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Light effects on the isotopic fractionation of skeletal oxygen and carbon in the cultured zooxanthellate coral, *Acropora*: implications for coral-growth rates

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Abstract

Skeletal isotopic and metabolic measurements of the branching coral *Acropora* cultured in constant conditions and subjected to two light intensities were revisited. We individually compared the data recorded at low light (LL) and high light (HL) for 24 colonies, all derived from the same parent colony. Metabolic and isotopic responses to the different light levels were highly variable. High light led to productivity enhancement, reduction of surface extension, doubling of aragonite deposited weight and increased $\delta^{18}O$ levels in all nubbins; responses in respiration and $\delta^{13}C$ were not clear. The partitioning of the colonies into two groups, one showing a $\delta^{13}C$ increase and the other a $\delta^{13}C$ decrease with increased light, revealed common behaviors. Samples showing an increase in $\delta^{13}C$ were associated with the co-variation of low surface extension and high productivity while samples showing a decrease in $\delta^{13}C$ were associated with the co-variation of higher surface extension and limited productivity.

This experiment, which allowed for the separation of temperature and light effects on the coral, highlighted the significant light influences on both skeletal $\delta^{18}O$ and $\delta^{13}C$. The high scattering of inter-colony $\delta^{18}O$ observed at one site could be due to the differing photosynthetic responses of symbiotic algal assemblages.

The $\delta^{13}C$ responses could also be related to differing algal distributions in different skeletal portions. Our results were compared to observations by Gladfelter on *Acropora cervicornis* (1982). Both set of results highlight the relationships between coral-growth rates, micro-structures and photosynthetic activity. It appears that extension growth and accretion are two separate growth modes, and accretion is light-enhanced while extension is light-repressed. There are multiple consequences of these findings for paleoclimatic reconstructions involving corals.

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1 Introduction

As early as 1972, Weber and Woodhead demonstrated that the variability in oxygen-isotope compositions ($\delta^{18}O$) of scleractinian coral skeletons was essentially due to sea-surface temperature (SST), although showing very negative values compared with the isotopic equilibrium (Epstein et al., 1953). Correlations of SST with aragonite $\delta^{18}O$ also differed among coral genera (Weber and Woodhead, 1972). Some years after, analysis of samples taken along the main growth axis of a coral head revealed that monthly $\delta^{18}O$ signals were correlated with seasonal SST and seawater $\delta^{18}O$ variations (Fairbanks and Dodge, 1979; McConnaughey, 1989a). This has been the working hypothesis for paleoclimatic reconstructions of SST from coral $\delta^{18}O$ measurements (e.g., Cole et al., 1993; Quinn et al., 1993; Dunbar et al., 1994). However, many heads of *Porites lobata* growing in close proximity at Clipperton Atoll in the eastern Pacific showed isotopic discrepancies of up to 0.4‰ (Linsley et al., 1999), equivalent to a 2°C isotopic effect for the same period using the $\delta^{18}O$/SST relationship estimated by Gagan et al. (1994). This discrepancy reached 1.28‰ (more than 6°C) for *Porites spp.* from the Gulf of Aqaba (Felis et al., 2003). It was concluded that ^{18}O concentrations were also colony dependent, this effect being commonly called the "vital effect" (Urey et al., 1951). Moreover, isotopic profiles may also change according to the axis sampled on a single coral head (Maier et al., 2004). Such sources of variability could strongly compromise the validity of $\delta^{18}O$ as an accurate environmental proxy.

Compared to the skeletal $\delta^{18}O$ signature, interpretation of the variability of the carbon isotopic ratio ($\delta^{13}C$) within coral skeletons has long been a matter of debate. $\delta^{13}C$ variability has therefore scarcely been considered for climatic purposes (Guzman and Tudhope, 1998). In contrast to $\delta^{18}O$, which was assumed to essentially depend on external factors, $\delta^{13}C$ has been generally considered as affected by coral physiology either via respiration rate (McConnaughey et al., 1997), or via the photosynthetic activity of the symbiotic zooxanthellae (Swart, 1983; McConnaughey, 1989a). Different observations led Goreau (1977) and Erez (1978) to propose two different models to ex-

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plain carbon isotopic fractionation. The first author observed that $\delta^{13}C$ increased with increased light level. Indeed, algae and coral were thought to extract their inorganic carbon from the same reservoir for both photosynthesis and calcification processes. As photosynthesis is the faster reaction, lighter carbon isotopes were used preferentially; thus, the reservoir enriched in ^{13}C caused a $\delta^{13}C$ increase with light (Goreau, 1977). The second author, after the observing the opposite, i.e., that $\delta^{13}C$ decreased with light, proposed that during intense photosynthetic activity, it was possible that corals incorporated depleted metabolic carbon into their skeleton (Erez, 1978).

To explain the positive correlation between oxygen and carbon ratio, McConnaughey (1989a,b) assumed that the kinetic isotopic fractionation was strongly linked with calcification rate. After these publications, several theoretical models were put forth explaining how the observed coral-skeleton isotopic fractionations were derived from a combination of kinetic and metabolic effects (Heikoop et al., 2000; McConnaughey, 2003; Omata et al., 2005).

Experiments conducted in the laboratory by Weil et al. (1981) showed a negative correlation between $\delta^{13}C$ and light, i.e., the supply of autotrophic energy in the coral *Montipora*. Conversely, field experiments conducted by Swart et al. (1996) exhibited only weak correlations between skeletal $\delta^{13}C$ and the supply of autotrophic energy, measured as the P/R ratio (photosynthesis/respiration). Grottoli and Wellington (1999) later found a negative correlation between skeletal $\delta^{13}C$ and the heterotrophic energy supply in the zooplankton and a positive correlation with light, i.e., the autotrophic energy supply. In addition, $\delta^{13}C$ variability seemed decoupled from coral growth (Grottoli, 2002). Among these studies, only the laboratory experiments of Weil et al. (1981) deciphered the relations between the light and temperature effects and could document the effect of a single factor on metabolic activity and thus provide clear responses for the isotopic fractionations of oxygen and carbon.

It has been generally assumed that the geochemical response derived from several colonies is more significant than data provided by a single colony. Thus, authors have usually considered averaged metabolic and chemical data from several colonies

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(Grottoli and Wellington, 1999; Reynaud-Vaganay et al., 1999; Reynaud-Vaganay et al., 2001; Grottoli, 2002; Suzuki et al., 2005). However, individual metabolic and isotopic responses can differ markedly. For instance, *Acropora* nubbins collected from a single parent colony and cultured in controlled SST conditions exhibited an inter-colony variability of 1‰ (Reynaud-Vaganay et al., 1999). This has been confirmed for cultured *Porites* sp. (Suzuki et al., 2005), which showed similar variability at various temperature settings. In culture experiments, as in the field, coral $\delta^{13}C$ showed larger inter-colony variability than $\delta^{18}O$, often ≥2‰ (Reynaud-Vaganay et al., 1999; Suzuki et al., 2005).

The present work is based on data previously published by Reynaud-Vaganay et al. (2001), which examined the effect of light on the mean skeletal isotopic signatures ($\delta^{18}O$ and $\delta^{13}C$) of several nubbins of *Acropora* sp. The effect of light was also measured on metabolic activities, such as photosynthesis, respiration, calcification rate and surface extension. In contrast with the preceding study, we now examined the individual coral responses. The results of this experiment conducted on *Acropora* were then compared with observations made by Gladfelter (1982) and we considered the possible relationship between skeletal growth and the relative roles of two crystalline microstructures. Finally, for climatic purposes, we compared the effects of a change in light intensity on a branched colony versus the time response of samples collected along the main growth axis of *Porites*.

2 Materials and methods

2.1 Biological materials

The experiment was conducted in the laboratory using colonies of the branching, zooxanthellate scleractinian coral, *Acropora* sp. Tips from 24 branches were sampled from a single parent colony. The specimens were glued onto glass slides (3×6×0.2cm) using underwater epoxy (Devcon®) as described by Reynaud-Vaganay et al. (1999),

and randomly distributed in two aquaria (15 L). The tanks were supplied with heated Mediterranean seawater (24°C) pumped from a depth of 50 m. The seawater renewal rate was approximately five times per day and the seawater was continuously mixed with a Rena® pump (6 L min^{-1}). Metal halide lamps (Philips HPIT, 400 W) provided light of 260 or 130 $\mu mol m^{-2} s^{-1}$ on a 12:12 photoperiod. Seawater was continuously aerated with outside air. The culture temperature (25°C) was controlled to within ±0.1°C using a temperature controller (EW, PC 902/T).

All colonies were initially cultured for six weeks under a light intensity of 130 $\mu mol m^{-2} s^{-1}$ (referred to as Low Light, LL). Thereafter, colonies were cultured for six additional weeks under a light intensity of 260 $\mu mol m^{-2} s^{-1}$ (High Light, HL). At the completion of each period and for the determination of isotopic composition, the newly deposited ring skeleton formed on the glass slide was collected with a scalpel (Reynaud-Vaganay et al., 1999), dried overnight at room temperature and stored in glass containers pending isotopic analysis.

2.2 Measurements of environmental parameters

Irradiance was measured using a 4-π quantum sensor (Li-Cor, LI-193SA) once a week (Table 1). Temperature (precision: ±0.05°C) was logged at 10-min intervals using a Seamon® temperature recorder.

Light intensities used in this experiment (130 and 260 $\mu mol photons m^{-2} s^{-1}$) correspond to 5.6 and 11.2 $mol m^{-2} d^{-1}$, respectively. Davies (1991) estimated that during a typical sunny day on a tropical reef a coral receives about 14.4 $mol m^{-2} d^{-1}$ of sunlight at a depth of 3 m in turbid water. On a cloudy day, the coral receives about 6.2 $mol m^{-2} d^{-1}$ of sunlight. Thus, the light intensities in our experiments imitate a range from quite low natural illumination levels to very strong natural illumination levels.

2.3 Photosynthesis and respiration

Photosynthesis and respiration were measured using the respirometry technique, which consisted of measuring the changes in oxygen concentration during the incubation.

The experimental sequence was identical for each coral: each nubbin was taken from the culture aquarium, placed in a Perspex chamber (240 mL) containing filtered seawater, for a 30-min pre-incubation in the light (130 or 260 μmolphotons$m^{-2}s^{-1}$, depending on culture condition). The colony was then incubated for 1 h in the same chamber to measure the rate of photosynthesis. The chamber was then flushed and the coral pre-incubated for 30 min in the dark and then for 1 h in the dark to measure the respiration rate. During the incubation, the medium was continuously agitated using a magnetic stirrer and was changed after each incubation.

The respirometric chamber was kept at 25°C in a thermostatic water bath. All incubations took place between 08:00 and 14:00 LT. The colonies were subsequently returned to the culture aquarium. Oxygen concentration was monitored in the chamber and recorded every 1 min using a data-logger (LI-1000, Li-Cor Inc.). Dissolved O_2 was measured using a Ponselle polarographic electrode calibrated daily against air-saturated seawater (100%) and a saturated solution of sodium sulfite (zero oxygen). Rates of net photosynthesis and respiration were estimated using a linear regression of O_2 against time. Photosynthesis and respiration values were then normalized to the skeletal surface area as estimated by the aluminum-foil technique (Marsh, 1970).

Gross photosynthesis was calculated using the following formula:

$$P_g = \frac{12 \times P_n}{24 \times R}$$

Where P_g is the gross photosynthesis, P_n the net photosynthesis and R the respiration rate.

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2.4 Calcification

Corals were weighed using the buoyant-weight technique (Jokiel et al., 1978; Davies, 1989) at the beginning and at the end of the experiment. The calcification rate was measured using the following formula:

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

Where G is the calcification rate, n is the number of the culture days, P_n is the dry weight after n days of culture and P_0 is the initial dry weight.

2.5 Isotopic measurements

The δ^{18}O values were calibrated against the isotopic values determined by conventional methods using an Optima-VG mass spectrometer. Results are given in the conventional notation, expressed as per mil (δ‰) against the V-PDB standard (Vienna Pee Dee Belemnite), where:

$$\delta(\text{sample}) = ((R_{\text{sample}} - R_{\text{Standard}}) - 1) \times 10^3$$

The external precision, estimated using an internal standard, was ±0.11 and 0.08‰ vs. V-PDB for carbon and oxygen, respectively. The reproducibility of carbon and oxygen isotopic measurements, calculated from replicate coral samples, was 0.10 and 0.08‰ vs. V-PDB, respectively.

3 Results

All data are given in Table 1. This new interpretation of the data reported in Table 1 was based on a comparison of the behavior of each nubbin. Although nubbins were collected from a single parent colony, all measured parameters were highly variable.

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Measurements of some parameters were lacking due to difficulties related with experimental conditions (Table 1). Herein, we examined metabolic and isotopic data provided by a single colony successively submitted to the two light conditions (from LL to HL; Fig. 1). We note that the surface extension, used to normalize the metabolic measurements, is roughly proportional to linear extension and thus essentially reflects the extension rate of a colony.

3.1 The effect of light

For most of the parameters, although the data are highly scattered, all nubbins showed a similar response. At HL, all nubbins showed an increase or a slight change in net productivity expressed in μmolcm^{-2}h^{-1} (Fig. 1a,b). The P_{net} variability was much higher at HL (the amplitude was >2μmolcm^{-2}h^{-1}) than at LL (0.5μmolcm^{-2}h^{-1}). Respiration variability was high for both light conditions, from −0.6 to −0.2μmolcm^{-2}h^{-1}. Calcification rate (in terms of percentage of growth per day) was always two- to threefold higher at HL than LL, while for each colony, surface extension was lower at HL than at LL (Fig. 1a,b). The range of surface extension was highly variable among the nubbins. Since LL and HL incubation duration are equivalent, we may compare these two. The same value of surface extension could be reached by a colony cultured either at LL or at HL (Fig. 1a,b). For δ^{18}O values, a colony developed at LL was always depleted in ^{18}O compared to the same colony grown at HL. The δ^{13}C change was more confusing, with half of the nubbins exhibiting higher values and the others lower values with the increase in light level (Fig. 1a,b).

We note that all the nubbins submitted to a single forcing exhibited common behaviors for P_{net}, calcification, surface extension and δ^{18}O (Fig. 1a). Despite their high variability, this observation strengthens the significance of the metabolic response of the colonies compared with a study considering only the averages across several nubbins. We conclude that the light effect is clearly separated from temperature influence for almost all metabolic parameters and δ^{18}O, except for respiration and δ^{13}C responses.

3.2 Partitioning into two groups

We divided the results from the nubbins into two groups: those showing a $\delta^{13}C$ increase from LL to HL (the expected response according to the global carbon-pool assumption, Goreau, 1977) and those showing depletion (Erez, 1978) (Fig. 1c). By examining the responses at HL, we realized that metabolic and isotopic values shown by these two groups presented common features (Fig. 1c). The averaged values calculated after partitioning were more significant than earlier values published for all the colonies (Fig. 1b) (Reynaud-Vaganay et al., 2001).

At LL, the two groups exhibited similar P_{net}, respiration, growth rate and $\delta^{18}O$ values, but values were more scattered for surface extension and $\delta^{13}C$ (Fig. 1c). At HL, only respiration and growth rate were comparable for the two groups (Fig. 1a,c, clear and dark bars). Colonies displaying higher $\delta^{13}C$ at HL (Fig. 1a,c, clear bars) showed higher photosynthetic activity and the most enriched $\delta^{18}O$ and $\delta^{13}C$ values (Fig. 1b). Surface extensions of the nubbins showing lower $\delta^{13}C$ (Fig. 1a, dark bar) were almost all greater than that measured on the nubbins showing higher $\delta^{13}C$ but always lower than at LL. For the two groups (Fig. 1a, clear and dark bars), the weight of colonies at least doubled during the incubation, while surface extension was reduced by ca. 40% (Fig. 1c). The $\delta^{18}O$ of all colonies decreased (Fig. 1c), the depletion being more pronounced for colonies showing an increase in $\delta^{13}C$ (Fig. 1a, clear bar).

We noticed that all intermediate values between the highest and lowest $\delta^{13}C$-differences between LL and NL were recorded. This explains why the difference in the $\delta^{13}C$ average (Fig. 1b) was not significant (Reynaud-Vaganay et al., 2001).

3.3 Duality of metabolic and isotopic responses at HL

The partitioning of the colonies into two groups underlines various, previously ignored, metabolic responses. At HL, the net productivities (and surface extensions) measured for the nubbins showing lower $\delta^{13}C$ were clearly lower (and higher) than the others

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(Figs. 1a,c and 2). It also appears that the $\delta^{18}O$ of those nubbins showing lower $\delta^{13}C$ were lower (Fig. 1b).

Comparing net productivity and surface extension at HL (Fig. 3), the highest photosynthetic activities corresponded with the lowest surface-extension values. Colonies showing higher $\delta^{13}C$ (dark symbols) displayed a linear correlation between net productivity and surface extension.

$$\text{surface} = -0.17 * P_{net} + 2.48 \text{ with } R^2 = 0.48 \quad \text{for } N = 7 \quad (1)$$

(where N is the number of nubbins)

$$\text{surface} = 0.01 * P_{net} + 0.25 \text{ with } R^2 = 0.46 \quad \text{for } N = 8 \quad (2)$$

(for the others)

The correlation coefficients were not significant ($P>0.95$); however they do not invalidate the suggestion of two different behaviors related to light intensity. Under HL, corals may display one of two behaviors linked with photosynthetic activity: either photosynthesis activity slightly increases and surface extension remains noticeable (although lower than at LL) or photosynthesis is clearly enhanced and surface extension strongly reduced. Such a relationship cannot be related to the metabolic normalization taking into account the surface of newly formed skeleton; indeed, the units of productivity are $\mu molO_2 m^{-2} s^{-.1}$.

By comparing photosynthetic activity and respiration, these two metabolic activities were correlated for colonies showing the highest photosynthesis ($P_{net}>0.6$) (Fig. 2). However, as there were only four data points, this relationship needs to be confirmed by additional experiments. There was no correlation between isotopic data and metabolic indicators (Fig. 2).

For $\delta^{13}C$ versus $\delta^{18}O$, there was no relation at LL (full circles) but at HL (empty circles) the relationship was significant ($P>0.95$):

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$$\delta^{13}C = 1.92 * \delta^{18}O + 3.41 \text{ with } R^2 = 0.86 \quad (3)$$

(Fig. 4).

We note that nubbin $\delta^{18}O$ values were also more positive in those showing a more positive $\delta^{13}C$ at HL than at LL (clear circles), whereas the other $\delta^{18}O$ values were roughly within the same range as for LL (Figs. 2 and 4). Indeed, at HL few $\delta^{18}O$ showed lower values than −3.0‰.

We highlighted the unexpected light effects on $\delta^{18}O$ (Reynaud-Vaganay et al., 2001). The discrepancies in metabolic behavior at HL exhibited by $\delta^{13}C$ variability were also not expected. We sought to explain how modifications in the host metabolism may impact chemical properties of the coral skeleton and to understand what could induce two distinct growth mechanisms.

4 Discussion

This experiment confirmed that calcification is light-enhanced (Goreau and Goreau, 1959; Chalker, 1981; Gattuso et al., 1999) because each nubbin showed an increased calcification rate with increased light. All nubbins at least doubled their initial weight, but the light effect on surface extension was the opposite to that of the calcification rate.

4.1 Metabolic imprint on skeleton chemistry

The culture technique used here allowed us to separate temperature from light effects on the skeletal isotopic signature and metabolic parameters. Clode and Marshall (2004) earlier tested the role of light on the calcification rate of a zooxanthellate (*Galaxea*) and azooxanthellate (*Dendrophyllia*) coral, using the ^{45}Ca technique. *Galaxea* and *Dendrophyllia* presented similar Ca incorporation versus temperature

over the range of 18–29°C, and they concluded that the calcification process was affected by temperature but was probably not associated with photosynthesis. This previous experiment, however, used different species displaying different metabolic specificities and temperature and light effects were mixed. Thus, it could not demonstrate that photosynthetic influences due to the presence of symbiotic algae were solely responsible. Other factors could explain the difference of Ca incorporation, e.g., temperature or differences in calcification rate among species. Conversely, our experiment avoided the temperature effect and examined the response of corals originating from one species, even generated by a single parent colony, thus all presenting identical metabolic and morphological characteristics.

As the experiment was conducted on nubbins originating from a single parent colony the isotopic scattering observed may be compared with inter and even intra-colony $\delta^{18}O$ variability exhibited by *Porites* collected at Clipperton (Linsley et al., 1999) or in Indonesia (Maier et al., 2004). Moreover, by considering isotopic effects of a single nubbin successively submitted to two light intensities we were able to follow skeletal $\delta^{18}O$ variation along a corallite representing mineral deposited during two successive months. The results obtained showed that by the average of several responses mask the significance of the individual behavior (Weil et al., 1981; Grottoli and Wellington, 1999; Grottoli, 2002).

4.2 Light effects on growth

Increased light was systematically associated with an increase of skeletal weight and a decrease of surface extension. The coupled effects generated an increase in skeletal density. *Acropora* usually does not show clear annual density bands; however, this experiment presents proof that light affects *Acropora* density. This could also be related to observations made on *Porites* most often producing low-density bands during winter (Lough and Barnes, 2000), but it is important to keep in mind that, in the field, light effects may be obscured by other factors such as temperature and/or reproductive cycles (Mendes, 2004).

The light effect observed can provide an explanation for data from the Caribbean Sea. When *Montastrea annularis* was submitted to unfavorable conditions (lower light and anthropogenic influence), they appeared to "sacrifice skeletal density while maintaining or increasing skeletal extension, despite having a lower calcification rate" (Cruz-Piñon et al., 2003; see also Carricart-Ganivet, 2004). Although we measured surface extension and not linear extension, these field observations are roughly in agreement with what we observed at LL. Is the extension an expression of growth compensation when luminosity is insufficient, or is there a competition between the two growth rates? At LL, the extension rate was always higher than at HL (Fig. 2), and, at HL, when photosynthesis was intense, extension was reduced (Fig. 3). Nubbins showing higher extension at LL seemed to roughly keep this specificity at HL without being able to produce strong photosynthetic activity. However, among these colonies, there was a positive correlation between extension and net productivity (Fig. 3).

The partitioning of the colonies into two groups stresses that the two growth features, surface extension rate and weight of deposited aragonite per time unit (calcification rate), are distinct processes (Fig. 1c). It has been noted previously that these two measurements of coral growth are not redundant and may provide complementary information (Scoffin et al., 1992). However, we wanted to understand why nubbins originating from one parent colony presented two different behaviors.

4.3 The role of zooxanthellae

Although we did not measure algal abundance, we may relate the highly variable photosynthetic activity to different densities of zooxanthellae. Fig. 3 indeed suggests that skeletons showing the maximal extension also had a low algal density, leading to a low P_{net}. Weber et al. (1976) stressed such a specificity in *Acropora cervicornis*. They observed that the abundance of symbiotic zooxanthellae increased from the tip to the base of a branch, density at the apical growth being much greater than at the side accretions. They associated this difference with rather confused isotopic behaviors for O and C. Although it would be expected that the coral zone was where mineralization

started, it was concluded that the apical part of a branch could be lacking zooxanthellae.

Earlier we noted that the $\delta^{13}C$ responses exhibited all intermediate values between the two extremes (Fig. 1). The metabolic responses, especially photosynthesis and surface extension, are not completely clear because our samples were a mixture of side and top skeletal portions. Indeed, we suggest that the aragonite collected on the glass slide (Fig. 5) integrated variable relative amounts of skeleton fragments characterized by the two growth strategies stressed by Weber et al. (1976), with metabolic and also isotopic measurements integrating the relative responses; this could also explain the large scatter of our data. We assumed that the highest O and C isotopic values were provided by samples essentially composed of skeleton portions rich in algae, corresponding to the sides of corallites or to the skeletal zone called inter-corallites, and thus having the ability to strongly photosynthesize, while the samples characterized by low $\delta^{13}C$ contained greater amounts of aragonite forming the corallite tip according to observations made by Weber et al. (1976).

It seems that at HL, when photosynthesis was clearly active, respiration proportionally decreased (Fig. 2). This observation could be due to a part of the respired CO_2 being used for photosynthesis, as in the carbon-translocation hypothesis of Muscatine and Porter (1977). However, we have no information to document a mechanism linking the metabolic activity of algae with coral metabolism leading to modifications in skeletogenesis.

4.4 Is there competition between photosynthetic activity and surface extension?

Figure 3, obtained with samples maintained at HL, highlights an inverse relationship between photosynthesis and surface extension. Indeed the group of colonies showing reduced photosynthesis and $\delta^{13}C$ decreases with the light increase also presented an increase in surface extension, while the group showing an active photosynthesis was characterized by the lowest extension. On one hand, this could illustrate competition

between surface extension and photosynthetic activity at HL, while, on the other hand, it could indicate that there are two biomineralization strategies: submitted to HL, some parts of the skeleton are dedicated to spatial growth and the others to strengthening skeletal structure. We note that the relative growth weight doubled in both cases.

4.5 Axial and lateral portions of a corallite

Gladfelter (1982, 1983, 1984) investigated the skeletal growth pattern of *Acropora cervicornis*. She described the wall of an axial corallite, surrounding the calyx, formed by vertical spines connected together tangentially and radially by a porous mineral (Gladfelter, 1982). She clearly identified two different growth units: massive, randomly oriented crystals, called fusiform crystals, and numerous needle-like crystals projecting in many directions from the fusiform crystals. She noted that the needles, which were gathered into bundles oriented perpendicularly to the spine, showed a progressive infilling of pore space from the tip of the corallite to the base. She deduced from these observations that skeletogenesis could be the result of two processes: the deposition of fusiform crystals and the progressive thickening of the initial framework by needle-like crystals. She attributed these two modes of deposition to a dichotomy in growth axes: the axial growth most often expressed as a linear extension resulting from the deposition of fusiform crystals, with lateral accretion by fibers ensuring the strengthening of the skeleton (Gladfelter, 1982, 1984). These investigations led her to conclude that the first step of skeletogenesis was performed during the night, while the main parameter influencing the second was the duration of sunlight per day (Gladfelter, 1983).

The differentiation of coral-skeleton growth relative to its shape could be a specific feature of branched corals such as *Acropora*. Skeletal structure has also been studied in *Porites* (Barnes and Lough, 1993). Like Gladfelter (1983), these authors also proposed skeletal development in two steps. Lough and Barnes (2000) observed different rates of extension and calcification between the top and sides of colonies in numerous massive *Porites* from the Great Barrier, which led them to attribute the observed

discrepancy to light availability. Also like Gladfelter (1982) they noted the coral growth dichotomy and they related the first, axial growth to high photosynthetic activity and the second, the thickening of colony sides, to a less active photosynthesis. Zooxanthellae distribution information such as that described by Weber et al. (1976), which is in agreement with our results, may be counter-intuitive. The upper surface of the coral receiving more direct light incidence could be supposed to be richer in algae than the sides of colonies, which receive less intense light.

SEM microstructural observations of several coral genera led Cuif and Dauphin (1998) to also suggest that coral-skeleton deposition operated into two successive steps. Also from SEM observations, Nothdurft and Webb (2007) underlined common features and discrepancies shown by several coral genera, the presence of two growth units and two growth axes appearing as common features to everyone. At another size scale, it has been demonstrated that calcification of *Stylophora pistillata* also operates by two mechanisms: first extension and secondly progressive filling-in of the previously deposited structure (Raz-Bahat et al., 2006).

We observe in Fig. 5 that higher surface extension (on the right) corresponds to new corallite formation and growth of the axial one, whereas lower surface extension (on the left) is associated with thickening of inter-polyp space, lacking any new polyps.

4.6 The link between light, skeletal microstructures and growth modes

Isotopic measurements conducted at the micrometer scale on cultured *Acropora*, similar to the colonies studied here, (Juillet-Leclerc et al., 2009) confirmed that the fusiform crystals stressed by Gladfelter (1982), abundant around the theca of *Acropora,* were identical with centers of calcification (COC) or early mineralization zones (EMZ) like those observed along the trabecula by other authors (Cuif and Dauphin, 1998, 2003; Raz-Bahat et al., 2006; Nothdurft and Webb, 2007).

Although the earlier observations and the present study are based on two different size scales, our experiment indicated that coral growth followed two main axes corresponding to two separate processes. Our results also suggest that extension is

light-repressed, whereas calcification, in terms of mineral to weight deposited, is light-enhanced. However, this demonstrated that, conversely to Gladfelter's assumption (1983, 2007), an increase in extension does not require low light. Photosynthetic activity of the lateral portion of a corallite, assumed to be less exposed to light, was more intense than at the corallite tips because the sides were likely richer in algae. The same paradox at the crystal scale was stressed in *Stylophora pistillata* observations by combining Field Emission Scanning Electron Microscopy (FESEM) and supplementary fluorescence techniques (Tambutté et al., 2007).

To date the intuitive zooxanthellae distribution (algae more numerous on the tip than on the sides of corallites) has been used to justify the isotopic sampling mode for climatic purposes by following the main growth axis and assuming that this corresponded to the maximum light effect over the growth period (McConnaughey, 1989a). Our results show the opposite. As shown in Fig. 5, there was a side where the newly deposited mineral corresponded essentially to accretion of fibers with some spines containing few COC but free of calyces, forming a thin and dense aragonite layer around the axial polyp, while on the other side the new mineral was rich in calyces with COC at their tip and also in the radial septae. Thus, the relative proportion between COC and fibers depends on the location of the nubbin section, which explains why superficial extension of several nubbins may be as different as noted during this experiment. Juillet-Leclerc et al. (2009) demonstrated that, in a colony grown at constant conditions, COC δ^{18}O is almost constant and centered on the lowest value, while fiber δ^{18}O varies over a 5‰-amplitude range, from the equilibrium value to the lowest one (corresponding to the COC values), the latter isotopic behavior likely being linked with kinetic processes. This could explain the high variability exhibited by nubbins from a single parent colony due to the highly variable relative crystal amounts. Additionally, the skeleton developed on the glass slide received identical light over the whole surface (which is not the case on the side of a wild colony), thus, the effect on the different portions of the skeleton is amplified.

We emphasize that, in the case of *Acropora*, the space between each calyx is more developed than in other genera such as *Porites* (Nothdurft and Webb, 2007). This skeletal difference may also likely serve to amplify the discrepancies between several nubbins.

4.7 Relationship between growth and isotopic values

We demonstrated that HL enhanced fiber formation. Therefore, knowing that $\delta^{18}O$ in fibers is always higher than $\delta^{18}O$ in COC, we suggest that the systematic positive $\delta^{18}O$ response to light increase was due to fiber enrichment in all the colonies. This conclusion is supported by the fact that the positive isotopic response was more pronounced in colonies responding actively to light through photosynthesis. Additionally, we may check that when photosynthesis was strongly active, $\delta^{18}O$ response was higher. Moreover, the oxygen reservoir was isotopically constant over this time, as the atoms came from seawater where they are abundant.

Meibom et al. (2006) showed that the COC $\delta^{13}C$ of *Colpophyllia* sp. were lower than that of the fibers. This explains why at HL, colonies strongly sensitive to photosynthesis (richer in zooxanthellae) exhibited higher values than the others. At LL, the $\delta^{13}C$ of colonies richer in COC would then be lower than those containing mainly fibers. Curiously, we observed the opposite. In addition, these nubbins showed lower $\delta^{13}C$ at HL. In the case of carbon, the origin of the atoms might vary between the two conditions. Indeed, Rollion-Bard et al. (2003a) demonstrated that $\delta^{18}O$ and $\delta^{13}C$ measured in a single sample at the micrometer scale were not always correlated. We previously noted that the results considered in the present study were formed by the integration of numerous isotopically heterogeneous microstructures. At the macro scale, $\delta^{18}O$ and $\delta^{13}C$ may thus be correlated. Therefore, to explain $\delta^{13}C$ variations, we must assume that, in addition to the fractionation due to kinetic processes, the carbon reservoir has to differ. Biological investigations using double labeling (^{14}C and ^{45}Ca) (Erez, 1978; Furla et al., 2000) demonstrated that in the case of active photosynthesis, the amount of carbon present in the symbiotic system is not sufficient to sustain physiological ac-

tivities, and this lack of carbon is compensated by an increase of seawater-uptake by the coral tissue. Considering that Dissolved Inorganic Carbon DIC seawater $\delta^{13}C$ is much higher than metabolic $\delta^{13}C$, skeletal $\delta^{13}C$ should be enriched, supporting the macroscale observations.

In addition, deposition processes of each crystal type differ as well as their isotopic signatures (Juillet-Leclerc et al., 2009). Therefore, we also can explain isotopic discrepancies existing between different nubbins. Some of our samples contained largely fibers, thus showing at HL an increase of $\delta^{18}O$ and $\delta^{13}C$ associated with the lower extension rate. For the others richer in COC, HL enhanced only the growth of the fibrous portion of the nubbin, increasing the $\delta^{18}O$ signature but decreasing the extension rate. The primary skeletal carbon origins then changed between LL and HL, and the global effect on the $\delta^{13}C$ values of "bulk" samples was variable.

4.8 $\delta^{18}O$ and $\delta^{13}C$ correlation

The high $\delta^{18}O$ and $\delta^{13}C$ correlations of seasonal samples and samples collected horizontally around *Pavona clavus* heads led McConnaughey (1989a,b) to assume kinetic isotopic fractionation in the coral skeleton. Simultaneously, the distributions of $\delta^{18}O$ and $\delta^{13}C$ in *Porites* aragonite sampled along the faster growing axis appeared to be caused by the relative variability of temperature and light. The two interpretations were consistent: the upper surface of the coral head received more sunshine, thus grew faster and showed depleted $\delta^{18}O$ while the lateral surfaces, receiving less light, exhibited a slower growth rate and higher $\delta^{18}O$ (McConnaughey, 1989a). Therefore, it has been concluded that when $\delta^{18}O$ and $\delta^{13}C$ were correlated this could indicate the skeleton deposition following a kinetic process; negative oxygen fractionation has been associated with high growth rate and high light, $\delta^{18}O$ being affected by temperature and $\delta^{13}C$ by photosynthesis. Our experiment highlights that it is not so simple.

$\delta^{18}O$ and $\delta^{13}C$ are both affected by light. However, light effects are not equivalent on all skeletal microstructures and thus the isotopic analyses are more or less impacted by the integration of micro-isotopic signatures. This leads to morphological isotopic inho-

mogeneity. Linear extension and calcification are distinct kinetic processes, responding differently to light. Thus, relationships between kinetics and isotopic fractionation and between kinetics and light are more complex: in *Acropora* sampled at the macroscale, the kinetic imprint on isotopic fractionation due to fiber growth is sensitive because fibers are the most numerous skeletal components. Only oxygen is purely fractionated through a kinetic process; $\delta^{13}C$ is determined by the combination of reservoir change and kinetic fractionation according to the major metabolic activity. We note that oxygen and carbon isotope correlation was essentially significant under HL because it was only in these conditions that photosynthesis activity and thus accretion, responsible for the O and C correlation, was really active (Fig. 4).

4.9 Consequences for paleoclimatic investigations

The present experiment performed on cultured *Acropora* highlights the behavior of coral clones, potentially inhabited by different concentrations of zooxanthellae and maintained under two light conditions. Each aragonite measurement includes an axial corallite plus some radial corallites. Our conclusions make sense only by comparing the same initial corallite subject to different conditions. Although morphology and micro-structure distribution differ between *Acropora* and *Porites*, the conclusions inferred from our study may be extended to the time series usually developed for *Porites* paleoclimatic studies.

The common sampling method used for paleoclimatic purposes systematically follows the major growth axis of the coral. By examining samples collected on a profile over time, we record the chemical response to environmental seasonal changes of corallites derived from identical clones and probably inhabited by similar symbiont assemblages. Additionally, this means that the sample profile corresponds to an area where COC constitute the largest portion of microstructures and where photosynthetic activity influence is reduced, in contrast to the initial assumptions commonly formulated. This confirms conclusions earlier proposed for *Acropora* and probably other genera (de Villiers et al., 1995; Cardinal et al., 2001), that extension is not linear during

the year and decreases with increasing light. Thus, we should take into account such a feature to correctly record and convert environmental changes in a given chronology. This emphasizes the importance of the sampling mode along corallites always being oriented along a similar direction during the study period and showing an apparent continuity by X-ray imaging.

In contrast with the commonly held belief, linear extension of coral does not respond positively to light. We cannot apply systematic corrections to $\delta^{18}O$ as proposed by Maier et al. (2004). However, we must assume that metabolic activity, essentially photosynthetic activity, is roughly reproducible each year, even when the algal assemblage is slightly modified over time. Two adjacent areas horizontally sampled on a single coral head may host different algal assemblages and algal concentrations and this could explain the great variability in isotope signals as reported by Linsley et al. (1999), Felis et al. (2003) and Maier et al. (2004).

At the millimeter scale, it would be difficult to observe simple relationships between isotopic signatures and metabolic activity due to the presence in the bulk sample of variable relative amounts of crystals characterized by different isotopic fractionations.

As light and temperature often vary in parallel but have opposite effects, the amplitude of $\delta^{18}O$ fluctuations due only to temperature will always be smoothed by the effect of light. This could explain the observed high variability of time series and calibrations (Wellington et al., 1996; Linsley et al., 1999; Maier et al., 2004). It could also explain the correlation of $\delta^{18}O$ versus SST showing lower absolute slope values than $-0.20‰/°C$ (Juillet-Leclerc and Schmidt, 2001; Suzuki et al., 2005). Thickening of the corallite wall could also smooth $\delta^{18}O$ by the addition of aragonite fibers. Due to a specific morphological organization, especially a reduced inter-corallite surface, this effect would likely remain limited for *Porites* compared with *Acropora*; sampling along the axis of maximum growth rate would also reduce this effect.

We note that linear extension and $\delta^{18}O$ are affected by light in the same way; the direct temperature effect and that caused through photosynthesis are opposite. It would be informative to compare $\delta^{18}O$ and linear-extension fluctuations over a year, but ex-

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tension variability is not easy to measure over a year. The light influence on $\delta^{13}C$ is more complex and depends on the corallite portion sampled.

Our findings help explain the contrasting observations made on different colonies (Goreau, 1977; Erez, 1978). However, on a *Porites* head, as sampling is centered along the main growth axis where COC are abundant, a clear $\delta^{13}C$ decrease is almost always recorded with light enhancement during the summer (Swart et al., 1996).

5 Summary

1. Although there is some additional evidence, we need to ensure that the features described are not restricted to cultured corals and /or the genus *Acropora*.

2. Rates of photosynthesis are not constant and equal on the surface of a coral skeleton. Zooxanthellae can also be more abundant on the sides than on the apex of corallites. Light enhancement leads to decreased extension rate and increased aragonite weight deposited. Thus, HL causes a skeleton-density increase. Although the absence of algae on the apical part of the skeleton is counter-intuitive, several lines of evidence suggest this conclusion.

3. Both $\delta^{18}O$ and $\delta^{13}C$ are impacted by light. In the field, light-induced increase in $\delta^{18}O$ is masked by the temperature effect. $\delta^{13}C$ may increase in the lateral corallite portion while simultaneously decreasing at the apex.

4. We did not observe a direct correlation between metabolism and isotopic data. This could be attributed to the systemic mixture of microstructures, for which the relative ratios varied in the measured samples.

5. Significant correlations between $\delta^{18}O$ and $\delta^{13}C$, likely due to kinetic deposition process of fibers (Juillet-Leclerc et al., 2009) are not directly related to classical growth-rate measurements, density and linear extension. The strong relationships

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between oxygen and carbon isotopic fractionation, growth rate and light are much more complex than previously thought.

6. Light influences on O and C isotopic ratios is part of a vital effect, essentially inducing high colony variability and also horizontal variability on the surfaces of colonies in some species (McConnaughey, 1989). This could be due to the variable abundance of zooxanthellae, variable light incidence and/or the sampled morphological parts of corallites.

7. Variability of isotopic ratios is significant over the time only when measurements are performed along individual successive specimens, in this case corallites.

These results provide evidence underlining the importance of the coral-algae symbiosis in coral-reef formation, especially concerning the strong and complex relationships between calcification and photosynthetic activity (Gattuso et al., 1999). Estimation of the relative importance of temperature and light effect on skeletal isotopic signatures remains for a future study.

Vital effects are particularly complex in corals due to the impact of the zooxanthellate metabolism on the chemical features of the coral skeleton and the collective growth of a colony of multiple organisms. However, as we highlighted here, the specific signature shown by each specimen from a single colony, inter- and intra-specimen variability of *Mytilus edulis* and *Pecten maximus* has still been stressed by Freitas et al. (2008).

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Table 1. Metabolic and isotopic measurements for each colony after 6 weeks of culture. Colonies presenting higher $\delta^{13}C$ at HL than at LL are reported in the green portion of the table.

# sample	Pnet (μmolcm^{-2}h^{-1})	Resp (μmolcm^{-2}h^{-1})	growth rate %/day	surf (cm^2)	$\delta^{18}O$‰vsPDB	$\delta^{13}C$‰vsPDB
260 μmolcm^{-2}h^{-1}						
22	0.57	-0.34		26.10	-2.83	-2.08
49	0.42	-0.25	0.66	8.30		-2.16
21	0.54	-0.45		19.20	-2.82	-1.88
23	0.53	-0.23		24.60	-3.00	-2.36
55	0.42	-0.26	0.66	13.60	-2.78	-2.17
66	0.29	-0.24	0.58	17.50	-2.93	-2.44
68	0.31	-0.33		10.00	-2.69	-1.66
48	0.47	-0.50	0.78	12.50	-2.93	-1.75
24	1.01	-0.65		8.30	-2.52	-1.50
50	0.94	-0.64	0.60	8.20	-2.21	-0.76
67	2.55	-0.31		5.10	-2.35	-0.97
57	1.34	-0.51	0.64	6.30	-2.98	-2.24
58	0.57	-0.24	0.63	8.20	-2.79	-2.09
26 bis	0.53	-0.29		14.00	-2.50	-1.49
26	0.26	-0.22		8.60	-2.66	-1.78
130 μmolcm^{-2}h^{-1}						
22	0.27	-0.37	0.31	36.80	-3.32	-2.05
49	0.47	-0.27	0.17	12.90	-2.99	-1.94
21	0.38	-0.28	0.33	41.70	-2.95	-1.26
23	0.22	-0.16	0.30	35.50	-3.16	-1.99
55	0.41	-0.62	0.20	14.90	-3.04	-2.01
66	0.13	-0.20	0.22	22.40	-3.07	-1.79
68	0.20	-0.40	0.37	16.20	-3.18	-1.41
48	0.34	-0.23	0.20		-3.28	-2.67
24	0.09	-0.44	0.33		-3.20	-1.99
50	0.51	-0.46	0.21	9.70	-3.00	-2.50
67	0.27	-0.54	0.23	12.10		
57	0.29	-0.17	0.02	17.70	-3.03	-2.53
58	0.29	-0.25	0.18	14.70	-3.29	-2.52
26 bis	0.36	-0.34	0.41	20.90	-3.06	-2.14
26	0.09	-0.15	0.40	25.40	-2.95	-2.03

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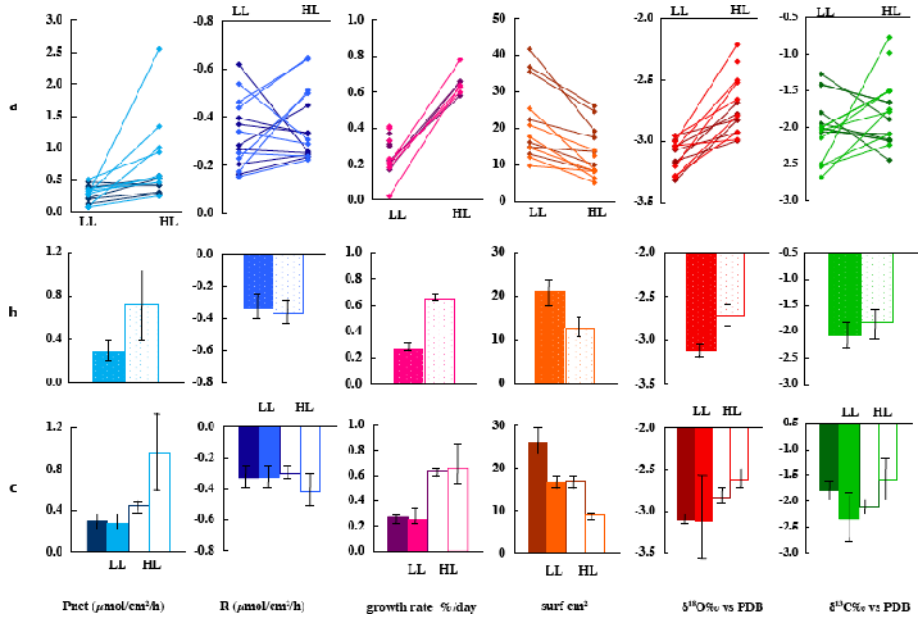

Fig. 1. Values of measured metabolic and geochemical parameters. **(a)** Individual values are plotted for high and low light. Clear (dark) bars correspond with colonies showing higher (lower) δ^{13}C at HL than at LL. **(b)** Results based on averaging all data. Full columns are associated with LL and empty ones with HL (plotted error: 2σ). **(c)** Averaged values for each light condition calculated after splitting nubbins into two groups: colonies showing higher (lower) δ^{13}C at HL than at LL are plotted with clearer (darker) bars (plotted error: 2σ).

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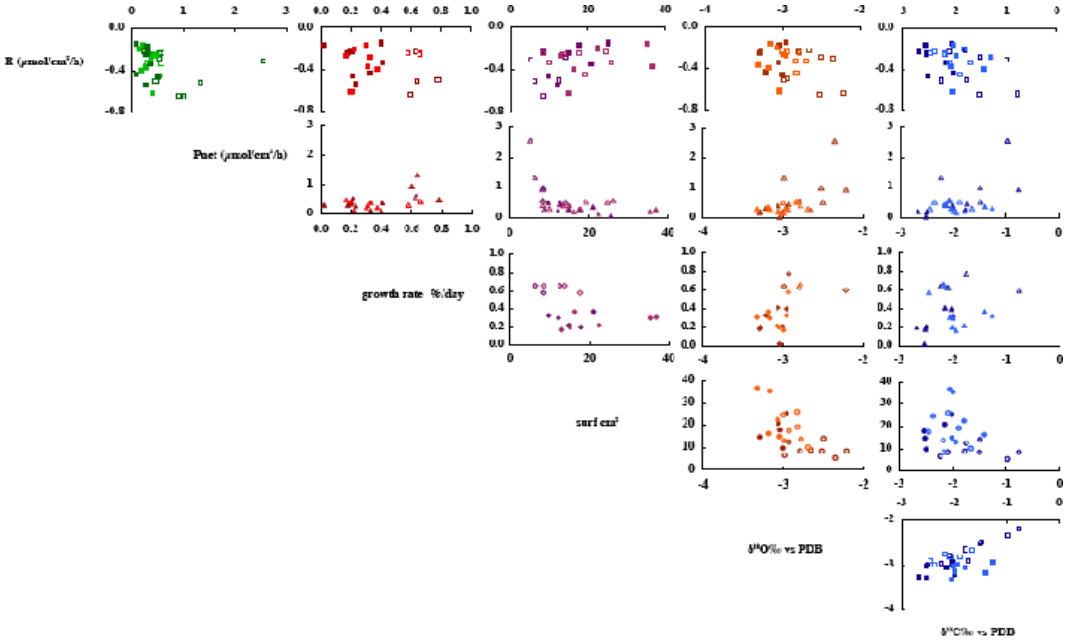

Fig. 2. Comparison of all measured metabolic and isotopic parameters. Colonies showing higher (lower) $\delta^{13}C$ at HL than at LL are plotted with clearer (darker) symbols. Full (empty) symbols are values measured at LL (HL).

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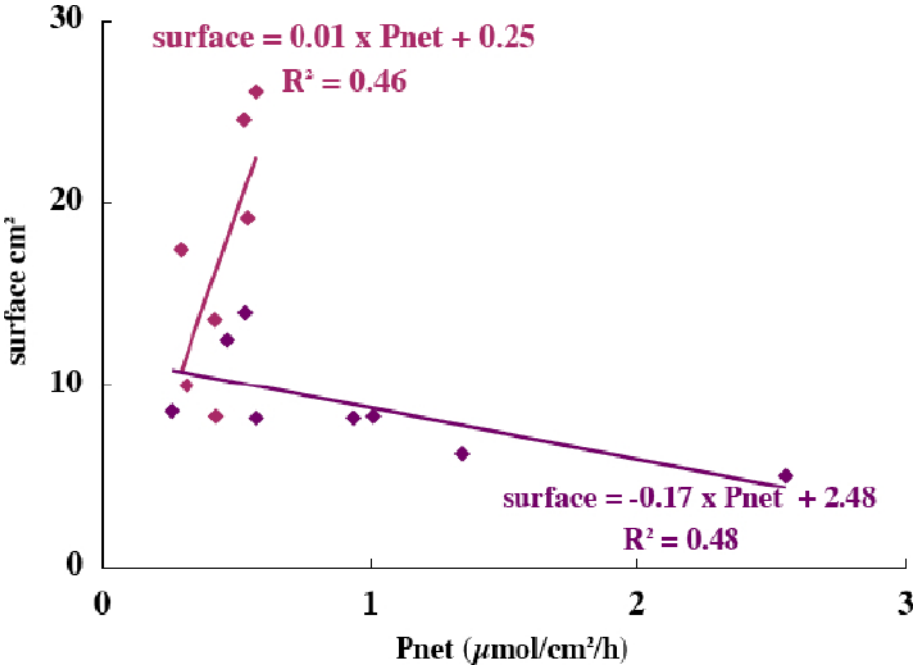

Fig. 3. Photosynthetic activity versus surface covered by new-formed aragonite at HL. Darker symbols correspond to colonies showing higher $\delta^{13}C$ at HL than at LL.
P_{net} values measured on colonies showing lower $\delta^{13}C$ than at LL, are limited to 1.6 mmol cm^{-2} h^{-1} and are associated with development on larger surface than colonies showing higher $\delta^{13}C$ than at LL and responding with higher photosynthetic activity, though all the nubbins, respectively almost doubled their skeleton weight.

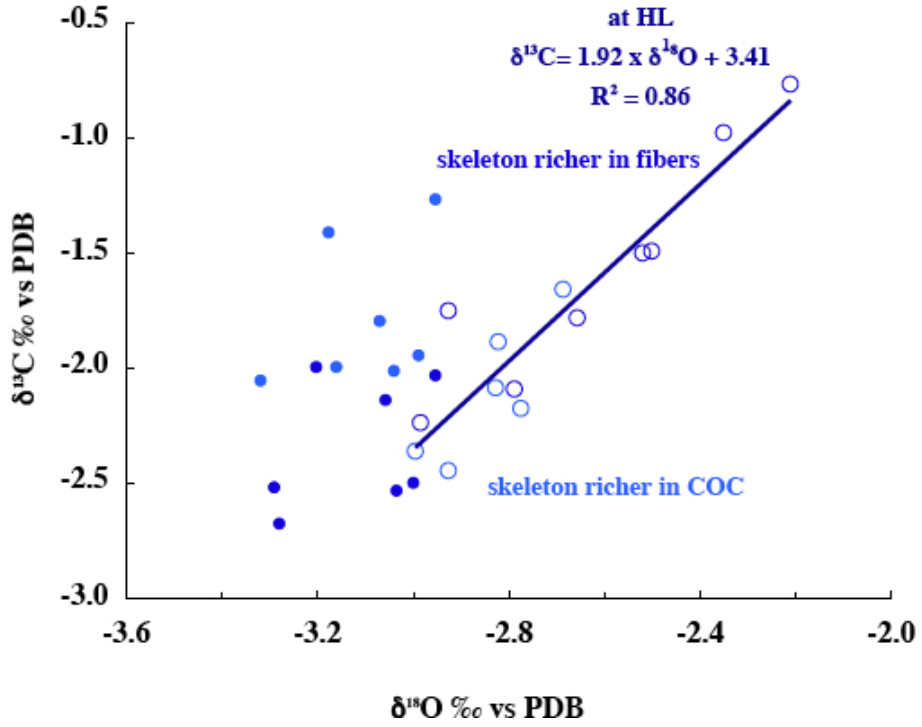

Fig. 4. $\delta^{18}O$ values of individual specimen plotted against $\delta^{13}C$. Data corresponding to LL are denoted by full symbols. First, we note that $\delta^{13}C$ of colonies richer in COC are lower than that of colonies richer in fibers for equivalent $\delta^{18}O$ values. Second, at HL, $\delta^{18}O$ and $\delta^{13}C$ are linearly correlated, the relationship assessed only from colonies showing higher $\delta^{13}C$ than at LL being likely most significant.

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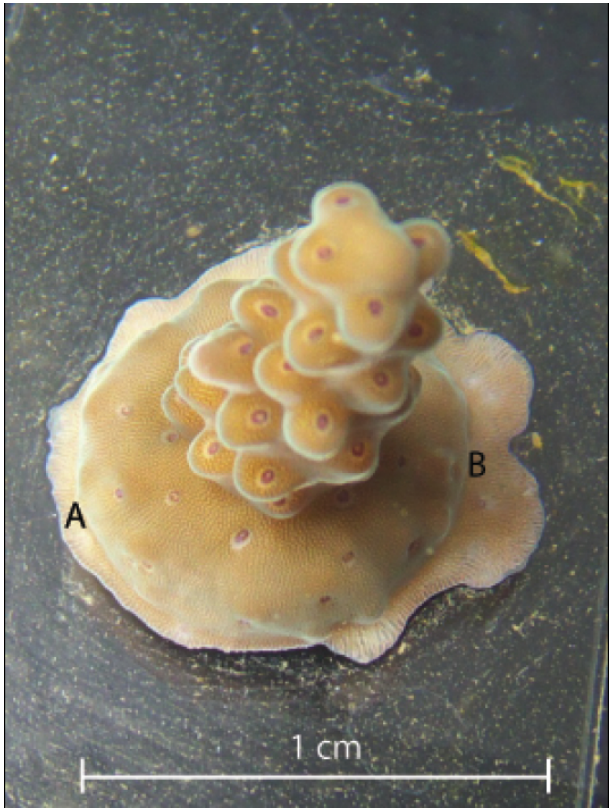

Fig. 5. *Acropora* nubbin after few week growth. New-formed aragonite is deposited on the colony and the glass slide. On side A, the layer of new mineral is reduced and thick, whereas on side B it extends further the initially stuck nubbin and new corallites are visible.

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