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

D. Dissard[1,2], E. Douville[1], S. Reynaud[3], A. Juillet-Leclerc[1], P. Montagna[4], P. Louvat[5], and M. McCulloch[2]

[1]Laboratoire des Sciences du Climat et de l'Environnement, UMR8212, UVSQ-CNRS-CEA, LSCE/IPSL, Av. de la Terrasse, 91198 Gif sur Yvette, France
[2]School of Earth and Environment, The University of Western Australia, 35 Stirling Highway Crawley WA 6009, Perth, Australia
[3]Centre Scientifique de Monaco, Avenue Saint-Martin, MC 98000, Monaco
[4]Institute of Marine Sciences – National Research Council, ISMAR-CNR, Via Gobetti 101 40129 Bologna, Italy
[5]Institut de Physique du Globe de Paris, Sorbonne Paris Cité, Université Paris-Diderot, UMR7154, CNRS, 1 rue Jussieu, 75238 Paris, France

Correspondence to: D. Dissard (delphine.dissard@uwa.edu.au)

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Abstract. The boron isotopic composition (δ^{11}B) of marine carbonates (e.g. corals) is increasingly utilised as a proxy for paleo-pH, with the strong correlation between δ^{11}B of marine calcifiers and seawater pH now well documented. However, the potential roles of other environmental parameters that may also influence both the boron isotopic composition and boron concentration into coral aragonite are poorly known. To overcome this, the tropical scleractinian coral *Acropora* sp. was cultured under 3 different temperatures (22, 25 and 28 °C) and two light conditions (200 and 400 µmol photon m^{-2} s^{-1}). The δ^{11}B indicates an increase in internal pH that is dependent on the light conditions. Changes in light intensities from 200 to 400 µmol photon m^{-2} s^{-1} seem to indicate an apparent decrease in pH at the site of calcification, contrary to what is expected in most models of light-enhanced calcification. Thus, variations in light conditions chosen to mimic average annual variations of the natural environments where *Acropora* sp. colonies can be found 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 an increase of about 0.02 pH-units, while no further δ^{11}B increase can be observed from 25 to 28 °C. This non-linear temperature effect complicates the determination of a correction factor. B / Ca ratios decrease with increasing light, consistent with the decrease in pH at the site of calcification under enhanced light intensities. When all the other parameters are constant, boron concentrations in *Acropora* sp. increase with increasing temperatures and increasing carbonate ion 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 environmental variables and/or species-specific responses.

1 Introduction

Increased atmospheric CO_2 concentrations from 280 (pre-industrial values) to 390 ppmv (2010) 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 (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 past 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 long-term changes in seawater 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., 2007, 2008, 2009; Wei et al., 2009; Douville et al., 2010), with its reliability recently 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, Hemming and Hanson (1992) proposed the following mechanism for boron substitution in the CO_3^{2-} site in carbonate:

$$CaCO_3 + B(OH)_4^- = Ca(HBO_3) + HCO_3^- + H_2O. \quad (R1)$$

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 in the use of boron isotope-pH proxy might be the potential incorporation of significant concentrations of seawater boric acid into biogenic calcium carbonate instead of only borate. This assumption was recently raised by two NMR studies, which measured proportions of trigonally coordinated boron in coral aragonite varying from 12 to 48 % (Klochko et al., 2009; Rollion-Bard et al., 2011). However, NMR technique cannot distinguish between boric acid directly incorporated from solution or derived from borate ion during adsorption and incorporation into boron-carbonate. 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. In addition, because the incorporation of seawater boric acid would shift the boron isotopic composition of coral skeletons to considerably higher values (McCulloch et al., 2012), it appears unlikely that the trigonal $B(OH)_3^-$ species detected in calcite and aragonite is directly derived from seawater.

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 (Trotter et al., 2011).

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 calcification 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 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 ($\delta^{11}B$) 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 in natural environments where *Acropora* sp. can be found (e.g. Nouméa lagoon; Quinn and Sampson, 2002). 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).

Table 1. Description of the three steps of the culture protocol.

	Step 1: Acclimation	Step 2: Experiment		Step 3: Recovery
Duration	9 weeks	15 weeks		10 weeks
Conditions	All nubbins were cultivated in the same aquarium at constant temperature (25 °C) and light conditions (200 µmol photons m^{-2} s^{-1}).	All nubbins were randomly distributed within the six culture tanks (2 nubbins per tank, except for condition 400, 28 where three nubbins were incubated)	**Mechanical breakage**	All nubbins were placed in the same aquarium under identical culture conditions (200 µmol photons m^{-2} s^{-1}) and 25 °C

2 Material and method

2.1 Experimental protocol

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.

The culture experiment was conducted following a three step protocol (see Table 1). Step 1: all the nubbins were cultivated in the same aquarium at constant temperature (25 °C) and light conditions (200 µmol photons 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) (two nubbins per tank, except for condition 400, 28, where three nubbins were maintained in culture). 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 photons m^{-2} s^{-1}, 25 °C) for step 3. This ensured the secretion of new skeleton through identical biological regulation (same organism) but allowed differentiation of aragonite precipitated during step 2 from step 3. Step 3 is referred to as "recovery period" in the following sections and lasted for ten additional weeks.

2.2 Experimental setup

Culture experiments were conducted using the culturing setup and protocol described by Reynaud et al. (1999), in which tips, sampled from a single parent colony, were adhered onto slides ($2.6 \times 6 \times 0.2$ cm, see Fig. 1) using underwater epoxy (Devcon), and randomly distributed in incubation tanks (30 l). At the end of step 1, the skeleton was only present until the limit of the glue, but never on the surface of the slide. Nubbins were then subsequently randomly distributed within the six culture tanks. At the completion of step 2, only the ring skeleton deposited on the slide 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 (Rena pump, 6 l min^{-1}) and maintained under ambient pCO_2 ($pCO_2 \approx 390$ ppm) prior to flowing into the culture aquaria. 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 12h:12h light dark photo-period. Irradiance was measured once a week using a 4π quantum sensor (Li-Cor, LI-193SA). The culture temperature was controlled within the culture tank using a temperature controller (±0.1 °C, EW, PC 902/T; Table 2). Temperatures and light intensities were chosen to mimic average annual variations in natural environments where the *Acropora* sp. parent colonies can be found (e.g. Nouméa lagoon; Quinn and Sampson, 2002). Salinity and pH were measured directly within the culture tanks (Table 2, 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 2 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.

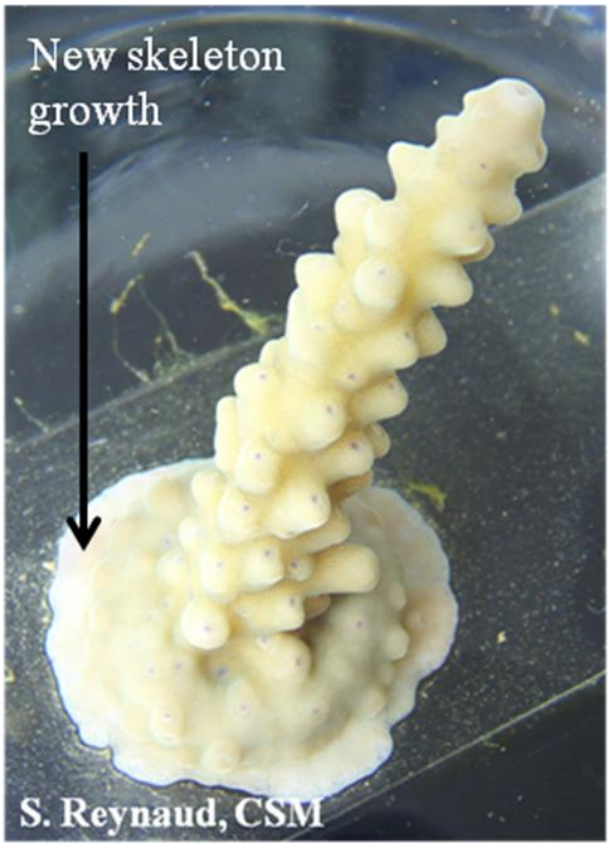

Fig. 1. Normal light picture of *Acropora* sp. tip adhered onto slides using underwater epoxy. (Slide width = 2.6 cm).

2.3 Metabolic measurements

Nubbins glued onto 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 in the same culture tank as their associated nubbin (Table 3).

Photosynthesis and respiration were measured once a month using the respirometry technique, which consists of monitoring the changes in oxygen concentration during incubation time (Griffith et al., 1987). 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 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 bubbler (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 weighed once a week using the buoyant weight technique (Jokiel et al., 1978; Davies, 1989). Calcification rates were calculated using the following formula (Reynaud et al., 2002):

$$G = \sqrt[n]{\frac{P_n}{P_0} - 1}, \quad (1)$$

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.

Table 2. 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 (high light) and LL (low light) 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

2.4 Geochemical measurements

For each experimental condition, elemental and isotopic measurements were performed on two replicates of separate coral nubbins incubated in the same culture conditions, except for condition 400, 28, where values presented are the average of three replicates.

Prior elemental and isotopic chemistries, coral powder was cleaned in order to remove organic matter. Coral aragonite was soaked for 12 h in 30 % hydrogen peroxide (Reynaud-Vaganay et al., 1999). The solution was subsequently filtered and rinsed repeatedly with MilliQ water through a membrane filtration (nucleopore polycarbonate with pores 0.45 µm in diameter) chemically compatible with hydrogen peroxide. Each filter was dried for 2 h at 40 °C, and the aragonite was then removed from the filter pending further preparation for geochemical analysis.

Table 3. Mean value and standard deviation of photosynthesis (Pnet, O_2 µmol cm^{-2} s^{-1}), respiration (R, O_2 µmol cm^{-2} s^{-1}), and calcification rate (g cm^{-2} yr^{-1}) at the end of step 2. Net photosynthesis and respiration are normalized relative to the colony's surface.

	Step 2				
	Light	Temperature	Photosynthesis net	Respiration	Calcification rate
	(µmol m^{-2} s^{-1})	(°C)	O_2 µmol cm^{-2} s^{-1}	O_2 µmol cm^{-2} s^{-1}	g cm^{-2} yr^{-1}
Mean value	200	22	0.19	−0.47	0.14
SD			±0.08	±0.1	±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	0.3
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	0.4
SD			±0.3	±0.25	±0.09

2.4.1 Boron isotope measurements

The boron isotopic composition was measured using a double focusing sector-field multi-collector-inductively coupled plasma mass spectrometer (ThermoScientific Neptune) at the Institut de Physique du Globe de Paris (IPGP, France), following the protocol described by Douville et al. (2010) that includes chemical separation of boron before its measurement (Table 4). The total quantity of material considered was 55 mg of powdered aragonite per samples. 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 on isotope composition (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 to 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; Table 4). 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 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 µmol 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 3). 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.

Table 4. δ^{11}B (‰) and B / Ca (µmol mol^{-1}) measured at the end of steps 2 and 3. For step 2 values represent mean calculation of measurements performed on 2 replicates per experimental condition, with the exception of condition 400 µmol m^{-2} s^{-1} and 28 °C where 3 replicates were considered. SD represents the combined standard deviation calculated based on the analytical uncertainty and the difference between the separate replicates. Due to sample size limitation, values presented for step 3 are based on a single sample per experimental condition; here SD represents only the analytical uncertainty. 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 $T = 22$ °C, 25 °C and 28 °C).

	Light (µmol m^{-2} s^{-1})	Temperature (°C)	δ^{11}B (‰)	Average δ^{11}B per exp. conditions (‰)	B / Ca (µmol mol^{-1})	Average B / Ca per exp. conditions (µmol mol)	δ^{11}B derived pH site of calcification (total scale)
Step 2							
Mean value	200	22	21.87	22.16	458	460	8.39
			22.46	±0.40	461	±9	
Mean value	200	25	23.25	23.1	480	485	8.42
			22.95	±0.26	491	±11	
Mean value	200	28	23.62	23.19	515	512	8.39
			22.76	±0.56	510	±10	
Mean value	400	22	21.90	21.76	458	455	8.36
			21.62	±0.25	452	±10	
Mean value	400	25	22.21	22.5	473	478	8.38
			22.78	±0.39	483	±11	
Mean value	400	28	22.50	22.67	498	487	8.35
			22.89	±0.26	480	±13	
			22.61		484		
Step 3							
Mean value	200	22	20.93		436		8.27
			±0.25		±13		
Mean value	200	25	20.07		442		8.2
			±0.25		±13		
Mean value	200	28	n.a.		n.a.		
Mean value	400	22	21.55; 21.73		443; 449		8.33; 8.31
			±0.25		±13		
Mean value	400	25	20.46		416		8.23
			±0.25		±13		
Mean value	400	28	n.a.		n.a.		

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 temperatures. 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 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, $pH_T = 8.02 \pm 0.02$, $S = 38 \pm 0.1$ and light conditions: 200–400 µmol photons m^{-2} s^{-1}, Table 4).

For the three temperature regimes, boron isotope compositions show a significant decrease with increasing

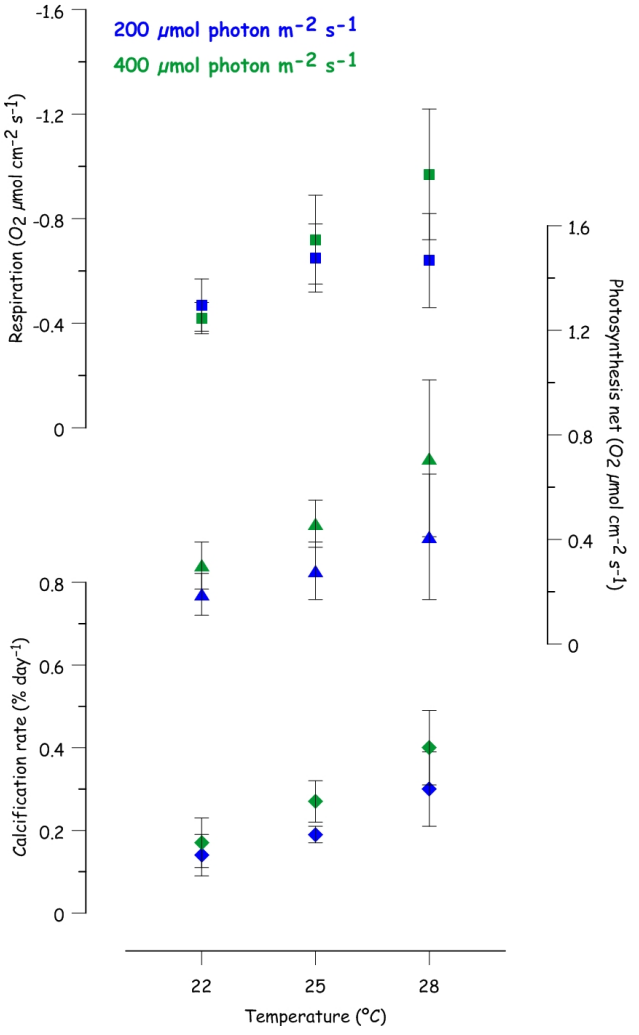

Fig. 2. Mean respiration (O_2 µmol cm^{-2} s^{-1}), photosynthesis (O_2 µmol cm^{-2} s^{-1}) and calcification (% day^{-1}) rates measured per experimental conditions and plotted vs. temperature (error bars represent SD).

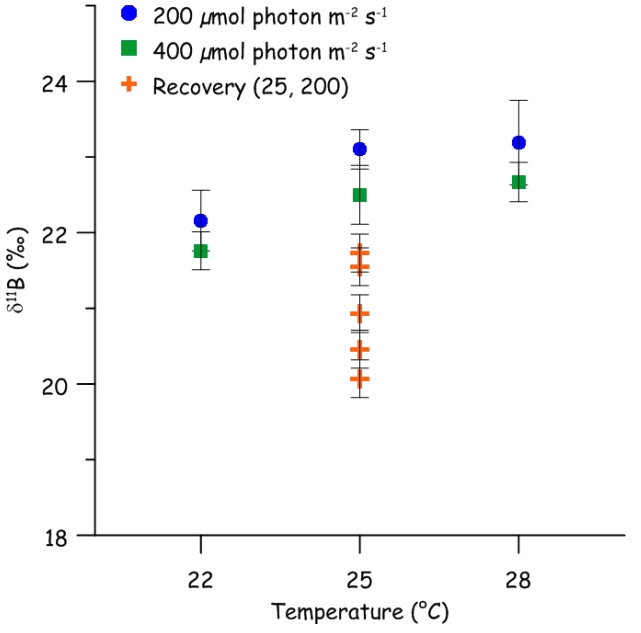

Fig. 3. Mean δ^{11}B (‰) measured at the end of step 2 on corals grown under 200 (blue dots) and 400 (green squares) µmol photon m^{-2} s^{-1}, plotted vs. temperature (°C). Orange crosses represent measurements performed at the end of step 3 (recovery experiment).

light (2-way ANOVA $F_{1,9} = 8.46$, $p = 0.0173$, see Table 5). With increasing light intensities from 200 to 400 µmol photons 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 4). These decreases can be considered statistically similar for each temperature condition (Table 5).

Boron isotope compositions show a significant increase with increasing temperatures between 22 and 25 °C for both light conditions (SCHEFFE test 22–25 °C, $p = -0.0671$, Table 5). Measured δ^{11}B varied between 22.16 ± 0.40 ‰ (22 °C) and 23.10 ± 0.26 ‰ (25 °C) under LL, and between 21.76 ± 0.25 ‰ (22 °C) and 22.50 ± 0.39 ‰ (25 °C) under HL conditions ($pH_T \approx 8.02$). For temperatures 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 5). δ^{11}B was measured to vary from 23.10 ± 0.26 ‰ at 25 °C to 23.19 ± 0.56 ‰ at 28 °C (at 200 µmol photons m^{-2} s^{-1}) and from 22.50 ± 0.39 ‰ at 25 °C to 22.67 ± 0.26 ‰ at 28 °C (at 400 µmol photons m^{-2} s^{-1}), with $pH_T \approx 8.02$ for both conditions.

For clarity, measured boron isotope compositions are plotted versus medium pH in Fig. 4. pH at the site of calcification reconstructed from δ^{11}B values (with $\alpha = 1.0272 \pm 0.0006$, $\delta^{11}B_{seawater} = 39.61$ ‰ and $B_T = 416$ µM from DOE, 1994) 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 δ^{11}B measured on *Acropora* sp. cultured under similar conditions. Hönisch et al. (2004) measured δ^{11}B values of *Acropora nobilis* of about 22.9 ‰ ($T = 27$ °C, $pH_T = 7.97$, and light condition of 300 ± 15 µmol photons m^{-2} s^{-1}), while Reynaud et al. (2004) report δ^{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 δ^{11}B data of Reynaud et al. (2004) and Hönisch et al. (2004) are plotted vs. pH in 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 δ^{11}B was measured with MC-ICPMS in our study, while Hönisch et al. (2004) and Reynaud et al. (2004) used the N-TIMS

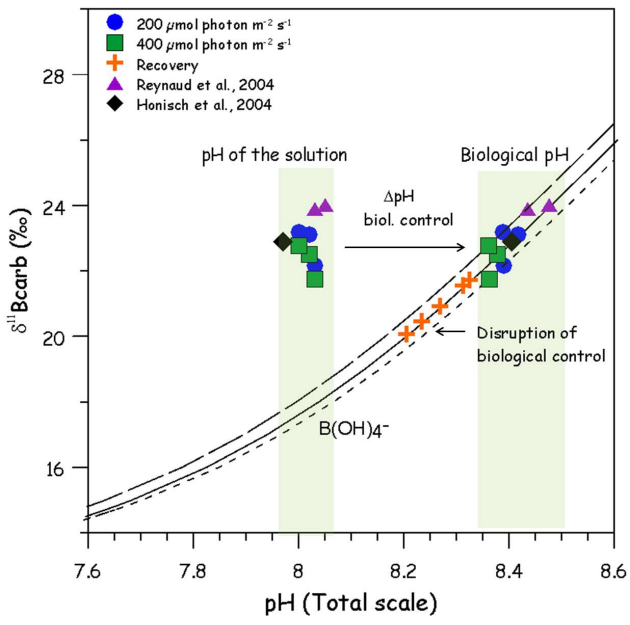

Fig. 4. Mean δ^{11}B (‰) measured at the end of step 2 on corals grown under 200 (blue dots) and 400 (green squares) photon m^{-2} s^{-1}, plotted vs. growth medium pH and reconstructed pH at the site of calcification using the fractionation factor determined by Klochko et al. (2006) ($\alpha = 1.0272 \pm 0.0006$; borate curve lines for $\delta^{11}B_{seawater} = 39.61$ ‰, $B_T = 416$ µM (DOE, 1994) and $T = 22$ °C (small dashed line), $T = 25$ °C (solid line), $T = 28$ °C (large dashed line)). Black diamond and purple triangles represent values measured by Hönisch et al. (2004) and Reynaud et al. (2004), respectively. Orange crosses represent mean δ^{11}B (‰) measured at the end of step 3 (recovery experiment) plotted vs. reconstructed site of calcification pH using the fractionation factor determined by Klochko et al. (2006) $\alpha = 1.0272 \pm 0.0006$.

(Negative Thermo-Ionization 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 δ^{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 4, 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 4, Fig. 5a).

B / Ca ratios show a significant decrease with increasing light (2-way ANOVA $F_{1,9} = 8.31$, $p = 0.0181$, see Table 5), with values varying from 460 ± 9, 485 ± 11, 512 ± 10 µmol mol^{-1} under LL and 455 ± 10, 478 ± 11 and 487 ± 13 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 5). 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 photons 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 environments 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 4, 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 vs. 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 natural environments (from ≈8.5 to 9.5 mmol mol^{-1}, Shirai et al., 2008).

4 Discussion

4.1 Metabolic measurements

In our study, photosynthesis, respiration and calcification rates increase with both increasing light and temperature (Fig. 2, Table 3). Overall, these observations are in good agreement with numerous previous studies made on corals from both culture experiments and natural environments (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). For example, for tropical corals, Iglesias-Prieto and Trench (1997) already reported an increase of photosynthetic activity with increasing light, while Reynaud et al. (2003) observed an increase in photosynthetic activity with increasing temperature. Similarly, Kajiwara et al. (1995) already recorded an increase in respiration rate with increasing temperature (*Acropora pulchra*), while Kuhl et al. (1995) calculated that coral respiration

Table 5. 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-way 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 on coral boron concentrations. Significant differences of temperature can be observed between 22 and 25 °C. Two additional 1-way ANOVA tests were performed to measure 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-way 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				
	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	F2,2	107.53	0.0178	sign.
HL 25–28	F2,2.5	12.22	0.052	non sign.

was 6 times higher under irradiance of 350 µmol m^{-2} s^{-1} than the dark respiration measured under otherwise identical conditions. Finally, coral calcification is well-known to increase with increasing light (Light Enhanced Calcification, LEC, Goreau, 1959; Chalker and Taylor, 1975; Chalker and Barnes, 1990; Moya et al., 2006), and has been observed to increase with temperature for several species of corals (within the the limits of biological sustainability, Coles and Jokiel, 1977, 1978; Reynaud et al., 2007).

It can therefore be considered that nubbins used 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 isotopes

Due to the high seawater renewal rate into our culture aquaria (5 times per day), seawater carbonate chemistry remained constant through the experiment for all conditions (Table 2). Therefore, it is assumed that in our study the variation of the skeletal δ^{11}B reflects pH-variations at the site of calcification (Trotter et al., 2011).

4.2.1 Boron isotope compositions

Measured *Acropora* sp. δ^{11}B vs. 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 δ^{11}B values measured in this study plot significantly above the curves and correspond to an increase in pH at 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 ($\delta^{11}B_{sw}$ = 39.61 ‰, B_T = 416 µM and pK_B corrected for temperature and salinity using Dickson, 1990). These increases in pH are in good agreement with the recent study of Venn et al. (2011) on tropical corals using live tissue imaging. They measured a pH increase from 0.2 to 0.5 pH units above ambient seawater under the calicoblastic

epithelium of *Stylophora pistillata* maintained at 25 °C. Using microsensors, Al-Moghrabi et al. (2001) and Al-Horani et al. (2003) measured a pH varying from 8.1 to 9.3 at 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 isotopes provide an average of several daily cycles (weeks). Furthermore, species-specific pH up-regulation 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 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; Chalker and Barnes, 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; Tambutté et al., 2011). Among others, several hypotheses involving modifications of carbonate chemistry inside the coelenterons have been suggested to be responsible for (part of) the LEC processes. Hence, symbionts have been suggested to affect the DIC equilibrium within coral tissues by (1) taking up CO_2 for photosynthesis (Goreau, 1959; McConnaughey and Whelan, 1997); or by (2) secreting OH^- as a by-product of the carbon concentrating mechanism (CCM) (Furla et al., 1998). Interestingly, both of these processes should lead to an increase in pH of the extracellular site of calcification with light enhancement. This is in contradiction with our result where a light enhancement of 200 µmol photons m^{-2} s^{-1} induces a decrease in boron isotopic signature, representing a decrease in pH at the site of calcification of about 0.03, 0.04 and 0.03 pH-units at 22, 25 and 28 °C, respectively (Table 4). So far, only few studies have reported on 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 at the site of calcification under light vs. dark conditions, independently of the analytical methods considered (e.g. microelectrodes, live tissue imaging). 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.

Recently, Krief et al. (2010) observed a decrease in calcification rate together with an increase in tissue biomass for tropical corals maintained under increased pCO_2 conditions. They argued that calcification stimulates zooxanthellae photosynthesis by enhancing CO_2 concentration within the coelenteron. Under high pCO_2 conditions, more CO_2 is available at the site of calcification, rendering less energy consuming the process concentrating Ca^{2+}, which allows the coral polyp to allocate more energy on tissue biomass. Similarly, Al Horani (2005) suggested 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. Increases in light intensities and consequently calcification rates should release more CO_2 at the site of calcification, and decrease internal pH with increasing light. However, although both these processes appear in line with our result where a decrease in pH at the site of calcification is related to increased calcification and photosynthesis rates, they rather seem to be consequences of the LEC processes rather than causes. At this stage, our results underline the urgent need of additional studies to better understand the LEC mechanisms in corals.

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 that 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 experiment 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. 4.2.4).

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 average annual light variations for tropical environment where *Acropora* sp. can be found (e.g. 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 photosynthetic activities do not significantly compromise $\delta^{11}B$-pH reconstructions for tropical corals. Due to analytical and sampling bias, paleo-pH reconstructions from corals are still broadly limited to a precision no better than ≈ 0.05 pH-units. Therefore, variations in light intensities on pH reconstructions for inter-annual resolution

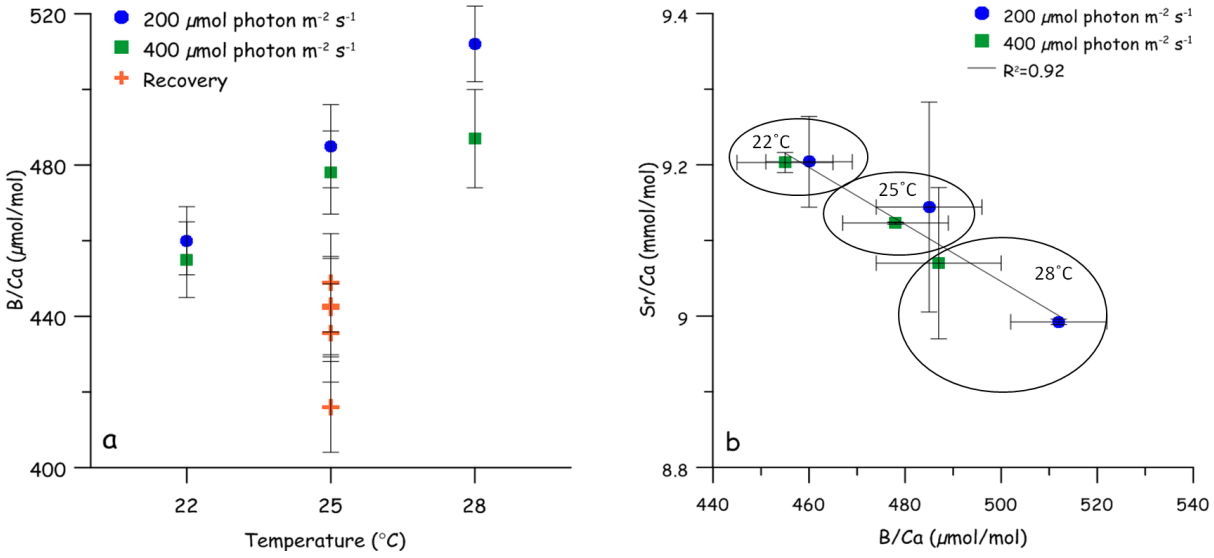

Fig. 5. (a) Mean B / Ca ratios (µmol mol^{-1}) measured at the end of step 2 on corals grown under 200 (blue dots) and 400 (green squares) photon m^{-2} s^{-1}, plotted vs. temperature. Measurements performed after step 3 (recovery experiment) are plotted on the same graph (orange crosses). **(b)**. Mean B / Ca ratios plotted vs. mean Sr / Ca ratios measured simultaneously (200 and 400 photon m^{-2} s^{-1} light conditions are represented by blue dots and green squares, respectively). Solid lines 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).

can be considered as negligible. Our results thus confirm the general applicability of the δ^{11}B-pH proxy as well as provide 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 5) and correspond to enhancements of 0.027 and 0.016 pH units at 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 4 and 5).

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 generated during calcification via Ca^{2+} ATPase activity was proposed to be responsible for the observed pH increase (and therewith increase in $[CO_3^{2-}]$) and Ca^{2+} concentration of coral site of calcification (Cohen and McConnaughey, 2003; McConnaughey and Whelan, 1997, Al-Horani et al., 2003). More recently, Herfort et al. (2008) reported that additions of $NaHCO_3$ to synthetic seawater proportionally increased the calcification rate of *Acropora* sp. This indicates 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. Carbonate system equilibria are such that 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 $[CO_3^{2-}]$. In turn, this could 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).

This appears in line with the linear increase in calcification rates with increasing temperature observed in our study. 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) at 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 when reconstructing pH further back in time where important SST changes have occurred.

This non-linear temperature effect complicates the determination of a potential correcting factor.

It should be noted here that, despite seeing in our study an increase in calcification rate under both increased temperature and increased light intensity, these conditions have the opposite effect on boron isotopic signatures. 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.

4.2.4 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 photons 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 compositions 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 2 (from 8.35 to 8.42), independent of the light and temperature conditions considered (Table 4, Figs. 3 and 4). These results indicate that the mechanical breakage applied at the end of step 2 induced 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 and δ^{11}B show comparable trends, although they were measured following different sample preparation and analytical techniques (ICP-QMS and MC-ICPMS, respectively). An increase in pH of the calcification site increases $B(OH)_4$ concentrations (Hershey et al., 1986; Hemming and Hanson, 1992). The boron concentration in the coral being proportional to the boron activity in the precipitating solution (Kitano et al., 1978; Vengosh et al., 1991; Hemming and Hanson, 1992), a decrease in pH at 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 are consistent with a decrease in pH at the site of calcification observed under enhanced light intensity.

4.3.2 Temperature effect

In our study, when all 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 and Finch, 2010; Trotter et al., 2011). To our knowledge, Trotter et al. (2011) is the only other study to report on the 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 setup 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 seawater might also impact coral biology and therewith boron 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 the impact of temperature difficult to unravel 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 $[CO_3^{2-}]$ (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, to date the influence of temperature on foraminifera B / Ca ratios is still subject to 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 is observed to increase with temperature in *Globorotalia inflata* (Yu et al., 2007), no temperature influence can be seen on *Neogloboquadrina pachyderma* (sinistral) (Hendry et al., 2009). Recently, culture experiments made by Allen et al., (2011) on the planktonic foraminifera *Orbulina universa*, did not indicate any temperature effect on B/Ca ratios (B/Ca values measured on shells grown between 17.7 and 26.5 °C agree within error with a slope statistically indistinguishable from zero). Considering the varying responses of foraminifera shell B/Ca ratios to temperature, Allen et al. (2011) recommend applying empirical, species-specific temperature calibrations for paleo-reconstructions.

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 been adequately identified yet. Additional species-specific experiments combining both cultured and naturally-grown 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 and Finch, 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 is presented elsewhere, see 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). 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 photons 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 4). 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. $\delta^{11}B$ values indicate higher pH at the site of calcification compared to ambient seawater pH, both under low light (LL) and high light (HL) conditions. Changes in light intensities from 200 to 400 µmol photon m^{-2} s^{-1} induces a decrease in pH at the site of calcification of about 0.03, 0.04 and 0.03 pH-units at 22, 25 and 28 °C, respectively. These light variations, chosen to mimic average annual variations in natural environments where *Acropora* sp. can be found, only biased pH reconstructions by about 0.05 units. Paleo-pH reconstructions from corals being still broadly limited to a precision no better than ≈ 0.05 pH-units, our observations support the idea that variations in light intensities on pH reconstructions for inter-annual resolution can be considered as negligible.

An increase of $\delta^{11}B$ 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 $\delta^{11}B$ increase occurred between 25 and 28 °C. This non-linear temperature effect complicates the determination of a correcting factor, underlining the need of additional culture experiments to better calibrate the impact of temperature on the pH- $\delta^{11}B$ proxy.

B/Ca ratios show a decrease with increasing light consistent with the decrease in pH at the site of calcification under enhanced light intensities observed with $\delta^{11}B$ 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 compositions 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 photons 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.

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