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Quantifying the pH 'vital effect' in the temperate zooxanthellate coral *Cladocora caespitosa*: Validation of the boron seawater pH proxy

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ABSTRACT

Boron isotopic and elemental systematics are used to define the vital effects for the temperate shallow water Mediterranean coral *Cladocora caespitosa*. The corals are from a range of seawater pH conditions (pH_T ~7.6 to ~8.1) and environmental settings: (1) naturally living colonies harvested from normal pH waters offshore Levanto, (2) colonies transplanted nearby a subsea volcanic vent system, and (3) corals cultured in aquaria exposed to high (700 μatm) and near present day (400 μatm) pCO_2 levels. B/Ca compositions measured using laser ablation inductively coupled mass spectrometry (LA-ICPMS) show that boron uptake by *C. caespitosa* cultured at different pCO_2 levels is independent of ambient seawater pH being mainly controlled by temperature-dependent calcification. In contrast, the boron isotope compositions ($\delta^{11}B_{carb}$) of the full suite of corals determined by positive thermal ionisation mass spectrometry (PTIMS) shows a clear trend of decreasing $\delta^{11}B_{carb}$ (from 26.7 to 22.2‰) with decreasing seawater pH, reflecting the strong pH dependence of the boron isotope system. The $\delta^{11}B_{carb}$ compositions together with measurements of ambient seawater parameters enable calibration of the boron pH proxy for *C. caespitosa*, by using a new approach that defines the relationship between ambient seawater pH (pH_{sw}) and the internally controlled pH at the site of calcification (pH_{biol}). *C. caespitosa* exhibits a linear relationship between pH_{sw} and the shift in pH due to physiological processes ($\Delta pH = pH_{biol} - pH_{sw}$) giving the regression $\Delta pH_{Clad} = 4.80 - 0.52 \times pH_{sw}$ for this species. We further apply this method ("ΔpH–pH_{sw}") to calibrate tropical species of *Porites*, *Acropora*, and *Stylophora* reported in the literature. The temperate and tropical species calibrations are all linearly correlated ($r^2 > 0.9$) and the biological fractionation component (ΔpH) between species varies within ~0.2 pH units. Our "ΔpH–pH_{sw}" approach provides a robust and accurate tool to reconstruct palaeoseawater pH_{sw} for both temperate and tropical corals, further validating the boron fractionation factor ($\alpha_{B3-B4} = 1.0272$) determined experimentally by Klochko et al. (2006) and the boron isotope pH proxy, both of which have been the foci of considerable debate.

1. Introduction

Rapidly rising levels of atmospheric CO_2 is not only causing global warming but, as a result of increased uptake of CO_2 into the ocean's surface waters, is also reducing seawater pH and carbonate ion

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concentrations (Caldeira and Wickett, 2003; Gattuso et al., 1999). This process, now commonly referred to as 'ocean acidification', has several important consequences. Firstly, the decreased efficacy of carbonate biomineralisation will have significant, although still poorly understood, ramifications for calcifying organisms and the broader trophic structure of the marine biosphere. In addition, lowering the carbonate saturation state is reducing the oceans' capacity to serve as major sinks for anthropogenic CO_2, which in turn will likely lead to even more rapidly increasing levels of atmospheric CO_2.

Whilst ocean acidification is a global-scale phenomenon, like greenhouse warming its effects are spatially and temporally variable. Thus a major uncertainty in predicting future outcomes is our lack of knowledge and understanding of how various ocean regions have already responded to the ~30% increase in atmospheric CO_2 over the last two centuries (Orr et al., 2001). Unlike temperature however, *in situ* records of changing carbonate chemistry are sparse and at most only span the last 3 decades (eg. HOTS, BATS, and ESTOC records).

One of the few means to overcome this limitation is the use of geochemical records of environmental change preserved within the carbonate skeletons of long-lived marine organisms. In particular, the boron isotope composition of marine biogenic carbonates ($\delta^{11}B_{carb}$) is a key quantitative proxy for determining long-term pre-instrumental records of ambient seawater pH. This is due to the essentially exclusive uptake of the borate ion ($B(OH)_4^-$) species from seawater during the secretion of biogenic carbonate in preference to boric acid ($B(OH)_3$), the relative proportions of these species in seawater being strongly pH dependent (Hemming and Hanson, 1992; Vengosh et al., 1991). Boron elemental abundance in marine carbonates should also theoretically be related to seawater pH but is confounded by a series of other factors such as temperature, salinity, the ratio of borate to carbonate in seawater, biological controls, and kinetic factors (Fallon et al., 1999; Hemming and Hanson, 1992; Montagna et al., 2007, 2009; Sinclair et al., 1998).

The $\delta^{11}B_{carb}$–pH relationship has been determined by culture experiments of foraminifers (Sanyal et al., 2001) and corals (Hönisch et al., 2004; Reynaud et al., 2004), which have also shown that significant physiological controls are imparted at the calcification site during biomineralisation. Such controls are attributed to the systematic offsets in measured $\delta^{11}B_{carb}$ from the theoretical borate curves, which are used to calculate the palaeoseawater pH from the $\delta^{11}B_{carb}$ compositions. Palaeoseawater pH reconstructions have mainly utilised foraminifers from ocean sediment cores, providing insights into natural changes in atmospheric CO_2 through the Quaternary glacial–interglacial cycle (e.g. Foster, 2008; Hönisch and Hemming, 2005; Hönisch et al., 2009; Sanyal et al., 1995, 1997). In the context of present-day ocean acidification driven by increasing anthropogenic CO_2 emissions, continuous records of seawater pH over decadal to centennial timescales have recently been determined from boron isotope analyses of massive long-lived shallow water *Porites* corals (e.g. Pelejero et al., 2005; Wei et al., 2009). Wei et al. (2009) have shown an apparent trend of decreasing $\delta^{11}B_{carb}$ thus seawater pH, from the 1950s that is coincident with the pronounced downward trend in the $\delta^{13}C$ record.

Here we investigate the boron systematics (B/Ca, $\delta^{11}B$) of the temperate coral *Cladocora caespitosa* as a potential archive of seawater pH. *C. caespitosa* is mainly found in the coastal shallow waters of the Mediterranean Sea and represents the main zooxanthellate coral in the region. Its growth rate is slow and individual corallites can reach lengths of many tens of centimeters with a temporal range of several hundred years, potentially providing continuous centennial-scale records of seawater pH. Recent studies (e.g. Montagna et al., 2007, 2009; Peirano et al., 2004; Silenzi et al., 2005) have identified *C. caespitosa* as a good candidate for climate and environmental reconstructions, with B/Ca, Sr/Ca, and Li/Mg ratios highly correlated to sea surface temperatures and thus ideal for palaeothermometry studies.

The Mediterranean is an ideal ocean basin to study the effects of anthropogenic CO_2 over the past few centuries. It is a semi-enclosed mid-latitude ocean basin that is well ventilated in most parts, hence likely to be in close equilibrium with current rapidly increasing levels of atmospheric CO_2. Its temperature structure (8 to 28 °C) is also well known and so the decreased solubility of CO_2 with increasing ocean temperatures is relatively well-constrained. Importantly, the Mediterranean also has high total alkalinity (~2.6 mmol kg^{-1}) (Schneider et al., 2007) and an unusually high carbonate saturation state, and therefore a high chemical potential to sequester CO_2 resulting in relatively larger decreases in seawater pH. However, recent aquaria experiments with elevated pCO_2 suggest that temperate water corals, including *C. caespitosa*, may be relatively insensitive to decreasing seawater pH (Ries et al., 2009, 2010; Rodolfo-Metalpa et al., 2010b). This is unexpected given that temperate corals generally exist close to their natural tolerance limit, with calcification being strongly influenced by temperature fluctuations (Rodolfo-Metalpa et al., 2008). Collectively these factors identify the Mediterranean as a key region where the competing interactions of declining seawater pH and increasing temperature, and the resultant impacts on calcifying organisms, will quickly become apparent (CIESM, 2008a,b).

2. Materials and methods

C. caespitosa was targeted for analysis given its longevity and widespread occurrence throughout the shallow temperate waters of the Mediterranean. It is a colonial species characterised by discrete corallites that synchronously grow as a thicket forming stable hemispherical mounds and large banks, below the compensation depth of photophilic algae or in turbid waters (Kühlmann, 1996; Morri et al., 1994). The relatively slow skeletal growth rates, which range from 1.3 mm yr^{-1} (Peirano et al., 1999) to 6.2 mm yr^{-1} (Kružiæ and Požar-Domac, 2003), produce long-lived corallites suitable for characterising climate change over centennial timescales, incorporating both anthropogenic and natural variability.

2.1. Coral collection and culturing

Samples of *C. caespitosa* were sourced from colonies growing under different conditions: i) in their natural environment (Levanto) under normal seawater conditions and pH, ii) in normal and naturally occurring low pH waters near subsea CO_2 vent systems (Ischia), and iii) in controlled aquaria (Villefranche-sur-Mer) at normal and altered pCO_2 levels. The corals were sourced from experiments and sampling trips previously undertaken at the following three sites (Fig. 1):

(1) Levanto (Ligurian Sea, Italy): two colonies were sub-sampled in October 2005 by SCUBA at depths of 18 and 27 m (Silenzi et al., 2005), within normal pH waters off the coast near Levanto (Ligurian Sea) and far from fluvial discharge. Seawater samples were collected at each depth adjacent to the coral heads, however this was undertaken more recently in March 2010 during cooler winter conditions (see Section 2.2).

(2) Island of Ischia (Tyrrhenian Sea, Italy): colonies were grown in both normal and low seawater pH near the subsea volcanic vent system south of Castello d'Aragonese off the island of Ischia, where a natural pH_T gradient ranges from 8.2 to 6.6 (Hall-Spencer et al., 2008). Colonies were removed from normal pH waters at this site in March 2008, stained with Alizarin Red S (~36 h) in aquaria to differentiate subsequent growth, then transferred to 2 stations for a period of 7 months: proximal to the vents (ISCH_S2) in low pH_T (~7.6) waters at ~3 m depth; and ~150 m from the vents (ISCH_Cont) in normal seawater pH at ~3 m depth to serve as experimental controls. Notably, these vents lack the toxic sulphur compounds that characterise the majority of marine vents, and are at mean

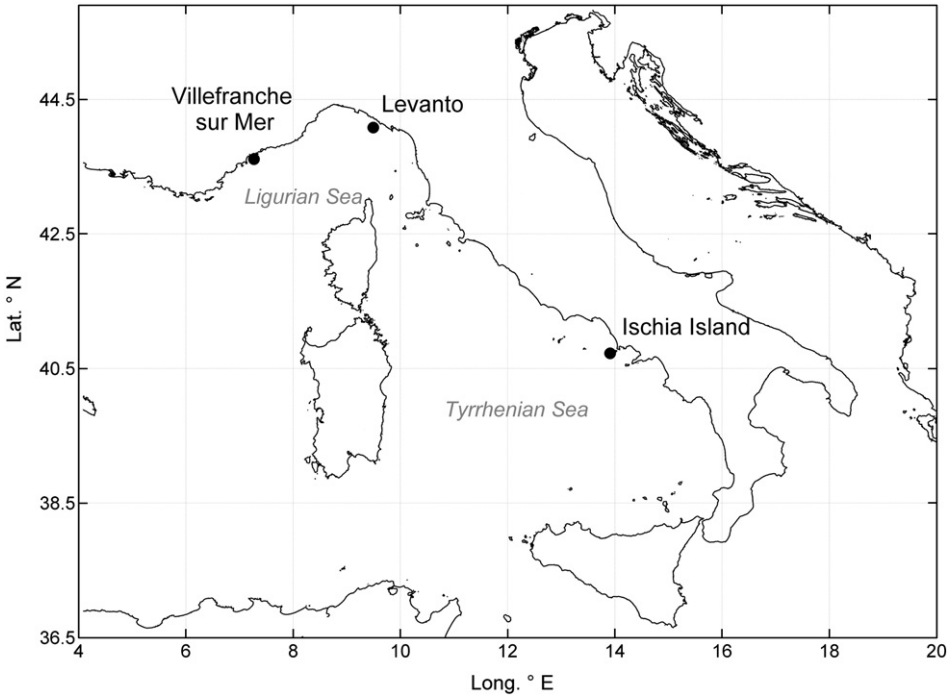

Fig. 1. Locality map of sites from where *C. caespitosa* colonies were sampled and analysed for this study.

Mediterranean salinity (38) with the same total alkalinity (2.5 mmol kg^{-1}) and seasonal temperature fluctuations (13 to 28 °C). Details of a similar transplantation experiment are reported in Rodolfo-Metalpa et al. (2010a).

(3) Bay of Villefranche (Ligurian Sea, France): two colonies were collected in July 2006 from the Bay of Villefranche at ~25 m depth, maintained in aquaria, stained with Alizarin Red S (September 2006), then grown for 10 months under controlled pCO_2 conditions. Two independent aquaria were set at constant pCO_2 levels of 400 µatm and 700 µatm by bubbling CO_2-free air and CO_2-enriched air in two header tanks continuously supplied by seawater from the Bay of Villefranche. Temperature, artificial light intensity and exposure simulated the natural seasonal fluctuations of the source waters. Sample collection and experimental setup are detailed in Rodolfo-Metalpa et al. (2010b).

2.2. Seawater measurements

Seawater temperatures, salinity, pH and carbonate parameters were measured and calculated for each of the study sites (Table 1) to enable calibration of the boron isotope pH proxy. Seawater temperatures were measured *in situ* using electronic temperature controllers of Corema, Camarsac, France (±0.1 °C) in the aquaria at Villefranche-sur-Mer, Hobo Onset loggers (±0.35 °C) at the Ischia stations, and a Pt 100 Delta-OHM probe (±0.25 °C) at the Levanto site. During the experimental periods seawater temperatures ranged between 13.3 and 22 °C at Villefranche (aquaria) and 14.8 to 26 °C at Ischia (vent site). The annual temperature range offshore Levanto during 2005 is between 12 and 24 °C at 18 m, and 12 to 23 °C at 27 m (Si.Di.Mar. data base, 2010: www.sidimar.tutelamare.it; Italian Ministry of Environment, Land and Sea), however the subsampled skeleton for boron isotope analysis (the low density band) was secreted during the summer months (April to October). Salinity values were sourced from relevant databases (Si.Di.Mar. data base, 2010: www.sidimar.tutelamare.it) or measured using an YSI 556 MPS multiparametric probe and taken as 38 for all sites which is typical for Mediterranean seawater.

Seawater pH and total alkalinity were measured according to Dickson et al. (2007). Water was collected in glass bottles adjacent to the corals and the pH measured immediately using a meter accurate to 0.01 pH units (Metrohm 826 pH mobile) calibrated using TRIS/HCl and 2-aminopyridine/HCl buffer solutions (Dickson et al., 2007). The samples were then filtered using GF/F Whatman (0.45 µm) membranes, poisoned with 0.05 ml of 50% $HgCl_2$ to avoid biological alteration, and stored in the dark at 4 °C pending analysis. Total alkalinity was measured as 3 replicate 20 ml sub-samples at 25 °C using a potentiometric titration system. Parameters of the carbonate system (pCO_2, CO_3^{2-}, HCO_3^-, and aragonite saturation state (Ω_{arag})) for the Ischia and Levanto samples were calculated from pH, TA, temperature, and salinity using CO2SYS (Lewis and Wallace, 1998) software, whereas the *Seacarb* R package (Lavigne and Gattuso, 2010) was used for the Villefranche aquaria samples. For further details of the aquaria methods see Martin and Gattuso (2009), and Rodolfo-Metalpa et al. (2010b) and for the CO_2 vent transplant experiment see Rodolfo-Metalpa et al. (2010a).

Seawater pH measurements for our sites are calibrated to the total scale (pH_T). Note that ambient pH of the Levanto seawater samples was determined by back-calculating to the mean temperature measurements of 20.8 °C (18 m) and 19.5 °C (27 m) that are relevant for the period of skeletal growth (April to October 2005), which is represented by the boron isotope compositions. Seasonal variations in seawater pH_T (at 25 °C) for the upper 30 m in the Ligurian Sea are reported to range between 7.95 and 8.11 (DYFAMED CNRS-INSU, Observatoire Océanologique de Villefranche-sur-Mer). However it is particularly important to note the very large range in seawater pH_T (~7 to 8) measured from March to September 2008 (n = 22 per site) at the subsea CO_2 vent site (S2) near Ischia.

2.3. B/Ca elemental analysis

B/Ca compositions were determined for two corallite pairs from each of the pCO_2 experiments (400 and 700 µatm) conducted at Villefranche-sur-Mer. Importantly, additional key parameters (temperature, seawater pH, total alkalinity, and calcification rate) were measured which enabled direct comparison of the ambient

Table 1
Mean ambient seawater temperatures and pH (total scale) measured for each of the study sites, and carbonate chemistry parameters calculated using *Seacarb* R package for the Aquaria samples and CO2SYS for the Ischia and Levanto samples. Carbonate parameter calculations are based on measured average total alkalinity, salinity (38), and pH_T values. Standard deviations (1SD) from the means are shown in parentheses.

Sample	Temp.°C	TA ($\mu mol\ kg^{-1}$)	pH_T	pCO_2 (μatm)	CO_2 ($\mu mol\ kg^{-1}$)	CO_3^{2-} ($\mu mol\ kg^{-1}$)	HCO_3^- ($\mu mol\ kg^{-1}$)	DIC ($\mu mol\ kg^{-1}$)	Ωarag
Aquaria seawater									
400 µatm	16.4	2513	8.09	390	13.8	219	1988	2221	3.31
	(2.6)	(29)	(0.02)	(24)	(1.1)	(17)	(48)	(34)	(0.28)
700 µatm	16.3	2514	7.87	701	24.8	145	2168	2339	2.19
	(2.6)	(28)	(0.02)	(39)	(2.5)	(15)	(44)	(33)	(0.24)
Ischia vent seawater									
Cont	20.6	2575	8.07	423	13.5	246	1980	2240	3.73
	(4.1)	(2)	(0.07)	(92)	(3.0)	(39)	(94)	(57)	(0.61)
S2	20.5	2575	7.62	1660	55.3	110	2309	2475	1.68
	(4.1)	(2)	(0.27)	(1169)	(40.4)	(53)	(125)	(109)	(0.81)
Levanto seawater									
18 m	13.3[a]	2551	8.15[a]	338	13.4	217	2021	2251	3.24
	20.76[b]		8.10[b]						
27 m	12.6[a]	2548	8.15[a]	339	13.1	221	2008	2243	3.32
	19.48[b]		8.10[b]						

[a] pH_T and measured temperatures for ambient seawater collected in March 2010.
[b] pH_T determined from back-calculations for mean measured summer temperatures (April–October 2005).

environmental conditions and B/Ca analyses. The corallites were soaked in 30% H_2O_2 for two days at room temperature in order to remove residual organic matter then dried in an oven at 50 °C for 8 h. The corallites were embedded in epoxy resin and longitudinally sectioned along the columella (the internal structure formed by the fusion of the septa) using a water-cooled saw. Two half-sections of the corallites for each experimental setup were cleaned with double-distilled water and methanol, then examined under a stereoscope to check the presence of any contaminant residues. The analyses targeted the outer thecal wall (septotheca) precipitated prior to and during the life of the experiment (September 2006 to July 2007), which was clearly differentiated by the Alizarin Red S marker. Due to the significantly higher concentration of lead in the Alizarin Red S stain compared to the coral skeleton, ^{208}Pb was analysed to provide precise chronological control, with the exact position of aragonite precipitation from the onset of the experiment determined by the large (six-fold) increase in ^{208}Pb concentrations.

2.3.1. Laser ablation ICP-MS

Concentrations of ^{11}B, ^{43}Ca and ^{208}Pb were determined using a 193 nm ArF excimer laser ablation system coupled to a Varian 820 inductively coupled plasma mass spectrometer at the Research School of Earth Sciences, The Australian National University. Surface contamination was removed with two pre-ablation scans prior to analysis using a 230 µm diameter spot. During analysis the laser was pulsed at 5 Hz and energy of 50 mJ in the form of a rectangular slit 200 µm long and 20 µm wide, with the short side parallel to the growth axis. The thecal wall was scanned continuously at 20 $\mu m\ s^{-1}$ from the youngest part of the coral calyx across the Alizarin Red S marker into the pre-experiment portion. The NIST 614 glass standard, an in-house *Porites* pressed-pellet standard, and the background system blank were analysed for 60 s before and after each sample run in order to calibrate the trace element profiles and correct for any long-term machine drift. This analytical protocol is similar to that reported in Montagna et al. (2007). The overall precision for B/Ca based on the uncertainties from compositional heterogeneity of the *Porites* standard and counting statistics is 3.8% (1σ standard deviation).

2.4. Boron isotope analysis

The uppermost skeleton just below the tissue zone was subsampled from corallites collected offshore Levanto (LEV_18m and LEV_27m). These subsamples are representative of the summer period (April to October) according to growth rates determined at this site (Silenzi et al., 2005), when the low density band is fully developed. Corallites from the aquaria (400 and 700 µatm) and Ischia CO_2 vent sites (ISCH_S2 and ISCH_Cont) were sampled above the Alizarin Red S marker, hence within the uppermost skeletal layers protected by the tissue zone. Only very small fractions could be subsampled from the Ischia corals as new skeletal growth was very limited (~1 to 2 mm) due to the short experimental timeframe (7 months). Analyses were restricted to the outer thecal wall (septotheca) in order to avoid potential contamination phases within the columella.

2.4.1. Chemical separation

Approximately 15 to 20 mg of carbonate powder was extracted from the corallites using a fine dental drill. Sample preparation followed that outlined by Wei et al. (2009), a variant of the di-cesium metaborate ($Cs_2BO_2^+$) technique that was recently optimised by Lemarchand et al. (2002) in order to analyse small volume samples. Organic matter from the coral samples was removed by pre-treating in 30% H_2O_2 at room temperature for several days. Calcium, a major constituent of corals, was removed by cation exchange chromatography using AG50W-X8 resin. The procedure of Wei et al. (2009) was modified to incorporate a final cation AG50W-X8 'clean up' process immediately following the 'double column' boron separation by anion Amberlite IRA 743 chromatography. This process seemed to reduce and sometimes eliminate the isobaric interferences on Cs_2BO_2 that was typically observed during ionisation mass spectrometry in the earlier work of Wei et al. (2009). CsCl and mannitol were added to the boron eluent then evaporated slowly at low temperature (<60 °C) under infrared light. Aliquots of each sample (~200 ng B) was loaded onto degassed single tantalum filaments in a graphite suspension following Xiao et al. (1988), and heated slowly to dryness under a ceramic heat lamp. A modern *Porites* coral from New Ireland, Papua New Guinea, was used as an in-house secondary standard (NEP) to monitor reproducibility of the complete wet chemistry and analytical procedure. The SRM 951 standard processed through the ion exchange protocol showed negligible blank contribution. All samples were processed and analysed in a number of sessions over a period of ~14 months.

2.4.2. Positive thermal ionisation mass spectrometry

Boron isotope compositions of the high mass borate (Cs_2BO_2) complexes were determined by thermal ionisation mass spectrometry

using a Finnigan TRITON housed at the Research School of Earth Sciences at the Australian National University. Where sample volume is not limiting (i.e. ~15 to 20 mg), the di-cesium metaborate ($Cs_2BO_2^+$) method has the significant advantage of utilising positive polarity thermal ionisation mass spectrometry (PTIMS) at high masses (308 and 309), which is considerably less affected by fractionation during ionisation than low mass (42 and 43) BO_2^- molecules analysed by negative thermal ionisation mass spectrometry (NTIMS). Since the study of Wei et al. (2009), the instrument has been retrofitted with a double Faraday collector array with fixed spacing enabling more precise measurements using static multi-collector mode of Cs_2BO_2. Simultaneous measurement of masses 308 and 309 is otherwise not possible using the conventional Faraday collection system in this earlier generation instrument due to the small relative mass difference.

The Faraday cups were configured to measure mass 285 ($^{133}Cs^{133}Cs^{19}F$) in L1, 301 ($^{133}Cs^{133}Cs^{35}Cl$) in H1, 303 ($^{133}Cs^{133}Cs^{37}Cl$) in H2, and masses 308 ($^{133}Cs^{133}Cs^{10}B^{16}O^{16}O$) and 309 ($^{133}Cs^{133}Cs^{11}B^{16}O^{16}O + ^{133}Cs^{133}Cs^{10}B^{16}O^{17}O$) respectively in the H3 and H4 double cup array, with the centre cup set at 295 (which cannot exceed 300 amu). Data was acquired in 1 to 2 blocks of 100 cycles when the intensity of 309 reached ~0.5 V. Cs_2Cl was used to monitor isobaric interferences (Cs_2CNO) from remnant organic matter that occasionally appeared on Cs_2BO_2 (mass 308) thereby lowering the 309/308 ratios during the initial stage of ion emission (Wei et al., 2009), which were subsequently vetted from the effected datasets.

An outlier correction was applied at 3-sigma limits to all ratios collected during analysis. The internal precision (2 standard errors of the mean) of the $\delta^{11}B$ measurements of the unknown samples range between 0.02 and 0.05‰, consistent with direct loads of the SRM 951 primary standard (0.05‰, n = 30) and slightly better than our NEP in-house coral secondary standard (0.08‰, n = 14). Our running mean of the SRM 951 is 0.40527 with an external precision of 0.27‰ at 95% confidence limits (i.e. 2 SD). The precision of the NEP coral relative to the SRM mean standard value is 0.31‰ (95% c.l.), which reflects the complete procedural reproducibility over the course of the analytical period. An oxygen isotope correction for ^{17}O (Spivack and Edmond, 1986) was applied to the measured 309/308 ratios of the coral unknowns (Eq. 1). Boron isotope ($^{11}B/^{10}B$) ratios of the unknowns were normalised to our SRM 951 mean value and are expressed in permil notation ($\delta^{11}B$) relative to the standard (Eq. 2):

$$^{11}B/^{10}B_{sample} = 309_{measured} / 308_{measured} - 0.00078 \quad (1)$$

$$\delta^{11}B = \left[\left(^{11}B/^{10}B\right)_{sample} \Big/ \left(^{11}B/^{10}B\right)_{standard} - 1 \right] \times 1000. \quad (2)$$

3. Results

3.1. B/Ca systematics and extension rates of C. caespitosa

B/Ca profiles of the four corallites grown at 400 µatm and 700 µatm CO_2 levels in the aquaria at Villefranche-sur-Mer, together with the measured temperatures of the ambient source waters and the borate/carbonate and borate/bicarbonate speciation are shown in Fig. 2. The B/Ca compositions of the two discrete corallites from each experiment are essentially identical so were combined to generate a single B/Ca profile for each pCO_2 treatment.

The profiles for both experiments display very similar trends and ranges in B/Ca values, with an average of 0.84 mmol mol^{-1} (±0.08 1 SD) for those grown at 400 µatm pCO_2 and 0.86 mmol mol^{-1} (±0.08 1 SD) for corals exposed to 700 µatm pCO_2. The B/Ca profiles generally follow the culturing temperature and the borate/carbonate ratios, notwithstanding some fine-scale discrepancies, the most significant being at the actively calcifying surface within the tissue zone. Both B/Ca profiles are inversely correlated with temperature and positively correlated with borate/carbonate ratios but are independent of ambient seawater pH and borate/bicarbonate ratios (see Section 4.4 for discussion). The inverse relationship between B/Ca ratios and temperature corroborates earlier findings (Montagna et al., 2007) that this elemental ratio in *C. caespitosa* is mainly controlled by temperature. Furthermore, the corallites from both 400 µatm and 700 µatm CO_2 experiments also show comparable linear extension, with averages of 4.2 ± 0.6 mm (1 SD) and 4.5 ± 0.4 mm (1 SD) respectively, indicating that decreasing ambient seawater pH, hence aragonite saturation state, is not controlling or limiting skeletal growth in this slow growing species (see also Rodolfo-Metalpa et al., 2010b).

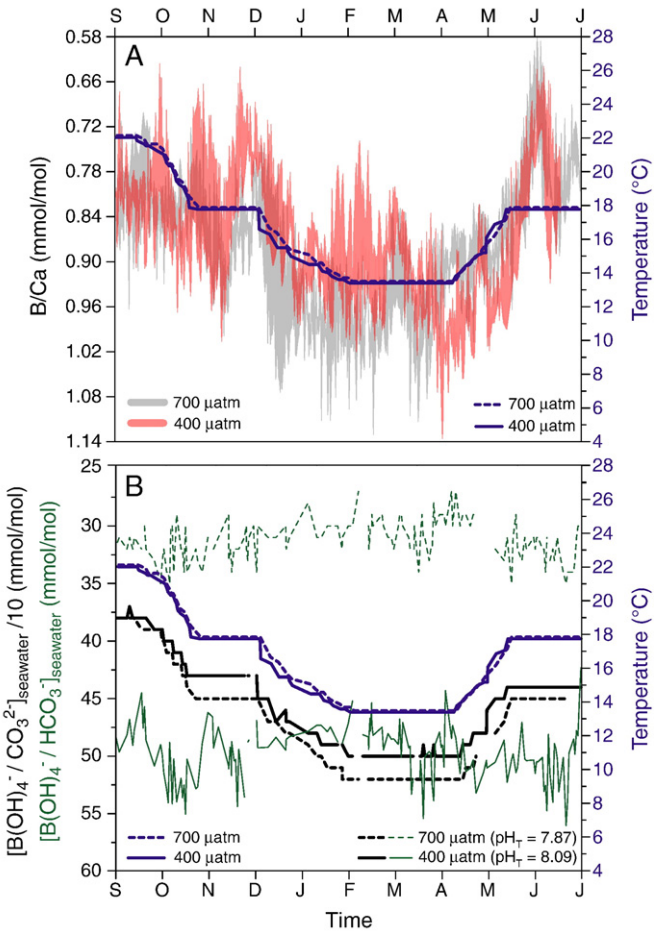

Fig. 2. Relationship between culture temperature, seawater borate/bicarbonate and borate/carbonate ratios and B/Ca composition of *C. caespitosa* grown in aquaria at normal (400 µatm; $pH_{sw} = 7.87$) and elevated (700 µatm; $pH_{sw} = 8.09$) pCO_2. B/Ca profiles of both pCO_2 experiments are essentially identical and track: (A) the measured seasonal temperature fluctuations (blue lines), and (B) the calculated borate/carbonate ratios (black lines) of ambient seawater. The correlation of B/Ca and temperature is a result of the strong temperature dependence of seawater borate/carbonate ratio. The lack of correlation between coral B/Ca and ambient pH_{sw} or borate/bicarbonate ratio (green lines), as commonly observed in foraminifer (e.g. Yu and Elderfield, 2007), is ascribed to a different uptake mechanism for borate in aragonitic corals compared to calcitic foraminifers (see text). Borate ion concentration of the tank seawater was calculated using the measured temperature, salinity, and pH, according to equation: $[B(OH)_4^-] = B_T/(1 + [H^+]/K_B)$, with B_T being the total boron concentration [416 × (salinity/35) µmol kg^{-1}; DOE, 1994] and K_B the dissociation constant of boric acid. The carbonate and bicarbonate ion concentrations were calculated from pH, TA, temperature, and salinity using the *Seacarb* R package (Lavigne and Gattuso, 2010). Note that heights of the profile bands represent the overall maximum and minimum values of a given datum measured from each corallite pair.

Table 2
Boron isotope compositions of *C. caespitosa* expressed as $^{11}B/^{10}B$ ratios (309/308) and delta values ($\delta^{11}B$) normalised to SRM 951, and ambient seawater pH calculations (pH_T) at the pH total scale. External precision for $\delta^{11}B$ measurements is 0.31‰ (2 SD) based on our in-house coral *Porites* standard (NEP).

Sample	Date analysed	309/308	$\delta^{11}B$	2 sem	pH_T	1 SD
Levanto corals						
LEV_18A (18 m)	19-Mar-10	4.1582	26.0	0.03	8.10	n/a
LEV_18B (18 m)	19-Mar-10	4.1557	25.4	0.03		
LEV_27A (27 m)	22-Mar-10	4.1611	26.7	0.03	8.10	n/a
LEV_27B (27 m)	22-Mar-10	4.1582	26.0	0.04		
Ischia vent corals						
ISCH_Cont	12-Nov-09	4.1578	25.9	0.03	8.07	0.07
ISCH_S2	12-Nov-09	4.1426	22.2	0.05	7.62	0.27
Aquaria corals						
CLA_400	20-Feb-09	4.1546	25.1	0.04	8.09	0.02
CLA_400	31-Aug-09	4.1556	25.4	0.04		
CLA_700	20-Feb-09	4.1511	24.3	0.02	7.87	0.02
CLA_700	31-Aug-09	4.1508	24.2	0.05		

3.2. Seawater pH and $\delta^{11}B$ compositions of *C. caespitosa*

The relationship between ambient seawater pH and the boron isotopic compositions of *C. caespitosa* ($\delta^{11}B_{carb}$), which cover a wide range of pH_T regimes (7.6 to 8.1), is shown in Table 2 and Fig. 3A. There is a clear trend of decreasing $\delta^{11}B$ in the coral skeletons with decreasing seawater pH, reflecting the strong pH dependence of the boron isotope system. $\delta^{11}B_{carb}$ compositions range from 26.7 to 22.2‰, with respective ambient seawater pH_T at our upper range of 8.1[1] and lowermost value of 7.6.

There is however some isotopic variability, in particular the Levanto corals that grew under natural conditions in normal pH waters. The measured $\delta^{11}B$ of 2 discrete corallites taken from each of the 2 colonies living at 18 and 27 m depths, shows intra-pair variability of 0.6 and 0.7‰ respectively, which is well outside the analytical error. Clearly this variability cannot be attributed to depth effects and could possibly represent compositional differences within contemporaneously precipitating skeleton. However, it is most likely that the intra-pair subsamples are not in fact temporally equivalent with the differences reflecting seasonal fluctuations in seawater pH. It suggests that a more rigorous and consistent subsampling protocol at annual resolution incorporating full seasonal cycles is necessary to minimise these potential variables. The overall range in $\delta^{11}B_{carb}$ composition is nonetheless broadly consistent with the other corals sampled from normal pH waters: (1) in aquaria with seawater maintained at pH_T of 8.09 and 400 µatm pCO_2 (CLA_400), and (2) at the reference station of the Ischia vent experiment with pH_T at 8.07 (ISCH_Cont).

The aquaria experiments simulated low and high pCO_2 scenarios (400 and 700 µatm), resulting in large differences in seawater pH that have clearly been recorded as distinct differences in the $\delta^{11}B$ compositions of the coral skeletons. Corals exposed to enhanced pCO_2 (700 µatm) hence lower pH_T (7.87) yielded measurably lower $\delta^{11}B$ compositions (24.3‰, n=2) than those grown under normal pCO_2 (400 µatm) and pH_T (8.09) conditions ($\delta^{11}B$=25.3‰, n=2). The replicate analyses were conducted 6 months apart and show good reproducibility, 0.1 and 0.3‰ respectively for the high and normal pCO_2 simulated conditions. It is noted that SIMS analyses (Reynaud et al., 2008) of equivalent subsamples extracted from the same coral nubbins could not resolve differences in isotopic compositions and yielded significantly higher $\delta^{11}B_{carb}$ measurements of 30.05‰ (±1.51 at 1 SD; 400 µatm CO_2) and 29.33‰ (±1.28 1 SD; 700 µatm CO_2) with large analytical errors (Fig. 3B). The cause of the aberrant SIMS data is unclear but may be related to standard normalisation or other analytical artefacts.

Our Ischia corals cover an even greater range in boron isotopic compositions and seawater pH. The nubbins subsampled from the parent colony and transferred to naturally low pH (pH_T=7.62) waters nearby the subsea volcanic vent off the island of Ischia, yielded the lowest measured $\delta^{11}B_{carb}$ value (22.2‰) of all of the *Cladocora* samples. This is 3.7‰ lower than the $\delta^{11}B$ composition (25.91‰) of the control sample that was taken from the same parent colony and relocated to the vent system where seawater pH is normal (pH_T=8.07).

4. Discussion

To effectively apply the boron isotope pH proxy it is critical to quantify the physiologically controlled 'vital effects' superimposed on the external (i.e. seawater) pH recorded during calcification, and calibrate the $\delta^{11}B$ compositions using ambient seawater pH measurements. These parameters, together with taxon-specific $\delta^{11}B$ versus pH calibrations for the temperate coral species (*C. caespitosa*) and a suite of tropical shallow water species reported in the literature, are defined below. We also discuss the implications of our combined boron elemental-isotopic dataset, which provide important insights into the environmental controls of coral calcification and the inherent capacity for *C. caespitosa* to adjust to changing seawater pH.

4.1. $\delta^{11}B$–pH calibration curves for *C. caespitosa*

The measurements of ambient seawater pH provide a means to compare the boron isotope data to both theoretical and experimental borate reference curves, and evaluate the relationship between the $\delta^{11}B$ compositions of *C. caespitosa* and changing seawater pH. If it is assumed that the $\delta^{11}B$ in carbonates is representative of the $B(OH)_4^-$ component in seawater, pH values can be calculated from the measured $\delta^{11}B_{carb}$ composition using the following equation (e.g. Zeebe and Wolf-Gladow, 2001):

$$\mathrm{pH} = pK_B - \log\left\{\left[\delta^{11}B_{sw} - \delta^{11}B_{carb}\right] / \left[\alpha_{B3-B4} \times \delta^{11}B_{carb} - \delta^{11}B_{sw} + 1000(\alpha_{B3-B4} - 1)\right]\right\} \quad (3)$$

where: $\delta^{11}B_{SW}$ and $\delta^{11}B_{carb}$ represent the $\delta^{11}B$ in seawater ($\delta^{11}B_{sw}$=39.5 from Foster 2008; Spivack and Edmond 1986) and in carbonate respectively, with pK_B being the dissociation constant of boric acid with a well defined value of 8.597 (Dickson, 1990), and $\alpha_{(B3-B4)}$ represents the fractionation factor for isotope exchange between $B(OH)_3$ and $B(OH)_4^-$ in seawater that is given by:

$$^{10}B(OH)_3 + {}^{11}B(OH)_4^- = {}^{11}B(OH)_3 + {}^{10}B(OH)_4^- \quad (4)$$

and:

$$\alpha_{B3-B4} = \left[{}^{11}B(OH)_3\right] \times \left[{}^{10}B(OH)_4^-\right] / \left[{}^{10}B(OH)_3\right] \times \left[{}^{11}B(OH)_4^-\right] \quad (5)$$

There are however several possible $\alpha_{(B3-B4)}$ values. The theoretically derived value of 1.0194 calculated at 25 °C (reported by Kakihana et al., 1977) is typically used in palaeoseawater pH calculations (e.g. Hönisch et al., 2004; Pelejero et al., 2005; Sanyal et al., 1996, 2001). However, depending on the choice of methods and parameters employed, the theoretical values could potentially range between ~1.020 and ~1.050 (Zeebe, 2005). Improved theoretical calculations have indicated a consistently higher range of $\alpha_{(B3-B4)}$ values, from 1.0260 to 1.0267 (Liu and Tossell, 2005; Oi and Yanase, 2001). A slightly higher value of 1.0272 (Klochko et al., 2006) determined directly by chemical equilibria experiments of artificial seawater has recently been considered to be

[1] Measured pH_T=8.15 at temperatures of 13.3 °C (18 m) and 12.6 °C (27 m). pH_T=8.1 was determined from back-calculations for summer temperatures 20.8 °C and 19.5 °C (see Section 2.2).

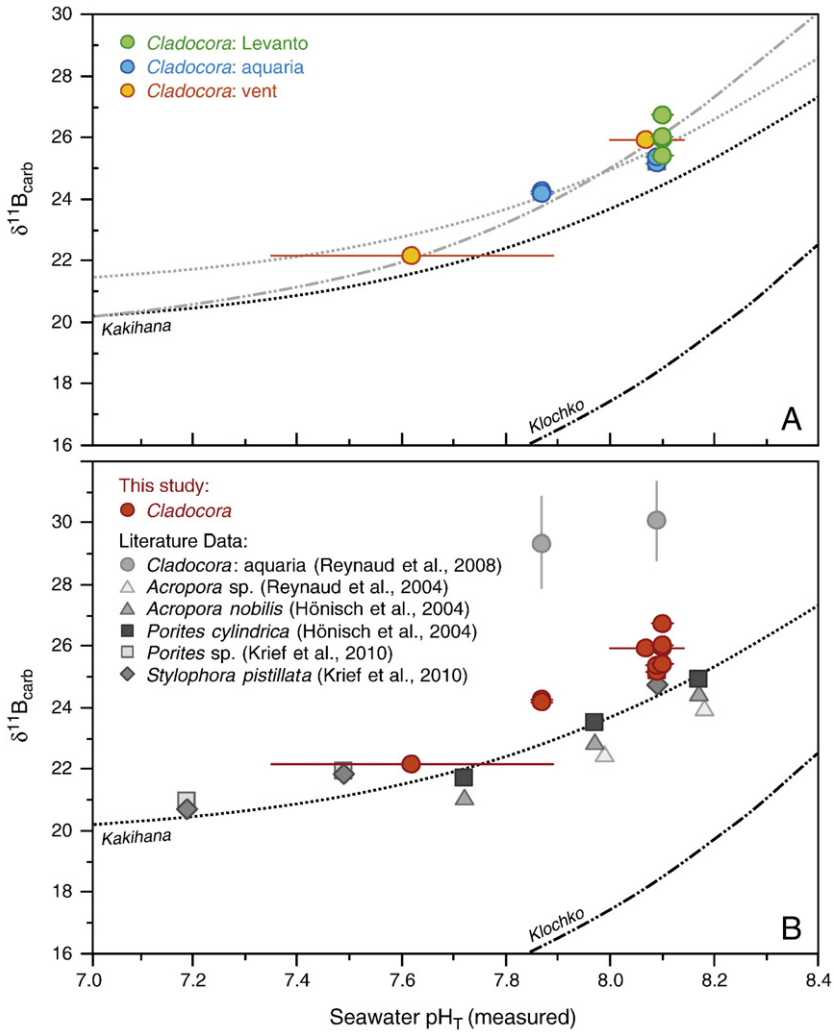

Fig. 3. Measured $\delta^{11}B_{carb}$ compositions relative to ambient seawater pH_T and borate reference curves. (A) *C. caespitosa* (this study) are positively offset from the Kakihana et al. (1977) and Klochko et al. (2006) calibration curves by ~1‰ and ~7.5‰ respectively. Respective 'best fit' offsets are shown by grey dotted and dashed lines; the vent samples are more closely aligned to the slope of the Klochko curve, whereas samples from higher seawater pH_T are nearest to the Kakihana curve. (B) Comparison of $\delta^{11}B$-pH_T for *Cladocora* and a suite of tropical shallow water corals from the literature. Note large differences between *Cladocora* PTIMS data from this study and ion microprobe SIMS analyses (grey circles: Reynaud et al., 2008) of subsamples from the same coral host grown in CO_2 controlled aquaria. Error bars at ±1 SD.

most applicable for $\delta^{11}B$–pH calibrations of foraminiferal calcite (Foster, 2008). The use of an appropriate $\alpha_{(B3-B4)}$ in calculating seawater pH from coral $\delta^{11}B$ is important as it effects not only the absolute calculated pH values but also has a second-order effect on the differential changes in pH (Wei et al., 2009).

The measured $\delta^{11}B$ of *C. caespitosa* and measured ambient seawater pH are plotted relative to the borate curves (Fig. 3) of Kakihana et al. (1977) and Klochko et al. (2006), the latter defined by a steeper gradient and markedly lower $\delta^{11}B$. Offsets in measured $\delta^{11}B_{carb}$ from these curves are typically interpreted as fractionation effects due to physiological processes (vital effects) imparted during precipitation of the carbonate skeleton, as commonly observed in different species of corals and foraminifers (e.g. Foster, 2008; Hönisch et al., 2004, 2007; Reynaud et al., 2004; Sanyal et al., 2001). However, applying this conventional approach to the *Cladocora* data gives inconsistent results; the relationship of the $\delta^{11}B_{carb}$ compositions to each of these curves, and by implication seawater pH, differ according to their ambient environment: the slope defined by the Ischia samples matches that of the steeper Klochko curve but with large positive offsets in $\delta^{11}B$ (~7.5‰), however when excluding the sample near the vent our overall dataset is closest to the Kakihana borate curve with only a small positive offset (~1‰).

It is clear that corals adjust their internal pH during calcification (Al-Horani et al., 2003), and it is necessary to consider that the effects of these biological processes on the partitioning of $B(OH)_3$ and $B(OH)_4^-$ within the secreted skeleton may vary between species and environmental regimes. However the specific physiological mechanisms controlling coral calcification and their influence on skeletal composition are poorly understood, although various models have been proposed (Adkins et al., 2003; Cohen and McConnaughey, 2003; Houlbrèque et al., 2009; Meibom et al., 2008; Sinclair, 2005). Difficulties in reconciling some geochemical data with these models however have led researchers to question the validity of the boron isotope pH proxy (Blamart et al., 2007; Rollion-Bard et al. 2010). Accordingly, biological controls imparted during biomineralisation and their effects on the $\delta^{11}B$ composition of coral skeletons need to be constrained in order to apply the boron isotope pH proxy.

Recent studies have attempted to discriminate the biological component of the calculated seawater pH from the boron isotope compositions of corals (Krief et al., 2010) and foraminifers (Rollion-Bard and Erez, 2010). This was achieved for the scleractinian corals (*Porites* sp. and *Stylophora pistillata*) by applying a boron fractionation factor (α_B) of 'best fit' based on the $\delta^{11}B_{carb}$ offset from both the Kakihana et al. (1977) and Klochko et al. (2006) borate curves. The somewhat different approach based on SIMS $\delta^{11}B$ measurements of the epibenthic foraminifer *Amphistegina lobifera* identifies the wide range in measured values as the physiological $\delta^{11}B$ contribution, which is especially

dependent on determining the lowermost value that defines initial (ambient) seawater pH. Thus, both of these approaches have significant limitations which lack robust quantification of the physiological pH component and $\delta^{11}B_{carb}$–seawater pH calibrations for the studied coral species.

4.2. Quantification of physiological controls: ΔpH versus seawater pH

Recent revisions of the theoretical calculations (Rustad et al., 2010) now clearly support the experimental calibration of Klochko et al. (2006) rather than that of Kakihana et al. (1977), and suggest there is no legitimate basis to accept Kakihana's lower boron fractionation $\alpha_{(B3-B4)}$ value. The dilemma is therefore to reconcile the apparent conflicts between our $\delta^{11}B_{carb}$ dataset and the boron calibration of Klochko et al. (2006).

Here we present a new approach that enables ambient seawater pH to be calculated from the measured $\delta^{11}B$ composition of *C. caespitosa*, however the superimposed biological fractionation first needs to be quantified. All of our pH calculations are based on $\alpha_{(B3-B4)} = 1.0272$, and pK_B is adjusted to the ambient seawater temperature and salinity for each sample site. The empirically derived boron isotope fractionation factor $\alpha_{(B3-B4)}$ of 1.0272 from Klochko et al. (2006) is thus assumed to be applicable to the internal biologically modulated pH environment (pH_{biol}) within which aragonite is precipitated at the site of calcification. The Klochko-derived pH (pH_{biol}) is thus calculated using pK_B and α values adjusted to ambient seawater temperature and salinity in Eq. (3), and is given by:

$$pH_{biol} = pK_B - \log\left\{\left[\delta^{11}B_{sw} - \delta^{11}B_{carb}\right] / \left[1.0272\delta^{11}B_{carb} - \delta^{11}B_{sw} + 1000(1.0272 - 1)\right]\right\}. \quad (6)$$

The superimposed biological pH overprint (ΔpH) imparted during calcification represents the difference between the measured ambient seawater pH (pH_{sw}) and the internal pH_{biol}:

$$\Delta pH = pH_{biol} - pH_{sw}. \quad (7)$$

The relationship of ΔpH to pH_{sw} for *C. caespitosa* (Fig. 4) defined by our data (Table 1) gives the following linear regression:

$$\Delta pH_{Clad} = 4.80 - 0.52 \times pH_{sw} \quad (8)$$

where: $r^2 = 0.92$

The excellent linear relationship (Fig. 4) implies that the biological offset for *C. caespitosa* is inversely proportional to the ambient seawater pH. This is an important finding and has implications for the calcification process. The slope of the curve (−0.5) implies that with decreasing seawater pH, the coral can only partially adjust its internal pH (pH_{biol}) so is unable to maintain a constant pH and hence aragonite saturation state at the site of calcification. The pH_{biol} is interpreted to represent the mean pH at the calcification site, which is broadly consistent with the average pH (range of 8.13 to 9.28) under the calicoblastic layer of *Galaxea fascicularis* that was measured *in situ* using micro-electrodes (Al-Horani et al., 2003). The linear relationship (Eq. (8)) extrapolates to the Klochko curve at approximately $pH_T = 9.2$, which we interpret to be the maximum pH_{biol} attainable by *C. caespitosa*.

Seawater pH can now be solved by iterative calculations using the calibration relationships given by Eqs. (6), (7), and (8). Notably these equations are valid for the large range of seawater pH_T values within which the corals grew, 7.62 to 8.10, that includes the very low pH_T (equivalent to pCO_2 ~1800 μatm) environment near the subsea vents off Ischia (ISCH_S2). This lowermost pH_T value (7.62 ± 0.27) is not well constrained given the large fluctuations in measured seawater pH_T (7.19 to 8.02), hence better controls on the lower range in pH (e.g. from a stable environment) would provide a more accurate calibration for this species. Recalculation of the *Cladocora* regression after removing the ISCH_S2 data gives: $y = 6.28 - 0.71x$ ($r^2 = 0.95$), thus an approximate increase in slope of 0.2 compared to the regression defined by Eq. (8). Accordingly, the accuracy of the iterative calculations of ambient pH_{sw} is dependent on the accuracy of the initial dataset and its relative offset from the regression (i.e. r^2 value) that defines the species calibration. Nevertheless, the iteratively calculated pH_T value of 7.55 for ISCH_S2 (Table 3) is well within

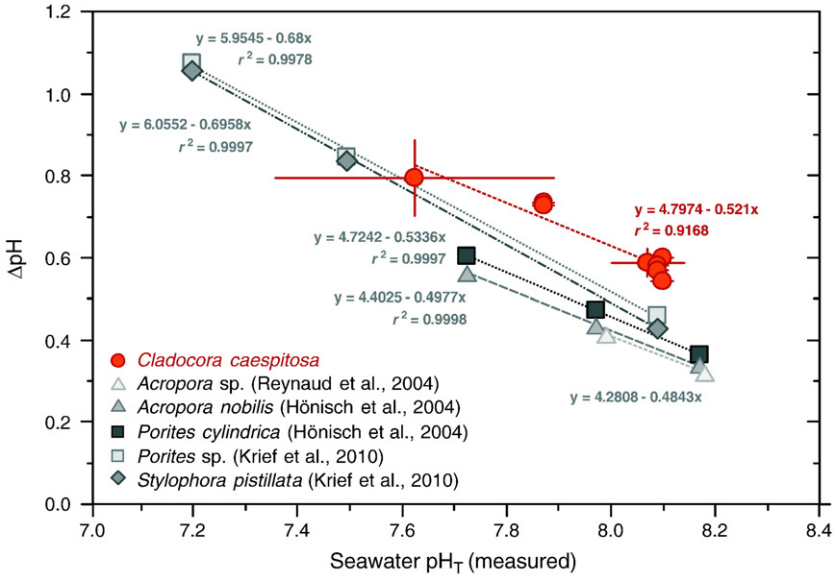

Fig. 4. Regressions for the temperate shallow water coral *C. caespitosa* (this study) and tropical species of *Porites, Acropora,* and *Stylophora* (Hönisch et al., 2004; Krief et al., 2010; Reynaud et al., 2004) that define the relationship between seawater pH and the biological pH component (ΔpH) imparted during calcification. ΔpH is calculated as the difference between pH_{biol}, based on the $\delta^{11}B$-derived pH using Klochko et al's fractionation factor ($\alpha = 1.0272$), and measured pH_{sw}. The resultant regressions define the species pH calibrations and can be used to iteratively calculate ΔpH and ambient seawater pH. Measurement errors for pH_{sw} are shown at 1 SD, and errors for ΔpH are calculated as the combined fractional errors (at 1 SD) for measured pH_{sw} and pH_{biol}.

Table 3
$\delta^{11}B$ compositions of *C. caespitosa*, measured seawater pH, and derived pH parameters. pH_{biol} is calculated using the Klochko et al. (2006) $\delta^{11}B$–pH calibration where $\alpha_B = 1.0272$ with pK_B adjusted for ambient temperature and salinity; ΔpH is the difference between pH_{biol} and measured pH_{sw}. Iterative calculations for pH_{sw} using the *Cladocora* calibration are within error of measured values.

Sample	$\delta^{11}B_{carb}$ (‰)	pH_T	1 SD	pH_{biol}	ΔpH	fractional error	Iterated pH_T	Iterated ΔpH
Levanto corals								
LEV_18A (18 m)	26.0	8.10		8.66	0.56	0.01	8.07	0.59
LEV_18B (18 m)	25.4			8.62	0.52	0.01	7.99	0.64
LEV_18 mean	25.7			8.64	0.54	0.01	8.03	0.62
LEV_27A (27 m)	26.7	8.10		8.72	0.62	0.01	8.19	0.53
LEV_27B (27 m)	26.0			8.68	0.58	0.01	8.10	0.58
LEV_27 mean	26.4			8.70	0.60	0.01	8.15	0.55
Ischia vent corals								
ISCH_Cont	25.9	8.07	0.07	8.66	0.59	0.03	8.06	0.60
ISCH_S2	22.2	7.62	0.27	8.42	0.80	0.09	7.55	0.86
Aquaria corals								
CLA_400	25.1	8.09	0.02	8.66	0.57	0.01	8.06	0.60
CLA_400	25.4			8.67	0.58	0.01	8.09	0.58
CLA_700	24.3	7.87	0.02	8.60	0.73	0.01	7.94	0.66
CLA_700	24.2			8.60	0.73	0.01	7.94	0.66

measurement error of ambient pH_T (7.62 ± 0.27), which shows that the *Cladocora* regression has effectively calibrated the boron isotope pH proxy for this coral species. Furthermore, the data clearly suggest that even within this hostile environment, proximal to the CO_2 vent site where the exposed skeleton of corals were being actively decalcified (Hall-Spencer and Rodolfo-Metalpa, 2008), the 'normal' physiological controls of pH and their boron isotopic composition are unperturbed.

4.3. Comparative analysis of *C. caespitosa* and tropical shallow water species

All of the $\delta^{11}B$ compositions of *C. caespitosa* are consistently higher than those of tropical shallow water species of *Acropora, Porites, and Stylophora* (Fig. 3B) reported in the literature (Hönisch et al., 2004; Krief et al., 2010; Reynaud et al., 2004). Following our "ΔpH–pH_{sw}" approach, we have calibrated their $\delta^{11}B$ compositions and the relationship between ΔpH and ambient seawater pH (Fig. 4, Table 4) as defined by the following equations:

Table 4
Derived pH parameters using our species calibrations defined herein for a suite of cultured tropical shallow water corals, and their $\delta^{11}B$ compositions with ambient seawater pH_T reported in the literature. Note Hönisch et al. (2004) data converted to total scale. pH_{biol} is calculated using the Klochko et al. (2006) $\delta^{11}B$–pH calibration where $\alpha_B = 1.0272$ with pK_B adjusted for ambient temperature and salinity; ΔpH is the difference between pH_{biol} and measured seawater pH_T. Iterative calculations for pH_T using the respective species calibrations are within error of measured values.

Sample	$\delta^{11}B_{carb}$ (‰)	pH_T	2 SD	pH_{biol}	ΔpH	Iterated pH_T	Iterated ΔpH
Hönisch et al. (2004)							
Acropora nobilis	21.10	7.72	0.31	8.28	0.56	7.72	0.56
Acropora nobilis	22.90	7.97	0.31	8.40	0.43	7.97	0.44
Acropora nobilis	24.50	8.17	0.31	8.51	0.34	8.17	0.34
Porites cylindrica	21.70	7.72	0.31	8.32	0.60	7.72	0.61
Porites cylindrica	23.50	7.97	0.31	8.44	0.47	7.97	0.47
Porites cylindrica	24.90	8.17	0.31	8.53	0.36	8.17	0.37
Reynaud et al. (2004)							
Acropora sp.	24.00	8.18	0.50	8.50	0.32	8.18	0.32
Acropora sp.	22.50	7.99	0.50	8.40	0.41	7.99	0.41
Krief et al. (2010)							
Porites sp.	25.24	8.09	0.25	8.55	0.46	8.11	0.44
Porites sp.	21.95	7.49	0.25	8.33	0.84	7.44	0.90
Porites sp.	20.97	7.19	0.25	8.27	1.08	7.22	1.04
Stylophora pistillata	24.76	8.09	0.25	8.52	0.43	8.10	0.42
Stylophora pistillata	21.84	7.49	0.25	8.33	0.84	7.47	0.86
Stylophora pistillata	20.70	7.19	0.25	8.25	1.06	7.20	1.04

Porites cylindrica (Hönisch et al., 2004)

$$\Delta pH_{Porc} = 4.72 - 0.53 \times pH_{sw} \quad (9)$$

where: $r^2 = 0.9997$

Acropora nobilis (Hönisch et al., 2004)

$$\Delta pH_{Acropn} = 4.40 - 0.50 \times pH_{sw} \quad (10)$$

where: $r^2 = 0.9998$

Acropora sp. (Reynaud et al., 2004)

$$\Delta pH_{Acrop} = 4.28 - 0.48 \times pH_{sw} \quad (11)$$

(defined by 2 data points only)

Porites sp. (Krief et al., 2010)
where: $r^2 = 0.998$

$$\Delta pH_{Por} = 5.96 - 0.68 \times pH_{sw} \quad (12)$$

S. pistillata (Krief et al., 2010)

$$\Delta pH_{Stylp} = 6.06 - 0.70 \times pH_{sw} \quad (13)$$

where: $r^2 = 0.9997$.

Like *C. caespitosa*, all calibrations of these tropical zooxanthellate corals are strongly linearly correlated (>0.9, Fig. 4). The gradients defining the biologically controlled shift in ΔpH relative to ambient seawater pH for *Acropora* sp. (−0.48), *A. nobilis* (−0.50), *P. cylindrica* (−0.53), are very similar to *Cladocora* (−0.52), whereas the slope of the calibrations for *Porites* sp. (−0.68) and *S. pistillata* (−0.70) are somewhat higher. Within the seawater pH range of the present study, the offset in ΔpH_{Clad} relative to these varied tropical species is approximately +0.2 pH units higher. However, given the high measurement error for the *Cladocora* datum of lowest pH (S2 $pH_T = 7.62 \pm 0.27$ 1SD; Table 1 and see Section 2.2), the slope of the *Cladocora* regression could in theory be closer to the MC-ICP-MS dataset, as also indicated by the regression on removal of this datum (see Section 4.2). This would further increase ΔpH_{Clad} relative to all of these tropical species hence *C. caespitosa* could have an even greater capacity to adjust its internal pH_{biol} in response to decreasing ambient pH_{sw}. It is also unclear however whether the apparent differences between the two tropical coral datasets represent taxon-specific vital effects, or are otherwise due to analytical biases between MC-ICPMS as used by Krief et al. (2010), and the NTIMS approach of Hönisch et al. (2004) and Reynaud et al. (2004).

4.4. Boron systematics and implications for calcification of *C. caespitosa*

The independence of the B/Ca composition from ambient seawater pH is somewhat unexpected as the concentration of borate ($B(OH)_4^-$) in seawater, the species taken up by corals, should theoretically decrease by ~35% under the lower seawater pH (and higher pCO_2) regime. However, both $B(OH)_4^-$ and CO_3^{2-} have very similar pH speciation relationships, thus changes in ambient pH results in a concomitant decrease in CO_3^{2-} hence to a first approximation the $B(OH)_4^-/CO_3^{2-}$ ratio is independent of pH (Fig. 2). Since the molar ratios of CO_3^{2-} and Ca^{2+} are essentially at unity in $CaCO_3$ a constant B/Ca ratio is implied at different pH values. The inverse correlation of B/Ca

ratio of *C. caespitosa* with ambient seawater temperature is therefore entirely consistent with the temperature dependence of the seawater borate/carbonate ratio: the coral B/Ca ratio is thus proportional to the borate/carbonate seawater ratio with an apparent temperature sensitivity of ~3% per °C, consistent with previous observations (Montagna et al., 2007). To account for the lack of correlation between B/Ca to varying pCO_2, as commonly observed in calcitic foraminifers, we suggest that the borate ion is probably tightly coupled to CO_3^{2-} rather than HCO_3^- during the process of internal adjustment of pH of the calcifying fluid and aragonite precipitation. This suggests that the borate ion may be directly incorporated into the aragonite coral structure maintaining its tetrahedral coordination (Sen et al., 1994) via a reaction of the type:

$$Ca^{2+} + 2B(OH)_4^- \rightarrow Ca(H_3BO_4) + B(OH)_3 + H_2O \quad (14)$$

or if a trigonal coordination is applicable via:

$$Ca^{2+} + 2B(OH)_4^- \rightarrow Ca(HBO_3) + B(OH)_3 + 2H_2O \quad (15)$$

rather than the surface exchange reaction of Hemming and Hanson (1992):

$$CaCO_3 + B(OH)_4^- \rightarrow Ca(HBO_3) + HCO_3^- + H_2O \quad (16)$$

which has been applied to trigonal coordination in the calcite lattice of foraminifers (e.g. Yu and Elderfield, 2007). Carbonate speciation must therefore be considered when applying the boron proxy to different carbonate polymorphs (i.e. calcite or vs aragonite).

The $\delta^{11}B$–pH_{sw} calibrations show that the physiological control on pH imparted during coral calcification (ΔpH) is greater in the temperate water species *C. caespitosa* than the faster growing tropical corals. This suggests that the slower growing temperate species has the ability to maintain a higher internal pH and hence ΔpH relative to ambient seawater pH. This is consistent with recent findings that calcification of *C. caespitosa* is insensitive to the higher pCO_2 treatment (700 μatm; Rodolfo-Metalpa et al., 2010b), and the similar growth rates of corallites from both aquaria experiments determined herein. As a consequence, in slow growing corals such as *C. caespitosa* that have a relatively low carbonate requirement, the concentration of carbonate ions may not be a major limiting factor even under relatively high pCO_2 concentrations (Rodolfo-Metalpa et al., 2010b). This indicates that for temperate corals, temperature controls on both the rate of aragonite formation as well as carbonate ion translocation to the site of calcification rather than seawater pH is an important mechanism controlling growth (see also Rodolfo-Metalpa et al., 2010b). Conversely for tropical zooxanthellate corals, in which calcification rates are much higher and are therefore more likely to be limited by internal carbonate ion concentration rather than temperature, the inability to fully compensate for lower external pH will directly limit calcification.

We also note that the boron isotope compositions of a number or calcitic foraminifers, *Globigerinoides ruber, Globigerinoides succulifer, Neogloboquadrina dutertrei, Cibicidoides wuellerstorfi,* and *Cibicidoides mundulus* lie on or near the Klochko borate curve (Foster, 2008), indicating that their internal and external pH are similar. This implies that these foram species lack the ability to adjust their internal pH thus, in contrast to *C. caespitosa* discussed herein, their rate of calcification will be directly linked to the changing ambient seawater saturation state. Accordingly, the greater the ability an organism has to modulate internal pH the less sensitive it will be to decreasing saturation state as atmospheric CO_2 increases.

5. Conclusions

This study clearly shows that the boron isotope proxy combined with our new calibration "ΔpH–pH_{sw}" method is a powerful tool for determining ambient seawater pH from measured $\delta^{11}B$ compositions of shallow water coral skeletons. It is however necessary to measure ambient seawater pH in order to calibrate the boron isotope pH proxy for any given species. The "ΔpH–pH_{sw}" approach constrains the relationships between biological pH, seawater pH, and the $\delta^{11}B$ compositions of *C. caespitosa*, as well as for several tropical shallow water corals (*P. cylindrica, Porites* sp., *A. nobilis*, *Acropora* sp. and *S. pistillata*) using published boron and seawater pH data. This new approach thus provides a means to effectively quantify the physiologically driven 'vital effect' on $\delta^{11}B$ imparted during calcification, but also validates the empirically constrained Klochko calibration for boron fractionation, both of which hitherto have been elusive and a source of considerable debate.

The "ΔpH–pH_{sw}" method appears to be robust across a large range of pH and environmental regimes and is applicable to both temperate and tropical corals. Our study shows that, contrary to expectations, the slow growing temperate coral *C. caespitosa* has the ability to adjust its internal pH over a wide range, but at a constant proportion to external changes in pH. Additionally, in contrast to calcitic forams, the B/Ca compositions are largely controlled by ambient temperature rather than seawater pH. These relationships and in particular quantifying the extent to which calcifiers can adjust their internal pH, have important implications for understanding the potential resilience of different species to the combined effects of ocean acidification and global warming in a rapidly increasing CO_2 world.

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