

# Light-Enhanced Calcification and Dark Decalcification in Isolates of the Soft Coral *Cladiella* sp. During Tissue Recovery

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**Abstract.** Light-enhanced calcification is a general characteristic of zooxanthellate corals, suggesting a link between calcification by the coral and photosynthesis by the zooxanthellae, but the relationship between zooxanthellae and coral hosts during this process has not been elucidated. We hypothesized that the effects of tissue injury on the coral fragments used in experiments studying calcification might obscure that link. To detect the effects of tissue injury on light-enhanced calcification, we measured calcification rates (sclerite formation) in the soft coral *Cladiella* sp. by the alkalinity anomaly method during a 36-day experiment following injury associated with coral fragmentation. In the 2 weeks after colony fragmentation, the calcification response did not show a relation with light intensity. The typical light-enhanced calcification pattern was not noticed until day 15 of tissue recovery. The calcification rate of this soft coral increased with light intensity and time of tissue recovery and was comparable to that of hard corals exposed to similar experimental conditions. However, *Cladiella* sp. decalcified in the dark. The diurnal calcification-decalcification cycles probably control sclerite size and shape.

## Introduction

Studies of the short-term effect of light on calcification rates of zooxanthellate scleractinian corals indicate that photosynthesis by symbiotic algae is involved in the process (see reviews by Barnes and Chalker, 1990; Gattuso *et al.*, 1999). Kawaguti and Sakumoto (1948) inferred that calci-

fication occurs in the light but not in the dark by measuring variations of levels of  $\text{Ca}^{2+}$  in the seawater in which they incubated the corals. Using  $^{45}\text{Ca}$ , Goreau (1959) also noted the favorable effect of light on coral calcification. Pearse and Muscatine (1971) introduced the concept of *light-enhanced calcification* in coral physiology and strongly suggested that zooxanthellae from the base of coral branches participate in the calcification of the tips of branches in *Acropora cervicornis*. Subsequent research has shown overwhelmingly that calcification rates are greater in the light than in the dark in various species of tropical and temperate scleractinian corals (Gattuso *et al.*, 1999) and that calcification is biologically controlled. The process depends upon (1) the calcifying cells in the aboral epidermis; (2) the active transport of calcium and inorganic carbon from the animal cells to the skeleton; and (3) the organic matter as a nucleating center of calcium carbonate crystals. Symbiotic algae may supply the coral with the necessary organic carbon and metabolic energy, they may create the optimum chemical microenvironment for crystallization of  $\text{CaCO}_3$ , or they may do both.

Scleractinian corals, coralline algae, and green algae are the main calcifying organisms of coral reefs. Symbiotic benthic foraminiferans and molluscs as well as bryozoans and echinoderms are also regarded as important reef calcifiers (Barnes and Chalker, 1990; Done *et al.*, 1996; Langer *et al.*, 1997; Gattuso *et al.*, 1999; Yamano *et al.*, 2000). The level of contribution by each of these taxa to reef carbonates varies with geographic region and is difficult to assess because different methods of study have been used in obtaining the data.

Soft corals (Octocorallia: Alcyonacea) are a major benthic component of Indo-Pacific reefs in particular, and they have a high level of ecological interaction through their

Received 10 August 2005; accepted 6 July 2006.

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Abbreviations: HL, high light; LL, low light; NL, no light.

production of secondary metabolites (Coll, 1992). Soft coral spiculite (the massive skeletons formed by sclerites cemented in aragonite) appears in fossil Quaternary coral reefs at various locations in the Indo-Pacific (Johnson and Risk, 1987; Accordi *et al.*, 1989; Cabioch *et al.*, 1995). Examples of spiculite in living *Sinularia* species are known (Konishi, 1981; Schumacher, 1997); however, today soft corals appear to provide only sand particles or cementing material.

The species-specific control of calcification in soft corals is implicit in the extensive use of sclerite morphology as key taxonomic characters (Bayer, 1981), but the underlying mineralization mechanisms in these corals remain largely unknown.

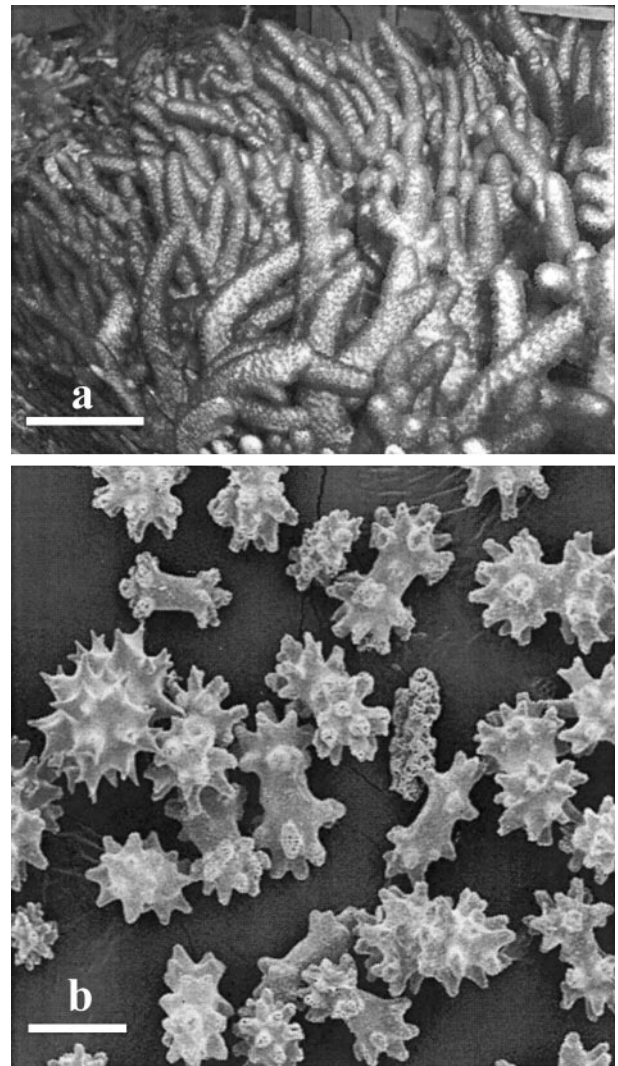
In a recent study (Tentori *et al.*, 2004) on growth and calcification of the tropical soft coral *Litophyton arboreum* during 49 days of tissue recovery from colony fragmentation, it was found that (1) tissue injury can affect the results of the investigation, and (2) under laboratory-controlled conditions, *L. arboreum* has a calcification rate comparable to those of some hermatypic corals. However, comparison across species should be taken only as a guide, particularly in cases in which calcification rates were obtained in experiments performed immediately after cutting and relocating the coral fragments from the reef (Goreau, 1959; Barnes, 1985; Dennison and Barnes, 1988).

There are fundamental differences between calcification of hard (scleractinian) and soft (alcyonacean) corals. Hard corals deposit calcium carbonate as aragonite crystals underneath their tissues, forming the well-known complex geological structures; in contrast, soft corals deposit calcium carbonate as high-magnesium calcite in the form of individual microscopic sclerites within the tissues of the colony (Konishi, 1981). Nevertheless, given their close systematic relationship, geographic distribution, and common association with zooxanthellae, we expected to find similar mechanisms of calcification between tropical hard and soft corals. In particular, it was of interest to determine whether soft corals exhibit light-enhanced calcification and to know if this calcification is affected by tissue injury associated with the removal of fragments from the parent colony. Our study suggests that a diurnal pattern of calcification/decalcification in *Cladiella* sp. may be typical of soft corals.

## Materials and Methods

### *The experimental organism*

*Cladiella* sp., in common with many alcyoniids, grows as thick carpet-like colonies that can extend to several square meters on the coral reef substratum (E.T., pers. obs.). The top surface of these colonies has lobes and fingerlike projections that are about 7 cm long and composed of hundreds of polyps that open to the external medium through tiny mouths 1–2 mm diameter (Fig. 1a). When disturbed, the coral contracts, changing color from dark mauve-brown to



**Figure 1.** *Cladiella* sp. (a) Colony reared at the Centre Scientifique Monegasque. Scale bar = 5 cm. (b) Sclerites diversity; smallest sclerites are found in the polyps; larger and well-defined halters (dumbbells) are more abundant toward the base and the interior of the colony. Scale bar = 25  $\mu\text{m}$ . Figure 1a photographed by E. Tambutté and used with permission.

gray. The skeleton of *Cladiella* consists of microscopic sclerites (Fig. 1b) distributed throughout the body, mainly within the mesoglea. The colonies used in this study were collected at 5–8 m depth in the Gulf of Aqaba, Jordan, and had been maintained in the aquarium of the Oceanographic Museum of Monaco for at least 3 years prior to this study. In this artificial environment *Cladiella* sp. is a relatively fast grower. Several fragments (5 to 6 cm in length) of one parent colony were cut on the same day and placed on plastic mesh trays in the experimental aquarium of the Centre Scientifique de Monaco under controlled conditions (semi-open circulation with a water exchange rate of 2%  $\text{h}^{-1}$ , 38 psu salinity,  $26 \pm 1$   $^{\circ}\text{C}$ ;  $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; photoperiod of 12h:12h light/dark). The fragments dis-

played the expansion and color typical of intact colonies within 2 h of fragmentation. Survival was 100%.

#### *Experimental setup*

Nine colony fragments selected at random from the pool were incubated for 4 h (0900 to 1300 hours) under one of three light intensities: high light (HL), low light (LL), and no light (NL) corresponding to 390, 90, and 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (metal halide lamps, Phillips HQI-TS 400 W). Each fragment was incubated at  $26 \pm 1^\circ\text{C}$  in 70 ml of filtered seawater (0.45- $\mu\text{m}$  Millipore, FSW). The incubation chamber consisted of a glass tube 3.5 cm in diameter, 8 cm tall, fitted with a plastic mesh floor under which a stirring bar was placed. Each tube was wrapped with the corresponding light filter. The set of incubating chambers was kept on a stirring plate in a water bath. A few minutes before the aquarium lights were switched on, we covered the plastic trays with light filters, allowing the *Cladiella* fragments to adapt to the experimental light intensity for 1 h before transferring them from the common tank into the individual incubation chambers. During incubation, the water was stirred with the magnet, providing constant and gentle movement of water around the coral (500 rpm); the incubation chamber was loosely covered with a plastic petri dish to avoid loss of water by evaporation. After incubation, the coral fragments were lightly dried on paper towels and their wet weight was measured before they were transferred back to the common tank. The same fragments were used throughout the experiment and exposed to the same light treatment so that individual responses could be followed for the 36 days of the experiment. Calcification rates were measured at 2, 9, 14, 19, 29, and 36 days (*recovery times*) after the corals were cut. All coral fragments were maintained in the same tank and under the same conditions between incubations.

#### *Calcification measurements*

The method employed to measure calcification rates is based on the alkalinity anomaly technique developed by Smith and Kinsey (1978); it uses a ratio of two equivalents of total alkalinity for each mole of  $\text{CaCO}_3$  precipitation. The ionic strength of the titrating acid (0.01 mol/l HCl) was adjusted by addition of high-grade NaCl (38.4 g  $\text{l}^{-1}$ , in agreement with Mediterranean seawater). The pH meter was calibrated on the day of use against pH 7.00 (ORION 9109107) and 4.01 (ORION 910104). The pH solutions, incubation samples, and titrating acid were equilibrated to  $25^\circ\text{C}$  in a water bath before being used. From each incubation medium, three replicate samples of about 20 ml were analyzed (sample volume was calculated from the sample weighed to the nearest 0.01 g); a final volume ratio of SW/HCl of about 20:8.5 was used for titration. FSW samples were refrigerated for up to 24 h before analysis. Alka-

linity determinations were performed with a Mettler DL70 automatic titrator. The total alkalinity (TA) was calculated as  $\text{mEq l}^{-1}$ , from the slope of the curve HCl vol/pH within the range of pH 3.0 to 4.2, using the Gran equation corrected for sulfate and fluorides (Hansson and Jagner, 1973). The calcification rates were normalized by protein content, skeletal mass, and wet weight.

#### *Measurement of proteins and skeletal mass*

To follow changes of calcification rates through time in each one of the coral fragments, it was necessary to keep the specimens intact. Their protein and skeletal contents were measured at the end of the last experiment (day 36 of tissue recovery). The coral samples were covered with 20 ml of 1 mol/l NaOH and heated at  $90^\circ\text{C}$  for 30 min, left to cool down for a few minutes, homogenized, and centrifuged at  $12,000 \times g$ ,  $22^\circ\text{C}$ , for 20 min. The supernatant was removed for protein analysis using Pierce MicroBCA reactant, measuring at 595 nm using beta gamma globulin standards. The NaOH-insoluble precipitate consisted of sclerites. The total skeletal weight was measured to the nearest 0.001 g from oven-dried samples ( $60^\circ\text{C}$  for 24 h).

To determine whether light intensity affected calcification rate, the responses to the three light treatments at each date of tissue recovery were analyzed by regression analysis. On day 29, only HL and LL samples were processed; these data were analysed by student's *t* test.

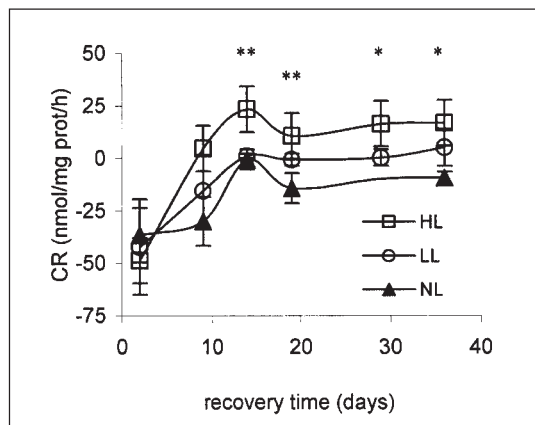
To determine whether calcification rate was affected by time of tissue recovery, the data of each light treatment throughout the study were analyzed by one-way analysis of variance.

## Results

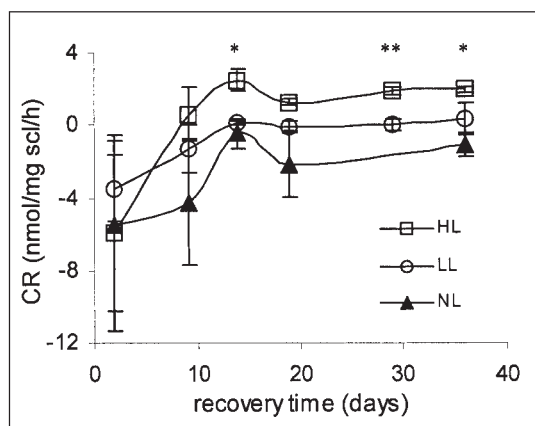
#### *Calcification rates*

Light-enhanced calcification was detected in *Cladiella* sp. 15 days after major tissue injury, when the coral fragments had recovered (Fig. 2a). The calcification rate increased with time of recovery under the high light (HL) and low light (LL) treatments normalized per protein content (HL  $P = 0.0116$ , LL  $P = 0.0095$ ). The no light (NL) treatment always resulted in decalcification; the decalcification rate decreased significantly with time of recovery (NL  $P = 0.0178$ ). The variation of the calcification (or decalcification) responses within treatments tended to be smaller as the coral fragments recovered.

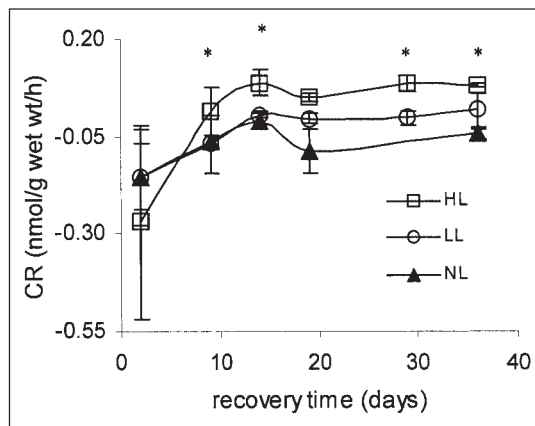
With different levels of significance, the same pattern of calcification rate was observed whether accretion of  $\text{CaCO}_3$  was normalized by protein content or by skeletal weight measured on day 36 of the study, or by coral wet weight measured on the day of each experiment (Fig. 2a–c).



a)



b)

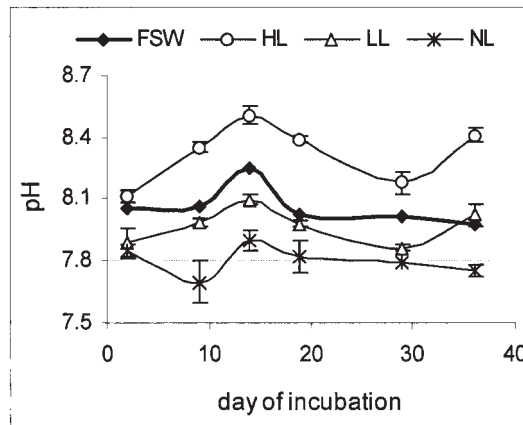


c)

**Figure 2.** *Cladiella* sp. Calcification rates through time of tissue recovery. Data normalized by (a) protein content; (b) sclerite dry weight, and (c) colony wet weight (mean ± SE). Values significantly different between light treatments are indicated by \* $P < 0.05$  or \*\* $P < 0.001$ .

*Alkalinity and pH of the incubation medium*

The seawater incubation medium had an alkalinity range of 2.549 to 2.594 mEq l<sup>-1</sup> and a pH range of 7.979 to 8.256.



**Figure 3.** *Cladiella* sp. pH of seawater at start (FSW) and end (HL, LL, NL) of incubation periods (mean ± SE). HL, high light; LL, low light; NL, no light.

After 4 h, according to the light intensity, with the coral fragments present, the seawater pH changed. The HL incubation medium had a higher pH than the original incubation medium (FSW). The NL incubation medium had a lower pH than FSW. The LL incubation medium showed the smallest changes of pH and tended to be lower than FSW (Fig. 3).

*Wet weight, calcium carbonate and protein content*

The colony fragments used were of similar dimensions. On completion of the study, their wet weight and protein content seemed well correlated, while the sclerite weight appeared to be independent (Table 1).

**Discussion**

*Choice of normalizing parameter*

Since the seminal work of Goreau (1959), protein content has been the most common parameter used for normalization of calcification estimates (see Appendix). The underlying assumptions are that protein represents biomass, that all biomass has the same calcifying potential, and that

**Table 1**

*Cladiella* sp. Constitution of the colonies on completion of the study, after 36 days of recovery

Treatment	Protein wt (mg)	Wet wt (g)	Sclerite wt (mg)
HL	37.4 ± 2.3	7.8 ± 1.4	318 ± 28
LL	34.0 ± 3.8	6.5 ± 2.1	351 ± 47
NL	29.1 ± 8.3	5.8 ± 2.2	321 ± 155

HL (high light) = 390; LL (low light) = 90; NL (no light) = 0 μmol m<sup>-2</sup> s<sup>-1</sup> (mean ± SE); n = 3 in all groups. The NL group had the lowest (0.080 g) and the highest (0.511 g) sclerite weights.

biomass runs parallel to the accretion surface of the skeleton. These assumptions may not apply to soft corals because a high proportion of their protein content is extracellular and because the sclerites are not homogeneously distributed in the colony (Tentori *et al.*, 2004).

Estimating calcification rates in terms of surface area has been attempted through a variety of methods (Appendix). Although these methods revealed valuable information, comparisons of such experimental data across research groups and calcifying taxa should be used with caution because the level of accuracy of surface area measurement does not match the level of accuracy of calcium carbonate measurement.

In the interests of comparing the calcification rates of *Cladiella* sp. to those of other calcifiers, and considering the impossibility of measuring the surface area of soft corals or their sclerites, we used protein as the normalizing parameter, keeping in mind that our data would underestimate the process. The analysis of protein content requires the destruction of the sample. The 36-day duration of the present study was a compromise between a time short enough to avoid significant changes in protein content but long enough to cover the required period of tissue recovery indicated in our previous study (Tentori *et al.*, 2004).

### Calcification

The calcification rates of several tropical and temperate calcifying organisms have been measured over the last few decades. The Appendix summarizes the investigations that employed protein, surface area, or both as normalizing parameters; the original data were recalculated for comparative purposes. The calcification rates fluctuate from 6.4 to 3680 and from 0.6 to 19.2 nmol CaCO<sub>3</sub> mg prot<sup>-1</sup> h<sup>-1</sup> for tropical and temperate species, respectively. The wide range of variation is in part due to the diverse experimental settings. Under similar measurement conditions, the calcification rates of the soft corals *Cladiella* sp. and *Litophyton arboreum* (23 and 49 nmol CaCO<sub>3</sub> mg prot<sup>-1</sup> h<sup>-1</sup>, respectively) are within the range of scleractinian calcification rates. These calcification rates are remarkably high and unexpected, suggesting that high calcification rates in daytime are not an exclusive characteristic of reef-building organisms and supporting the observations reported by Goreau and Goreau (1959).

The calcification rates of *Cladiella* were measured on colony fragments maintained in the same aquarium conditions, the only difference being the incubation under high, low, or no light initiated an hour prior to the alkalinity measurements. The results indicate that, once recovered from tissue injury, the effect of light intensity on calcification is immediate.

### Decalcification

Decrease of calcification rates in the dark is common in scleractinian corals, but decalcification is not (Gattuso *et al.*, 1999). Kawaguti and Sakumoto (1948) noted “intake of Ca<sup>2+</sup>” in all corals exposed to light and “output of Ca<sup>2+</sup>” in all corals exposed to dark. They argued that the skeleton formation was favored by the alkaline pH (8.84 to 9.15) of the incubating medium, which was assumed to be result of photosynthesis by zooxanthellae in the coral; correspondingly, the drop in pH (8.00 to 7.80) in the dark was the reason for the “resolution of the skeleton [sic].” We interpret such *intake* and *output* as calcification and decalcification, respectively. Chisholm (2000) observed dark decalcification in coralline algae incubated at various depths, and explained this as a result of previous light exposure or the acidification caused by cell respiration. It is not clear how “previous light exposure” could cause decalcification. Our results agree with Chisholm’s latter explanation. It is also possible that the tissue recovery verified visually underwater was overestimated and that decalcification was due to tissue injury.

Although the calcification rates of soft corals in the light are comparable to those of some tropical scleractinian corals, the net calcification rate in a 24-h cycle would be much lower if dark decalcification was taken into account. Our preliminary experiments on *Sarcophyton* sp. and *Sinularia* sp. indicated that these corals also decalcify in the dark; they also showed that the level of calcification at a given light intensity is affected by the previous treatment.

Octocoral sclerites consist of calcite and organic matter. Their main role is traditionally seen as physical deterrence to predation (Coll, 1992; Van Alstyne *et al.*, 1994; West, 1997). According to Bengston (2004), marine invertebrate skeletons with a poor organic matter structure are not the strongest. Bengston (2004) also states that the physiological cost of producing the mineral is smaller than that of producing the organic matrix. In this sense, and assuming that predation stress is reduced in the colder months in temperate waters, the loss of sclerite mass and sclerite organic matter during winter in the gorgonian *Leptogorgia virgulata* (Kingsley *et al.*, 1990) fits the physical deterrence model. However, a daily cycle of sclerite formation as suggested in this study would be a physical adaptation of little value in tropical waters. Our decalcification results must be linked to a different mechanism—one that is possibly related to the 2-h cycles of movement of Ca<sup>2+</sup> between seawater, tissues, and sclerites (Velimirov and King, 1979) in the gorgonian *Eunicella papillosa*.

### Tissue recovery

The experimental data presented in the Appendix clearly confirm the light-enhanced calcification concept. Our study shows an additional aspect of calcification studies not con-

sidered before: the light-enhanced calcification is unmistakable only when the experimental coral fragments have had time to heal (Fig. 2a–c). The delayed response on calcification obtained in this study is in agreement with the delayed response on cellular growth observed in the soft coral *Litophyton arboreum* following tissue injury (Tentori *et al.*, 2004). These findings suggest that other processes take over the metabolic energy reserves of the coral immediately after tissue injury.

Meszaros and Bigger (1999) investigated the mechanism of wound healing in the zooxanthellate gorgonian *Plexaura fusifera* and found that amoebocytes from the mesoglea accumulated at the wound site by migration rather than by cell division. These amoebocytes later differentiated into epithelial cells. Furthermore, while the process of wound healing was noticeable for 4 to 7 days, it took 2 weeks to complete. Tissue repair and metabolic energy status in scleractinian corals (Kramarsky-Winter and Loya, 2000; Houlbrègue *et al.*, 2003) could also explain the increased calcification rates of *A. formosa* after a one-week recovery period (Barnes, 1985) shown in the Appendix.

#### pH measurement

We detected a drop of pH and decalcification as the typical response to dark conditions (Fig. 3); however, corals under the high light treatment exhibited increases of pH and decalcification in the first few days of recovery (Fig. 2a–c). If the increase of pH indicates that photosynthesis was not interrupted in the high and low light treatments, the recorded decalcification results suggest that in order for carbonates to be deposited, metabolic energy needs to be available.

#### Acknowledgments

The Centre Scientifique de Monaco made available the research funds and infrastructure to conduct this work. Francesca Marubini helped us with the progress of the methodology employed. The Musée Océanographique de Monaco (Nadia Ounais and Pierre Gilles) kindly supplied the biological material. Eric Tambutté produced the photograph used in Figure 1. E. Tentori was supported by a study leave from Central Queensland University, Australia, and the hospitality of the CSM.

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## Appendix

## Calcification rates of corals from the literature

SPECIES	METHOD rec; inc; PAR; temp	CALCIFICATION RATES (LIGHT/DARK)		REFERENCE
		Original data	(A) Recalculated as nmol CaCO <sub>3</sub> mg prot <sup>-1</sup> h <sup>-1</sup>	
<b>TROPICAL CALCIFIERS</b>				
<b>Scleractinian Corals</b>				
	<sup>45</sup> Ca	μg Ca/mg N/h		
<i>Pocillopora damicornis</i>	24 h rec; 30h inc;	10.3/6.8	41.2/27.2	Goreau (1959)
<i>Acropora prolifera</i>	2 × 20 Watt	12.4/7.2	49.6/28.8	
<i>Porites divaricata</i>	lamps PAR; 25–	9.8/5.0	39.2/20.0	
<i>Oculina diffusa</i>	28.5°C	1.6/0.8	6.4/3.2	
	<sup>45</sup> Ca	μg Ca/mg N/h		
<i>Acropora palmata</i>	ND rec; 4–8h inc;	50.9/4.0	203.6/16.0	Goreau & Goreau (1959)
<i>Porites porites</i>	natural light; 26–	25.1/7.9	100.4/31.6	
<i>Montipora annularis</i>	28°C	7.3/0.3	29.2/1.2	
	<sup>45</sup> Ca	μg Ca/cm <sup>2</sup> /24 h		
<i>Stylophora pistillata</i>	0.5–2 h rec; 24h	44.0/50.0		Rinkevich & Loya (1984)
Tips	inc; 400–700		4.58/5.21	
Base	PAR; ND temp	6.3/6.0	0.66/0.63	
<i>Acropora formosa</i>	Alkalinity anomaly few hours–1 week rec; 1.1–1.3 h inc; 212–1335 PAR; 25–29°C	μmol/mg prot/h <sup>a</sup> 0.370/0.294 <sup>b</sup> 0.500/0.157 <sup>c</sup> 0.698/0.261	370.6/294.4 500.9/157.7 698.9/261.6	Barnes (1985)
<i>Acropora formosa</i>	Alkalinity anomaly 24 h rec; 2 h inc; shaded to >150 PAR; ambient temp.	μmol/h/mg prot 3.68/1.84	3680/1840	118.33/59.16 Dennison & Barnes (1988)
<i>Stylophora pistillata</i>	<sup>d45</sup> Ca & <sup>e</sup> Alkalinity anomaly 1 month rec; 1 h inc; 175 PAR; 26°C	nmol/mg prot/h <sup>d</sup> 58.5/— <sup>e</sup>	58.5/— 343/—	Tambutté <i>et al.</i> (1995)
<i>Stylophora pistillata</i>	Alkalinity anomaly Weeks rec: 2 h inc; 300 PAR; 27°C	nmol CaCO <sub>3</sub> /mg prot/h 10.3/4.6	10.3/4.6	Gattuso <i>et al.</i> (2000)
<i>Stylophora pistillata</i>	<sup>45</sup> Ca several weeks rec; 2–180 min inc; 250 PAR; 25°C several weeks rec; 2–180 min inc; 250 PAR; 25°C	nmol h <sup>-1</sup> mg prot <sup>-1</sup> 49.25/12.31	49.25/12.31	Furla <i>et al.</i> (2000)

Calcification rates of corals from the literature (*continued*)

SPECIES	METHOD rec; inc; PAR; temp	CALCIFICATION RATES (LIGHT/DARK)		REFERENCE	
		Original data	(A) Recalculated as nmol CaCO <sub>3</sub> mg prot <sup>-1</sup> h <sup>-1</sup>		(B) Recalculated as μmol CaCO <sub>3</sub> dm <sup>-2</sup> h <sup>-1</sup>
<b>TROPICAL CALCIFIERS</b>					
<b>Scleractinian Corals</b>					
<i>Porites compressa</i>	Alkalinity anomaly 2 weeks rec; ND inc; <sup>f</sup> 698; <sup>g</sup> 150; <sup>h</sup> 81PAR; 26.2°C	mg CaCO <sub>3</sub> cm <sup>-2</sup> d <sup>-1</sup> <sup>f</sup> 1.94 <sup>g</sup> 1.03 <sup>h</sup> 0.58		80.83 42.92 24.17	Marubini <i>et al.</i> (2001)
<b>Octocorals</b>					
<i>Litophyton arboreum</i>	<sup>45</sup> Ca 2 month rec; 2 h inc; 200 PAR; 27°C	nmoles mg prot <sup>-1</sup> h <sup>-1</sup> 47.95/—	47.95/—		Tentori <i>et al.</i> (2004)
<i>Cladiella</i> sp.	Alkalinity Anomaly 36 days rec; 4 h inc; 400 PAR; 26°C	nmoles mg prot <sup>-1</sup> h <sup>-1</sup> 23.3/—6.87	23.3/—6.87		This work
<b>Hydrozoan Coral</b>					
<i>Millepora complanata</i>	<sup>45</sup> Ca ND rec; 4–8 h inc; “sunny” PAR; 26–28°C	μg Ca/mg N/h 35.7/4.7	142.8/18.8		Goreau & Goreau (1959)
<b>Coralline Algae</b>					
<i>Porolithon</i> sp.	<sup>45</sup> Ca 24 h rec; 30 h inc; 2 × 20 Watt lamps PAR; 28°C	μg Ca/mg N/h 8.8/3.3	35.2/13.2		Goreau (1959)
<i>Neogoniolithon brassica- florida</i>	Alkalinity anomaly “visible” rec; ND inc; 1516 PAR at 0 m depth; 23–30°C	mmol/m <sup>2</sup> /h 8.49/—0.57 7.51/1.13 3.14/0.37		84.9/—5.7 75.1/11.3 31.4/3.7	Chisholm (2000)
<i>Hydrolithon onkodes</i>	0 m	9.57/—0.35		95.7/—3.5	
<i>N. conicum</i>	0 m	3.45/0.26		34.5/2.6	
	6 m	2.07/0.20		20.7/2.0	

Calcification rates of corals from the literature (*continued*)

SPECIES	METHOD rec; inc; PAR; temp	CALCIFICATION RATES (LIGHT/DARK)		REFERENCE	
		Original data	(A) Recalculated as nmol CaCO <sub>3</sub> mg prot <sup>-1</sup> h <sup>-1</sup>		(B) Recalculated as μmol CaCO <sub>3</sub> dm <sup>-2</sup> h <sup>-1</sup>
<b>TEMPERATE CALCIFIERS</b>					
<b>Scleractinian Corals</b>					
<i>Astrangia danae</i>	Alkalinity anomaly min 3 weeks rec; 3 h inc; 400 PAR; <sup>i</sup> 27; <sup>j</sup> 15; <sup>k</sup> 6.5°C	μmolCaCO <sub>3</sub> dm <sup>-2</sup> h <sup>-1</sup> <sup>i</sup> 51.2/34.5 <sup>j</sup> 14.9/13.7 <sup>k</sup> -1.2/-0.1		51.2/34.5 14.9/13.7 -1.2/-0.1	Jacques <i>et al.</i> (1983)
<i>Plesiastrea versipora</i>	<sup>45</sup> Ca min 1 week rec; 4 h inc; 300 PAR; 15.5 °C	nmoles mg prot <sup>-1</sup> h <sup>-1</sup> 7.38/— μmol CaCo <sub>3</sub> / dm <sup>2</sup> /h 9.70/6.58	7.38/—	9/6.58  9.79/6.58	Howe & Marshall (2002)
<b>Octocorals (azooxanthellate)</b>					
<i>Eunicella papillosa</i>	<sup>45</sup> Ca ND rec; 12–24 h inc; NA; 14°C	μg Ca/mg N/h 4.8/—	19.2/—		Velimirov & King (1979)
<i>Corallium rubrum</i>	<sup>45</sup> Ca 1 day rec; 15 min to 48 h inc; NA; 18°C	nmol/mg prot/day 15.05	NA/0.62		Allemand & Grillo (1992)
<i>Corallium rubrum</i>	<sup>45</sup> Ca 1 day rec; 1–5 d inc; D; 18°C	nmol/mg prot/day (tips) 19.9 (base) 9.7	NA/0.83 NA/0.40		Allemand & Bénazet- Tambutté (1996)

## ABBREVIATIONS

Under Methods: rec, recovery time; inc, incubation time; PAR, photosynthetic active radiation ( $\mu\text{m m}^{-2} \text{s}^{-1}$ ). Table as a whole: NA, not applicable; ND, no data.

## NOTES

**Goreau (1959), Goreau and Goreau (1959), Velimirov & King (1979).** Recalculation (A) based on the assumption that 1 mg N = 6.25 mg prot (Lehninger, 1978).

**Rinkevich & Loya (1984).** Experiments run with 24 h continuous light exposure. SA calculated from photograph sketches.

**Barnes (1985).** Temperature: <sup>a</sup>25.9, <sup>b</sup>25.2; <sup>c</sup>29.4°C. Recovery time: <sup>a</sup>a few hours; <sup>b</sup>and <sup>c</sup>1 week. PAR: <sup>a</sup>1335, <sup>b</sup>212; <sup>c</sup>258.

**Dennison and Barnes (1988).** Experiments performed in austral summer; Recalculation (A) based on reported data of average surface area of 3.11 cm<sup>2</sup> per mg protein. SA = SA calices + SA branches.

**Tambutté *et al.* (1995).** <sup>d</sup>CR = -243.6 + 0.88 °CR. (CR = calcification rate.)

**Gattuso *et al.* (2000).** Units should be “nmol” not “mmol” (Gattuso, pers. comm.).

**Marubini *et al.* (2001).** SA calculated assuming branch tips are regular geometric forms.

**Jacques *et al.* (1983).** SA calculated from projected area of photography.

**Howe & Marshall (2002).** SA extrapolated from 24.4 mm diam. cores.

**Tentori *et al.* (2004).** Recalculation (A) includes “polyps” and “stems” data from original.

**Chisholm (2000).** Light calcification reported as “Gross calcification = (C light + C dark)”; SA extrapolated from 36 mm diam. cores.

**Allemand and Bénazet-Tambutté (1996).** Calcification rate data include axial skeleton and sclerites.

<sup>e,f,g,h,i,j</sup>Indicates correspondence between columns.