeletons (Barnes, 1973). A higher dark inhibition of organic matrix synthesis *vs.* $CaCO_3$ deposition may be explained by the lack of photosynthates as organic matrix precursors (Cuif et al., 1999) or by some other unknown process. In either case, this suggests a close relationship between calcification, organic matrix synthesis and photosynthesis. These results are in agreement with the hypothesis of Barnes et al. (1989, 1990) and Taylor et al. (1993), who suggested a cyclic deposition of skeleton leading to the formation of skeletal banding in scleractinian corals.

Feeding did not affect the $CaCO_3$/aspartate deposition in the light. This result suggests a close coupling between organic matrix synthesis and $CaCO_3$ deposition in the light. This coupling appears less strict in the dark where feeding induces a slight decrease of the ratio. Since it has been previously shown that the organic matrix synthesis is a prerequisite step for calcification (Allemand et al., 1998), the increase in the rates of calcification in fed corals might therefore be induced by an increase in the rates of feeding-induced organic matrix synthesis. Corals may derive some important source of amino acid and/or energy for their growth from external food supplies. Another conclusion can be drawn from the comparison of the ratio between starved/fed and light/dark treatments. Since these ratios were different from each other, we suggest that autotrophy and heterotrophy do not affect calcification in the same way.

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