Effect of feeding on the carbon and oxygen isotopic composition in the tissues and skeleton of the zooxanthellate coral *Stylophora pistillata*

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ABSTRACT: The effect of feeding on the carbon isotopic composition of zooxanthellae, animal tissue and skeleton was investigated in the scleractinian coral *Stylophora pistillata*. Two sets of corals were grown with filtered seawater under controlled conditions. One group of colonies was fed *Artemia* sp. nauplii and compared to a control group that was starved. Fed corals exhibited higher concentrations of chlorophyll (60% more), soluble protein (4 times more) and calcification rates (29% more) than starved colonies. The net photosynthetic rate was higher in starved than in fed corals (18.53 ± 6.99 and 6.78 ± 2.06 µmol O_2 cm^{-2} h^{-1} respectively), whereas dark respiration was not significantly different (8.74 ± 2.27 and 6.66 ± 0.40 µmol O_2 cm^{-2} h^{-1}). The average δ^{13}C value of *Artemia* sp. nauplii used for feeding was –12‰. δ^{13}C was significantly heavier in zooxanthellae than in animal tissues, for both fed (–10.1 vs –11.7‰) and starved colonies (–10.9 vs –13.2‰). *Artemia* sp. carbon was incorporated into the coral tissue as shown by the heavier δ^{13}C in fed than in starved colonies (–11.7 to –13.2‰, respectively), although there was no difference in the δ^{13}C of the zooxanthellae fraction. Skeletal δ^{13}C was similar in fed and starved colonies (mean –4.6‰). Skeletal δ^{18}O composition was, however, significantly different between the 2 treatments (–4.24 to –4.05‰ for fed and starved colonies, respectively), which may have been due to differences in the calcification rates of fed and starved corals. These data are used to establish a conceptual model of the carbon flow between the various compartments of a symbiotic coral. It suggests that the skeletal δ^{13}C is not sensitive to heterotrophic food supply.

KEY WORDS: Coral · Carbon · Oxygen · Isotopic composition · Feeding · Primary production · Respiration · Calcification

INTRODUCTION

Corals are known to flourish in oligotrophic tropical water and can be considered to be 'mixotrophic' (both auto- and heterotrophic) organisms. They are able to fix inorganic carbon through the photosynthetic activity of their dinoflagellate symbionts, the zooxanthellae

(Rahav et al. 1989, Muscatine 1990). They may also derive a fraction of their energy either through predation on bacterioplankton (Farrant et al. 1987, Sorokin 1991, Ferrier-Pagès et al. 1998) and zooplankton (Sorokin 1991, Lewis 1992, Sebens et al. 1996) or through the use of dissolved organic matter (Sorokin 1973, Al-Moghrabi et al. 1993). Such heterotrophic nutrition was suggested to be predominant in deep waters, where rates of photosynthesis are low (Muscatine et al. 1989, Anthony & Fabricius 2000). The relative impor-

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tance of autotrophy and heterotrophy in the nutrition of corals has been a source of considerable controversy (Edmunds & Davies 1986, Sebens et al. 1996). The use of stable isotopes provides insight into this problem (Risk et al. 1994, Sammarco et al. 1999). The carbon isotopic signature (δ^{13}C) of the tissues is a tracer of food sources (Peterson & Fry 1987, Risk et al. 1994) and enables quantification of carbon fluxes between trophic levels (Rau et al. 1992). The δ^{13}C values of consumers are usually similar to those of their diet (Rau et al. 1983). Muscatine et al. (1989) were the first to investigate the effects of depth and availability of particulate organic matter on the carbon isotopic composition of animal and algal fractions. They reported depletion in heavy isotopes with depth and suggested that deep corals were more heterotrophic than corals living in shallow waters. A few subsequent studies investigated the relationship between environmental parameters and the stable isotope composition of coral tissues (Risk et al. 1994, Yamamuro et al. 1995).

The carbon and oxygen isotopic compositions of coral skeletons are also widely used as indicators of environmental parameters (Cole & Fairbanks 1990, Dunbar et al. 1994). The main physiological processes that potentially alter skeletal δ^{13}C are photosynthesis and its variation with light (e.g. Weil et al. 1981, Swart et al. 1996, Juillet-Leclerc et al. 1997, Reynaud-Vaganay et al. 2001), respiration (Swart et al. 1996) and feeding (Felis et al. 1998, Grottoli & Wellington 1999). Most studies have demonstrated that an increase in the rate of photosynthesis induces an increase in skeletal δ^{13}C (Swart et al. 1996, Juillet-Leclerc et al. 1997, McConnaughey et al. 1997, Reynaud-Vaganay et al. 2001). However, feeding could have an opposite effect since zooplankton carbon typically has a low δ^{13}C signal compared to the skeleton. Therefore, the interpretations of skeletal δ^{13}C value have to take into account these 2 processes.

Despite an isotopic disequilibrium with seawater, aragonite δ^{18}O is generally considered to be a reliable recorder of sea surface temperature in corals (e.g. Weber & Woodhead 1972, Leder et al. 1996, Reynaud-Vaganay et al. 1999). However, it also reflects rainfall influence on seawater δ^{18}O (e.g. Dunbar & Wellington 1981, Cole & Fairbanks 1990, Linsley et al. 1994, Le Bec et al. 2001) and it has been shown to vary with linear extension rate (Land et al. 1975, McConnaughey 1989, Allison et al. 1996). Only 1 study has investigated the effects of feeding on skeletal δ^{18}O (Grottoli & Wellington 1999) because feeding affects the calcification and extension rates of corals.

The aims of this study were to investigate the carbon sources and fluxes in the zooxanthellate scleractinian coral *Stylophora pistillata* and to study how feeding influences these sources. This analysis is based on the stable isotopic composition of the animal and algal tissues, as well as that of the skeleton, during controlled experiments.

MATERIALS AND METHODS

Experimental set up. The experiment was performed in the laboratory using colonies of the branching zooxanthellate scleractinian coral *Stylophora pistillata* (Esper 1797), collected in the Gulf of Aqaba (Red Sea, Jordan) and maintained in the aquaria of the Oceanographic Museum of Monaco. Sixty-eight 'nubbins' (small live coral samples) were obtained by cutting terminal portions of branches from a single parent colony and gluing these fragments onto glass slides using underwater epoxy (Devcon®) as described by Reynaud-Vaganay et al. (1999). The slides were then haphazardly distributed in 4 tanks (30 l). The tanks were continuously supplied with filtered (0.45 µm) Mediterranean seawater (salinity 38.5) pumped at 50 m depth, aerated and heated to 27°C using a temperature controller (EW, PC 902/T) and continuously mixed with a Rena® pump (6 l min^{-1}). The renewal rate was approximately 5 times d^{-1}. Light (380 µmol m^{-2} s^{-1}) was provided by metal halide lamps (400 W, Phillips HPIT) on a 12:12 h photoperiod. This intensity corresponds to saturating light levels measured at 5 to 6 m depth.

The nubbins kept in 2 of the tanks were fed 5 g of *Artemia salina* nauplii, mixed with an Ultra Turrax (T-25), 3 times wk^{-1} for 12 wk. The corals were transferred to a different aquarium (no mixing or seawater renewal) during the 2 h feeding period and were checked for effective feeding. The same *Artemia* stock was used throughout the experiment, in order to have an identical isotopic composition of the food during the whole experiment. This condition makes easier the interpretation of the effect of feeding on the isotopic composition of the coral tissue and skeleton. These nubbins will be referred to as 'fed colonies'. The remaining nubbins were maintained without any food during the 12 wk incubation, and will be referred to as 'starved colonies'.

Measurement of environmental variables. Temperature (accuracy ±0.05°C) was logged at 10 min intervals using a Seamon® temperature recorder. Salinity and irradiance were measured using a conductivity meter (Meter LF196) and a 4π quantum sensor (LI-193SA, Li-Cor), respectively.

Calcification rate. Corals were weighed at the beginning and end of the experiment according to the buoyant weight technique (Jokiel et al. 1978), using a Mettler AT 261 balance (accuracy ±0.01 mg). Calcification rates were calculated using the following formula:

$$G \;=\; \sqrt[n]{\frac{P_n}{P_0}} - 1$$

where G is the calcification rate (% d^{-1}), n the time (d), P_n the weight (g) after n days of culture and P_0 the initial weight (g).

Net photosynthesis and respiration. Oxygen metabolism was measured on fed and starved colonies (n = 4 for each). Each nubbin was taken from the culture aquarium and placed in a plexiglass chamber (240 ml) for a 30 min pre-incubation period in the light (380 µmol photons m^{-2} s^{-1}) or in the dark. Rates of photosynthesis and respiration were then monitored for 1 h. The incubation medium (seawater filtered on 0.45 µm membranes) was continuously agitated using a magnetic stirrer and changed after each incubation. The respirometric chamber was kept at 27°C in a thermostated water bath. Incubations took place between 8:00 and 14:00 h. Light was provided by a 400 W metal halide lamp (Philips). Irradiance was measured using the 4π quantum sensor described above. Oxygen concentration was measured using a Ponselle polarographic electrode calibrated daily against air-saturated seawater and a saturated solution of sodium sulfite (zero oxygen). Oxygen concentration was recorded every min by a data logger (Li-1000, Li-Cor). Rates of net photosynthesis were estimated from the slope of the linear regression of O_2 concentration against time. Data were normalized to the chlorophyll content and the surface area measured with the aluminum foil technique (Marsh 1970). At the end of the incubations, colonies were frozen and kept at –20°C pending determination of chlorophyll a (chl a), chlorophyll c_2 (chl c_2) and soluble protein.

Measurement of chlorophyll and protein contents. Chl a and chl c_2 were extracted twice from the coral in 100% acetone (24 h at 4°C). The extracts were centrifuged at 5400 $\times$ g for 10 min at 4°C and the absorbances were measured at 630, 663 and 750 nm. Chlorophyll concentrations were computed according to the spectrophotometric equations of Jeffrey & Humphrey (1975). Proteins were solubilized in NaOH (1 N) at 90°C for 30 min. Samples were then neutralized with HCl (1 N), and the total soluble protein content was measured using the Bradford method (Bradford 1976) with the Coomassie protein assay reagent (Ref. 23200 Pierce). Optical density was read at 595 nm using a microplate reader (Multiskan® Bichromatic). Bovine gamma globulin (BGG) was used as a standard.

Sample preparation for isotopic analysis. *Isolation of zooxanthellae and host tissue fractions:* The sampling glassware was pre-combusted at 480°C for at least 6 h. Each coral was placed in a 100 ml beaker containing 10 to 20 ml of filtered seawater. Tissue was removed from skeleton with an 'air pick' (air under pressure) and homogenized with a Potter tissue grinder.

The homogenate was then centrifuged at 2800 $\times$ g for 5 min at 4°C to pellet most of the zooxanthellae. The supernatant was centrifuged again at least 2 times for 10 min to pellet residual zooxanthellae (Muscatine et al. 1989), transferred to 50 ml Pyrex tubes and frozen (–20°C) pending subsequent analysis. Pellets of zooxanthellae were re-suspended, washed 3 times with filtered seawater to avoid any tissue contamination and frozen. Before isotopic measurements, tissue, zooxanthellae and *Artemia* sp. samples were treated overnight with 0.05 M H_3PO_4 to remove carbonates (Muscatine et al. 1989) and freeze-dried using a Heto (CT 60) lyophilizer.

Skeleton: At the end of the experiment, the skeleton deposited on the glass slide was removed with a scalpel (Reynaud-Vaganay et al. 1999), dried overnight at room temperature and stored in glass containers. Following the treatment described by Boiseau & Juillet-Leclerc (1997), each skeleton sample was ground and ultrasonicated for 1 min. The skeletal powder was then soaked in hydrogen peroxide (30% v:v) for 12 h to eliminate the organic matter, filtered on Nuclepore® polycarbonate membranes (0.4 µm) and dried at 40°C for 4 h.

Stable isotope measurements. *Organic carbon:* $\delta^{13}C$ was determined for coral tissue, zooxanthellae, and *Artemia* fractions. Samples were homogenized by grinding, weighed into tin capsules and analyzed on a PDZ Europa 20-20 mass spectrometer with an ANCA-SL combustion system. The instrument was operated in a continuous flow mode, in which the samples were subjected to an automated Dumas combustion followed by chromatographic separation of the resulting gases. Carbon isotopic ratios were referenced against standard material obtained from the US National Institute of Standards and Technology and adjusted to match the mass of the samples. The carbon reference material was sucrose (NIST SRM#8542). The fact that the reference material was in solid form permitted standards to be carried through the same automated analytical process as the samples. The value of $\delta^{13}C$ for each sample was calculated as follows:

$$\delta^{13}C \;=\; \left(\frac{(^{13}C/^{12}C)_{\text{sample}}}{(^{13}C/^{12}C)_{\text{standard}}} - 1 \right) \times 10^3 \qquad (1)$$

In practice, there was enough material for at least 3 analyses on each tissue sample and at least 5 analyses on each sample of zooxanthellae. The precision of the isotopic measurements (expressed as the standard deviation [SD]) was 0.2‰ for $\delta^{13}C$.

Carbon from seawater dissolved inorganic carbon (DIC) and seawater oxygen: Carbon isotopic composition of DIC in aquaria seawater was measured in samples collected once a wk and poisoned with $HgCl_2$ (1 ml of saturated $HgCl_2$ in 60 ml of seawater) to pre-

vent any further biological activity. The results of these measurements are also expressed in conventional $\delta^{13}C$ notation (Eq. 1). The reproducibility of carbon isotope measurements from seawater was $\pm0.07‰$ (SD). The oxygen isotopic composition of aquaria seawater samples was measured on a Finnigan MAT 252 and the results expressed according to the following formula:

$$\delta^{18}O = \left(\frac{(^{18}O/^{16}O)_{sample}}{(^{18}O/^{16}O)_{standard}} - 1 \right) \times 10^3 \qquad (2)$$

The reproducibility of the seawater $\delta^{18}O$ measurements was 0.05‰ (SD).

Skeleton: A subsample of 100 µg of aragonite powder was dissolved in 95% H_3PO_4 at 90°C. The $\delta^{13}C$ and $\delta^{18}O$ of the CO_2 gas evolved was analyzed using a VG Optima mass spectrometer with a common acid bath. The isotopic data are expressed in the conventional delta notation given above (Eqs. 1 & 2) relative to a PDbelemnite (PDB) standard. The intra-sample reproducibility combined with instrumental precision is 0.04‰ (SD) for $\delta^{18}O$ and 0.05‰ (SD) for $\delta^{13}C$ from skeletal material. As seawater $\delta^{18}O$ and $\delta^{13}C$ were constant over the time, the true change in skeletal $\delta^{13}C$ and $\delta^{18}O$, resulting solely from physiological and kinetic processes, can be calculated by subtracting

Table 1. *Stylophora pistillata*. Biomass and physiological variables measured at the end of the experiment for fed and starved colonies of Chl *a*: chlorophyll *a*; DW: dry weight

	Fed	Starved	n	p (*t*-test)
Protein mg (g DW)$^{-1}$	5.7 ± 0.7	1.3 ± 0.4	4	0.002
Chl *a* µg (g DW)$^{-1}$	12.7 ± 0.6	7.7 ± 0.4	4	0.006
Chl *c$_2$* µg (g DW)$^{-1}$	1.9 ± 0.3	1.2 ± 0.2	4	0.04

these values from the skeletal isotopic composition (Hut 1987, Bemis & Spero 1998).

Statistical analysis. The effect of feeding on biomass and physiological variables was assessed using *t*-tests, preceded by tests for normality. The degrees of freedom (df) are also given. All statistical analyses were carried out using the statistical package JMP 3.1.6 (SAS Institute). Results are reported as mean ± standard error of the mean (SE), and n is the sample size.

RESULTS

Physiological measurements

The amount of soluble protein was significantly different between treatments: fed colonies contained 4.5 times more protein than starved colonies (Table 1). The pigment content (chl *a* and *c$_2$*) was also significantly higher in fed than in starved corals.

The rate of net photosynthesis normalized to chl *a* was significantly lower in fed than in starved colonies (Fig. 1A). These rates normalized to surface area were not significantly different (p = 0.1, df = 5; Fig. 1B). Respiration rates normalized to surface area were not significantly different between treatments (p = 0.4, df = 6; Fig. 1B).

The rates of calcification measured after 12 wk of experiment were significantly different between treatments (p = 0.008, df = 55) with a higher rate in fed than in starved colonies (0.22 vs 0.17% d^{-1}; Fig. 2).

Seawater $\delta^{18}O$ and $\delta^{13}C_{DIC}$

The oxygen and carbon isotopic composition of seawater ($\delta^{18}O$ and $\delta^{13}C_{DIC}$) remained relatively constant, both during a diel cycle (1.28 ± 0.01‰ vs standard mean ocean water [SMOW], n = 3, and 0.95 ± 0.06‰ vs PDB, n = 4 respectively) and during the course of the experiment (1.29 ± 0.02‰ vs SMOW, n = 13, and 0.82 ± 0.09‰ vs PDB, n = 10).
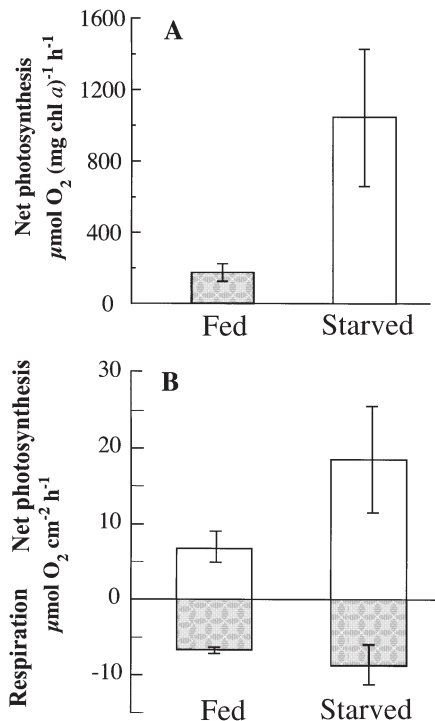

Fig. 1. *Stylophora pistillata*. (A) Net photosynthetic rate (µmol O$_2$ [mg chlorophyll (chl) *a*]$^{-1}$ h^{-1}) of fed and starved colonies. Mean ± SE, n = 4. (B) Net photosynthetic and respiration rates (µmol O$_2$ cm^{-2} h^{-1}) of fed and starved colonies. Mean ± SE, n = 4
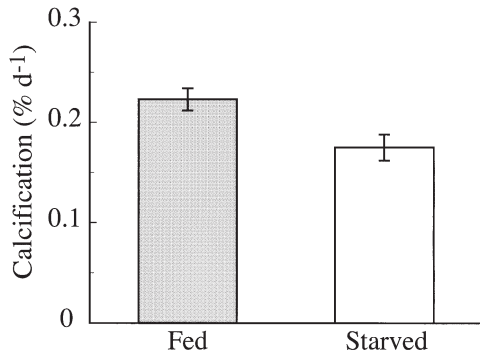
Fig. 2. *Stylophora pistillata.* Calcification rate (% d^{-1}) measured during the 12 wk of culture for fed and starved colonies. Mean ± SE, n = 33 for fed corals and n = 24 for starved corals

Fig. 3. *Stylophora pistillata.* δ^{13}C values of tissues and zooxanthellae for fed and starved colonies of (mean ± SE). n = 28 for fed colonies and n = 18 for starved corals. PDB: PDbelemnite standard

Isotopic composition of prey, coral tissue and zooxanthellae

The nauplii of *Artemia salina* used for coral feeding exhibited δ^{13}C values of –12.1 ± 0.2‰, within the range measured for the animal fraction. The δ^{13}C signature was significantly higher in zooxanthellae than in coral tissues (Fig. 3), for both fed (–10.11 ± 0.39‰ vs –11.69 ± 0.39‰) and starved colonies (–10.85 ± 0.36‰ vs –13.19 ± 0.23‰; *t*-test, p = 0.02, df = 41 and p < 0.001, df = 19, respectively). The δ^{13}C of host tissues were significantly higher in fed than in starved colonies (*t*-test, p = 0.02, df = 40; Fig. 3), whereas there was no significant difference for the zooxanthellae fraction (*t*-test, p = 0.2, df = 23).

Skeletal isotopic composition

There was no significant difference in skeletal δ^{13}C between treatments (*t*-test, p = 0.5, df = 37; Fig. 4). The average skeletal δ^{13}C was –4.6‰. Skeletal δ^{18}O, however, was significantly lower in fed than in starved colonies (–4.24 vs –4.05 ‰, *t*-test, p < 0.001, df = 38; Fig. 4). There was no relationship between skeletal δ^{13}C or δ^{18}O and the calcification rate measured for fed and starved colonies (data not shown).

DISCUSSION

Feeding effects on physiological variables

Feeding induced significant changes in several of the physiological parameters measured. The 29% increase in the calcification rate measured in fed corals confirms previous results showing higher calcification rates in fed than in starved corals (Jacques &
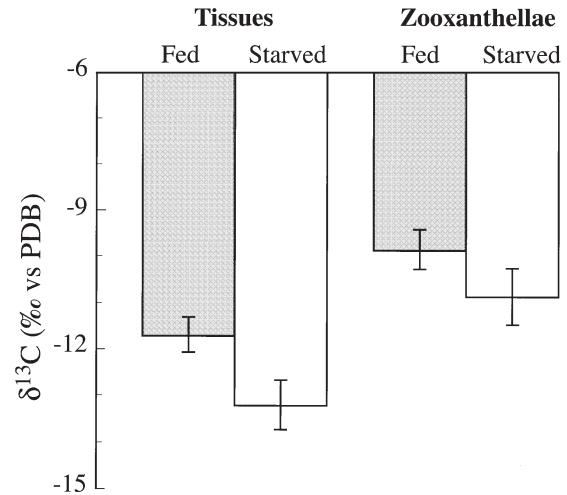
Pilson 1980, Kim & Lasker 1998, Anthony & Fabricius 2000). Fed colonies also exhibited higher levels of soluble protein, chl *a* and chl c_2 than starved corals, in agreement with some previous results (Stambler et al. 1991, Titlyanov et al. 1999). The increase in chlorophyll content may be due to an increase in symbiotic zooxanthellae densities following a food supply or to an increase in the amount of chlorophyll content per alga itself (Titlyanov et al. 1999). Since feeding altered the chlorophyll concentration per unit of skeletal weight, photosynthesis expressed per chloro-
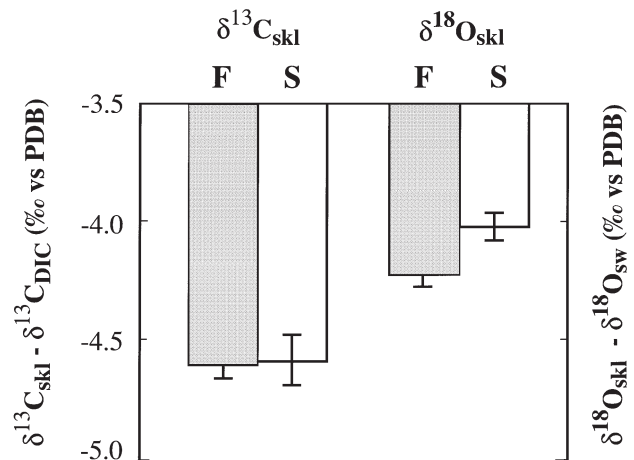

Fig. 4. *Stylophora pistillata.* Skeletal (skl) δ^{13}C and δ^{18}O in fed (F) (n = 24 and 25, respectively) and starved (S) colonies (n = 15 and 14, respectively). Mean ± SE. DIC: dissolved inorganic carbon; SW: seawater

phyll content was also different between treatments, but lower in fed than in starved corals, suggesting that the photosynthetic efficiency is lower when corals are fed. The rate of respiration was not affected by feeding, in contrast to the study of Jacques & Pilson (1980), who reported respiration rates 4 times higher in fed than in starved corals. This lack of stimulation of respiration by feeding may seem puzzling because some of the energy expenditures, such as calcification, were higher in fed corals. However, increased calcification does not necessarily imply a higher tissue growth rate and respiration. Moreover, the energetic cost of calcification is unknown in corals; its contribution to the rate of respiration could be limited and within the resolution limit of the respirometric technique used. Although this technique (respiration chambers with a vortex motion) is still widely used in coral ecophysiology experiments (Anthony & Fabricius 2000), it is known to create a boundary layer effect on mass transfer into the corals, which may have underestimated respiration rates (Dennison & Barnes 1988, Patterson et al. 1991). Moreover, Anthony & Fabricius (2000) did not find any increase in the respiration rates of corals fed suspended particulate matter. In our study, respiration rates were not measured during feeding, but the day after. It is therefore possible that a transient increase in the respiration rates has been missed.

Skeletal $\delta^{18}O$

Only one study has investigated the relationship between skeletal $\delta^{18}O$ composition and nutrition (Grottoli & Wellington 1999). These authors found no change in skeletal $\delta^{18}O$ with feeding, which disagrees with the results found in the present study. The skeletal $\delta^{18}O$ of *Stylophora pistillata* was significantly lower in fed than in starved colonies (–4.24 vs –4.05‰, respectively). This difference may, however, partly result from an indirect effect of the calcification rate. We explain this in terms of McConnaughey's kinetic isotopic disequilibria model. McConnaughey (1989) indeed reported an inverse relationship between linear extension and skeletal $\delta^{18}O$ in *Pavona clavus*. He found that kinetic isotope disequilibria tend to be fairly consistent in rapidly growing parts of photosynthetic corals. He therefore suggested that an oxygen isotopic disequilibrium occurs due to discrimination against the heavier isotopes during CO_2 hydration in the calcifying fluid, just before calcification. Such an inverse relationship between skeletal $\delta^{18}O$ and linear extension rate has also been reported in corals by 2 other investigators (Land et al. 1975, Allison et al. 1996). Allison et al. (1996) stated that this relationship is rarely apparent

unless the range of linear extension rates is large. The increase of 29% in calcification rate in fed corals measured in the present study may have induced the observed depletion of the skeletal $\delta^{18}O$.

Skeletal $\delta^{13}C$

Skeletal $\delta^{13}C$ values have been considered a good indicator of changes in irradiance in laboratory experiments (Weil et al. 1981) and of changes in DIC of seawater (Swart et al. 1996). Therefore, most paleoclimatic studies have considered corals to be mainly autotrophic for carbon. However, the following processes have also been shown to alter the skeletal $\delta^{13}C$: nutrition (Felis et al. 1998, Grottoli & Wellington 1999, Grottoli 2000), respiration (Swart et al. 1996) and coral spawning (Kramer et al. 1993, Gagan et al. 1994, 1996). Recently, it has been suggested that foraminiferal $\delta^{13}C$ can also be affected by the seawater pH (Spero et al. 1997) and temperature (Bemis et al. 2000). In the present experiment, light, seawater temperature and pH, $\delta^{13}C_{DIC}$ and the rate of respiration remained constant. No spawning event was observed. Therefore, the skeletal $\delta^{13}C$ signal could only have been altered by feeding.

In a previous study, Grottoli & Wellington (1999) found that skeletal $\delta^{13}C$ was affected by zooplankton. Reduction of zooplankton induced an increase in skeletal $\delta^{13}C$ of at least 0.5‰. In the present study, no significant difference was measured in skeletal $\delta^{13}C$ between fed and starved colonies of *Stylophora pistillata*. This could be explained by the relatively short duration of the experiment (12 wk), compared to the experiment of Grottoli & Wellington (1999), where corals were maintained 1 yr under feeding or starvation conditions. Feeding could also have a different impact on skeletal composition according to the coral species (*Pavona* sp. in Grottoli & Wellington 1999), but this remains to be tested. Another explanation is that the value of $\delta^{13}C$ of the *Artemia* sp. prey used in this study (–12‰) was high compared to values measured in natural zooplankton (–20‰ in Land et al. 1975 or –22‰ in Spero 1992). The change induced by feeding might therefore have been too small to be detectable. Moreover, in our experiment, feeding increased calcification rate, which itself increases skeletal $\delta^{13}C$ (Reynaud-Vaganay et al. 2001). Therefore, the effect of feeding on carbon fractionation may have been overwhelmed by the opposite effect of calcification. Grottoli (2000) concluded that changes in light accounted for almost 80% of the variation in $\delta^{13}C$ in the coral skeleton. In the present experiment, light was constant, which could explain the lack of difference between fed and starved corals.

Tissue $\delta^{13}C$

The $\delta^{13}C$ values of zooxanthellae and animal tissue of *Stylophora pistillata* (–10 to –13‰) were in the same range of values measured by Muscatine et al. (1989) on the same species at 1 m depth (–13 to –14‰), by Risk et al. (1994) on several other species (–10 to –16‰) and by Swart et al. (1996) on *Montastrea annularis* (–11.5 to –15.5‰). The consistently lighter carbon isotopic signature in animal tissues relative to the zooxanthellae is in agreement with differences (ca. 2‰) found in previous studies (Muscatine et al. 1989, Risk et al. 1994, Swart et al. 1996). These observations are compatible with a significant translocation of photosynthate from zooxanthellae to the host with an isotopic fractionation of more than 1‰ (Rau et al. 1983). The carbon isotopic signature of the animal tissue reflected a significant impact from the ingestion of prey ($\delta^{13}C$ = –12‰) because $\delta^{13}C$ of the tissue was higher in fed ($\delta^{13}C$ = –11.7‰) than in starved corals ($\delta^{13}C$ = –13.2‰).

$\delta^{13}C$ values of fed and starved colonies of *Stylophora pistillata* were comparable to those measured by Muscatine et al. (1989) for a set of corals sampled at 1 m depth. These authors have, however, measured higher $\delta^{13}C$ values for the same corals at greater depths, which are presumed to be more heterotrophic. Muscatine et al. (1989) explained this difference by a higher ratio of photosynthesis/respiration in surface than in deep water. In the high-light conditions used here, the photosynthate should be only slightly isotopically lighter than its carbon source due to the decrease in fractionation when photosynthesis is elevated and CO_2 levels are low (Laws et al. 1997). However, the source of carbon for photosynthesis is complicated by the fact that the carbon can be supplied from bicarbonate in the DIC pool in tissues or from metabolic CO_2 (this isotopic value was not measured in the present study, but due to fractionation should be lighter than the coral tissue it is derived from). The availability of carbon for photosynthesis provides a feedback from the isotopic effects of feeding on coral tissue to the isotopic signature of the zooxanthellae. Therefore, under low light, the relatively lighter isotopic carbon values in animal fraction (as compared to high light conditions) can result from lower photosynthetic rates or a greater reliance on particulate feeding (Muscatine et al. 1989).

Inferred carbon flow in experimental system

A schematic interpretation of carbon flow between the different reservoirs of a zooxanthellate scleractinian coral is shown in Fig. 5. However, it must be kept in mind that this work was performed with a single species and must be completed with other observations.

Seawater bicarbonate ($\delta^{13}C \approx 0.8‰$) is subject to various processes (dehydration into CO_2, diffusion through the ectodermal and endodermal layers) before reaching the vicinity of zooxanthellae (Furla et al. 2000). Isotope fractionation is involved during each of these processes, with the larger fractionation (–7‰) occurring during dehydration (Deuser & Degens 1967). CO_2 subsequently goes through the animal and algal layers and reaches the zooxanthellae (average $\delta^{13}C \approx$ –10.5‰). A large fraction of the algal photosynthate is then translocated to the animal cells ($\delta^{13}C$ of ca. –11.7 and –13.2‰, respectively, in fed and starved colonies), is used for mitochondrial respiration and can be incorporated into the skeleton. It is assumed (Muscatine et al. 1989) that the $\delta^{13}C$ of metabolic CO_2 that mostly influences skeletal $\delta^{13}C$ is similar to, and probably slightly lighter than, that of the tissue from which it is derived (average value of –12.5‰ in the present study). Some mixture of this carbon and bicarbonate from seawater produces a skeletal $\delta^{13}C$ value of –4.6‰. Here, the ability of prey carbon to affect isotopic values of the entire colony is emphasized as the isotopically light carbon is selectively translocated back to the coral tissue. In the present work, the carbon isotopic difference between host tissue and algae was lower in fed than in control colonies (1.6 vs 2.3‰). Because natural zooplankton typically have an isotopic composition lighter than that of the food used here, the
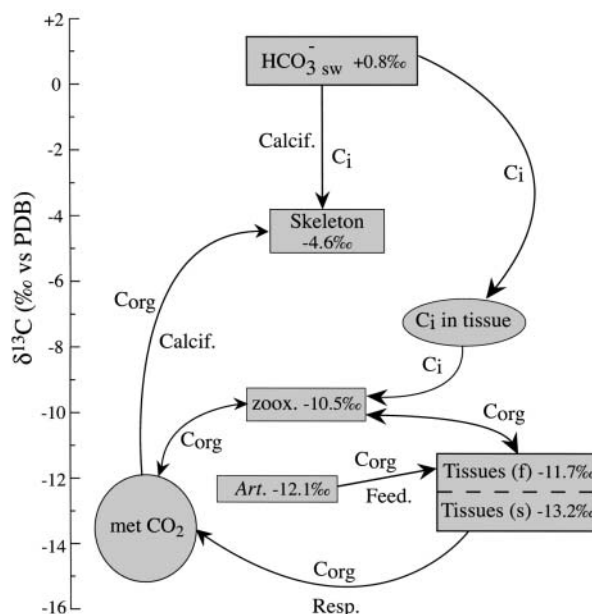

Fig. 5. *Stylophora pistillata*. Model of carbon flow between several compartments. Square boxes represent measured pools, whereas rounded boxes represent inferred isotopic values. *Art.*: *Artemia*; calcif.: calcification; C_i: inorganic carbon; C_{org}: organic carbon; feed.: feeding; met.: metabolic; resp.: respiration; zoox.: zooxanthellae

zooxanthellae using metabolic CO_2 derived from natural zooplankton may be significantly isotopically lighter that the values that we measured. Therefore, the carbon isotopic signature of coral tissue may be a useful indicator of the feeding strategy of *Stylophora pistillata*, as has been found by other investigators (Muscatine et al. 1989, Risk et al. 1994). In high light, the zooxanthellae would tend to be carbon-limited, and the relatively heavy isotopic signal would be transferred to the animal tissue via the translocated carbon. In low light, the animal tissue would largely be limited to carbon derived from prey that would impart a lighter isotopic signature to the animal tissue.

Few studies have investigated in the same experiment the effect of feeding on carbon isotopic composition of the tissues, the zooxanthellae and the skeleton of a scleractinian coral. Most of the existing data were obtained on corals collected *in situ*, where several environmental parameters vary at the same time. Laboratory experiments such as the one presented in this paper allow better control of the physico-chemical parameters and therefore a better understanding of the various factors in isolation. Our work suggests that prey was a significant source of carbon for *Stylophora pistillata*. Skeletal $\delta^{13}C$ was not different between fed and starved colonies; the isotopic consequence of feeding could be obscured by the higher calcification rate in fed corals. It is suggested that the use of skeletal $\delta^{13}C$ should be carefully considered prior to using it as a proxy of paleoproductivity. Skeletal $\delta^{18}O$ was different between the 2 groups of corals, confirming a possible relationship between ^{18}O fractionation and coral growth.

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