Global Change Biology (2003) **9,** 1660–1668, doi: 10.1046/j.1529-8817.2003.00678.x

Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral

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Abstract

We show here that CO_2 partial pressure (pCO_2) and temperature significantly interact on coral physiology. The effects of increased pCO_2 and temperature on photosynthesis, respiration and calcification rates were investigated in the scleractinian coral *Stylophora pistillata*. Cuttings were exposed to temperatures of 25 °C or 28 °C and to pCO_2 values of ca. 460 or 760 µatm for 5 weeks. The contents of chlorophyll c_2 and protein remained constant throughout the experiment, while the chlorophyll *a* content was significantly affected by temperature, and was higher under the 'high-temperature–high-pCO_2' condition. The cell-specific density was higher at 'high pCO_2' than at 'normal pCO_2' (1.7 vs. 1.4). The net photosynthesis normalized per unit protein was affected by both temperature and pCO_2, whereas respiration was not affected by the treatments. Calcification decreased by 50% when temperature and pCO_2 were both elevated. Calcification under normal temperature did not change in response to an increased pCO_2. This is not in agreement with numerous published papers that describe a negative relationship between marine calcification and CO_2. The confounding effect of temperature has the potential to explain a large portion of the variability of the relationship between calcification and pCO_2 reported in the literature, and warrants a re-evaluation of the projected decrease of marine calcification by the year 2100.

Keywords: calcification, coral, global change, photosynthesis, pCO_2, temperature

Received 21 December 2002; revised version received and accepted 15 May 2003

Introduction

The response of ecosystems to global environmental change, and its retroaction on climate and human societies, is one of the major challenges facing science. The partial pressure of CO_2 (pCO_2) increases in the atmosphere due to anthropogenic inputs of carbon dioxide. It has increased by 32% between 1880 and 2000 (280 vs. 370 µatm; Houghton *et al*., 2002). This has important consequences on the Earth's climate, including air temperature, which has risen by 0.6 °C between 1880 and 2000. These past and predicted changes of atmospheric pCO_2 and temperature have prompted several studies of the response of terrestrial organisms and ecosystems. For example, the free-air CO_2 enrichment (FACE) and free-air temperature increase (FATI) experiments have provided considerable information on the response of terrestrial plants and communities to climatic changes. However, interactions between environmental parameters must be investigated because they vary in combination. Studies of interactions between CO_2 enrichment and climate have begun at the community level in terrestrial ecosystems quite some time ago, for example by combining the FACE and FATI approaches (Nijs *et al*., 1996). A model demonstrates that the increase in global net ecosystem production since 1861 will decline as the CO_2 fertilization effect becomes saturated and is diminished by changes in climatic factors (Cao & Woodward, 1998). Recent experiments in terrestrial community highlight the need for a multifactor experimental approach in

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understanding ecosystem responses to global change (Morgan, 2002; Shaw *et al*., 2002).

The atmosphere and surface ocean being tightly coupled, the environmental changes observed long ago in the atmosphere are now also distinguishable in the ocean. Several reports demonstrated that the concentration of inorganic carbon (Brewer *et al*., 1997) and temperature (Levitus *et al*., 2000) of the surface ocean were significantly higher during the late 20th century than during the previous 50–200 years. It is likely that these changes will continue well into the 21st century as pCO_2 increased at a rate of $0.39\%\,yr^{-1}$ during the period 1990–2000 (CDIAC, 2002), compared with an average increase of $0.24\%\,yr^{-1}$ during the period 1880–1990 (Mauna Loa record; Houghton *et al*., 2002). However, considerably less information is available on the response of the marine realm than of the terrestrial realm. Numerous biotic and abiotic factors control the structure and function of marine ecosystems. Their synergistic or antagonistic interactions are poorly known because physiological responses are primarily investigated by manipulating one parameter at a time.

Coral reef ecosystems are negatively affected by the increase of both temperature and pCO_2. Increased temperature leads to the loss of zooxanthellae, the unicellular algae that live symbiotically within the animal cells, or to the decrease in chlorophyll content per algal cell. The frequency and geographical extent of this response, referred to as coral bleaching, have increased considerably, and coral bleaching is predicted to become annual within 30–50 years (Hoegh-Guldberg, 1999). It is now well established that calcification of corals and coral communities is controlled by the saturation of seawater with respect to aragonite (Gattuso *et al*., 1999; Langdon *et al*., 2000; Leclercq *et al*., 2000; Leclercq *et al*., 2002; Marubini *et al*., in press):

$$\Omega_{arag} = \frac{[Ca^{2+}][CO_3^{2-}]}{K'_{sp}},$$

where K'_{sp} is the stoichiometric solubility product of the aragonite form of $CaCO_3$. Since $[Ca^{2+}]$ is about 100 times that of $[CO_3^{2-}]$ and is a near-conservative element of seawater over 10^4 years, Ω_{arag} is largely determined by $[CO_3^{2-}]$. The increase of pCO_2 in the surface ocean profoundly affects the seawater carbonate system. One of the changes is a decrease in the concentration of one of the building blocks of calcium carbonate, the carbonate ions:

$$CO_2 + CO_3^{2-} + H_2O \rightarrow 2HCO_3^-.$$

Several papers have used the experimental evidence published recently in an attempt to predict the future response of calcification by corals and coral communities to elevated pCO_2 and its biogeochemical significance (Gattuso *et al*., 1999; Kleypas *et al*., 1999). The consensus opinion is that calcification will decrease by 14–30% by 2100 (Gattuso *et al*., 1999; Kleypas *et al*., 1999). However, these predictions are hampered by the great variability of the responses to elevated pCO_2 reported so far (-3% to -79%; Table 1).

The aim of the present study is to gain a better understanding of the effect of global environmental changes on the physiology of scleractinian corals in order to reach better predicting capabilities on their response to future changes. The effects of increases of pCO_2 and temperature of magnitudes similar to those

Table 1 Predicted changes of calcification in corals, coral communities and other calcifying ecosystems during the period 1880–2100

Species or community	Percent change	Reference
Stylophora pistillata	−3	Gattuso *et al*. (1998)
Porites porites	−19	Marubini & Thake (1999)
Okinawa reef flat	−79	Ohde & van Woesik (1999)
Biosphere 2 (reef mesocosm)	−65	Langdon *et al*. (2000)
Coral community (reef mesocosm)	−27	Leclercq *et al*. (2000)
Coral community (reef mesocosm)	−19	Leclercq *et al*. (2002)
Turbinaria reniformis	−13	Marubini *et al*. (2003)
Galaxea fascicularis	−16	
Pavona cactus	−18	
Acropora verweyi	−18	
Biosphere 2 (reef mesocosm)	−85	Langdon *et al*. (2003)
Stylophora pistillata (at 25 °C)	+5	Present study
Stylophora pistillata (at 28 °C)	−50	Present study

Values of the aragonite saturation state of 4.9 and 3 were used for the years 1880 and 2100, respectively (Gattuso *et al*., 1999).

expected between 2000 and 2100 were investigated in combination for the first time.

Materials and methods

Biological material

Colonies of the branching scleractinian coral *Stylophora pistillata* (Esper, 1797) were grown for more than 5 years in aquaria (pCO_2 = 500 µatm, temperature = 27 °C, irradiance = 350 µmol $m^{-2} s^{-1}$ and photoperiod = 12 : 12). Forty nubbins from the same parent colony were prepared 2 weeks prior to the experiment, suspended on nylon strings (Al-Moghrabi *et al*., 1993) and kept under controlled conditions (pCO_2 = 430 µatm, temperature = 25 °C, irradiance = 380 µmol $m^{-2} s^{-1}$ and photoperiod = 12 : 12). Each piece of coral displayed similar forms and sizes.

Experimental setup

Mediterranean seawater pumped at 55 m depth (salinity = 38.0 and temperature ≈ 13 °C) was continuously supplied to two 150 L tanks (Fig. 1). It was heated to 24 °C and its pCO_2 was adjusted to the desired level as described below. The experimental setup also comprised four incubation tanks (0.4 m × 0.3 m × 0.2 m; volume = 24 L), which were continuously supplied with seawater from the two larger tanks. The seawater renewal rate was six times per day, and the seawater was constantly mixed with a Rena® pump (6 L min^{-1}) (Rena Corporation, Charlotte, NC 28273, USA). Light, provided by two metal halide lamps (Powerstar HQI-T 400W/D, Osram GmBH, Germany), ran on a 12 : 12 photoperiod. The irradiance was measured with a 4π quantum sensor (LI-COR, LI-193SA) and adjusted to 380 µmol photons $m^{-2} s^{-1}$. The temperature was controlled in each tank to within ±0.1 °C using a temperature controller (Eliwell, PC 902 T, Invensys Plc., London, UK) connected to submersible 300 W heaters.

Four culture conditions were used: 450 µatm–25.3 °C (referred to as 'normal pCO_2, normal temperature'), 470 µatm–28.2 °C ('normal pCO_2, high temperature'), 734 µatm–25.1 °C ('high pCO_2, normal temperature') and 798 µatm–28.3 °C ('high pCO_2, high temperature'). All colonies were initially kept for 2 weeks under 'normal pCO_2, normal temperature'. After this initial period, 10 colonies were randomly dispatched in each of the four tanks and the experiment ran for five more weeks.

Control of seawater pCO_2

Seawater pCO_2 was adjusted before flowing into aquaria using a pH controller (R305, Consort Inc., Topac Inc., St. Hingham, USA) connected to pH electrodes (Orion, model 8102SC, Orion Research Inc., Boston, USA) as described by Leclercq *et al*. (2000). The electrodes were calibrated every 2 days using the Sea Water Scale (SWS) buffers TRIS and AMP (DOE, 1994). The pH in seawater is a function of total alkalinity (*TA*) and CO_2 concentration. Since the seawater used in this experiment had a known and constant *TA* (2.6 mEq kg^{-1}), seawater pH was manipulated to reach

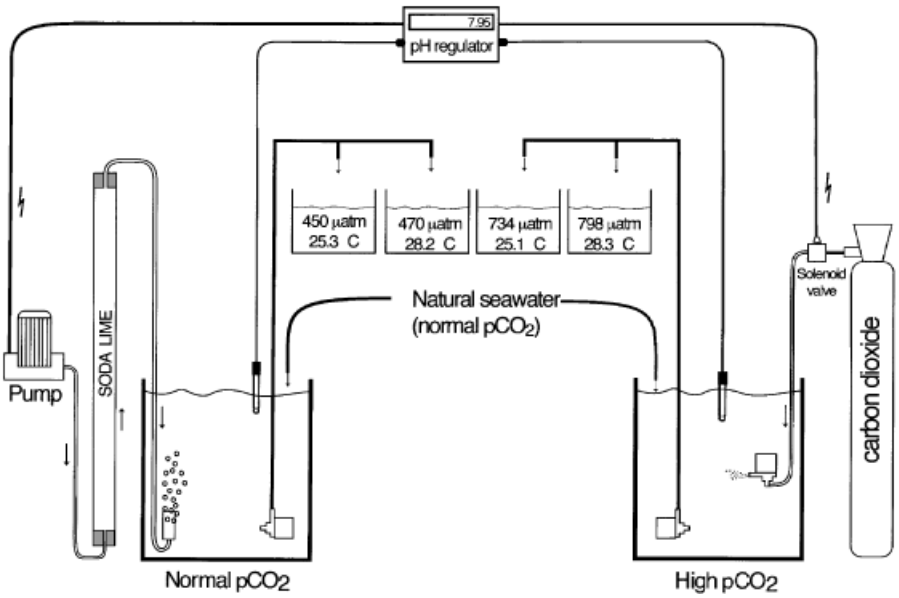

Fig. 1 Experimental setup used to control pCO_2 in aquaria.

Table 2 Temperature and parameters of the carbonate system in each aquarium

	T (°C)	pCO_2 (µatm)	pH (SWS)	CO_2 (mmol kg^{-1})	HCO_3^- (mmol kg^{-1})	CO_3^{2-} (mmol kg^{-1})	DIC (mmol kg^{-1})
Aq. 1	25.3	450	8.04	0.013	1.94	0.27	2.22
Aq. 2	28.2	470	8.02	0.012	1.91	0.28	2.21
Aq. 3	25.1	734	7.86	0.021	2.12	0.20	2.33
Aq. 4	28.3	798	7.83	0.021	2.10	0.20	2.33

T: temperature; DIC: dissolved inorganic carbon; SWS: Sea Water Scale. The values reported are averages of several thousands of data collected during the 5 weeks following the perturbation. The total alkalinity was constant and equal to 2.6 mEq kg^{-1}.

a fixed value of pCO_2. pH modifications were achieved by bubbling seawater with either pure CO_2 (to increase pCO_2) or with CO_2–free air (to decrease pCO_2). For the normal pCO_2 treatment, the pH controller was plugged to an air pump (900 L h^{-1}) that ran when pH fell below 8.09, thus bubbling CO_2–free air in the Mediterranean seawater used for the renewal until pH = 8.11 was obtained (Fig. 1). CO_2–free air was obtained by passing it through a 2 m long 'CO_2 scrubber' that consisted of a Perspex cylinder filled with soda lime (R.P. Normapur AR, VWR International). For the high pCO_2 treatment, the pH controller opened a solenoid valve when pH rose above 7.89, thus injecting pure CO_2 from a 35 kg bottle to seawater until pH = 7.87 was reached. The values of pH and temperature were stored every 5 min on a data-logger (LI-COR, LI-1000).

The seawater supply had a CO_2 partial pressure above the average value of atmospheric pCO_2 at the time of measurement (368 µatm; CDIAC, 2002). It was approximately 550 µatm due to the warming of seawater from 13 °C (at the depth of pumping) to 25 °C or 28 °C (in the aquaria) and, to a lesser extent, to CO_2 generated by bacterial respiration during the transit time (2 h) from the inlet of the underwater pump to the delivery into the experimental tanks. Some CO_2 had to be removed from the incoming seawater, as described above, in order to obtain the 'normal-pCO_2' condition. Results of the pCO_2 regulation (Table 2) show that, due to the relatively short residence time of the water in the two larger tanks, pCO_2 remained above 368 µatm in the 'normal-pCO_2' condition, even after being bubbled with CO_2–free air.

Photosynthesis and respiration

Net photosynthesis (P_n) and dark respiration (R) were measured on three colonies taken in each of the four tanks. The first measurements took place 1 week after the experiment began, when all colonies were kept in the same condition (normal pCO_2 and normal temperature), and twice a week after the perturbation and for the remaining duration of the experiment (5 weeks). Three Perspex chambers (240 mL) were used simultaneously in a thermostated water bath. They were filled with the seawater used in each treatment and were illuminated with a metal halide lamp at 380 µmol m^{-2} s^{-1}. The incubation medium was continuously agitated using a magnetic stirrer. Each incubation was processed as follows: adaptation to light (20 min), P_n measurement (30 min), adaptation to dark (20 min) and R measurement (30 min). The chamber was flushed between each measurement. Dissolved O_2 was measured using a polarographic electrode (Ponselle Viraflay, France) calibrated daily against air-saturated seawater and a saturated solution of sodium sulfite (zero oxygen). Oxygen concentration was monitored in the chamber and its average value was stored every 1 min using a data-logger (LI-1000, Li-COR). The rates of net photosynthesis and respiration were estimated by regressing O_2 against time. All incubations took place between 08:00 and 14:00 hours.

At the end of the experiment, five colonies from each treatment, including those used for respirometric measurements, were frozen pending subsequent measurement of the total protein content, chlorophyll *a* and c_2 concentrations and surface area. The total surface of each colony was measured using the aluminum foil technique (Marsh, 1970). Chlorophyll *a* and c_2 were extracted twice in 100% acetone at 4 °C during 24 h. The concentrations were calculated using spectrophotometric equations (Jeffrey & Humphrey, 1975). Proteins were solubilized in NaOH (1 N) at 90 °C for 30 min. The samples were then neutralized with HCl (1 N) and the total protein content was measured using the Bradford method (Bradford, 1976) with the Coomassie protein assay reagent (Ref. 23200, Pierce, Rockford, IL, USA). The optical density was read at 595 nm using a microplate reader (Multiskan® Bichromatic, Labsystems, Helsinki, Finland). Bovine gamma globulin was used as a standard.

Calcification

The skeletal dry weight was measured every week by weighing each colony using the buoyant weight

technique (Jokiel *et al.*, 1978; Davies, 1989). The rate of calcification was calculated using the following formula:

$$G = \sqrt[n]{\frac{W_n}{W_0}} - 1,$$

where G is the calcification rate, n is the time (days), W_n is the skeletal dry weight (g) after n days of culture and W_0 is the skeletal dry weight (g) measured the previous week.

Cell-specific density

At the end of the experiment, three nubbins from each treatment were used to determine the average number of zooxanthellae per animal cell (cell-specific density, *CSD*). Corals were crushed with a hammer, placed in a 50 mL flask and macerated by agitation (Muscatine & Cernichiari, 1969). Intact host cells were then processed and counted as described in Muscatine *et al.* (1998). Maceration, sampling and counting were carried out between 10:00 and 16:00 hours, and cells were observed within 1 h of maceration. Host cells containing one or more endosymbiotic algae were recognized by the presence of host cytoplasm and a host cell nucleus (DAPI staining). Approximately 330 host cells from each colony were observed and ranked according to the number of zooxanthellae (from one to eight) that each contained. The cell-specific density was estimated as

$$CSD = \frac{\sum (f_i \times r_i)}{\sum f_i},$$

where f_i is the frequency of occurrence of host cells in each rank (r_i).

Statistical analysis

Analyses of variance (ANOVA) were performed using JMP 3.1 (SAS Institute Inc., Cary, NC, USA) for Macintosh computers. Results are expressed as mean $\pm$ standard error of the mean (SE), unless mentioned otherwise. n is the sample size and P is the probability.

Results

Proteins and chlorophyll contents

The amount of proteins measured on each nubbin at the end of the experiment was not significantly affected by pCO_2 or by temperature (ANOVA, $P = 0.4$ and 0.7). Its average value was 0.72 ± 0.03 mg protein cm^{-2}. Since this parameter was not affected by the culture conditions, it was used to normalize net photosynthesis and respiration.

The chlorophyll c_2 normalized per surface area was not affected by pCO_2 (ANOVA, $P = 0.3$) or by temperature (ANOVA, $P = 0.1$), and its average value was 1.34 ± 0.06 µg cm^{-2}. The chlorophyll c_2 normalized per unit protein was also not affected by pCO_2 (ANOVA, $P = 0.98$) or by temperature (ANOVA, $P = 0.2$).

The content of chlorophyll *a* normalized per unit protein was not significantly affected by pCO_2 (ANOVA, $P = 0.3$), but was affected by temperature (ANOVA, $P = 0.02$). The average values were 2.90 ± 0.21, 3.46 ± 0.22, 2.59 ± 0.31 and 3.99 ± 0.38 µg (mg prot.)$^{-1}$, respectively, for 'normal pCO_2, normal temperature', 'normal pCO_2, high temperature', 'high pCO_2, normal temperature' and 'high pCO_2, high temperature'. The content of chlorophyll *a* normalized per surface area was also significantly affected by temperature (ANOVA, $P = 0.005$).

Cell-specific density

The *CSD* was significantly affected by the treatments (Fig. 2): it increased under 'high pCO_2' (ANOVA, $P < 0.001$) without being affected by the change in temperature (ANOVA, $P = 0.4$). It was 1.32 ± 0.01 at 'normal temperature, normal pCO_2', 1.40 ± 0.03 at 'high temperature, normal pCO_2', 1.70 ± 0.03 at 'normal temperature, high pCO_2' and 1.66 ± 0.04 at 'high temperature, high pCO_2'. The number of zooxanthellae per animal cell was different when pCO_2 increased. There was a dominance of singlets (one zooxanthella per animal cell) over doublets or triplets (two or three zooxanthellae per animal cell) under 'normal pCO_2', with approximately 70% of singlets and 30% of doublets. The frequency distribution changed at elevated pCO_2 with 47% of singlets, 41% of doublets and

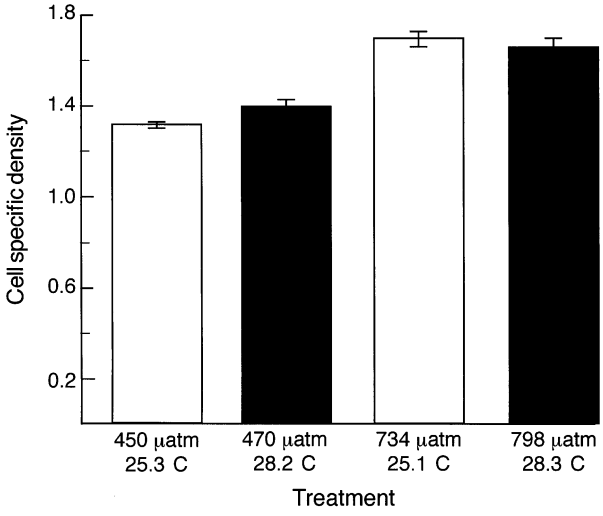

Fig. 2 Cell-specific density of *S. pistillata* at each experimental condition (mean $\pm$ SE); $n = 3$ for each treatment.

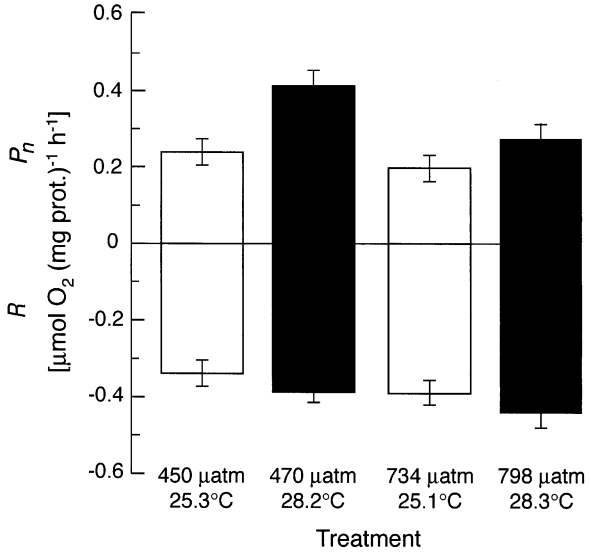

Fig. 3 Rates of net photosynthesis (P_n) and respiration (R) of *S. pistillata* at each experimental condition (mean ± SE); $n = 5$ for each treatment.

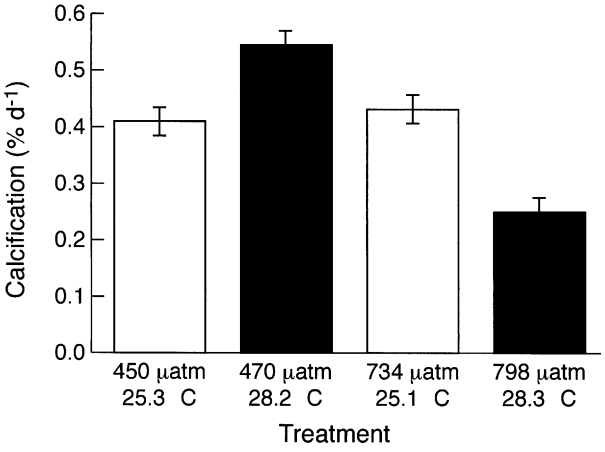

Fig. 4 Calcification rate (% day^{-1}) of *S. pistillata* at each experimental condition (mean ± SE); $n = 5$ for each treatment.

11% of animal cells containing more than two zooxanthellae.

Photosynthesis, respiration and calcification

The P_n of each colony measured during the 5 weeks subsequent to the perturbation did not vary with time (repeated measures ANOVA, $P = 0.15$). Therefore, only the average value obtained for each colony during this period was considered in the present study. P_n normalized per unit protein was affected by temperature (ANOVA, $P = 0.0005$; Fig. 3) and pCO_2 (ANOVA, $P = 0.009$). It was higher at high than at normal temperature, and lower at high than at normal pCO_2. Its values were 0.24 ± 0.03 and 0.41 ± 0.04 µmol O_2 (mg prot.)$^{-1}$ h^{-1}, respectively, at 'normal temperature, normal pCO_2' and 'high temperature, normal pCO_2'. Under high pCO_2, P_n values were 0.20 ± 0.03 and 0.27 ± 0.04 µmol O_2 (mg protein)$^{-1}$ h^{-1}, respectively, at normal and elevated temperature. Dark respiration was not affected by temperature (ANOVA, $P = 0.12$; Fig. 3) or by pCO_2 (ANOVA, $P = 0.11$) and was, on average, 0.39 ± 0.02 µmol O_2 (mg protein)$^{-1}$ h^{-1}.

The rate of calcification of each colony did not vary significantly after the perturbation (repeated measures ANOVA, $P = 0.3$). Therefore, the average value obtained for each colony during the period subsequent to the perturbation was used. The calcification rate was significantly affected by the treatment (ANOVA, $P < 0.0001$ for pCO_2 and $P = 0.3$ for temperature; Fig. 4). The significant interaction ($P < 0.001$) between pCO_2 and temperature demonstrates that there was a response to a change in temperature, but that it differs depending on the level of pCO_2. The increase in temperature induced an increase of calcification at normal pCO_2 (0.41 ± 0.02 vs. 0.54 ± 0.03% day^{-1}), whereas it led to a decrease of calcification at elevated pCO_2 (0.43 ± 0.02 vs. 0.27 ± 0.02% day^{-1}).

Discussion

The effect of elevated pCO_2 on calcification in corals and coral communities is now well documented (Gattuso *et al.*, 1999; Ohde & van Woesik, 1999; Langdon *et al.*, 2000; Leclercq *et al.*, 2000, 2002; Langdon *et al.*, 2003; Marubini *et al.*, 2003), but the interacting effects of pCO_2 and temperature have never been considered. Our results show that this interaction is significant in calcification.

The fact that the rate of dark respiration remained constant when pCO_2 increased is in agreement with the study of Langdon *et al.* (2003). However, dark respiration also remained constant when temperature increased, which differs from other works (Jokiel & Coles, 1990; Kajiwara *et al.*, 1995). Kajiwara *et al.* (1995) increased the temperature by 10 °C (20 °C to 30 °C). It is likely that the increase in temperature used in our experiment (+3 °C) was too small to lead to a detectable increase in the rate of respiration. Leclercq *et al.* (2002) have shown that the dark community respiration was slightly affected by variations of pCO_2 and was higher under the high pCO_2 condition.

The increase of photosynthesis with increasing temperature under normal pCO_2 is in agreement with previous studies performed on scleractinian corals

(Coles & Jokiel, 1977; Coles & Jokiel, 1978; Kajiwara *et al*., 1995). Kajiwara *et al*. (1995) showed that the rate of photosynthesis of *Acropora pulchra* increases up to 28 °C and decreases at higher temperature. Elevated pCO_2 did not stimulate photosynthesis, which even decreased slightly. Langdon *et al*. (2003) also showed that net community production did not change in response to elevated pCO_2. Corals are known to rely on bicarbonate for photosynthesis (Burris *et al*., 1983; Goiran *et al*., 1996). The increase in pCO_2 results in higher concentrations of dissolved CO_2 and bicarbonate (Table 2), but the increase of the bicarbonate reservoir in which corals pump carbon for photosynthesis is likely too small (9–10%) to lead to a measurable increase of photosynthesis. Elevated pCO_2 has only a stimulatory effect on CO_2 users, such as seagrasses, which rely on dissolved molecular CO_2 for photosynthesis (Zimmerman *et al*., 1997).

The *CSD*, however, increased in response to an increase in pCO_2 and was not affected by temperature. It was identical at both temperatures (under normal pCO_2) and equal to 1.4. The same value has been reported earlier in the same species (Muscatine *et al*., 1998), and indicates that there is a dominance of singlets over doublets or triplets under normal pCO_2. This seems to be the standard condition of the symbiosis. The *CSD* increased to 1.7 under elevated pCO_2, suggesting a comparatively higher rate of algal division compared to the division of animal cells. A change in *CSD* indicates a disruption of the delicate balance between the growth rate of algal and animal cells.

The rate of calcification declined immediately after the rise in pCO_2 and did not change afterwards, demonstrating that no acclimation process occurred. This is in agreement with two previous studies: Marubini & Atkinson (1999) and Langdon *et al*. (2000). Marubini & Atkinson (1999) reported that the decrease of calcification in response to changes in the carbonate system is immediate and reversible in the scleractinian coral *Porites compressa*. Also, Langdon *et al*. (2000) found that the response of the community calcification rate of the Biosphere 2 ocean is not significantly different during short-term (days) and long-term (months) changes in Ω_{arag}.

The stimulation of calcification by the elevation of temperature up to a threshold has already been demonstrated (Clausen & Roth, 1975; Coles & Jokiel, 1978; Kajiwara *et al*., 1995; Reynaud-Vaganay *et al*., 1999; Lough & Barnes, 2000). For example, the rate of calcification of *A. pulchra* peaks at 28 °C (Kajiwara *et al*., 1995) and the linear extension rate of *Acropora* sp. and *S. pistillata* increases until 27 °C and declines at higher temperature (Reynaud-Vaganay *et al*., 1999). These results show that the colonies of *S. pistillata* used in the present experiment had an optimal temperature that is, under normal pCO_2, close to 27–28 °C. As suggested by the study of Clausen & Roth (1975), this optimal temperature could be the result of a long-term exposure to 27 °C, the temperature at which corals are grown in our culture facility. The calcification of colonies maintained at elevated temperature (ca. 28.2 °C) declined by 50% in response to increased pCO_2 (Fig. 4). This is in accordance with previous reports. However, calcification was not affected by elevation of pCO_2 in colonies maintained at normal temperature (ca. 25.2 °C). The latter result is not in agreement with several papers that describe a negative relationship between calcification and CO_2, or a positive relationship with the aragonite saturation state (Gattuso *et al*., 1998; Marubini & Atkinson, 1999; Langdon *et al*., 2000, 2003; Leclercq *et al*., 2000, 2002; Marubini *et al*., 2003). However, in some of these studies, Ω_{arag} has not been changed by manipulating pCO_2, but by changing the Ca^{2+} concentration (Gattuso *et al*., 1998) or by the addition of acid (Marubini & Thake, 1999; Marubini *et al*., 2003) or sodium bicarbonate (Marubini & Atkinson, 1999). Moreover, except for the study of Gattuso *et al*. (1998), the other experiments were not performed on *S. pistillata*. These results demonstrate that pCO_2 and temperature significantly interact to control calcification in scleractinian corals, which could explain the large range of responses to elevated pCO_2 reported in corals and coral communities (Table 1).

The physiological basis of the different responses at two temperatures remains unclear. However, it does not result from an indirect effect of temperature on the seawater carbonate chemistry. Indeed, the change of pH and aragonite saturation state (Ω_{arag}) due to increased temperature was similar at both pCO_2 conditions (ΔpH: -0.02 to -0.03; $\Delta\Omega_{arag}$: 0.18–0.25) and approximately 10 times lower than the changes resulting from increased pCO_2 (Table 2).

These results are of major interest from a predictive point of view. Several studies investigated the physiological relationship between calcification and pCO_2 or the aragonite saturation state, and predicted a decline of calcification of coral and coral communities by up to 65% between the preindustrial rate and the year 2100 (Table 1). The consensus opinion is that the calcification of tropical marine organisms and coral communities will decrease by an average of 18–37% (Gattuso *et al*., 1999) or 17–35% (Kleypas *et al*., 1999) between the preindustrial time and the year 2100. However, none of these studies considered the effect of the forecast increase in temperature and its interaction with pCO_2 on photosynthesis and calcification. Our results

demonstrate that the rate of calcification of *S. pistillata* could decrease by 50% between the years 2000 and 2100. This temperature effect must be taken into consideration in subsequent investigations of future changes of coral physiology and reef metabolism. The present predictions must be re-evaluated as our results suggest that the decrease in the rate of calcification at the end of the century could be much higher than the forecast due to the synergistic effects of temperature and pCO_2.

There is a pressing need to manipulate environmental parameters in concert in order to determine the response of coral calcification to global environmental changes. Antagonistic responses to an increase in both pCO_2 and temperature were already reported in terrestrial ecosystems (Cao & Woodward, 1998): the increase in pCO_2 and temperature interact positively to enhance the net primary production in the northern latitudes, whereas elevated pCO_2 decreases the net primary production in the tropics.

Light and water motion are other environmental parameters that should be investigated together with pCO_2 and temperature to predict the response of calcification of corals and coral communities to global environmental changes. The interacting effects of temperature and pCO_2 could equally affect the present prediction of future changes of calcification in planktonic calcifiers, such as coccolithophorids (Riebesell *et al*., 2000). Another important challenge, which has received little attention so far, is whether long-term (years to decades) physiological acclimation and adaptation have the potential to mitigate acute physiological responses (Gattuso & Buddemeier, 2000).

Acknowledgements

Her coauthors dedicate this paper to the memory of Samantha Romaine-Lioud; she is sorely missed. Thanks are due to Nadia Ounais and the staff of the public aquarium of the Oceanographic Museum (Monaco) for their support. We are grateful to R. Buddemeir, J. Kleypas and M. Shick for comments on a draft version of this manuscript, to two anonymous referees and the topic editor for their constructive comments.

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