Effect of feeding on the carbon isotopic composition of the zooxanthellate coral *Stylophora pistillata*

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INTRODUCTION

Corals are known to flourish in oligotrophic tropical water, and can be considered as "mixotrophic" (both auto- and heterotrophic) organisms. They are able to fix inorganic carbon through the photosynthetic activity of their zooxanthellae. They may also derive a fraction of their energy through the predation of bacterioplankton and zooplankton or through the use of dissolved organic matter. The relative importance of autotrophy and heterotrophy in the corals' nutrition has been a source of considerable controversy. The use of stable isotopes provides insight into this problem (Risk *et al*., 1994; Sammarco *et al*., 1999). The carbon isotopic signature ($\delta^{13}C$) of the tissues is a tracer of food sources and enables quantification of carbon fluxes between different trophic levels.

The carbon isotopic composition of coral skeletons is also widely used as indicator of environmental parameters. The main physiological processes that potentially alter skeletal $\delta^{13}C$ are photosynthesis and its variation with light (Swart *et al*., 1996; Juillet-Leclerc *et al*., 1997, Reynaud-Vaganay *et al*., 2001), respiration (Swart *et al*., 1996), and feeding (Grottoli & Wellington, 1999). Most studies have demonstrated that an increase in the rate of photosynthesis induces an increase in skeletal $\delta^{13}C$ (Swart *et al*., 1996; Juillet-Leclerc *et al*., 1997; Reynaud-Vaganay *et al*., 2001). However, feeding could have an opposite effect since zooplankton carbon typically has a low $\delta^{13}C$ signal compared to the skeleton. Therefore, the interpretations of skeletal $\delta^{13}C$ value have to take into account these two processes.

The aims of this study are to investigate the carbon sources and fluxes in the zooxanthellate scleractinian coral (*Stylophora pistillata*), and also 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 of the skeleton, during controlled experiments.

MATERIALS AND METHODS

The experiment was conducted in the laboratory using colonies of *Stylophora pistillata*. The tips were glued on glass slides as described by Reynaud-Vaganay *et al*. (1999). All the tanks were continuously supplied with filtered (0.45 µm) Mediterranean seawater. Half of the "nubbins" were fed 3 times a week during 12 weeks with 5 g of *Artemia salina* nauplii. At th end of the experiment, the skeleton deposited on the glass slide was then removed with a scalpel.

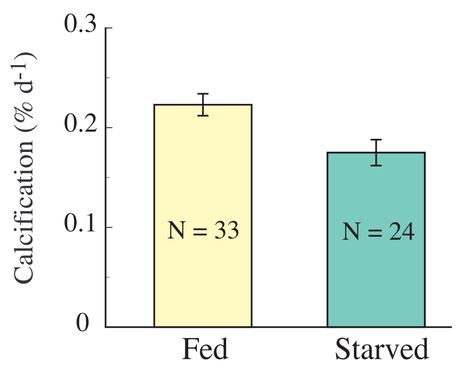
Tissue was removed from skeleton with an "air pick", and homogenized with a Potter tissue grinder. The homogenate was then centrifuged at 2800 g for 5 min at 4°C to pellet most of the zooxanthellae. The supernatant was centrifuged again at least two times for 10 min to pellet residual zooxanthellae (Muscatine *et al*., 1989), and frozen.

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* samples were treated overnight with 0.05 M H_3PO_4 to remove carbonates and freeze-dried

$\delta^{13}C$ of tissue, zooxanthellae, and *Artemia*. 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 carbon reference material was sucrose (NIST SRM#8542). The precision of the isotopic measurements was 0.2‰.

Skeletal $\delta^{13}C$: 100 µg of aragonite powder was dissolved in 95% H_3PO_4 at 90°C. The $\delta^{13}C$ of the CO_2 gas evolved was analyzed using a VG Optima mass spectrometer. The intra-sample reproducibility combined with instrumental precision is 0.05‰ (sd). As seawater $\delta^{13}C$ wa constant over the time, by subtracting these values from the skeletal isotopic composition, the true change in skeletal $\delta^{13}C$ resulting solely from physiological and kinetic processes, can be calculated (Bemis & Spero, 1998).

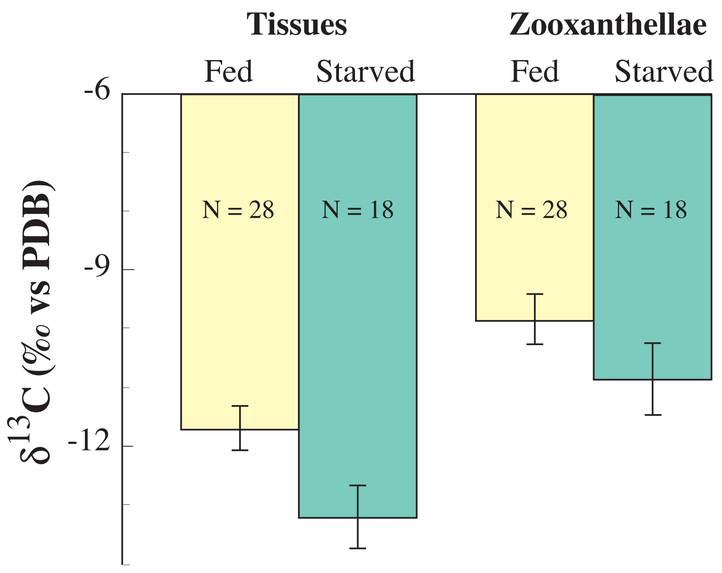
RESULTS



Calcification rate (% d^{-1}) of *Stylophora pistillata* measured during 12 weeks of culture for fed and starved colonies. Mean ± standard error of the mean.

- ✔ The rate of calcification increases by 29% in fed corals.
- ✔ The amount of protein /g DW is 4.4 higher in fed colonies than in starved colony.
- ✔ The chlorophyll /g DW is 1.6 times higher in fed corals compared to starved corals.

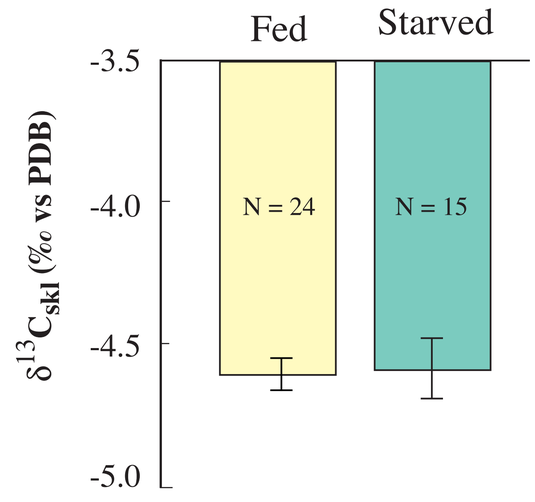
The increase in chlorophyll content may be due to an increase in symbiotic zooxanthellae densities following a food supply or to an increase in the amount of chlorophyll content per alga itself.



$\delta^{13}C$ values of tissues and zooxanthellae for fed and starved colonies of *Stylophora pistillata* (mean ± standard error of the mean).

- ✔ 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‰) compared to starved corals ($\delta^{13}C$ =-13.2‰).
- ✔ There was no significant difference for the zooxanthellae fraction between fed and starved corals.

These results 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).



Skeletal $\delta^{13}C$ in fed and starved colonies of *Stylophora pistillata*. Mean ± standard error of the mean.

There was no significant difference in skeletal $\delta^{13}C$ between treatments

Grottoli & Wellington (1999) however found that a reduction of zooplankton induced an increase in skeletal $\delta^{13}C$. The difference between these two results can be explained:

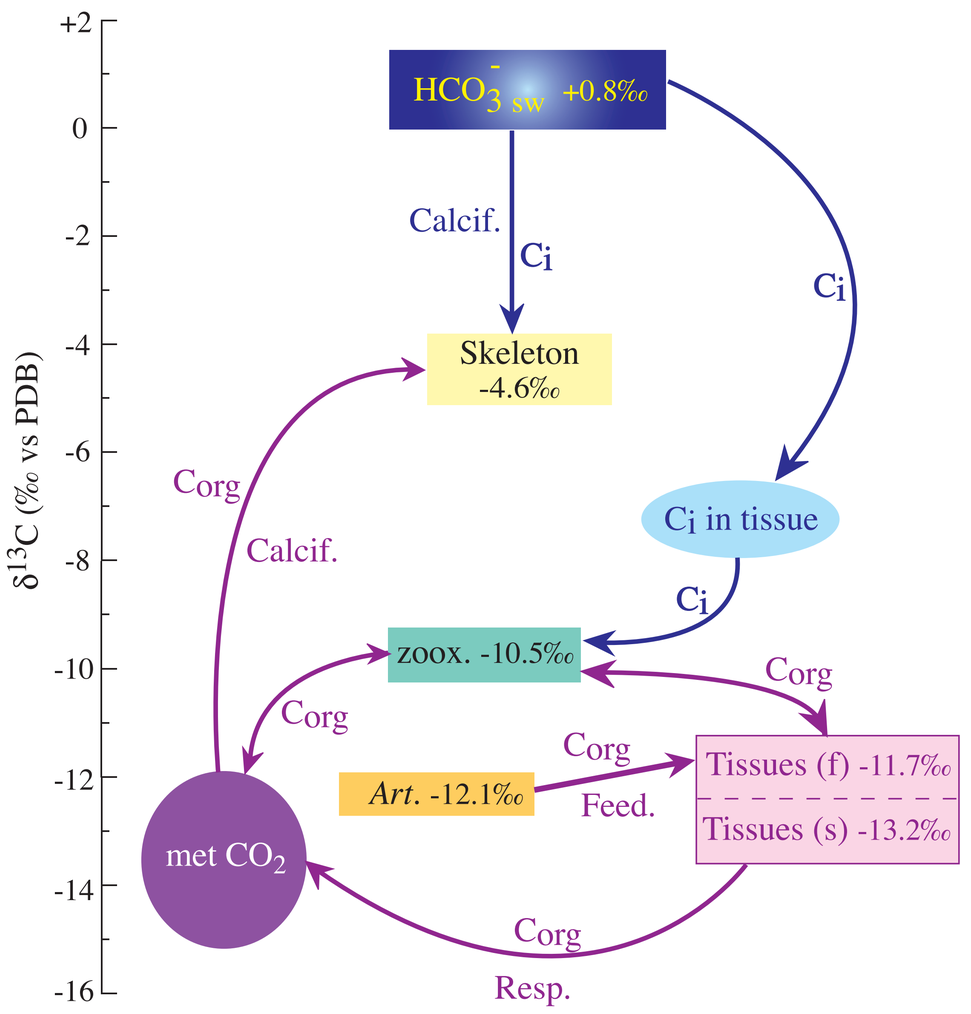
- ✔ by the short duration of this experiment (12 weeks), compared to one year in Grottoli & Wellington (1999).
- ✔ by the coral species (*Pavona* sp. in Grottoli & Wellington, 1999).
- ✔ the value of $\delta^{13}C$ of the *Artemia* prey used (-12‰) was high compared to values measured in natural zooplankton (-22‰ in Spero, 1992). The change induced by feeding might have been too small to be detectable.
- ✔ feeding increases 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.

MODEL

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. 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), used for mitochondrial respiration, and can be incorporated into the skeleton.

The $\delta^{13}C$ of metabolic CO_2 that mostly influences skeletal $\delta^{13}C$ is similar to 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 of the food used here, the zooxanthellae using metabolic CO_2 derived from natural zooplankton may be significantly isotopically lighter that the values that we measured. 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.



Model of carbon flow among several compartments in *Stylophora pistillata*. Square boxes represent measured pools, whereas circles represent inferred isotopic values. (Ci: inorganic carbon, Corg: organic carbon, calcif.: calcification, resp.: respiration, feed.: feeding, zoox.: zooxanthellae, sw: seawater, Art.: *Artemia*, f: fed, s: starved, met.: metabolic).

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Acknowledgements

Thanks are due to N. Lebec for her help with the mass spectrometer, B. Mace for carbon isotopic analyses, and to H. J. Spero for measurements of carbon isotopic composition of *DIC* in aquaria seawater. Thanks are also due to P. Furla and D. Allemand for fruitful discussions, and to the staff of the Monaco public aquarium for providing corals. This work was partially supported by NSF grant #9819151 to R. Sambrotto.