

Study of calcification during a daily cycle of the coral *Stylophora pistillata*: implications for ‘light-enhanced calcification’

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Summary

This work, performed on the scleractinian coral *Stylophora pistillata*, aims at providing new information on the ‘light-enhanced calcification’ process. In a first step, in controlled conditions of culture and constant light supply, we studied the diurnal cycle of calcification. We determined that calcification rates are constant during the day and the night with a 2.6-fold difference between day and night calcification rates. We also showed that the photosynthetic rate is constant throughout the day when a constant light intensity is applied. Our results on free-running experiments in prolonged conditions of light or dark suggest that calcification is not regulated by an endogenous circadian rhythm. In a second step, using a kinetic isotopic

approach with ^{45}Ca , we characterized the transition stages between day and night and *vice versa*. Under our experimental conditions, the lag-phase necessary to switch from the light to the dark calcification rate is the same as the lag-phase necessary to switch from the dark to the light calcification rate. We discuss our results in the context of two hypotheses of the light-enhanced calcification process: (1) the role of photosynthesis on the pH in the coelenteron and (2) the role of photosynthesis in supplying precursors of the organic matrix.

Key words: light-enhanced calcification, calcification, photosynthesis, kinetic approach, light/dark, radioisotope, *Stylophora pistillata*.

Introduction

The large majority of calcium carbonate precipitated every year in the ocean results from organisms that photosynthesize, either directly (coccolithophorids) or indirectly *via* the establishment of a symbiosis (foraminifera, scleractinian corals). Among these calcifying photosynthetic organisms, scleractinian corals are one of the fastest calcifiers of the ocean. These animals are the origin of the most important bioconstruction of the world, the coral reefs, which lie between 30°N and 30°S latitude. Most of these scleractinian corals have developed an endosymbiotic relationship with photosynthetic unicellular dinoflagellates, *Symbiodinium* sp., commonly referred as zooxanthellae, which include at least eight distinctive clades (Pochon et al., 2006). The presence of these symbionts in corals has a crucial effect on the metabolism of the host (for reviews, see Muscatine, 1990; Furla et al., 2005).

As early as 1948, it has been shown that scleractinian corals calcify faster in the light than in the dark (Kawaguti and Sakumoto, 1948); a phenomenon largely confirmed by Goreau (Goreau, 1959) and subsequently called light-enhanced calcification (LEC). However, after more than a half century of research, the mechanism underlying this process remains largely unknown, although a number of hypotheses have been

proposed (Barnes and Chalker, 1990; Cohen and McConnaughey, 2003; Allemand et al., 2004).

(1) Modification of the dissolved inorganic carbon equilibrium within coral tissues caused by CO_2 uptake for photosynthesis. The modification of carbonate chemistry inside the coelenteron can act on calcification on three different ways. According to Goreau, photosynthesis lowers the CO_2 partial pressure in the calcification site (Goreau, 1959), and thus favours calcium carbonate precipitation. According to McConnaughey and Whelan, the production of H^+ by calcification favours CO_2 formation to the detriment of the other dissolved inorganic carbon forms (McConnaughey and Whelan, 1997). Dinoflagellates absorb this CO_2 and thus favour calcium carbonate precipitation. According to Furla et al., the OH^- resulting from the carbon-concentrating mechanism (CCM) present in oral layers of cells may neutralize the H^+ produced by calcification (Furla et al., 1998), thus allowing calcification to proceed.

(2) Removal of inhibiting substances, such as phosphates (Simkiss, 1964).

(3) Production of energy by photosynthesis (Chalker and Taylor, 1975) or release of O_2 (Rinkevich and Loya, 1984).

(4) Synthesis of compounds necessary for organic matrix synthesis (Muscatine and Cernichiaro, 1969). It has been shown

that the composition of the organic matrix is different between symbiotic and non-symbiotic corals (Cuif et al., 1999). Zooxanthellae translocate photosynthates to the host (Muscatine and Cernichiaro, 1969). However, if zooxanthellae participate in the synthesis of the organic matrix, they only supply organic precursors, as organic matrix synthesis is only performed by calicoblastic cells (Puverel et al., 2005).

Although the involvement of light in coral calcification has probably been studied more extensively than any other environmental variable, effects of light remain one of the most poorly understood, for several reasons (Buddemeier and Kinzie, 1976). One of them is the possible existence of an endogenous circadian rhythm, which would modify any potentially monotonic relationship between light and growth (Vandermeulen and Muscatine, 1974; Chalker, 1977; Roth et al., 1982). A circadian rhythm is a biological rhythm in which the period is about 24 h. It is qualified as 'endogenous' if it is internal to the organism rather than due to a variation of an environmental factor (Hirota and Fukada, 2004).

The aim of this study was to provide new information on the light-enhanced calcification phenomenon, using the zooxanthellate scleractinian coral *Stylophora pistillata* as a model, by investigating, under controlled conditions, the time course of calcification at different hours of the day, and after light to dark and dark to light transitions. We used ^{45}Ca as a tracer, which can provide an extremely sensitive and precise measure of short-term calcification rates (Buddemeier and Kinzie, 1976) and has been validated for physiological studies in *S. pistillata* (Tambutté et al., 1995). In a first step, we studied variations of calcification during the day and the night and during free-running experiments, in order to determine if calcification rates are under the influence of an endogenous circadian rhythm. Photosynthetic rates were also measured during the day. In a second step, we compared for the first time the rates of calcification during transitions between light and dark and *vice versa*.

Materials and methods

Biological material

Coral maintenance

Experiments were conducted in the laboratory using microcolonies of the branching zooxanthellate scleractinian coral *Stylophora pistillata* (Esper 1797). Parent colonies and microcolonies, consisting of 1.5 cm long branch tips (Tambutté et al., 1995), were stored in a 300-l aquarium, supplied with seawater from the Mediterranean Sea (exchange rate 2% h⁻¹) under controlled conditions: semi-open circuit, temperature 26.0±0.2°C, salinity 38.2‰, light 175 μmol photons m⁻² s⁻¹ (using fluorescent tubes; Custom Sea Life®, California, USA) on a 12 h:12 h light:dark photoperiod, with the light period beginning at 9:00 h. Corals were fed three times weekly in the evening with a mix of *Artemia salina* nauplii, frozen adults of *Artemia salina* and frozen krill.

Experimental protocol

Measurement of calcification rates

In order to determine the light intensity necessary to obtain

saturation rates, calcification rates were measured under different light intensities. Varied light intensity was obtained with a screen placed between the light source and the samples. Microcolonies were adapted to the light intensity for 30 min before each experiment. Calcification rates were measured using the protocol described previously (Tambutté et al., 1995; Tambutté et al., 1996). Briefly, the microcolonies were placed in plastic holders and incubated for 1 h in 10-ml beakers containing 240 kBq of ^{45}Ca (as $^{45}\text{CaCl}_2$; New England Nuclear®, Boston, MA, USA) dissolved in seawater, filtered using 0.45 μm Millipore membranes (filtered seawater; FSW). Samples (100 μl) of seawater were removed during each incubation for determination of specific radioactivity. Incubations were performed in the same conditions of temperature (26.0±0.2°C) and light (175 μmol photons m⁻² s⁻¹) as in the maintenance experimental aquaria. A magnetic stirring bar maintained water movement during the experiment. At the end of the incubation, tissues were completely dissolved in 1 mol l⁻¹ NaOH at 90°C for 10 min. The supernatant was collected in a separate tube. The skeleton was rinsed first in 1 ml NaOH, then twice in FSW, and finally twice in H₂O milliQ. The first rinse solution was added to the tissue fraction, the remaining four were discarded since they did not contain proteins. Finally the skeleton was dried and dissolved in 6 mol l⁻¹ HCl. Samples of this skeletal fraction were counted with 4 ml of Ultima Gold AB (Packard, Boston, MA, USA). Emissions were measured using a liquid scintillation counter (2100 TR Packard, Tricarb). Protein content of each tissue fraction was measured using the BCA Protein Assay Kit (Uptima, Montluçon, France), based on the colorimetric determination of the amount of proteins (Lowry et al., 1951). The standard curve was established with bovine serum albumin. Results are expressed as nmol Ca h⁻¹ mg⁻¹ of protein.

To study the calcification rate during day and night, measurements of ^{45}Ca incorporation were performed at different hours of the day (9:30, 11:30, 13:30, 15:30, 17:30, 19:30 h) and the night (23:30, 3:30, 7:30 h) under saturating light, according to the protocol described above. Six microcolonies were used for each point of the daily cycle.

Measurement of photosynthetic rates

Photosynthetic rates were measured at different hours of the day (9:30, 11:30, 13:30, 15:30, 17:30, 19:30 h). Before each experiment, the oxygen sensor was calibrated against air-saturated seawater (100% oxygen) and a saturated solution of sodium dithionite (zero oxygen). Each microcolony was placed in a respirometric glass chamber containing a Strathkelvin 928 electrode (Glasgow, UK) for 15 min under saturating light (175 μmol m⁻² s⁻¹). The temperature was maintained constant at 26.0±0.2°C using a water bath. The incubation medium was homogenized with a magnetic stirring bar. Oxygen was monitored every 10 s on an acquisition station (Strathkelvin 928 oxygen system, version 2.0). Surface area of each microcolony was measured according to the wax technique (Stimson and Kinzie, 1991). Results are expressed as μmol O₂ cm⁻² h⁻¹.

Night and day free-running experiments

Free-running experiments were carried out to identify any possible endogenous circadian rhythm. Calcification rates were measured after 16 and 20 h of constant dark and light conditions, according to the protocol described above.

Transition between light and dark calcification rates

To study transitions between light and dark or dark and light, a cumulative kinetic isotopic approach was chosen. Twenty microcolonies were placed in plastic holders and incubated, as described above, in 10-ml beakers containing 240 kBq of ^{45}Ca dissolved in FSW. Every 10 min, one microcolony was sampled and the incorporated ^{45}Ca was counted according to the protocol described above. Three experiments per each transition were performed. Results are expressed as $\text{nmol Ca h}^{-1} \text{mg}^{-1} \text{protein}$.

Statistical analysis

Paired Student's *t*-tests and one-way ANOVA were used for statistical analysis (software Jump 5.1, SAS Institute, Cary, USA). Results were considered statistically significant at $P < 0.05$. Results are reported as mean \pm standard error of the mean (s.e.m.).

Results

Determination of the saturating light intensity for calcification

In order to determine the saturating light intensity for calcification, we performed ^{45}Ca incorporation under different light intensities. As can be seen in Fig. 1, we determined that at $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, optimal calcification rate was obtained. We thus chose to perform the experiments under $175 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, which is the intensity used in our laboratory to maintain corals in culture. The rate of calcification obtained for saturating light intensity is also defined as the 'calcification capacity' (Chalker, 1977).

Daily cycle of calcification

Calcification rates were measured at different hours of the day under a constant intensity of $175 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and during the night under constant darkness. The daily evolution of calcification rates is shown in Fig. 2. There is no significant variation of the calcification rate during the night (one-way ANOVA, $P = 0.43$), with a constant value of $60.76 \pm 2.84 \text{ nmol Ca h}^{-1} \text{mg}^{-1} \text{protein}$. Similarly, there is no significant variation of the calcification rate during the day under conditions of constant light (one-way ANOVA, $P = 0.86$), with a value of $159.86 \pm 4.13 \text{ nmol Ca h}^{-1} \text{mg}^{-1} \text{protein}$. Nevertheless, calcification rates were significantly different between day and night: light calcification rates being 2.6 times higher than dark calcification rates (paired *t*-test, $P < 0.001$). The same results were obtained when data were

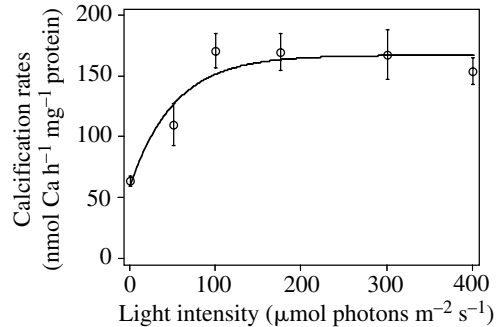


Fig. 1. Effect of light intensity on calcification rates of *Stylophora pistillata* microcolonies. Values are mean \pm s.e.m., $N = 3$.

standardized per g dry mass skeleton (respectively $1290.16 \pm 89.15 \text{ nmol Ca h}^{-1} \text{g}^{-1} \text{dry mass skeleton}$ at night; and $3208.61 \pm 124.25 \text{ nmol Ca h}^{-1} \text{g}^{-1} \text{dry mass skeleton}$ during the day). Since there was no significant variation of the calcification rate during the day and during the night, we will use without distinction the terms day or light, and night or dark.

Daily cycle of photosynthesis

Photosynthetic rates were measured at different hours of the day. The daily evolution of photosynthetic rates is shown in Fig. 3. There is no significant variation of photosynthetic rates during the day under constant conditions of light (one-way ANOVA, $P = 0.94$), with a stable value of $0.51 \pm 0.02 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$. The same result was obtained when data were standardized per mg of protein ($0.60 \pm 0.02 \mu\text{mol O}_2 \text{ mg}^{-1} \text{protein h}^{-1}$) or per g dry mass of skeleton ($8.26 \pm 0.27 \mu\text{mol O}_2 \text{ g}^{-1} \text{dry mass skeleton h}^{-1}$).

Night and day free-running experiments

This set of experiments was performed to investigate the possible existence of an endogenous circadian rhythm. For this purpose, free-running experiments in the dark or in the light were carried out during periods of 12, 16 and 20 h (Fig. 4A,B).

Results for dark experiments are shown in Fig. 4A. After

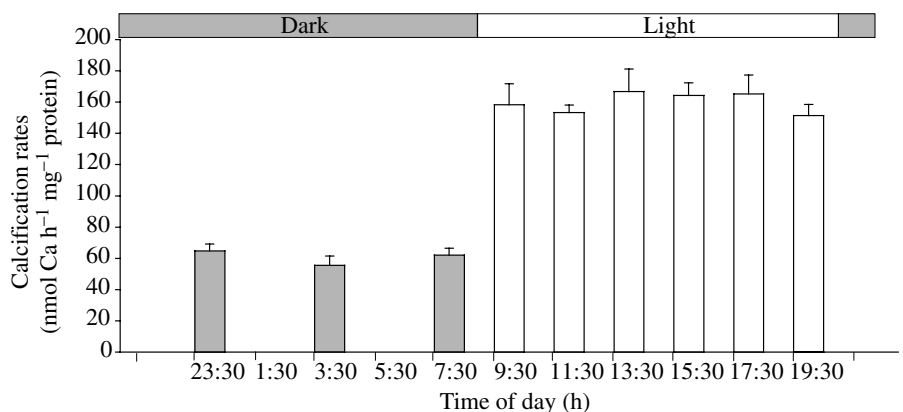


Fig. 2. Daily calcification rates of *Stylophora pistillata* microcolonies under controlled conditions of light intensity: 12 h:12 h light:dark; light intensity = $175 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, dark intensity = $0 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Values are mean \pm s.e.m., $N = 6$.

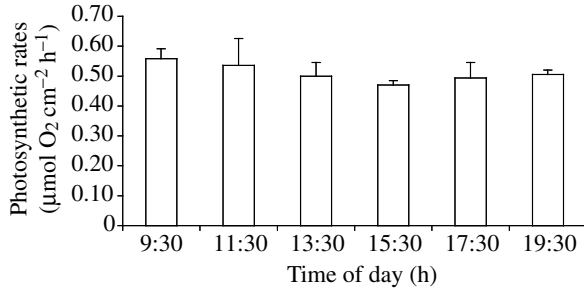


Fig. 3. Daily photosynthetic rates of *S. pistillata* over a 12 h period of constant light intensity ($175 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Values are mean \pm s.e.m., $N=3$.

12 h of dark, the calcification rate was not significantly different from the mean value obtained during the daily cycle (t -test, $P=0.83$). After 16 and 20 h of dark, calcification rates were not significantly different from the value obtained after 12 h (t -test, $P=0.82$ and $P=0.70$, respectively).

Results for light experiments are shown in Fig. 4B. After 12 h of light, the calcification rate was not significantly different from the mean value obtained during the daily cycle (t -test, $P=0.65$). After 16 and 20 h of light, calcification rates were not significantly different from the value obtained after 12 h (t -test, $P=0.60$ and $P=0.67$, respectively).

Transition from dark to light (and vice versa)

In order to determine the time-course of transition of the calcification rates from dark to light and *vice versa*, ^{45}Ca uptake was measured using a cumulative kinetic isotopic approach

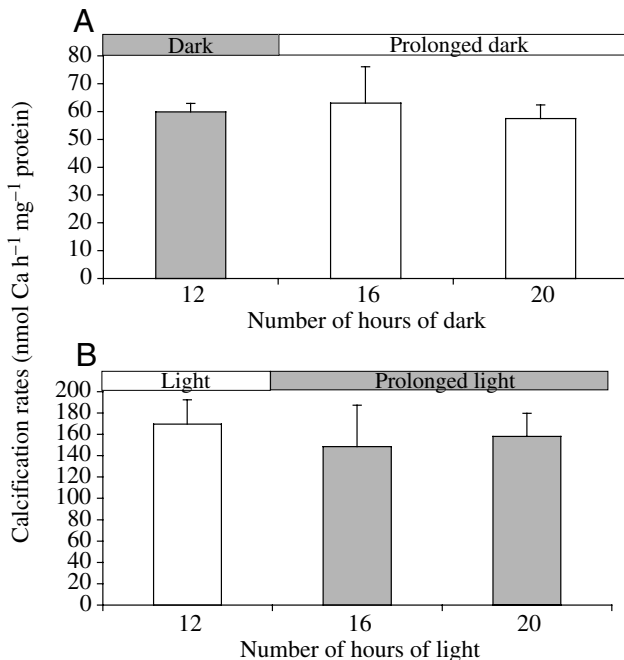


Fig. 4. Free-running experiment showing calcification rates of *S. pistillata* over prolonged exposure to (A) dark or (B) constant light conditions ($175 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Values are mean \pm s.e.m., $N=3$.

before and after switching the light on or off (Fig. 5A,B). In such a graphic representation, a steady slope of the regression line means that the calcification rate is constant over the time, whereas a slope break means that the calcification rate changes over the time.

Transition from dark to light

When corals are shifted from dark to light (Fig. 5A), the calcification rate is constant before switching on the light and at the end of the kinetics (points are well lined up), whereas it varies during the first hour when the light is switched on (points are scattered), thus proving the existence of a lag-phase.

To mathematically characterize this lag-phase, we chose a regression analysis that depends upon the intersection of two regression lines, one for the dark condition and one for the light condition. We chose the first five points and the last five points of the kinetics because points were lined up (so calcification rates were stable, as demonstrated above). The regression line of the first five points in the dark condition is: $y=1.03x+111.49$, $r^2=0.88$ and the regression line of the last five points in the light condition is: $y=2.50x+76.32$, $r^2=0.91$. Intersection between the two regression lines corresponds to 25 min after switching on the light, indicating that 25 min are necessary to reach the constant light calcification rate after a dark period. This is confirmed by the ratio between the slope of the first regression

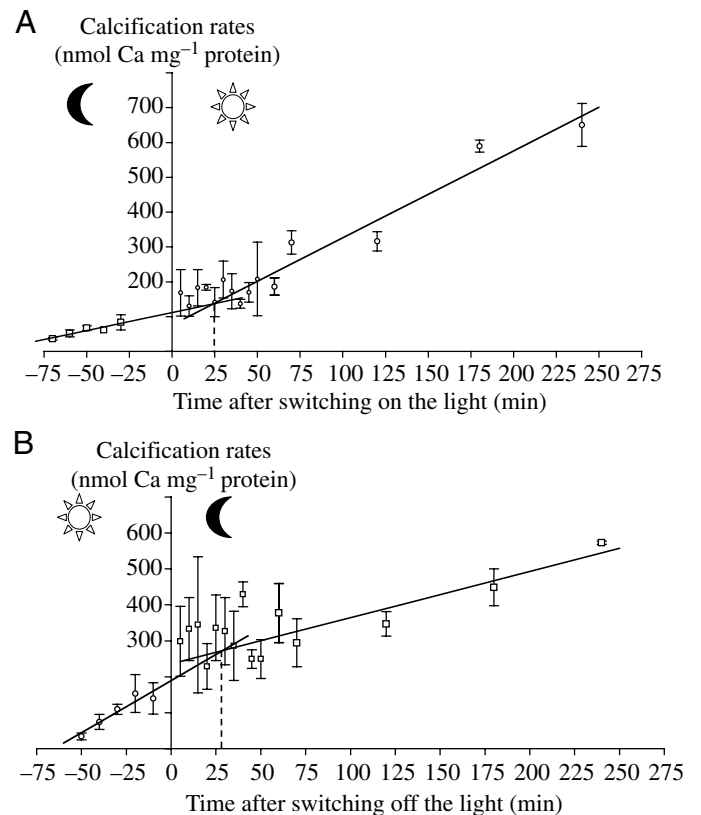


Fig. 5. Transitions between dark and light conditions using a cumulative kinetic isotopic approach: (A) from dark to light and (B) from light to dark conditions. Light = $175 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Values are mean \pm s.e.m., $N=3$.

line and the slope of the last regression line, which is about 2.4. This means that the light calcification rate is about 2.4 times higher than the dark calcification rate, which is close to the value obtained for the daily cycle.

Transition from light to dark

A similar experiment and mathematical treatment were performed for the opposite transition (Fig. 5B). The regression line of the first five points in the light condition is: $y=2.90x+189.24$, $r^2=0.89$ and the regression line of the last five points in dark condition is: $y=1.28x+235.86$, $r^2=0.83$. Intersection of the two regression lines corresponds to 25–30 min after switching off the light. This experiment shows that 25–30 min are necessary to reach the constant dark calcification rate after a light period. This is confirmed by the ratio between the slope of the first regression line and the slope of the last regression line, which is about 2.3. This means that the light calcification rate is about 2.3 times higher than the dark calcification rate, which is close to the value obtained for the daily cycle.

Discussion

Despite the plentiful literature on the enhancement of calcification by light (for reviews, see Gattuso et al., 1999; Allemand et al., 2004), this phenomenon still remains enigmatic. One of the reasons is that the existence of an endogenous circadian rhythm could modify the relationship between light and growth (Vandermeulen and Muscatine, 1974; Chalker, 1977; Roth et al., 1982). Thus in most coral calcification studies, measurements of calcification rates are made at a given time of the day in order to avoid possible variations caused by endogenous circadian rhythms (Buddemeier and Kinzie, 1976; Tambutté et al., 1995; Tambutté et al., 1996; Houlbrèque et al., 2004). However until now, there has never been direct evidence of such rhythms in corals. Most studies have been performed under natural daylight conditions. In these conditions, different results have been obtained: the rates either vary (Vandermeulen et al., 1972; Clausen and Roth, 1975; Barnes and Crossland, 1978) or remain constant during the day (Chalker, 1977; Hidaka, 1991). However, in such experiments, the variations of light intensity due to natural daylight conditions can hide endogenous circadian rhythms. Moreover, to be qualified as endogenous, the rhythm must persist under constant conditions and during free-running experiments.

In the present study, we measured calcification rates during a daily cycle of a 12 h:12 h light:dark periods under constant conditions of light, and we performed free-running experiments under prolonged conditions of dark and light.

Rate of calcification and photosynthesis at constant light intensity throughout a daily cycle

Under a constant light intensity of $175 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, corresponding to the intensity of the saturating calcification rate, our results show that there is no variation of calcification rate in *Stylophora pistillata* during the day and during the night (Fig. 2). An experiment performed on *Pocillopora damicornis* (Clausen

and Roth, 1975) showed a tendency towards a decrease in calcification rates between 9:00 and 15:00, but the authors specified that the difference between light and dark calcification rates was no significant. Chalker performed an experiment on the coral *Acropora cervicornis* (Chalker, 1977) and concluded that there was a circadian rhythm, but a statistical analysis of the data would have been necessary to confirm this study.

As for calcification rates, in our experiments, the photosynthetic rate remained constant throughout the day when a constant light intensity was applied (Fig. 3). In addition to studies on calcification, many studies have been performed on the daily rhythm of photosynthesis in zooxanthellae. However, most of the experiments were done under natural daylight (Chalker and Taylor, 1978; Porter, 1980; Hoegh-Guldberg and Jones, 1999; Jones and Hoegh-Guldberg, 2001; Levy et al., 2004) and thus no endogenous rhythm could be detected. Few experiments have been performed under constant light intensity (Chalker, 1977; Muller-Parker, 1984). In these conditions, a daily rhythm was detected in the freshly isolated dinoflagellate *Gymnodinium microadriaticum* (Chalker, 1977) and in the sea anemone *Aiptasia pulchella* (Muller-Parker, 1984). However, as for calcification, Chalker's data (Chalker, 1977) were not analysed statistically. Moreover, it was suggested that the circadian rhythm in photosynthesis could be species specific (Muller-Parker, 1984). The rates of photosynthesis of *S. pistillata* measured over a 24 h period and under constant light remain constant (Ferrier-Pagès et al., 1998). These results and ours show that in controlled constant conditions of culture and experimentation, there is no variation during a daily cycle of both calcification and photosynthesis in *S. pistillata*.

In natural conditions of light, i.e. with light intensity fluctuating during the daily cycle, it has often been noticed that the photosynthetic rates were different between the afternoon and the morning, for the same light intensity (Vollenweider, 1965; Schanz and Dubinsky, 1988; Levy et al., 2004). This phenomenon, called the 'hysteresis effect', was not observed in our experiments, suggested that it is not linked to the photosynthetic apparatus but probably determined by an environmental parameter such as light variation throughout the day.

Rate of calcification at constant light intensity under free-running conditions

Whereas the rate of calcification remains stable under constant light intensity, our results confirm that there is a sharp difference between the day and the night calcification rates with a ratio of about 2.6. Consequently, we checked whether this difference persisted when the corals were submitted to longer periods of light or dark, i.e. if this difference was due to an endogenous circadian rhythm or just relied on light regime. We thus performed free-running experiments, under prolonged periods of dark or light conditions. After 16 and 20 h in the dark or in the light, the calcification rates were the same as during the 12 h period of night or light. These results confirm that the LEC phenomenon is only due to a light-tempered parameter and not to an endogenous circadian rhythm.

In most coral calcification studies, measurements of calcification rates are made at a given time of the day in order to avoid possible variations caused by endogenous circadian rhythms (Buddemeier and Kinzie, 1976; Tambutté et al., 1995; Tambutté et al., 1996; Houlbrèque et al., 2004). In this study we determined that, when *S. pistillata* is maintained under constant conditions of light intensity, calcification rates are constant throughout the day and the night. Thus in this study we could perform experiments on the effect of light on calcification without being hampered by endogenous circadian rhythms.

Time-course of transitions between light/dark or dark/light calcification rates

The analysis of 28 publications on LEC shows that light/dark ratios of calcification range from negative values to 127 with a median ratio of 3 (Gattuso et al., 1999). This wide range of variations is attributed to the wide range of environmental and biological conditions during the experiments. For *S. pistillata*, under constant conditions of culture and experimentation (175 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 12 h:12 h light:dark photoperiod, temperature 26°C and salinity 38.2‰) the calcification rate that we measured in the light is about 2.6 times higher than the calcification rate in the dark. This value is in the range of the mean ratio values for LEC (cf. above) and similar to that shown before in *S. pistillata* (Furla et al., 2000). In the present study we made a fine analysis of the time necessary to switch from the light calcification rate to the dark calcification rate and *vice versa*, under constant conditions of light intensity.

Under constant conditions of culture and experiment, we have underlined the existence of a lag-phase, which is similar for both transitions. The time necessary to switch from one calcification rate to the other is about 25–30 min. Once stabilized, the rate of calcification remains stable for at least 12 h, under constant conditions.

For the transition between light and dark, the dark calcification rate of *Acropora formosa* is significantly increased during the first 20 min of dark by prior incubation in the light (Roth et al., 1982). We observed a lag-phase of similar duration. Roth et al. supposed that this lag-phase corresponds to the use of cellular reserves accumulated during light (Roth et al., 1982). However, for the opposite transition between dark and light, this hypothesis cannot be applied to explain the lag-phase observed.

The transition from dark to light conditions has been slightly more studied than the opposite transition. A lag-phase of 35–40 min has been observed before inorganic carbon deposition was stimulated in the skeleton of *Acropora acuminata* (Barnes and Crossland, 1978). These authors suggested that this lag-phase was an artefact arising from the dilution of ^{14}C by an unlabelled pool of dissolved inorganic carbon in the tissues. However, we find in the present study the same results with ^{45}Ca , for which it has been demonstrated that transport from sea water to the skeleton is rapid, with no lag-phase, and does not involve an intracellular pool (Tambutté et al., 1996). We can thus conclude that the lag-phase that we

observed during this transition cannot be due to an artefact arising from the dilution of calcium.

The presence of a lag-phase was also observed during the transition from dark to light in *S. pistillata* using $\text{H}^{14}\text{CO}_3^{2-}$ as a marker (Furla et al., 2000). These authors suggest that this lag-phase, which was about 10 min, corresponds to the time necessary, during the day, to produce OH^- by photosynthesis in order to titrate the H^+ produced by calcification (Furla et al., 2000) and thus to allow calcification to proceed. A study on the polarity of the tentacle of the sea anemone *Anemonia viridis* demonstrated that light induces a pH increase of the coelenteron, whereas darkness induced an acidification (Furla et al., 1998). This pH gradient across the tentacle becomes maximal after 20–30 min of saturating light (Furla et al., 1998), which is consistent with the lag-phase of transition that we obtained in this study. Such an hypothesis can also explain the lag-phase obtained during the transition from light to dark, since it could correspond to the time necessary to deplete the OH^- accumulated during the day (by diffusion of the OH^- out of the tentacle).

Based on the results of Allemand et al., a second hypothesis can also be proposed (Allemand et al., 1998). These authors found that the time required to absorb, transport, incorporate the amino acid precursor (aspartic acid) into organic matrix, and finally to export and incorporate it into the skeleton takes about 20 min. We can thus suggest that this time, which is similar to the length of the lag-phase found in the present study, corresponds to the time required for photosynthates to be transported to the calciblastic epithelium where organic matrix synthesis take place (Puverel et al., 2005) and then be exocytosed towards the skeleton. In this case, organic matrix would not only differ quantitatively between light and dark (Houlbrèque et al., 2004) but also qualitatively. Specific organic matrix proteins synthesized from photosynthates could thus play a role in the light-enhanced calcification mechanisms as previously suggested (Muscatine and Cernichiaro, 1969). This hypothesis is supported by the fact that the amino acid composition differs between zooxanthellate and non-zooxanthellate corals (Cuif et al., 1999). This hypothesis can also explain the lag-phase observed for the transition between light and dark, since the time necessary to obtain a stable dark rate of calcification could correspond to the synthesis of the last organic matrix proteins using photosynthates as precursors after switching off the light, and to their transport to the site of skeletogenesis.

To conclude, we have demonstrated that under controlled conditions of constant light, there is no endogenous circadian rhythm of calcification and photosynthesis and that light-enhanced calcification is dependent on the light regime only. Thus, in these conditions, we determined that a similar lag-phase exists between the transitions from light to dark or from dark to light calcification rates. At least two hypotheses from the literature can fit with our results: the role of photosynthesis on the pH in the coelenteron and the role of photosynthesis in supplying precursors of organic matrix. In a next step, our aim will be to study the biochemical composition of the organic

matrix during the day and during the night. Another approach will also be to determine the differential expression of genes in light and in dark conditions.

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