Biogeosciences Discuss., 9, 5969–6014, 2012
www.biogeosciences-discuss.net/9/5969/2012/
doi:10.5194/bgd-9-5969-2012

Biogeosciences Discussions

Light and temperature effect on δ^{11}B and B/Ca ratios of the zooxanthellate coral *Acropora* sp.: results from culturing experiments

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Received: 26 April 2012 – Accepted: 1 May 2012 – Published: 25 May 2012

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Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

The boron isotopic composition (δ^{11}B) of marine carbonates (e.g. corals) has been established as a reliable proxy for paleo-pH, with the strong correlation between δ^{11}B of marine calcifiers and seawater pH being now well documented. However, further investigations are needed in order to better quantify other environmental parameters potentially impacting boron isotopic composition and boron concentration into coral aragonite. To achieve this goal the tropical scleractinian coral *Acropora* sp. was cultured under 3 different temperature (22, 25 and 28 °C) and two light conditions (200 and 400 µmol photon m^{-2} s^{-1}). The δ^{11}B indicates an internal increase in pH from ambient seawater under both light conditions. Changes in light intensities from 200 to 400 µmol photon m^{-2} s^{-1} could bias pH reconstructions by about 0.05 units. For both light conditions, a significant impact of temperature on δ^{11}B can be observed between 22 and 25 °C corresponding to enhancements of about 0.02 pH-units, while no further δ^{11}B increase can be observed between 25 and 28 °C. This non-linear temperature effect complicates the determination of a correcting factor. B/Ca ratios decrease with increasing light, confirming the decrease in pH at the site of calcification under enhanced light intensities. When all the other parameters are maintained constant, boron concentrations in *Acropora* sp. increase with increasing temperature and increasing carbonate ions concentrations. These observations contradict previous studies where B/Ca in corals was found to vary inversely with temperature suggesting that the controlling factors driving boron concentrations have not yet been adequately identified and might be influenced by other seawater variables and species specific responses.

1 Introduction

Increased atmospheric CO_2 concentrations from 280 (pre-industrial values) to 380 ppmv (actual) have decreased surface ocean pH by approximately 0.1 unit (Orr et al., 2005). Estimates of future atmospheric pCO_2, as summarised in the IPCC report

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(IPCC, 2007), suggest a further decrease of 0.3 pH units by the end of the century (Caldeira and Wickett, 2003, 2005), with poorly known consequences on marine environments and ecosystems (Gattuso and Hanson, 2011). Knowledge of seawater carbonate ion concentration ($[CO_3^{2-}]$) and pH provides crucial insights into the ocean's role in regulating the global carbon cycle. However, unlike other key climatic indices such as temperature and salinity, seawater pH has, until recently, seldom been recorded in marine observations, and hence the need for a reliable proxy record to increase the accuracy of reconstructing pH. The boron isotopic composition ($\delta^{11}B$) of marine carbonates has been established as a proxy for paleo-pH (e.g., Spivack et al., 1993; Palmer and Pearson, 2003; Pelejero et al., 2005; Hönisch et al., 2008, 2009; Wei et al., 2009; Douville et al., 2010), with its reliability being validated by species specific calibrations (e.g., Trotter et al., 2011).

Boron dissolved in seawater is mainly present in the form of boric acid ($B(OH)_3$) and borate ion ($B(OH)_4^-$) and their relative abundance is strongly pH dependent (Hershey et al., 1986). Because of the isotopic equilibrium fractionation between the two aqueous boron species, the isotopic compositions of boric acid and borate ion are also pH dependent. Based on the observation that modern marine carbonates reflect the isotopic composition of borate, Vengosh et al. (1991) proposed the following mechanism for boron substitution in the CO_3^{2-} site in carbonate:

$$CaCO_3 + B(OH)_4^- = Ca(HBO_3) + HCO_3^- + H_2O$$

The pK_B value (stoichiometric equilibrium constant between $B(OH)_3$ and $B(OH)_4^-$ in seawater) determined by Dickson (1990) has been confirmed by Roy et al. (1993). The fractionation factor (α) between the two aqueous boron species has been a subject of much debate (Zeebe et al., 2005). Recently, using a spectrophotometric approach, Klochko et al. (2006) determined that α in seawater has a value of 1.0272 ± 0.0006 (Salinity = 35, $Boron_{Total} = 0.01\,mol\,kg^{-1}$). One caveat however, in the use of boron isotope-pH proxy, is the potential incorporation of significant concentrations of boric acid into biogenic calcium carbonate, instead of only borate. This assumption was

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recently raised by two studies, which measured proportions of boric acid in coral aragonite varying from 12 to 48 % (Klochko et al., 2009; Rollion-Bard et al., 2011). Because the incorporation of such proportions of boric acid would shift the boron isotopic composition of coral skeletons to considerably higher values, it appears unlikely that the trigonal $B(OH)_3^-$ species detected in calcite and aragonite is directly derived from seawater. Recently, Tossell et al. (2005) and Klochko et al. (2009) have pointed out the possibility of a "chemosorption stage" where $B(OH)CO_3^-$ isomers may form on the surface before breaking down into either BO_3 or BO_4 in natural carbonates. In turn, this may result in internal boron isotope redistribution which would allow both BO_3 and BO_4 to be incorporated while preserving the internal (site of calcification) $\delta^{11}B$ isotopic composition. The biologically-mediated pH regulation at the site of calcification, via seawater isolation and active modification, likely superimposes a biological regulation and accounts for the observed species-specific isotopic signatures.

So far, no light effect has been reported to impact boron isotopic composition on aragonitic corals (Hönisch et al., 2004). However, variations in light are known to impact symbiont algae photosynthesis and calcification rate (Dubinski et al., 1983; Gattuso et al., 1999; Reynaud et al., 2004; Juillet-Leclerc et al., 2012), which in turn might significantly impact the carbonate chemistry of the micro-environment and therewith the B isotopic composition. This highlights the need for species-specific calibrations in order to better understand the controlling factors of $\delta^{11}B$ in biogenic carbonates.

Alternatively, the recognition of the importance to perform accurate pH reconstruction has warranted the use of a multi-proxy approach to resolve past deep-water $[CO_3^{2-}]$. Recently, Yu and Elderfield (2007) and Yu et al. (2007) proposed that the B/Ca ratio in foraminiferal calcite can be use as a proxy for seawater $[CO_3^{2-}]$. The basic assumption behind this proxy is that the B/Ca ratio in foraminifera is a function of the ratio of $[B(OH)_4^-/HCO_3^-]$ in seawater, with the later being pH dependent. Although recent studies tend to confirm that seawater carbonate chemistry is the dominant environmental parameter controlling B/Ca ratios into foraminiferal calcite (while temperature appears to be only a secondary factor, Foster, 2008; Yu et al., 2010), no similar assumptions

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can be made at present for aragonitic corals. On the contrary, several studies have reported that the B/Ca ratio in tropical and temperate corals can be robust at documenting SST variations in certain locations (e.g., Hart and Cohen, 1996; Sinclair et al., 1998; Fallon et al., 1999, 2003; Montagna et al., 2007). Nevertheless, after measuring B concentrations in tropical *Porites* sp. coral cores from the Great Barrier Reef, Fallon et al. (2003) concluded that coral B/Ca ratio had to be influenced by factors other than temperature. Further investigations are therefore needed in order to better understand the parameters regulating the boron concentration into coral aragonite.

In this study, we present the boron isotopic composition (δ^{11}B) and the elemental ratio (B/Ca) measured on the zooxanthellate coral *Acropora* sp., cultured under three different temperature conditions (22, 25 and 28 °C) and two light intensities (200 and 400 µmol photon m^{-2} s^{-1}) corresponding to annual variations observed in Nouméa lagoon where the colonies were collected. Newly formed aragonite was analysed for boron isotopes using Multi Collector-Inductively Coupled Plasma Mass Spectrometry (MC-ICPMS), and for B/Ca ratios by Inductively Coupled Plasma-Quadrupole Mass Spectrometry (ICP-QMS).

2 Material and method

2.1 Experimental set-up

The culturing experiment was performed at the Scientific Centre of Monaco (CSM). The tropical branching zooxanthellate coral *Acropora* sp. was chosen for its strong aptitude to grow under culture condition and its large representation throughout the world coral communities.

Culture experiments were conducted following the protocol described by Reynaud et al. (1999), in which tips were sampled from a single parent colony, adhered onto slides (2.6 × 6 × 0.2 cm, see Fig. 1) using underwater epoxy (Devcon®), and randomly distributed in incubation tanks (30 l). On completion of the experiment, the ring skeleton

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deposited on the side was removed with a scalpel, dried overnight at room temperature, and stored in containers pending geochemical analyses. By sampling only the material covering the slide, this method allows easy collection of newly formed aragonite, ensuring it was precipitated under the targeted culture conditions. The experimental tanks were continuously supplied with Mediterranean seawater (salinity 38) pumped from 55 m depth and heated at a constant temperature (21 or 27 °C) in two common tanks maintained under ambient pCO_2 ($pCO_2 \approx 390$ ppm), prior to flowing into the culture aquaria (Rena® pump, 6 l min^{-1}). The seawater renewal rate in each tank was approximately five times a day. Light was provided by metal halide lamps (Phillips HPIT, 400 W), on a 12 h : 12 h light dark photo period. Irradiance was measured once a week using a 4π quantum sensor (Li-Cor, LI-193SA) (Table 1). The culture temperature was controlled to within ± 0.1 °C using a temperature controller (EW, PC 902/T). Temperature and light intensities were chosen to mimic average annual variations in natural environments where the *Acropora* sp. parent colonies originated (Nouméa lagoon in winter: 200 µmol photon m^{-2} s^{-1}, 22 °C; and summer: 400 µmol photon m^{-2} s^{-1}, 28 °C, Quinn and Sampson, 2002). Salinity, pH and total alkalinity (TA) were measured directly within the culture tanks (Table 1, all pH_T values in this manuscript are reported in Total scale). pH was measured using a glass combination electrode (Orion 8103SC) calibrated on the Total Scale using Tris/HCl and 2-aminopyridine/HCl Buffer solutions with a salinity of 38 and prepared according to Doe (1994) (accuracy ± 0.003 pH units). For TA measurements, seawater samples were filtered through 0.45 mm membranes, poisoned with Mercury Chloride and stored in a cool and dark place pending analyses (Doe, 1994). TA was determined using a titration system (TIAMO, TITRANDO 888, Metrohm), with a reproducibility of 3 µmol kg^{-1}. Salinity was measured using a conductimeter (HQ14, Hach Lange). Due to the strong seawater renewal rate in the culture tanks, no impact of light on seawater carbonate chemistry could be observed. Therefore, Table 1 represents the average values of High Light (HL) and Low Light (LL) measurements performed for each temperature condition. Corals were fed twice a week with *Artemia salini* nauplii during the course of the experiment. Nubbins glued onto

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slides could not fit in the incubating chamber, therefore, all metabolic measurements such as respiration, photosynthesis, and calcification rate were conducted simultaneously on small fragments from the same parent colony hung on nylon wire (Al-Moghrabi et al., 1993) and cultured under similar conditions.

2.2 Experimental protocol

Nubbins sampled from a single *Acropora* sp. parent colony were adhered onto slides and associated individually to a coral fragment hung on nylon wire for metabolic measurements. The culture experiment was conducted following a three steps protocol. Step 1: All the nubbins were cultivated in the same aquarium at constant temperature (25 °C) and light conditions (200 µmol photon $m^{-2} s^{-1}$). This step lasted for 9 weeks and is considered as the acclimation period. Step 2: The nubbins were subsequently randomly distributed within six tanks under six different conditions (200, 22; 200, 25; 200, 28; 400, 22; 400, 25 and 400, 28), where the first number indicates the light intensity (µmol photons $m^{-2} s^{-1}$) and the second one the temperature (°C). This second step, which is considered as the experiment itself, lasted for 15 weeks. At the end of step 2, all the newly precipitated aragonite forming a ring on the slide (lateral growth) was carefully collected with a scalpel, and all nubbins were placed under identical culture conditions (200 µmol photon $m^{-2} s^{-1}$, 25 °C) for step 3. This ensured the secretion of new skeleton through identical biological regulation (same organism) but allowing differentiation of aragonite precipitated during step 2 from step 3. Step 3 is referred to as "recovery period" in the following manuscript and lasted for ten additional weeks.

2.3 Metabolic measurements

Photosynthesis and respiration were measured once a month using the respirometry technique, which consists in monitoring the changes in oxygen concentration during incubation time. Each fragment of coral hung on nylon wire was placed in a respirometric chamber (50 ml) filled with seawater for 20 min and exposed to light intensity

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corresponding to the culture conditions. The nubbins were subsequently placed in the dark for 20 min to measure the respiration rate and then returned to the culture aquarium. The medium was continuously agitated during measurements using a magnetic stirrer, and replaced after each incubation. The respirometric chamber was thermostated to be kept at constant temperature. All incubations took place between 08:00 and 14:00 LT. Oxygen concentration was monitored in the chamber using an oxygen electrode (Strathkelvin 928) calibrated daily against air-saturated seawater (100 %) and a nitrogen-bulled (zero oxygen). Rates of net photosynthesis and respiration were estimated using a linear regression of O_2 against time. Photosynthesis and respiration values were then normalized to the skeletal surface area estimated using the wax technique (Simson and Kenzie, 1991). In order to determine calcification rates, corals were weighted once a week using the buoyant weight technique (Jokiel et al., 1978; Davies, 1989). Calcification rate were calculated using the following formula:

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

Where G is the calcification rate, n is the number of the culture days, P_n is the dry weight after 15 days of culture and P_0 is the initial dry weight.

2.4 Geochemical measurements

For each experimental condition, elemental and isotopic measurements were performed on two replicates, except for condition 400, 28, where values presented are the average of three replicates.

2.4.1 Boron isotopes measurements

The boron isotopic composition was measured using a double focusing sector-field multi-collector-inductively coupled plasma mass spectrometer (ThermoScientific

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Neptune) at the Institut de Physique du Globe de Paris (IPGP, France), following the protocol described by Douville et al. (2010) that includes a chemical separation of boron before its measurement. Direct injection high efficiency nebuliser (d-DIHEN) was used for sample introduction (Louvat et al., 2011). The direct injection technique allowed a strong reduction of the analytical blank contribution (lower than 0.5 ‰ of the sample signal for each isotope). Instrumental mass fractionation and drift of the $^{11}B/^{10}B$ ratio with time was corrected by standard-sample-standard bracketing. Each sample was measured three times successively, with resulting relative standard deviation being systematically in agreement with the external reproducibility of 0.25 ‰ (2σ) deduced from repeated analyses of boric acid standard NBS-951 and North Atlantic Seawater Standard NASS-V (Louvat et al., 2011; Douville et al., 2010). Mean $\delta^{11}B$ values measured on standards chemically prepared by following identical procedures than those applied to samples were: 39.53 ± 0.11 ‰, 24.42 ± 0.10 ‰ and 0.14 ± 0.20 ‰ at 2σ for NASS-V, JCp-1 and NBS 981, respectively. These isotopic compositions are well comparable with the recently published values of Foster et al. (2010) and Wang et al. (2010).

2.4.2 Boron concentrations

B/Ca concentrations were determined using quadrupole ICP-MS Xseries[II] (Thermo Fisher Scientific) at the Laboratoire des Sciences du Climat et de l'Environnement (LSCE, France). Analyses were calibrated against carbonate standards JCp-1 (coral), JCt-1 (clam) and Aragonite AK. Sample and standard solutions were systematically adjusted to 100 ppm Ca through dilution, in order to (1) avoid dominant Ca signal increasing salt deposition on cones and affecting therewith ICP-MS stability, and (2) adjust the Ca concentrations being introduced in the ICP-QMS, allowing control of Ca matrix effects on trace elements analysis (Harding et al., 2006; Bourdin et al., 2011). To monitor and correct for instrumental drift, standards JCp-1 and JCt-1, and Aragonite AK were analysed every five and ten samples, respectively. Instrumental calibration was achieved using standard solution for each element and by routinely measuring carbonate standards (JCp-1, JCt-1 and Aragonite. AK.). When considering repetitive

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B/Ca measurements of the JCp-1 standard, analytical uncertainties were calculated to be 3 % at 2σ (Douville et al., 2010), with a mean value of 459 $\mu mol\,mol^{-1}$. These results are in good agreement with the previously published value of Okai et al. (2004).

3 Results

3.1 Metabolic measurements

All nubbins survived the experiment despite the variations in light and temperature. Metabolic rates (respiration, photosynthesis and calcification) were monitored for each experimental condition in order to assess the impact of these two environmental parameters on coral growth (Fig. 2, Table 2). The values plotted in Fig. 2 are averaged measurements, per experimental conditions, performed on all nubbins hung on nylon wire and cultured during 15 weeks.

The increase in both temperature and light induces an enhancement of the calcification rate. A linear increase of the calcification rate with temperature is observed under both LL and HL conditions.

Net photosynthesis was significantly influenced by light and temperature (ANOVA, $P = 0.004$ and $P = 0.001$, respectively), with no interaction between the two factors (ANOVA, $P = 0.4$). For the three temperatures considered in this study (22, 25 and 28 °C), photosynthesis rates are always significantly higher under HL compared to LL conditions.

Although results appear less straightforward, respiration rates were also affected by light and temperature (ANOVA, $P = 0.0050$ and $P < 0.00001$, respectively), with an interaction between the two parameters ($P = 0.0012$). Under HL conditions, a linear increase of the respiration rate is observed with increasing temperature. Under LL conditions, an increase is observed between 22 and 25 °C. No further enhancement is observed between 25 and 28 °C. While no significant change is observed at 22 °C between the different light conditions, increase in light induces an enhancement of the

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respiration rate at 25 and 28 °C. As previously observed for the calcification rate, the impact of light on both photosynthesis and respiration rates increases with increasing temperatures.

3.2 Boron isotopes measurements

The boron isotope compositions measured on the two replicates per experimental conditions (except for condition 400, 28, where three replicates were considered) were averaged for each experimental condition and plotted against temperature (Fig. 3). The mean δ^{11}B vary from 21.76 to 23.19 ‰ (T = 22–28 °C, pH_T = 8.02 ± 0.02, S = 38 ± 0.1 and light conditions: 200–400 µmol photon m^{-2} s^{-1}, Table 3).

For the three temperature regimes, boron isotope compositions show a significant decrease with increasing light (2 ways ANOVA $F_{1,9}$ = 8.46, p = 0.0173, see Table 4). When increasing light intensities from 200 to 400 µmol photon m^{-2} s^{-1}, boron isotope compositions measured in our study, show a decrease of 0.4, 0.6 and 0.5 ‰ for 22, 25 and 28 °C, respectively (Table 3). These decreases can be considered statistically similar for each temperature condition (Table 4).

Boron isotope compositions show a significant increase with increasing temperature between 22 and 25 °C for both light conditions (SCHEFFE test 22–25 °C, p = −0.0671, Table 4). Measured δ^{11}B varied between 22.16 ± 0.42 ‰ (22 °C) and 23.10 ± 0.22 ‰ (25 °C) under LL, and between 21.76 ± 0.20 ‰ (22 °C) and 22.50 ± 0.40 ‰ (25 °C) under HL conditions (pH_T ≈ 8.02). For temperature between 25 and 28 °C no significant variation of the δ^{11}B could be observed, for any of the light conditions (SCHEFFE test 25–28 °C, p = 0.650, Table 4). δ^{11}B was measured to vary from 23.10 ± 0.22 ‰ at 25 °C to 23.19 ± 0.61 ‰ at 28 °C (at 200 µmol photon m^{-2} s^{-1}) and from 22.50 ± 0.40 ‰ at 25 °C to 22.67 ± 0.20 ‰ at 28 °C (at 400 µmol photon m^{-2} s^{-1}), with pH_T ≈ 8.02 for both conditions.

For clarity, measured boron isotope compositions are plotted versus medium pH on Fig. 4. pH of the site of calcification reconstructed from δ^{11}B values (with α = 1.0272 ±

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0.0006, $\delta^{11}B_{seawater}$ = 39.61 ‰ and B_T = 416 µM) are plotted on the same graph. The difference between medium and reconstructed pH is illustrated by the arrow and referred to as ΔpH biological control.

Two other studies report on $\delta^{11}B$ measured on *Acropora* sp. cultured under similar conditions. Hönisch et al. (2004) measured $\delta^{11}B$ values of *Acropora nobilis* of about 22.9 ‰ (T = 27 °C, pH_T = 7.97, and light condition of 300 ± 15 µmol photon m^{-2} s^{-1}), while Reynaud et al. (2004) report $\delta^{11}B$ of *Acropora* sp. varying between 24.0 and 23.9 ‰ (T = 25–28 °C, pH_T = 8.03–8.05, S = 38 and light = 380 ± 20 µmol photons m^{-2} s^{-1}). For comparison, the $\delta^{11}B$ data of Reynaud et al. (2004) and Hönisch et al. (2004) are plotted versus pH on Fig. 4. For similar ambient pCO_2 conditions, boron isotope compositions are comparable for *Acropora* sp. and *Acropora nobilis.* This observation is of great interest as $\delta^{11}B$ was measured with MC-ICPMS in our study, while Hönisch et al. (2004) and Reynaud et al. (2004) used the N-TIMS (Negative Thermo-Ionazation Mass Spectrometry) approach. Therefore, in light of our results it appears that results obtained by MC-ICPMS and N-TIMS analytical techniques are comparable and allow accurate and reproducible $\delta^{11}B$ measurements of aragonitic corals.

Boron isotope composition measured on nubbins after the end of step 3 (200 µmol photons m^{-2} s^{-1}, 25 °C), displayed strong scattering with lower values varying from 20.07 to 21.73 ‰ (Table 3, Figs. 3 and 4). No straightforward pattern of these values with the experimental conditions of step 2 could be observed.

3.3 Elemental concentrations

The mean coral B/Ca ratios obtained for each experimental condition (step 2) are plotted in Fig. 5a. Values vary between 460 and 512 µmol mol^{-1} (Table 3, Fig. 5a).

B/Ca ratios show a significant decrease with increasing light (2 ways ANOVA $F_{1,9}$ = 8.31, p = 0.0181, see Table 4), with values varying from 460 ± 2, 485 ± 8,

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512 ± 4 µmol mol^{-1} under LL and 455 ± 5, 478 ± 7 and 487 ± 10 µmol mol^{-1} under HL for 22, 25 and 28 °C, respectively.

Increasing temperature induces an increase in B concentrations between 22 and 25 °C, for both light conditions (SCHEFFE test 22–25 °C, $p = -7.309$, Table 4). A further enhancement can be observed between 25 and 28 °C under LL (one way ANOVA, $F_{2.2} = 107.53$, $p = 0.0178$) whereas no significant boron concentration increase can be considered between 25 and 28 °C under HL (one way ANOVA, $F_{2,2.5} = 12.22$, $p = 0.052$).

Hönisch et al. (2004) measured B/Ca ratios of *Acropora nobilis* from coral culturing experiments of 54.3, 57.9 and 63.0 ppm of boron at $pH_T = 8.17$, 7.97 and 7.72, respectively ($T = 27$ °C, and light condition of 300 ± 15 µmol photon m^{-2} s^{-1}). These results, which correspond to B/Ca of 503.3, 531.9 and 593.2 µmol mol^{-1}, respectively (using Ca = 40%), are well comparable with our study for similar pCO_2 conditions (532 vs. 478 to 512 µmol mol^{-1}, respectively). Similarly, B/Ca ratios measured on *Acropora nobilis* from natural environment have been measured to fluctuate between 474 and 612 µmol mol^{-1} (Shirai et al., 2008) using similar ICP-MS facilities.

B/Ca ratios measured after the end of step 3 (recovery), vary between 416 and 446 µmol mol^{-1} (Table 3, Fig. 5a). These values are lower than those measured at the end of step 2, independently of the light and temperature conditions considered.

For comparison, B/Ca ratios were plotted versus Sr/Ca ratios measured simultaneously (Fig. 5b). Sr/Ca ratios vary between ca. 8.99 and 9.20 mmol mol^{-1}. These results are in good agreement with ICP-MS values measured on *Acropora nobilis* from the natural environment (from ≈ 8.5 to 9.5 mmol mol^{-1}, Shirai et al., 2008).

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4 Discussion

4.1 Metabolic measurements

In our study, photosynthesis, respiration and calcification rates increase with both increasing light and temperature (Fig. 2, Table 2). These observations are in good agreement with numerous previous studies on tropical and temperate corals (e.g., Chalker and Taylor, 1975; Coles and Jokiel, 1977; Jokiel and Coles, 1977; Dubinsky et al., 1983; Jacques et al., 1983; Reynaud-Vaganay et al., 2001; Al-Horani et al., 2003; Rodolfo-Metalpa et al., 2008).

Photosynthetic activity increases with increasing light for the three considered temperatures. Similar observations were already made for tropical corals which were observed to change zooxanthellae density and/or pigment concentration in order to adapt to varying light levels (e.g., Falkowski and Dubinsky, 1981; Iglesias-Prieto and Trench, 1997). Photosynthetic activity also increases with increasing temperature under both light conditions. Due to potential bleaching of coral colonies with increasing temperature, the impact of temperature on photosynthesis has been extensively studied. A decrease in photosynthetic efficiency is indeed often observed after a heat stress (e.g., Iglesias-Prieto et al., 1992; Warner et al., 1996, 1999; Hill et al., 2004). However, the temperature range in our study 22–28 °C can be considered as non stressful for tropical species like *Acropora* sp. (e.g., Reynaud et al., 2003; Ferrier-Pages et al., 2007). Under such a temperature fluctuation, photosynthesis has been observed to increase with increasing temperature (e.g., Kajiwara et al., 1995; Reynaud et al., 2003).

Under LL, respiration does not vary (Fig. 2), while under HL, it is linearly correlated with temperature. Kajiwara et al. (1995) already recorded such a behavior for *Acropora pulchra,* which was interpreted as an increase in the algal respiration with raising temperature (Karako-Lampert et al., 2004). Although no difference can be observed at 22 °C, respiration increases with increasing light at 25 and 28 °C. Falkowski et al. (1984) first suggested that colonies growing under HL obtain all the reduced carbon needed for animal respiration from photosynthesis by symbiotic zooxanthellae. Later on, Kuhl

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et al. (1995) calculated that coral respiration was 6 times higher under irradiance of 350 $\mu mol\,m^{-2}\,s^{-1}$ than the dark respiration measured under identical conditions.

Calcification is observed to increase with increasing light. This effect is commonly referred as "light enhanced calcification" (LEC, Goreau, 1959; Chalker and Taylor, 1975; Barnes and Chalker, 1990). Although the mechanism is still not entirely understood, it is clear that calcification and photosynthesis are related (see Sect. 4.2.3., Gattuso et al., 1999; Allemand et al., 2004; Moya et al., 2006). Calcification is also enhanced with temperature both at LL and HL, as previously observed for several species of corals (Coles and Jokiel, 1977, 1978; Reynaud et al., 2007).

Overall, the coral metabolic responses to varying environmental parameters (light and temperature) are in agreement with observations previously made on corals from both culture experiments and natural environment. It can be considered that nubbins measured in this study were well acclimatized to culture conditions and should present geochemical signatures representative of the environmental parameters maintained in the culture conditions.

4.2 Boron isotope

Due to the important seawater renewal rate into our culture aquaria (5 times per day), seawater carbonate chemistry remained constant through the experiment, for all conditions (Table 1). Therefore, it is assumed that in our study the variation of the skeletal $\delta^{11}B$ reflects pH at the site of calcification (Trotter et al., 2011).

4.2.1 Boron isotopes compositions

Measured *Acropora* sp. $\delta^{11}B$ versus measured ambient seawater pH are plotted relative to the borate ion curves determined for each experimental temperature at 22, 25 and 28 °C using the fractionation factor determined by Klochko et al. (2006) $\alpha = 1.0272 \pm 0.0006$ (salinity = 35, B_T = 416 µM and $\delta^{11}B_{seawater}$ = 39.61 ‰, Fig. 4). The $\delta^{11}B$ values measured in this study plot significantly above the curves and correspond

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to an increase in pH of the site of calcification of about 0.36, 0.40 and 0.39 pH units under LL, and 0.33, 0.36 and 0.35 pH units under HL, for 22, 25 and 28 °C, respectively. These increases in pH are in good agreement with the recent study of Venn et al. (2011) on *Stylophora pistillata* using live tissue imaging. They measured a pH increase from 0.2 to 0.5 pH units above ambient seawater under the calicoblastic epithelium. Using microsensors, Al-Moghrabi et al. (2001) and Al-Horani et al. (2003) measured a pH varying from 8.1 to 9.3 in the site of calcification of *Galaxea fascicularis*. Although this pH enhancement appears somewhat higher, those measurements remain compatible with the internal pH values calculated in our study representing average values for calcification over ~15 weeks (see methods). Hence, in contrast to the microsensor or live tissue imaging approaches, which give instantaneous values, boron isotope provides an average of several daily cycles (weeks). Furthermore, species-specific upregulation mechanisms may account for the observed differences (Hönisch et al., 2004; Krief et al., 2010; Trotter et al., 2011).

4.2.2 Light effect

To obtain a more reliable boron isotope pH proxy, it is critical to better quantify the "vital effect" superimposed by the coral on ambient seawater pH during calcification. It has been long known that light influences the rate of coral calcification. This effect is commonly referred as "light enhanced calcification" (LEC) (e.g., Goreau, 1959; Chalker and Taylor, 1975; Barnes and Chalker, 1990). However, despite numerous studies performed on the coral-zooxanthellae symbiotic relationship, the mechanisms linking photosynthesis of the symbionts to coral calcification remain largely unknown (e.g., Gattuso et al., 1999; Allemand et al., 2004; Moya et al., 2006). Modifications of carbonate chemistry inside the coelenterons have been hypothesized to be responsible for (part of) the LEC processes. Following Moya et al. (2006), three mechanisms listed below have been described, which might explain LEC effect via changes in internal carbonate chemistry: 1) Decrease in pCO_2 in the site of calcification due to CO_2 uptake for photosynthesis (Goreau, 1959); 2) Neutralization of H^+ produced by

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calcification by the OH^- resulting from the carbon concentrating mechanism (CCM) (Furla et al., 1998); and 3) Production of H^+ by calcification favouring CO_2 formation easily absorbed by dinoflagellates, and therewith calcium carbonate precipitation (McConnaughey and Whelan, 1997). Interestingly, the first two processes should induce an increase in pH of the extracellular site of calcification with light enhancement. On the contrary, the third mechanism might lead to a decrease in pH with increasing light intensity, as more CO_2 should be released at the site of calcification.

To date, only a few studies have reported internal pH measurements of the coral site of calcification. Al-Moghrabi et al. (2001), Al-Horani et al. (2003) and Venn et al. (2011) all reported an increase in pH in the site of calcification under light vs. dark conditions, independently of the analytical methods considered (e.g. microelectrodes, live tissue imaging). Moreover, Kuhl et al. (1995) measured a decrease in pH in the outer 0.1 mm of coral tissue (*Favia* sp.) with decreasing light intensities. However, to our knowledge, no study reports on variation in pH of coral site of calcification under various light intensities.

In our study, when translated into pH values, a light enhancement of 200 µmol photons $m^{-2} s^{-1}$ induces a decrease in pH of the site of calcification of about 0.03, 0.04 and 0.03 pH-units at 22, 25 and 28 °C, respectively (Table 3). Krief et al. (2010) observed a decrease in calcification rate together with an increase in tissue biomass (measured by protein concentration) for *Porites* sp. and *Stylophora pistillata* maintained for 14 months under increased pCO_2 conditions. They argue that the inverse response of skeleton deposition and tissue biomass to changing pCO_2 conditions seems to be consistent with the hypothesis that calcification stimulates zooxanthellae photosynthesis by enhancing CO_2 concentration within the coelenterons. Under high pCO_2 conditions, more CO_2 is available in the site of calcification rending less energy consuming the process concentrating Ca^{2+}, as more protons are available for the Ca^{2+} ATP-ase enzyme. This allows the coral polyp to allocate more energy on tissue biomass. This is in good agreement with hypothesis 3 described above (McConnaughey and Whelan, 1997) and our measurements: the decrease in pH at the

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site of calcification is related to increased calcification and photosynthesis rates. However, this appears to be more a consequence than a cause and does not explain the impact of light intensity on internal pH and calcification rates.

More recently, Al Horani (2005) argued that higher respiration rates stimulated by algal photosynthesis under enhanced light conditions, increase ATP availability and therewith coral Ca^{2+} ATPase activity and calcification rates. This in turn could account for the increase in pH of the site of calcification measured in the light vs. dark conditions (Al-Horani et al., 2003; Venn et al., 2011). Increases in light intensities and consequently calcification rates should release more CO_2 in the site of calcification, and decrease internal pH with increasing light, as measured in our study.

To date, Hönisch et al. (2004), is the only other study to report on the impact of light on coral boron isotope composition (*Porites compressa*). Although variation in light intensities considered in their study (from 540 to 1210 µmol photons m^{-2} s^{-1}) is 3 times greater than in our experiment, $\delta^{11}B$ remained overall not affected. It might be argued than different species react differently to changes in light intensities, as for example the slow calcifying massive *Porites* sp. might present differences in metabolic fractionation when compared with the fast-growing branched *Acropora* sp. Nevertheless, it should be noted that the total duration of Hönisch et al. (2004) culturing experiments was 57 days, before which, five days were allocated to corals acclimation. In our study, corals were allowed for acclimation during 9 weeks (63 days), while the experiments itself (step 2) lasted for 15 weeks (105 days). The length of the acclimation period and of the experiment can play a significant role as stressed corals may present disruption in metabolic control and therewith, differing isotopic signatures (see Sect. 4.2.5).

Our results indicate that pH at the site of calcification seems to be related to photosynthetic rates. However, changes in light intensities from 200 to 400 µmol photon m^{-2} s^{-1}, representing global annual light variations for the tropical environment where *Acropora* sp. parent colonies were sampled (Nouméa lagoon, Quinn and Sampson, 2002), have only biased pH reconstructions by about 0.05 units. These observations support the idea that changes in light and therewith symbionts

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photosynthetic activities do not significantly compromise δ^{11}B-pH reconstructions for tropical corals. Due to analytical but also sampling bias, paleo-pH reconstructions are still broadly limited to a precision no better than ≈ 0.05 pH-units. Therefore, variations in light intensities on pH reconstructions can be considered as negligible. Our results thus confirm the general veracity of the δ^{11}B-pH proxy and as well as providing new insights on the mechanism of internal pH regulation in corals.

4.2.3 Temperature effect

In our study, δ^{11}B increases with increasing temperature between 22 and 25 °C. When considering analytical uncertainties/reproducibility (from 0.20 to 0.42 ‰), these δ^{11}B increases of 0.94 (LL) and 0.74 ‰ (HL), remain small but statistically significant (Table 4) and correspond to enhancements of 0.027 and 0.016 pH units in the site of calcification, respectively. However, no significant variations can be observed between 25 and 28 °C with δ^{11}B varying from 0.09 (LL) and 0.17 ‰ (HL) (Fig. 3, Tables 3 and 4). These results differ from our metabolic data, which show a linear increase in calcification rate with increasing temperature for both light conditions (R^2 = 0.95 and 0.99 for LL and HL, respectively). As mentioned above, corals can enhance pH under the calicoblastic epithelium (e.g., Venn et al., 2011). A mechanism involving the removal of protons from the site of calcification via Ca^{2+}ATPase was proposed to be responsible for the observed pH increase (Cohen and McConnaughey, 2003; McConnaughey and Whelan, 1997; Al-Horani et al., 2003). Ip et al. (1991) and Isa et al. (1994) demonstrated the existence of a Ca^{2+}ATPase activity in the homogenates of coral tissues. Subsequently, McConnaughey and Whelan (1997) suggested that the protons generated during calcification were removed by a Ca^{2+}ATPase activity, resulting in a pH enhancement (and therewith increase in $[CO_3^{2-}]$) and Ca^{2+} concentration of coral site of calcification. Zoccola et al. (2004) localized a Ca^{2+}ATPase in the epithelium of *Stylophora pistillata*, confirming this hypothesis.

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More recently, Herfort et al. (2008) reported that additions of $NaHCO_3$ to synthetic seawater, proportionally increased the calcification rate of *Acropora* sp. until the concentration exceeded four times that of seawater (8 mM). These observations indicate that the concentrations of carbonate species ($[HCO_3^-]$ and/or $[CO_3^{2-}]$) rather than calcium, are the limiting factor of coral calcium carbonate precipitation. By definition, CO_2 is more soluble in cold water. Hence, an increase in temperature leads to a decrease in $[CO_2(aq)]$ and a subsequent decrease in $[HCO_3^-]$ and increase in $[CO_3^{2-}]$ (for instance, under similar conditions an increase in temperature from 5 °C to 25 °C leads to a $[CO_3^{2-}]$ increase of 90 %). In turn, this might explain why, within a certain species-specific temperature range, coral calcification rates have been observed to increase with increasing temperature (e.g., Coles and Jokiel, 1977, 1978; Reynaud et al., 2007). Moreover, if temperature can influence carbonates availabilities as a result of chemical re-equilibration, for corals operating below their thermal optimum, increased temperature was also suggested to enhance zooxanthellae photosynthesis and thereby increase the pH of the site of calcification (McConnaughey and Whelan, 1997; Al-Horani et al., 2003).

Linear increase in calcification rates with increasing temperature observed in our study agrees with these hypotheses. Nevertheless, if the internal pH reconstructed from boron isotopic signature shows an increase between 22 and 25 °C, no significant pH enhancement can be observed between 25 and 28 °C. Our data may thus suggest that once the pH and hence the carbonate saturation state of the aragonite (Ω) in the site of calcification passes a certain threshold value (independently whether the carbonate enrichment is a result of a metabolic and/or chemical process), the enzyme system works at a constant rate, which does not require any further pH increase and therewith becomes unreactive to additional temperature enhancement (in the limits of biological sustainability). In terms of proxy reconstruction, this corresponds to an enhancement of about 0.02 (HL)–0.03 (LL) pH-units between 22 and 25 °C, while no significant impact can be observed between 25 and 28 °C. This impact remains small but could become significant while reconstructing pH further back in time, where

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important SST changes have occurred. This non-linear temperature effect complicates the determination of a potential correcting factor.

4.2.4 Comparison impact of light vs. temperature

Herfort et al. (2008) reported that a dramatic increase in *Acropora* sp. calcification rates with increased [HCO_3^-] of the growth media could be monitored in the dark, although these were lower than in the light for a given [HCO_3^-]. They therefore concluded that calcification was light triggered but not light dependent. As mentioned previously, increase in calcification rate with increased light conditions has been suggested to increase CO_2 concentration in the site of calcification (Al-Horani et al., 2003) and may account for the decrease in internal pH with increasing light intensities measured in our study. It could be argued that the increase in calcification rate with increasing temperatures does not correspond to a decrease in δ^{11}B and therewith in internal pH as observed under enhanced light conditions. However, one should keep in mind that these two environmental parameters might influence calcification on many different levels. Temperature, for example, is known to impact metabolic processes (e.g. enzyme activity), which in turn might account for the observed differences in pH regulation. At this stage calcification mechanisms need to be better understood to fully quantify light and temperature effect on δ^{11}B-pH proxy.

4.2.5 Boron isotope composition after recovery experiment

Boron isotopic composition determined at the end of step 3 (recovery period), does not show any correlation with recovery period culture conditions (200 µmol photon m^{-2} s^{-1}, 25 °C). Moreover, no straightforward correlation between boron isotopic compositions measured at the end of step 3 can be made with any of the previous step 2 culture conditions. When translated into pH values using Klochko et al. (2006) fractionation factor, almost all boron isotopic composition measured at the end of step 3 present lower pH results (from 8.20 to 8.36) compared to pH values obtained at the end of step

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2 (from 8.35 to 8.42), independently of the light and temperature conditions considered (Table 3, Figs. 3 and 4). These results indicate that the mechanical breakage applied at the end of step 2 induced a significant stress to the organisms, which led to a disruption of pH enhancement at the site of calcification. In terms of proxy reconstruction this finding highlights the fact that corals submitted to strong stress factors (e.g. storms, bleaching etc.) should not be considered for paleo-environmental reconstructions.

4.3 Boron concentrations

4.3.1 Light effect

B/Ca ratios show comparable trends than δ^{11}B to light variations although boron concentrations and boron isotope compositions were measured following different sample preparation and analytical techniques (ICP-QMS and MC-ICPMS, respectively). An increase in pH of the calcification site makes $B(OH)_4$ the dominant species in the site of calcification (Hershey et al., 1986; Hemming and Hanson, 1992), and the boron concentration in the coral is proportional to the boron activity in the precipitating solution (Kitano et al., 1978; Vengosh et al., 1991; Hemming and Hanson, 1992). Therefore, a decrease in pH of the site of calcification under increased light intensity should induce a decrease in $[B(OH)_4^-]$ and therewith lower B concentrations to be recorded in the coral aragonite. Our results confirm the decrease in pH at the site of calcification observed under enhanced light intensity.

4.3.2 Temperature effect

In our study, when all the other parameters are maintained constant, boron concentrations in *Acropora* sp. increase with increasing temperature and $[CO_3^{2-}]$. So far, B/Ca in corals was found to vary inversely with temperature (Hart and Cohen, 1996; Sinclair et al., 1998; Fallon et al., 2003; Montagna et al., 2007; Allison et al., 2010; Trotter et al., 2011). To our knowledge, Trotter et al. (2011) is the only other study to report on the

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impact of temperature on B/Ca ratios of cultured shallow water corals. They cultured the Mediterranean coral *Cladocora caespitosa* under different temperature conditions and observed, in contradiction to our study, a negative correlation with seawater temperature. Nevertheless, in the experimental set-up used by Trotter et al. (2011), corals were not maintained under constant temperature conditions, but supplied with natural unfiltered seawater following seasonal temperature fluctuations (for complete experimental protocol see Rodolfo-Metalpa et al., 2010). With the exception of pCO_2, which was held constant, irradiance as well as the photoperiod were changed according to their seasonal values measured at ca. 20 m depth in the Bay of Villefranche, where corals had been originally collected. Changes in food availability following seasonal fluctuations as corals fed from the unfiltered sea-water, might also impact coral biology and therewith B incorporation. Therefore, B/Ca ratios measured by Trotter et al. (2011) might well follow changes in calcification, respiration and/or photosynthesis rates of the coral-symbiont assemblage, triggered by seasonal changes in temperature, light, and feeding regime, rendering difficult to unravel the impact of temperature from that of other parameters that vary concomitantly.

Interestingly, Trotter et al. (2011) pointed out that the observed correlation of B/Ca with temperature seemed to be the result of the strong temperature dependence of seawater $[B(OH_4^-)]/[CO_3^{2-}]$. In their study, increase in temperature correlates with increasing calcification rates (Rodolfo-Metalpa et al., 2010), increasing seawater $[CO_3^{2-}]$ (decreasing $[B(OH_4^-)]/[CO_3^{2-}]$) and decreasing B/Ca ratios in the coral skeleton, while $[B(OH_4^-)]/[HCO_3^-]$ remains insignificantly correlated to either B/Ca ratios or temperature. Due to the relative proportion of $[B(OH_4^-)]$ vs. $[CO_3^{2-}]$ and/or $[HCO_3^-]$ in seawater, carbonate forms are expected to be the main driver of variations in $[B(OH_4^-)]/[CO_3^{2-}]$ and $[B(OH_4^-)]/[HCO_3^-]$ ratios. In our study, increases in temperature from 22 to 28 °C induce an increase in carbonate ion concentration from 236 to 268 µmol kg^{-1}, while bicarbonate concentration decreased from 1966 to 1892 µmol/kg^{-1}. Therefore, increases in temperature are correlated to increase in calcification rate, increase in $[CO_3^{2-}]$

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(decrease in $[B(OH_4^-)]/[CO_3^{2-}]$) but contrarily to Trotter et al. (2011), increase in B/Ca ratios in the coral skeleton. Interestingly, the influence of temperature on foraminifera B/Ca ratios is currently subject of debate.

For planktonic foraminifera for example, while certain studies report a positive relationship between the partition coefficient ($K_D = [(B/Ca)_{CaCO3}]/[B(OH)_4^-/HCO_3^-]_{sw}$) and temperature (*Globorotalia inflata*, *Globigerina bulloides* from coretop samples, and *Globigerinoides ruber* from downcore, Tripati et al., 2009; Yu et al., 2007), others observed a negative K_D–T (°C) relationship (*Globigerinoides sacculifer*, *G. ruber*, and *Neogloboquadrina dutertrei* from coretop; Foster, 2008). Similarly, when B/Ca ratios increase with temperature in *Globorotalia inflata* (Yu et al., 2007), no temperature influence can be observed on *Neogloboquadrina pachyderma* (sinistral) (Hendry et al., 2009). These observations suggest that the controlling factors and processes driving boron transport to the calcification site and its incorporation into marine calcium carbonates, have not yet been adequately identified. Additional species-specific experiments combining both culture experiment and natural environment samples are necessary to improve our understanding and therewith the use of B/Ca as an environmental proxy.

4.3.3 B/Ca vs. Sr/Ca ratios

B/Ca ratios in corals have been reported to be well correlated with Sr/Ca ratios (Hart and Cohen, 1996; Sinclair et al., 1998; Fallon et al., 2003; Montagna et al., 2007; Allison et al., 2010). Sr/Ca in corals is considered to be primarily controlled by seawater temperature (Sinclair et al., 1998), following a negative correlation (e.g., Quinn and Sampson, 2002; Corrège, 2006; DeLong et al., 2007; Cohen and Thorrold, 2007; Goodkin et al., 2007). This agrees with our study where Sr/Ca ratio decreases with increasing temperature (R^2 = 0.89; Interpretation of the Sr/Ca values will be presented elsewhere: Juillet-Leclerc et al., 2012). A strong negative correlation is observed between Sr/Ca and B/Ca (R^2 = 0.92), which contradicts the positive correlation measured in previous studies on corals from natural environments (e.g., Allison and Finch 2010).

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These observations enlighten: (1) The significant impact of temperature on B (and Sr) concentrations when seawater pH is maintained constant; and (2) The (negatively) coupled incorporation of B and Sr into *Acropora* sp.

4.3.4 B/Ca ratios after recovery experiment

Boron concentrations determined at the end of step 3, do not present any correlation with growth culture conditions under which they grew during the 10 weeks of the recovery period (200 µmol photon $m^{-2} s^{-1}$, 25 °C). Also, no straightforward correlation between boron concentrations measured at the end of step 3 can be made with any of the previous step 2 culture conditions. B/Ca ratios measured after the recovery period are all significantly lower than those at the end of step 2, independently of the experimental conditions considered (see Table 3). These results agree with the observations made for boron isotope composition and tend to confirm that the mechanical stress applied to the coral between step 2 and step 3, led to a perturbation of the pH enhancement process at the site of calcification.

5 Conclusions

We investigated the impact of light and temperature on boron isotope composition and boron concentration of *Acropora* sp. skeleton from nubbins maintained under culture experiments. Metabolic measurements indicate that photosynthesis, respiration and calcification rates increase with increasing light and temperature. δ^{11}B values indicate higher pH at the site of calcification compared to ambient seawater pH, both under Low Light and High Light conditions. Changes in light intensities from 200 to 400 µmol photon $m^{-2} s^{-1}$ could bias pH reconstructions by about 0.05 units. A increase of δ^{11}B is observed between 22 and 25 °C, which corresponds to enhancements of 0.027 and 0.016 pH units at the site of calcification, for LL and HL, respectively. However, no further δ^{11}B increase occurred between 25 and 28 °C. This non-linear

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temperature effect complicates the determination of a correcting factor, underlining the need of additional culture experiment to better calibrate the impact of temperature on the pH-δ^{11}B proxy.

B/Ca ratios decrease with increasing light confirming the decrease in pH at the site of calcification under enhanced light intensities observed with δ^{11}B signatures. When all the other parameters are maintained constant, boron concentrations in *Acropora* sp. increase with increasing temperature and increasing carbonate ions concentrations. These observations contradict previous studies where B/Ca in corals was found to vary inversely with temperature. This suggests that the controlling factors driving boron concentrations have not yet been adequately identified and might be influenced by other seawater variables and species specific responses.

Boron isotopic composition and boron concentrations determined at the end of step 3 do not present any correlation with environmental parameters of step 3 culture conditions (200 µmol photon m^{-2} s^{-1}, 25 °C) nor with any of the previous step 2 culture conditions. Nevertheless, when translated into pH, recovery boron isotopic compositions and boron concentrations are all consistent with decreased pH values, independently of the light and temperature conditions considered. These results indicate that physical stress applied to the organisms before beginning of step 3 led to a disruption of the pH enhancement at the site of calcification. This highlights the fact that corals submitted to strong stress factors (e.g. storms, bleaching events etc.) should not be considered for paleo-environmental reconstructions.

Acknowledgements. This work was supported by the CEA – CNRS and USVQ (national project INSU/ASTICO), and by Australian Research Council (ARC) grant DP0986505 and the ARC Centre of Excellence in Coral Reef Studies. It was also partially funded by the Framework 7 European Project on Ocean Acidification (EPOCA grant agreement number 211384). Paolo Montagna is grateful for financial support from the Marie Curie International Outgoing Fellowship (MEDAT-ARCHIVES). This is LSCE contribution 4800. The authors would like to thank Guillaume Tisserand for his help with coral culturing experiments. We are grateful to Louise Bordier for helping with elemental and isotopic samples preparation and measurement, and Michael Holcomb for editing the manuscript and for his help with statistical issue.

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The publication of this article is financed by CNRS-INSU.

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Impact of light and temperature on coral Boron incorporation

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Table 1. Mean temperature (°C), ALK (μeq kg^{-1}), pH (Total scale) and salinity of the culture media, measured during step 2 of the experiment. Measurements performed in culture tank submitted to HL and LL conditions were averaged and are presented under a single value per temperature conditions.

	Temperature (°C)	ALK (μeq kg^{-1})	pH (Total scale)	Salinity
Mean value	22	2537.67	8.03	38
SD	±0.1	±3.06	±0.02	±0.1
Mean value	25	2536.5	8.02	38
SD	±0.1	±5.92	±0.01	±0.1
Mean value	28	2536	8	38
SD	±0.1	±6.12	±0.01	±0.1

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Table 2. Mean value and standard deviation of photosynthesis (P_{net}, O_2 µmol cm^{-2} s^{-1}), respiration (R, O_2 µmol m^{-2} s^{-1}) and, calcification rate (Ca rate), at the end of step 2. Net photosynthesis and respiration are normalized relative to the colony's surface.

	light µmol photon m^{-2} s^{-1}	temperature °C	P_{net} O_2 µmol cm^{-2} s^{-1}	R O_2 µmol cm^{-2} s^{-2}	Ca rate *
mean value	200	22	0.19	−0.47	0.14
SD			0.08	0.10	0.05
mean value	200	25	0.28	−0.65	0.19
SD			0.11	0.13	0.02
mean value	200	28	0.41	−0.64	0.30
SD			0.24	0.18	0.09
mean value	400	22	0.30	−0.42	0.17
SD			0.09	0.06	0.06
mean value	400	25	0.46	−0.72	0.27
SD			0.09	0.17	0.05
mean value	400	28	0.71	−0.97	0.40
SD			0.30	0.25	0.09

* at the end of the step 2.

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Table 3. δ^{11}B (‰) and B/Ca (μmol mol^{-1}) measured at the end of step 2 and 3. For step 2 values represent mean calculation of measurements performed on 2 replicates per experimental condition at the exception of condition 400 μmol m^{-2} s^{-1} and 28 °C where 3 replicates were considered. Due to sample limitation, values presented for step 3 are based on a single sample per experimental condition. Reconstructed pH at the site of calcification using the boron isotope values and the fractionation factor determined by Klochko et al. (2006): $\alpha = 1.0272 \pm 0.0006$ ($\delta^{11}B_{seawater} = 39.61$ ‰, $B_T = 416.0$ μM and T = 22, 25 and 28 °C).

	Light (μmol m^{-2} s^{-1})	Temperature (°C)	δ^{11}B (‰)	B/Ca (μmol mol^{-1})	δ^{11}B derived pH site of calcification (Total scale)
Mean value	200	22	22.16	460	8.39
SD			0.42	2	
Mean value	200	25	23.10	485	8.42
SD			0.22	8	
Mean value	200	28	23.19	512	8.39
SD			0.61	4	
Mean value	400	22	21.76	455	8.36
SD			0.20	5	
Mean value	400	25	22.50	478	8.38
SD			0.40	7	
Mean value	400	28	22.67	487	8.35
SD			0.20	10	
	Light (μmol m^{-2} s^{-1})	Temperature (°C)	δ^{11}B (‰)	B/Ca (μmol mol^{-1})	δ^{11}B derived pH site of calcification (Total scale)
Mean value	200	22	20.93	436	8.30
SD			0.25	13	
Mean value	200	25	20.07	442	8.20
SD			0.25	13	
Mean value	200	28	n.a.	n.a.	
SD					
Mean value	400	22	21.65; 21.73	443; 449	8.35; 8.36
SD			0.25	13	
Mean value	400	25	20.46	416	8.23
SD			0.25	12	
Mean value	400	28	n.a.	n.a.	
SD					

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Table 4. Statistical analyses were performed with SAS version 72. A full factorial analyse was first conducted showing no significant interactions between light and temperature variations on boron isotope compositions. Subsequently, two ways ANOVA tests were applied showing both significant impact of light and temperature on δ^{11}B. The light impact can be considered similar between the three temperature conditions. An additional SCHEFFE test was applied to test for inter comparison of temperature conditions. Significant differences of temperature on coral boron isotope composition can be observed between 22 and 25 °C and 22 and 28 °C, while isotopic signatures can be considered similar between 25 and 28 °C. Identical statistical tests were applied to boron concentrations, showing both significant impact of light and temperature. Significant differences of temperature can be observed between 22 and 25 °C for both light conditions. Two additional 1 way ANOVA were performed to test the impact of temperature increase from 25 to 28 °C, per light condition independently. This temperature increase induces a significant increase in boron concentrations under LL, while no significant increase can be observed under HL conditions.

	Statistics	**for B isotope compositions**		
Full factorial: no significant interactions 2 ways ANOVA				
	Degree of freedom	**F value**	**p-value**	
Impact of light on δ^{11}B	Light F1,9	8.46	0.0173	sign.
Impact of temperature on δ^{11}B	Temp F2,9	11.64	0.0032	sign.
Scheffe test for temperature				
22–25			−0.0671	sign.
25–28			0.650	non sign.
22–28			−0.1850	sign.
	Statistics	**for B concentrations**		
Full factorial: no significant interactions 2 ways ANOVA				
	Degree of freedom	**F value**	**p-value**	
Impact of light on δ^{11}B	Light F1, 9	8.31	0.0181	sign.
Impact of temperature on δ^{11}B	Temp F2, 9	27.61	0.0001	sign.
Scheffe test for temperature				
22–25			−7.309	sign.
25–28			0.597	almost sign.
22–28			−23.6910	sign.
1 way ANOVA				
LL 25–28	F 2,2	107.53	0.0178	sign.
HL 25–28	F2,2.5	12.22	0.052	non sign.

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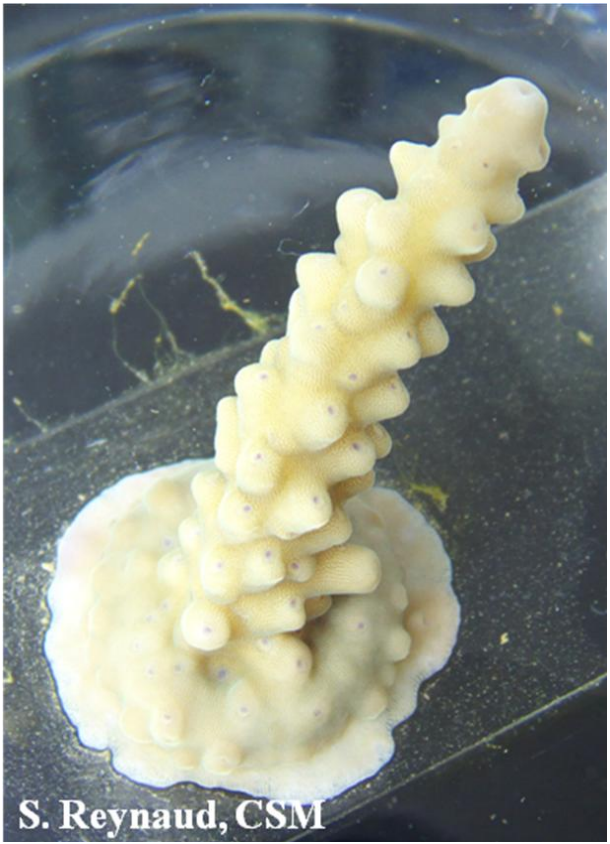

Fig. 1. Normal light picture of *Acropora* sp. tip adhered onto slides using underwater epoxy. (Slide width = 2.6 cm).

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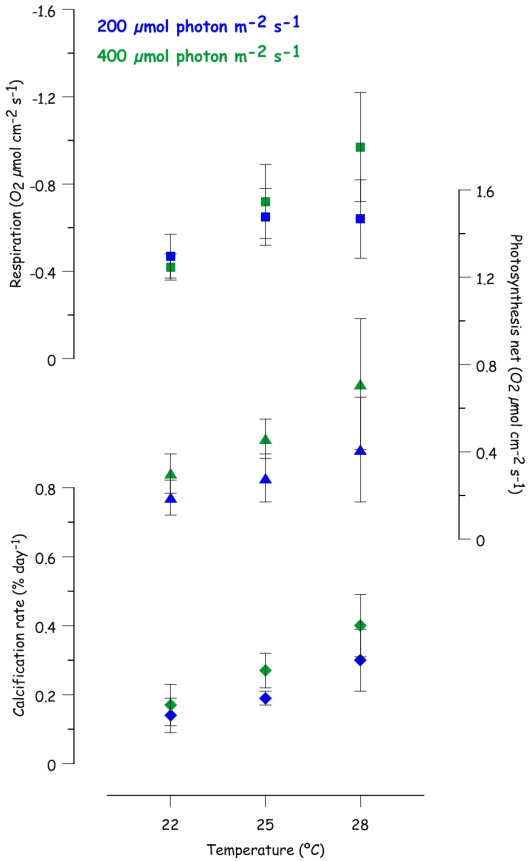

Fig. 2. Mean respiration (O_2 µmol cm^{-2} s^{-1}), photosynthesis (O_2 µmol cm^{-2} s^{-1}) and calcification (% day^{-1}) rates measured per experimental conditions and plotted versus temperature (error bars represent SD).

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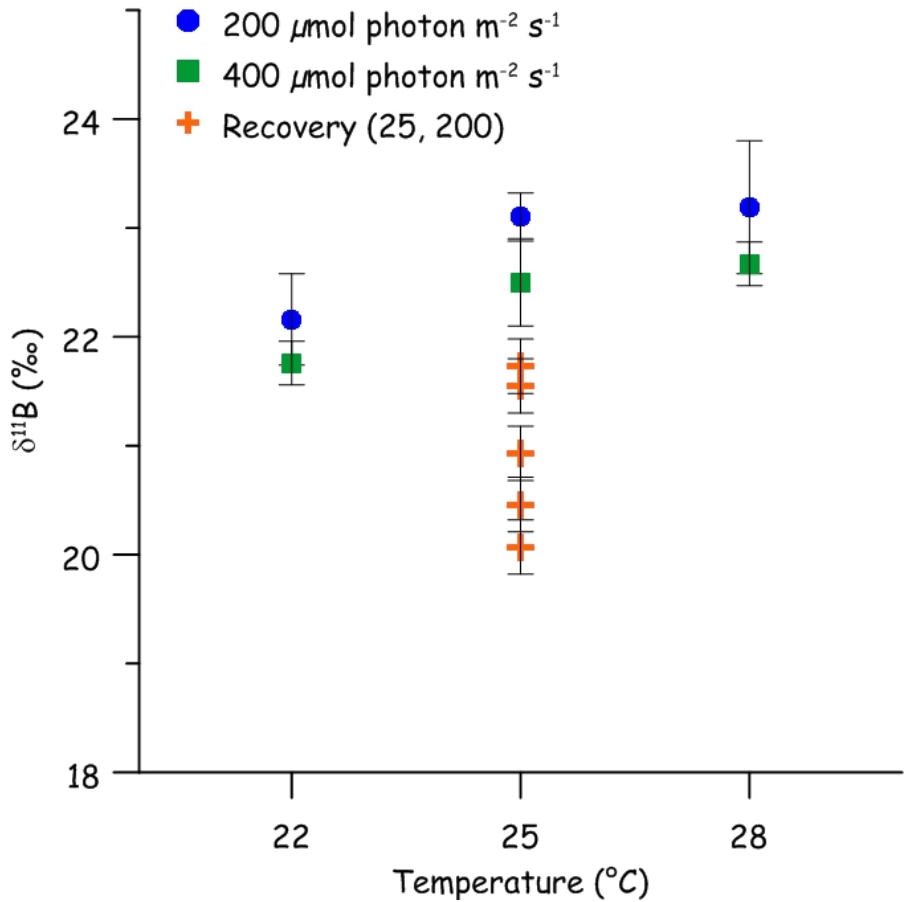

Fig. 3. Mean δ^{11}B (‰) measured at the end of step 2 on corals grown under 200 (blue dots) and 400 (green squares) µmol photon m^{-2} s^{-1}, plotted versus temperature (°C). Orange crosses represent measurements performed at the end of step 3 (recovery experiment).

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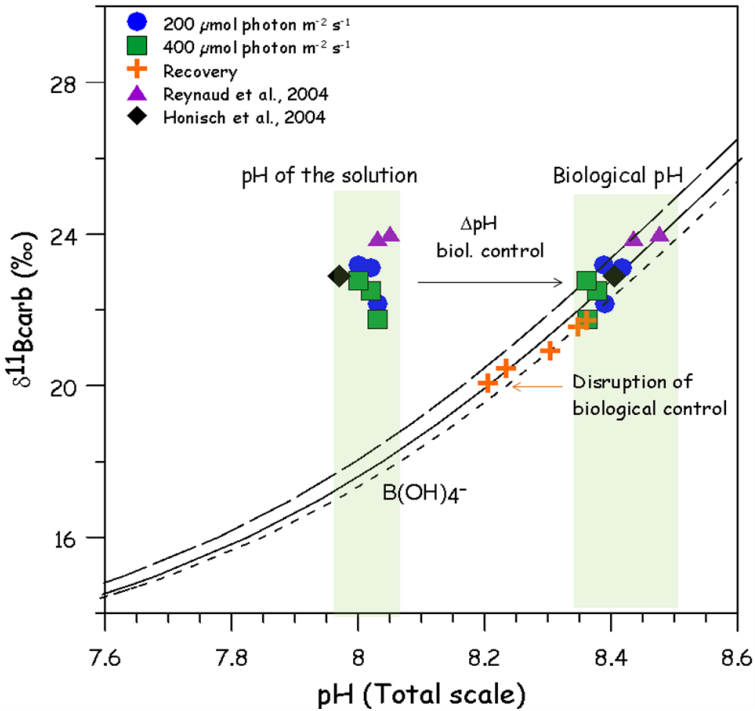

Fig. 4. Mean δ^{11}B (‰) measured at the end of step 2 on corals grown under 200 (blue dots) and 400 (green squares) photon m^{-2} s^{-1}, plotted versus growth medium pH and reconstructed pH in the site of calcification using the fractionation factor determined by Klochko et al. (2006) ($\alpha = 1.0272 \pm 0.0006$; borate curve lines for $\delta^{11}B_{seawater} = 39.61$ ‰, $B_T = 416$ µM and $T = 22$ °C (small dashed line), $T = 25$ °C (full line), $T = 28$ °C (large dashed line)). Black diamond and purple triangles represent value measured by Hönisch et al. (2004) and Reynaud et al. (2004), respectively. Oranges crosses represent mean δ^{11}B (‰) measured at the end of step 3 (recovery experiment) plotted versus reconstructed site of calcification pH using the fractionation factor determined by Klochko et al. (2006) $\alpha = 1.0272 \pm 0.0006$.

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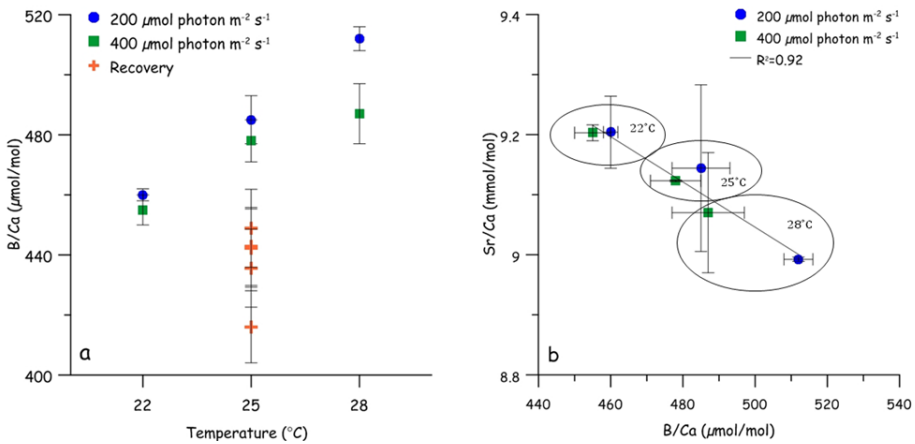

Fig. 5. (a) mean B/Ca ratios (µmol mol^{-1}) measured at the end of step 2 on corals grown under 200 (blue dots) and 400 (green squares) photon m^{-2} s^{-1}, plotted versus temperature. Measurements performed after step 3 (recovery experiment) are plotted on the same graph (orange crosses). **(b)** mean B/Ca ratios plotted versus mean Sr/Ca ratios measured simultaneously at the end of STEP 2 (200 and 400 photon m^{-2} s^{-1} light conditions are represented by blue dots and green squares, respectively). Full line represent linear regression calculated using all values (R^2 = 0.92). Interpretation of the Sr/Ca values will be presented elsewhere (Juillet-Leclerc et al., 2012).

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