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pH control on oxygen isotopic composition of symbiotic corals

Claire Rollion-Bard *, Marc Chaussidon, Christian France-Lanord

CRPG-CNRS, BP 20, 54501 Vandoeuvre-lès-Nancy Cedex, France

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Abstract

Boron, carbon and oxygen isotopic compositions were determined at the micrometre scale by high-resolution ion microprobe in a sample of modern coral (massive hermatypic coral, *Porites lutea*). The ion probe data show for B and O much larger isotopic variations at the micrometre scale than those measured at the millimetre scale by conventional techniques: $\delta^{18}O_{PDB}$ values range from -10.6 ± 0.9‰ to -0.2 ± 0.5‰ and $\delta^{11}B$ values range from $+18.6\pm1.5$‰ to $+30.6\pm1.6$‰. By contrast, $\delta^{13}C$ values show the same range of variations, from -4.6 ± 0.65‰ to -2.2 ± 0.67‰ at the micrometre and millimetre scales. The range of $\delta^{11}B$ values indicates that significant pH variations, from $\approx$7.1 to $\approx$9.0, are present at the sites of calcification. The largest $\delta^{18}O$ variations correspond to the highest $\delta^{11}B$ values, i.e. to the highest pHs. This measurement of pH allows modelling the oxygen isotopic fractionation occurring during aragonite precipitation. Taking into account the rate of O isotopic equilibrium between dissolved carbonate species (H_2CO_3, HCO_3^- and CO_3^{2-}) and water via the two reactions of hydration and hydroxylation, the full range of $\delta^{18}O$ values measured at the micrometre scale can be modelled for residence times of dissolved carbonates in the calcifying fluid ranging between $\approx$1 h and at maximum $\approx$12 h. The pH controls the $\delta^{18}O$ of the growing carbonate through the relative fractions of dissolved carbonate species and through the kinetics of their isotopic equilibration with water via hydration and hydroxylation. The so-called 'vital effect' systematically observed for $\delta^{18}O$ in corals can thus be understood as representing an average of rapid pH variations due to coral biology during coral growth. Selectively measuring $\delta^{18}O$ values in the zones of coral skeletons that have low $\delta^{11}B$ values (i.e. formed at low pH) should significantly improve the quality of palaeoclimatic reconstructions based on $\delta^{18}O$ values.

Keywords: coral; ion microprobe; vital effect; $\delta^{18}O$; $\delta^{13}C$; $\delta^{11}B$; pH

1. Introduction

Stable isotopic compositions ($\delta^{13}C$ and $\delta^{18}O$) of zooxanthellate coral skeletons measured at the millimetre scale are widely used to reconstruct high-resolution records of climate variability into the past [1,2]. However, these isotopic compositions differ from those calculated for inorganic

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* Corresponding author. Tel.: +33-3-83-59-42-36; Fax: +33-3-83-51-17-98.

E-mail addresses: rollion@crpg.cnrs-nancy.fr (C. Rollion-Bard), chocho@crpg.cnrs-nancy.fr (M. Chaussidon), cfl@crpg.cnrs-nancy.fr (C. France-Lanord).

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equilibrium between aragonite–calcite and seawater. This depletion relative to inorganic aragonite isotopic equilibrium can reach 4‰ for $\delta^{18}O$ and 10–15‰ for $\delta^{13}C$ [3]. The processes responsible for this offset, known as the 'vital effect', are not yet fully understood and species-specific empirical calibrations between sea surface temperature (SST) and $\delta^{18}O$ are always required. These calibrations may introduce significant uncertainties in SST due to the variations in the slopes and intercept values for SST-$\delta^{18}O$ calibrations observed for different colonies of the same species. For example, for the species *Porites lutea*, the slopes of the calibrations range from 0.131‰/°C [4] to 0.189‰/°C [5]. Thus 1‰ variation in coral $\delta^{18}O$ can be interpreted as a variation of temperature of either 7.6°C or 5.3°C according to the empirical calibration used.

Two major sources for this offset in isotopic composition can be anticipated, namely (i) kinetic processes and (ii) pH variations at the sites of calcification. (i) Large isotopic variations of coral aragonite are expected if the skeleton carbonate precipitation is fast enough to allow precipitation of HCO_3^- and/or CO_3^{2-}, formed by CO_2 hydration and hydroxylation, before their isotopic equilibration with water [6]. This results in a depletion of ^{18}O in carbonate. Since the skeleton calcification rate is the lowest during night-time [7], different $\delta^{18}O$ between parts of the skeleton precipitated during the night and during the day can be expected. In reality, this process is implied by the micrometre scale Sr/Ca variations observed by ion microprobe in coral skeleton [8]. (ii) pH variations at the sites of calcification can theoretically induce significant variations in the $\delta^{18}O$ of carbonates, which would then result in an apparent O isotopic disequilibrium between carbonate and seawater. In fact, the three dissolved carbonate species (H_2CO_3, HCO_3^- and CO_3^{2-}) have different $\delta^{18}O$ values at equilibrium with seawater and the amount of each species taken from the solution during carbonate precipitation is governed by pH [9,10]. Such a pH effect has been proposed to be partly responsible for the vital effect observed for $\delta^{18}O$ in foraminifera [11,12].

Up to now, the effects of these two processes, rapid variations in calcification rates and variations in the pH of the calcifying fluid, on the $\delta^{18}O$ of the coral skeleton have not been assessed in detail. This is due first to technical limitations in measuring $\delta^{18}O$ values in coral skeleton on the micrometre scale. The micrometre is in fact the scale at which isotopic variations due to rapid changes in coral growth rates ($\approx$1 cm/year, i.e. $\approx$15 μm/12 h) would be recorded. Second, a tool independent of O isotopes is needed to trace pH variations during coral growth. Though $\delta^{11}B$ variations in corals have not been proven to give a direct access to seawater pH, they are certainly a promising tool to track the pH variations at the sites of carbonate precipitation [13]. High precision O and B isotopic analyses by ion microprobe can obviously overcome these two technical limitations.

To look for a pH-$\delta^{18}O$ dependence and for $\delta^{18}O$ variations at the scale of a few hours in coral skeleton, we have performed B and O stable isotopic measurements on a micrometre scale using a high radius ion microprobe in a sample of modern zooxanthellate coral from New Caledonia. Carbon isotopes were also analysed to monitor the influence of metabolic ^{13}C-depleted CO_2 on carbon isotopes vital effect in coral skeleton.

2. Sample

The sample studied is a 22-mm-long fragment of a massive coral *P. lutea* collected at the Boulari Reef in New Caledonia in 1994 (166°3°E, 22°3°S, 2.5 m below mean sea level). This sample was donated by E. Bard and different samples of the same colony and of different colonies (modern, Holocene and Pleistocene) of the same species were studied in details for Sr/Ca, U/Ca, $\delta^{18}O$ and $\delta^{13}C$ by Castellaro (1999) [14]. For modern colonies, precisions of ±1°C and ±3°C were obtained for the Sr/Ca SST and for the U/Ca and $\delta^{18}O$ SST calibrations, respectively. A 3-mm-thick section of the sample was cut parallel to the axis of maximum growth and polished for ion probe analyses (see Section 3). The counterpart of this section was analysed for C and O isotopes by phosphoric acid digestion and classical gas source mass spectrometry at CRPG-CNRS in order to get a millimetre scale spatial resolution to compare with ion probe results.

Environmental parameters are recorded at Amédée Islet near the Boulari Reef by an IRD station. The average monthly SSTs were recorded since 1985. The maximum SST is observed in February (25.5°C in average) and the minimum SST in August (20.8°C in average), resulting in an amplitude of 4.7°C. The average monthly SSS (sea surface salinity) reaches a maximum value in October (36.0‰) and a minimum value in March (35.5‰). Thus the SSS has a weak average annual amplitude of only 0.5‰.

3. Analytical techniques

3.1. Ion probe instrumental settings

Ion probe analyses were performed at CRPG-CNRS (Nancy, France) using the Caméca ims 1270. C and O isotopes were analysed as C^- and O^- ions produced by a bombardment by a Cs^+ primary beam (5 nA intensity for O and 30 nA intensity for C) of 30–50 μm diameter, while an O^- primary beam of similar size was used for the analysis of B isotopes as B^+ ions. Mass resolutions of 5000 and 2000 were used for C-O and B isotope analyses, respectively. O and C isotopes were measured in multicollection mode using two off-axis Faraday cups (L′2 and H1) for oxygen isotopes and using one off-axis Faraday cup (L′2) and the central electron multiplier for carbon isotopes, while B isotopes were analysed in monocollection using the central electron multiplier. The gains of Faraday cups L′2 and H1 were calibrated at the beginning of the analytical session using the Caméca built-in amplifier calibration routine. Typical ion intensities of 4×10^9 and 2×10^7 counts per second (cps) were obtained on the $^{16}O^-$ and $^{12}C^-$, so that an internal 1σ error of ±0.1–0.2‰ was reached after a few minutes of counting. For boron isotopes, the typical ion intensities of $^{11}B^+$ were 4×10^4 cps so that the internal 1σ error was ±0.6‰ after several minutes of counting. The average external reproducibility, as estimated from replicate measurements of carbonate standards, was ±0.4‰ for oxygen isotopes, 0.65‰ for carbon isotopes and 0.9‰ for boron isotopes (Fig. 1). The stability of the instrument was monitored by multiple measurements of the aragonite standard (Arg). The exter-

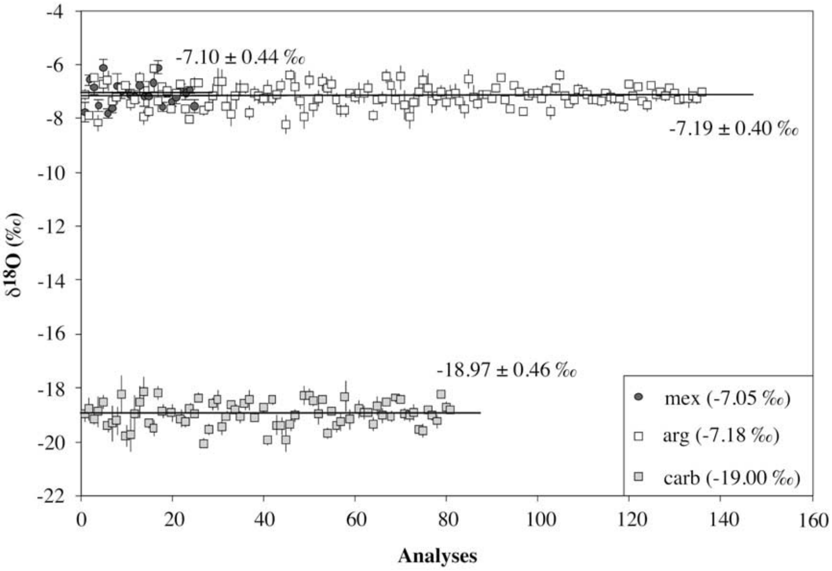

Fig. 1. Long-term reproducibility (4 years) of oxygen isotopic measurements on carbonate standards (two calcites, mex and carb, and one aragonite, arg). Horizontal lines represent the mean values of all analyses.

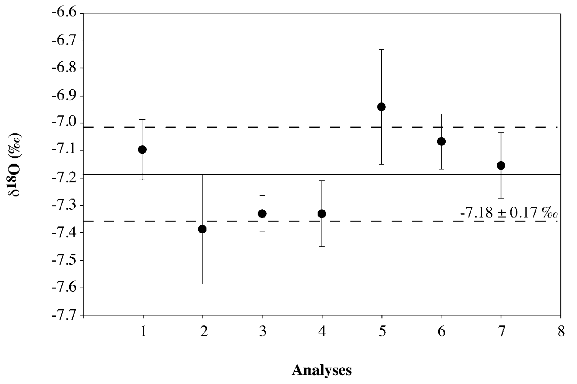

Fig. 2. Reproducibility for oxygen isotopic measurements for the aragonite standard over one analytical session of 8 h. No instrumental drift is observed.

nal reproducibility obtained on this standard during an entire 1-day analytical session can reach ±0.17‰ (Fig. 2). This value is similar to the uncertainty of individual standard measurements, showing that there is no significant instrumental drift.

During this study, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{11}B$ were measured successively. This order was chosen because oxygen and carbon isotopes are both analysed with a Cs^+ primary source, while boron isotopes are analysed with a O^- primary source that might contaminate the sample for oxygen isotopic measurements.

3.2. *Calibration of instrumental mass fractionation (IMF) for ion microprobe and matrix effect*

The IMF was monitored and determined using various carbonate standards that were analysed conventionally for O, C and B isotopes: two calcite standards ($\delta^{18}O_{PDB} = -19.00 \pm 0.04$‰ and $\delta^{13}C = -5.39 \pm 0.01$‰; $\delta^{18}O_{PDB} = -7.05 \pm 0.02$‰ and $\delta^{13}C = -6.37 \pm 0.04$‰), and one aragonite ($\delta^{18}O_{PDB} = -7.18 \pm 0.11$‰, $\delta^{13}C = 8.17 \pm 0.04$‰ and $\delta^{11}B = +23 \pm 1$‰). The homogeneity of the standards for oxygen, carbon and boron isotopes was tested by multiple ion probe measurements along different profiles.

A systematic difference of 2.8‰ for O and of 4.5‰ for C was observed between calcite and aragonite IMFs, aragonite being more fractionated. The values of IMF determined on the aragonite standards were used for the correction of the data collected on the coral.

3.3. *Orthophosphoric acid digestion and mass spectrometry*

A piece of the coral studied was analysed for O and C isotopes by mass spectrometry after an orthophosphoric acid digestion. Each sample, located according to the growth axis, was placed in a glass tube and an acid digestion under vacuum at 25°C [9] leads to carbonate dissolution and liberation of CO_2. To correct the sample data, international standards were measured during the analytical session (NBS 18, NBS 19, IAEA-CO-1 and IAEA-CO-8). For oxygen isotopes, the fractionation factor during the acid digestion is not the same between calcite and aragonite [15], so the coral samples were corrected with the aragonite fractionation. The reproducibility for oxygen and carbon isotopes is ±0.1‰.

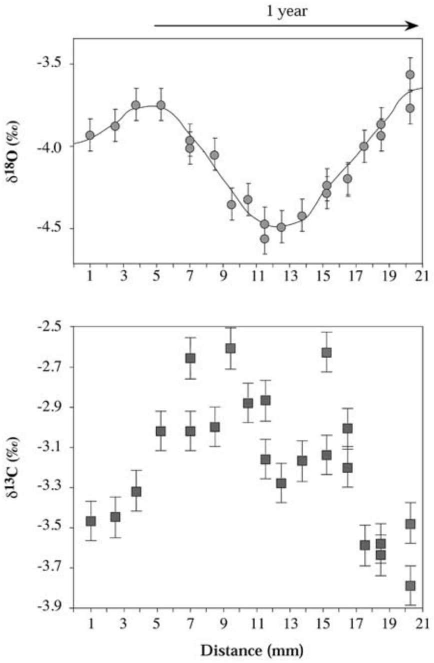

Fig. 3. Millimetre scale $\delta^{18}O$ and $\delta^{13}C$ values obtained by acid digestion and mass spectrometry versus sampling distance for the *P. lutea* sample. The growth rate of this coral, determined from seasonal variations observed in millimetre scale $\delta^{18}O$ measurements covering 2 years of growth, is 15 ± 1 mm/year.

4. Results

4.1. Millimetre scale $\delta^{18}O$ and $\delta^{13}C$ variations

At the millimetre scale, O and C isotopic compositions show variations within a 1‰ range, respectively between −4.56 ± 0.10‰ and −3.57 ± 0.10‰ and between −3.79 ± 0.10‰ and −2.63 ± 0.10‰ (Fig. 3). The δ^{18}O values show cyclic variations over a 22-mm-long section, in agreement with the local seasonal temperature variations of 4.7°C. They indicate an average growth rate of the coral of 15 ± 1 mm/yr. The δ^{13}C data do not show such a clear seasonal variation and are not correlated with δ^{18}O. In azooxanthellate corals, respiration and kinetic fractionation are the only processes affecting the oxygen and carbon isotopic compositions, which are thus positively correlated. In zooxanthellate corals, the photosynthesis combined with respiration, obscures the relationship between δ^{18}O and δ^{13}C, which can even disappear [2]. This is what is observed in the present sample.

4.2. Micrometre scale $\delta^{18}O$ variation along the growth axis

Oxygen isotopic ion probe data show, on a micrometre scale, very large variations that have never been observed using conventional sampling methods (Fig. 4). Within 100 μm the δ^{18}O can change from −10.6 ± 0.9‰ to −0.2 ± 0.5‰. Such a δ^{18}O range is much too large to result from changes in seawater temperature or salinity. The highest ion probe δ^{18}O values correspond to isotopic equilibrium with seawater according to the aragonite-water isotopic fractionation of Grossman and Ku (1986) [16] and for seawater temperatures of 20.8–25.5°C and seawater salinity of 35.55‰. The lowest δ^{18}O values demonstrate that some parts of the skeleton were precipitated in large isotopic disequilibrium with seawater. Despite the extreme variability, the mean ion probe δ^{18}O (−4.4 ± 1.9‰) is within the δ^{18}O range measured on the millimetre scale. A more detailed comparison between millimetre and micrometre scale isotopic variations can be obtained by inte-

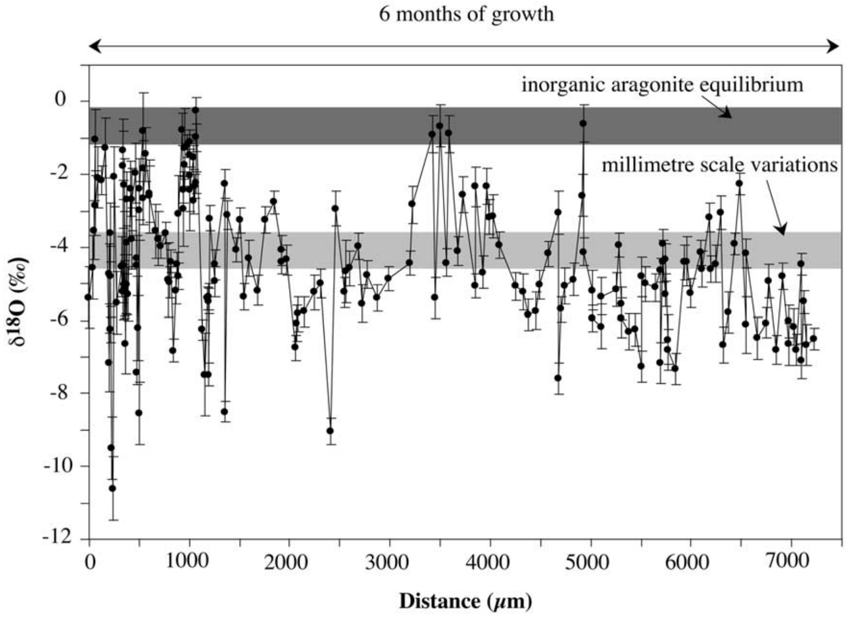

Fig. 4. δ^{18}O values measured by ion microprobe along the growth axis of the coral with a spatial resolution of 30–50 μm (black dots) corresponding to the size of the primary beam. The ion probe profile corresponds to ≈6 months of growth. The dark field corresponds to the range of δ^{18}O values calculated for inorganic isotopic equilibrium of aragonite with seawater [16] for a SST ranging between 20.8 and 25.5°C [14]. The light grey field corresponds to the range of δ^{18}O values measured on the millimetre scale. The 3–5‰ difference between the two fields represents the 'vital effect'.

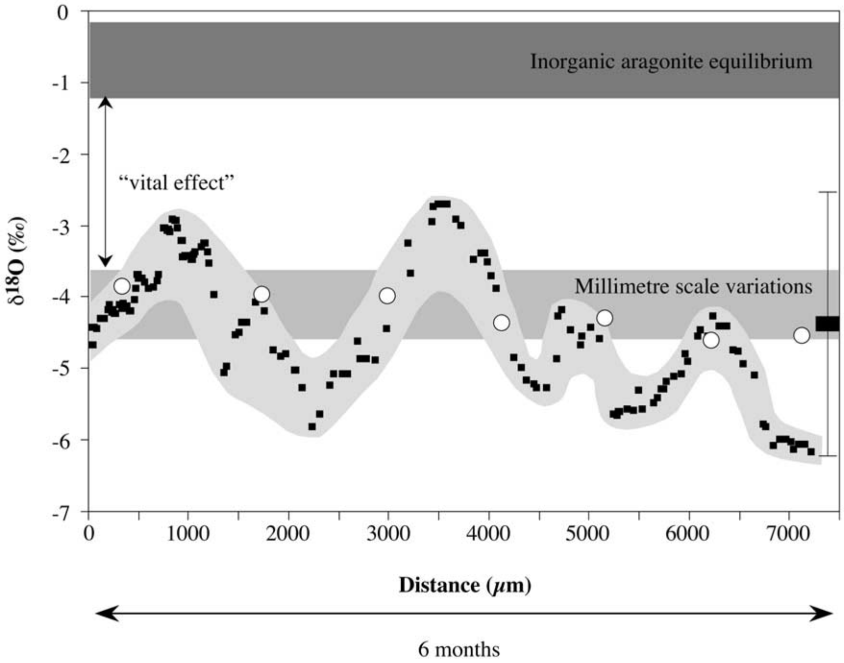

Fig. 5. $\delta^{18}O$ values measured by ion microprobe along the growth axis of the coral with an artificially degraded spatial resolution (black squares) obtained by smoothing the ion microprobe data using a sliding mean over 400 μm. The black rectangle represents the ion probe $\delta^{18}O$ mean value. The open circles correspond to millimetre scale classical $\delta^{18}O$ measurements made on the counterpart of the section used for ion microprobe analysis. The position of the millimetre scale data relative to the micrometre scale data is only approximate because some material was lost during sawing and polishing of the sample. The dark field and the light grey field are as in Fig. 4.

grating ion probe data over growth intervals of 400 μm using sliding means of the micrometre scale data (Fig. 5). Doing so, the range of the $\delta^{18}O$ data set is reduced to ≈ 4‰ and an apparent monthly periodicity is observed. Such a frequency could be related to that of dissepiments formation. This hypothesis cannot be checked further because the dissepiments disappeared during polishing of the sample. Both data sets, on millimetre and micrometre scales, are thus in agreement but the micrometre scale data set gives insights into processes that cannot be observed otherwise.

Because such a large variation in oxygen isotopic analyses has never been measured by conventional techniques, special attention was paid to all the possible artefacts that could influence the $\delta^{18}O$ measurements by ion probe. First, the lack of relationship between the intensity of H_2O^- and $\delta^{18}O$ ruled out fluid inclusions that might be

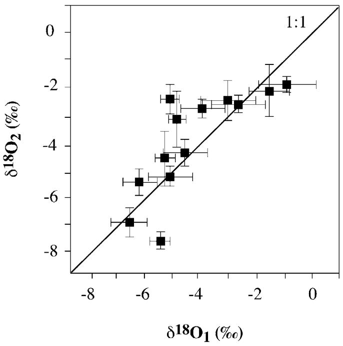

Fig. 6. Duplicate ion microprobe $\delta^{18}O$ measurements performed in the same ion probe spots during different analytical sessions ($\delta^{18}O_1$ stands for the first session and $\delta^{18}O_2$ for the other ones). All the data points, which cover a 6‰ range, are reproducible within ±0.5‰ except for three analyses. This apparent discrepancy is likely due to the small-scale heterogeneity of the coral compared to the depth of the ion probe spots (see text).

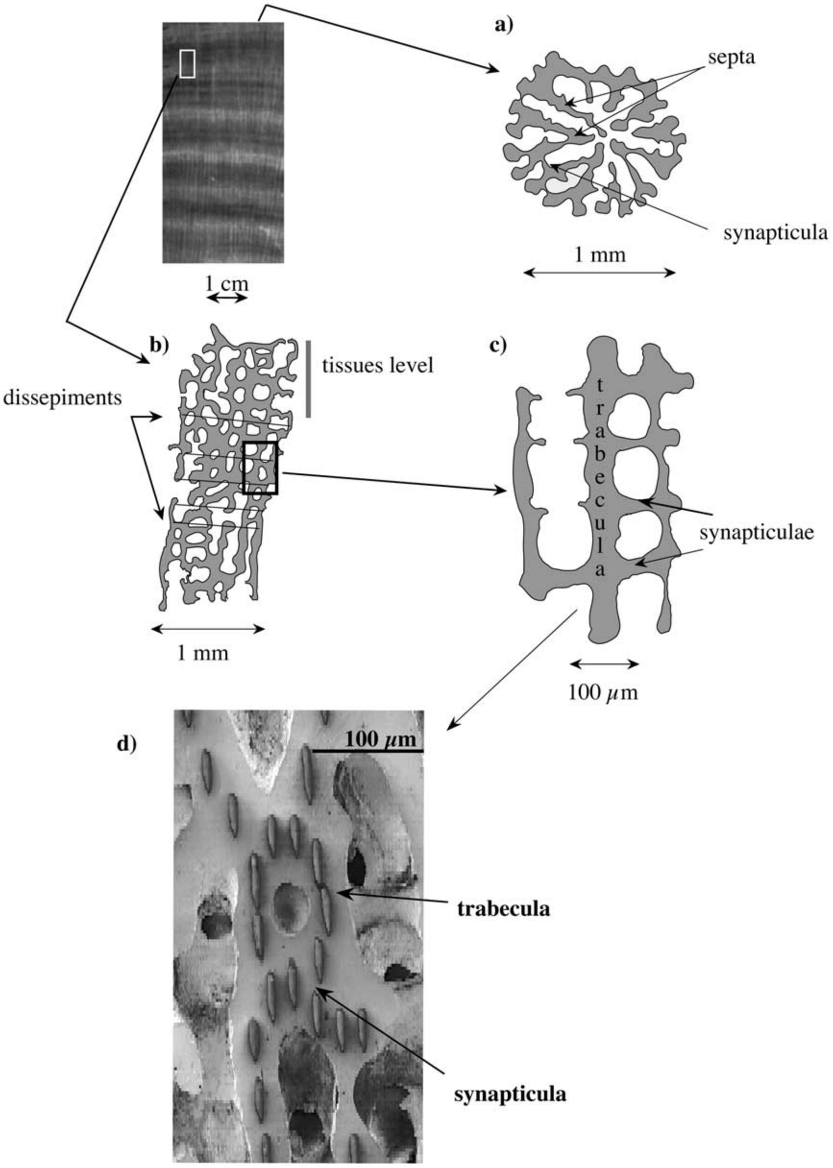

Fig. 7. Views of different sections of the *Porites* skeleton, from the macro-architecture to the micro-architecture, modified after [14]. The first picture is an X-radiograph of coral skeleton showing high- and low-density band couplets at annual resolution. The transversal section of a *Porites* corallite (a) shows the septa, which are radial elements directed toward the polyp centre. Septa can be linked by lateral outgrowths called synapticula. The dissepiments, which are horizontal elements formed every month by the coral, are visible on a longitudinal section (b). A magnification of a part of the longitudinal section shows the trabecula, vertical parts, and the synapticula, horizontal parts (c). The typical scale of the ion microprobe measurements (d) is that of trabecula and synapticula.

opened under the ion probe beam as being a cause of the observed δ^{18}O variation. Secondarily, the lack of correlation between δ^{13}C and δ^{18}O variations indicates that traces of organic matter had no influence on the δ^{18}O values. Finally, δ^{18}O measurements duplicated in the same ion probe spots during different analytical sessions are consistent within errors at the exception of three points (Fig. 6). The difficulty in duplicating exactly all the measurements can be simply understood as due to the microscopic isotopic heterogeneity of the corals since the ion probe primary beam makes holes of up to 1 µm depth so that zones with different $\delta^{18}O$ values can be successively tapped by the beam.

4.3. *Micrometre scale $\delta^{18}O$, $\delta^{13}C$, and $\delta^{11}B$ variations in relation to the coral microstructure*

The details of the relationships between the variations of B-C-O isotopic compositions have been investigated on a selected area of the polished

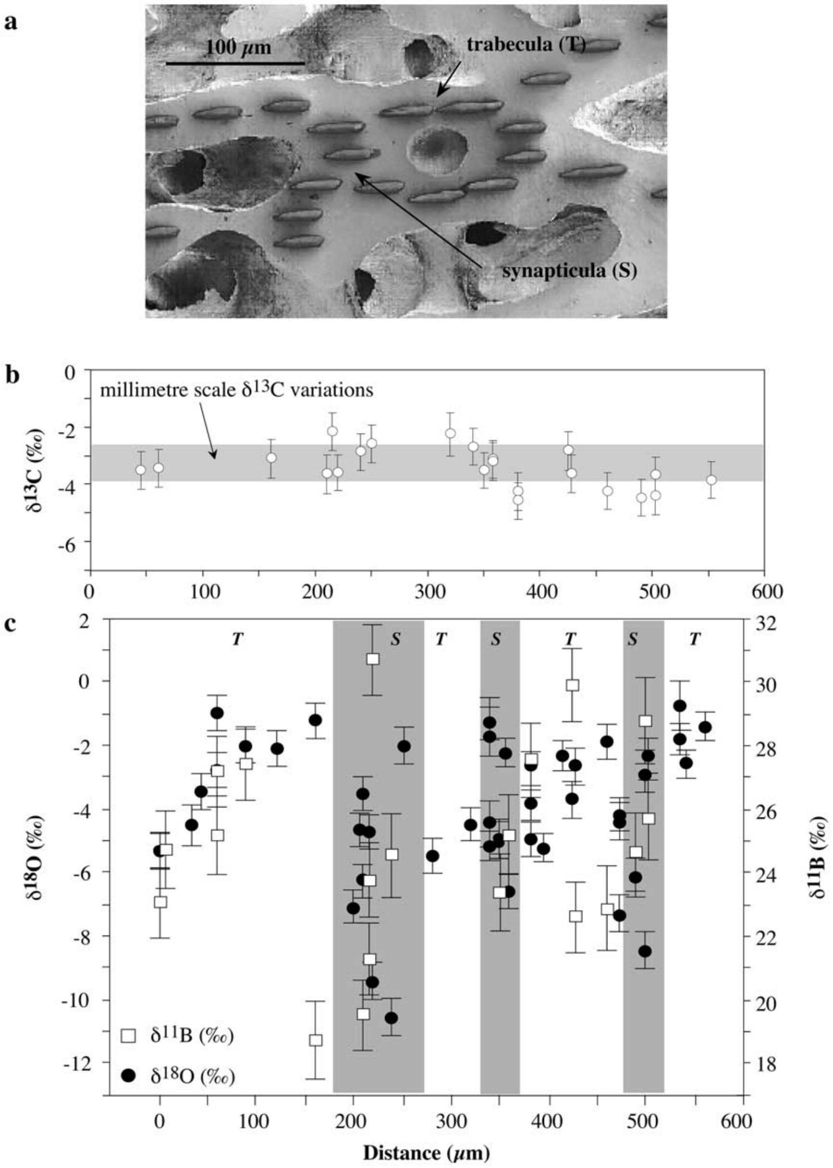

Fig. 8. $\delta^{13}C$ (open dots) (b), $\delta^{11}B$ (open squares) and $\delta^{18}O$ (solid dots) (c) values measured by ion microprobe in a zone of *P. lutea* where the microstructure of the coral was clearly observed with trabeculae (T) and synapticulae (S) (a). For $\delta^{11}B$ and $\delta^{18}O$ data (c), their relations with respect to the microstructure of the coral are also shown (synapticulae in grey area and trabeculae in white area). The ion probe spots are ≈30 µm × 15 µm in size.

section where the typical microstructure of *P. lutea* can be clearly observed (Fig. 7). While the micrometre scale $\delta^{13}C$ values do not show a large variation (from $-4.6 \pm 0.65‰$ to $-2.2 \pm 0.67‰$) and are all within the $\delta^{13}C$ range measured on the millimetre scale (Fig. 8b), large variations, up to 12‰ and 10‰, are observed for $\delta^{11}B$ and $\delta^{18}O$ values, respectively (Fig. 8c). $\delta^{18}O$ values range from $-10.6 \pm 0.9‰$ to $-0.2 \pm 0.5‰$. $\delta^{11}B$ values range from $18.6 \pm 1.5‰$ to $30.6 \pm 1.6‰$ with a mean value of 24.7‰, which is within the range of variation observed for modern corals (23.3–25.5‰) [17]. With respect to the structure of the coral, the lowest $\delta^{18}O$ values are associated with the synapticulae (Fig. 8c). No relationship exists between $\delta^{13}C$ and $\delta^{18}O$, but a relationship seems to exist between $\delta^{11}B$ and $\delta^{18}O$ (see Fig. 10), with the lowest $\delta^{18}O$ values associated with the highest $\delta^{11}B$ values.

5. Discussion

5.1. B isotopic evidence for pH variations at the sites of calcification

Recent studies of boron isotopes in carbonates suggest that $\delta^{11}B$ can be an important proxy for pH. This is due to the fact that the distribution of the two dissolved boron species, $B(OH)_3$ and $B(OH)_4^-$, is pH dependent and that a large B isotopic fractionation exists in solution between these species, $B(OH)_3$ being enriched in ^{11}B by ≈20‰ relative to $B(OH)_4^-$ at 20°C [18]. The B isotopic equilibration between the two species can be considered instantaneous [19]. The $\delta^{11}B$ of carbonate is related to pH because B incorporation occurs predominantly from seawater $B(OH)_4^-$ [20,21]. This relation is, however, not so straightforward since experiments [22] have shown that some foraminifera (e.g. *Orbulina universa*) precipitate carbonates with an additional B isotopic fractionation of up to 3.3‰ relative to seawater $B(OH)_4^-$, while others (e.g. *Globigerinoides sacculifer*) do not show this effect [23]. Brachiopods also seem to fractionate B relative to dissolved $B(OH)_4^-$ [24]. Data on corals [13,17] do not point to such an additional fractionation but this issue remains unclear since there are no experiments coupling in situ pH measurements and micrometre scale $\delta^{11}B$ measurements.

In the following, we assume that no additional B isotopic fractionation occurs during coral calcification and we use in our modelling the 'classical' $\delta^{11}B$–pH curve [23]:

$$\mathrm{pH} = 8.8 - \log\left(\frac{\delta^{11}B_{sw} - \delta^{11}B_c}{\alpha_{3/4}^{-1}\delta^{11}B_c - \delta^{11}B_{sw} + 1000 \times (\alpha_{3/4}^{-1} - 1)}\right)$$

where $\delta^{11}B_c$ stands for the boron isotopic composition of the carbonate, $\delta^{11}B_{sw}$ for the boron isotopic composition of modern seawater (39‰), and $\alpha_{3/4}$ is the boron fractionation factor between $B(OH)_3$ and $B(OH)_4^-$ ($\alpha_{3/4} = 0.98$ [18]).

Using this calibration, the present ion probe $\delta^{11}B$ data suggest that significant pH variations occur at the sites of calcification, between 7.1 and 9.0. Similar ranges in pH have been directly measured by microsensors between 7.5 and 9.3 in the colenteron for several corals [25,26] and in calicoblastic layers for a single polyp (from 8.13 to 9.29) [27]. The highest pH is associated with periods of illumination, which correspond to a high photosynthetic activity of zooxanthellae resulting in higher CO_2 consumption, while the lowest pHs are measured during the night [26]. Another source of pH variation during calcification is the uptake of H^+ ions via the exchange with Ca^{2+} ions. The periods of high calcification rate (day-time) are associated with more important exchange between Ca^{2+} and H^+ and thus result in higher pH in the ECF (extracytoplasmic calcifying fluid).

5.2. Effect of pH on equilibrium oxygen isotopic fractionation

The relative proportions of the dissolved carbonate species are pH dependent. At low pH, the major species is H_2CO_3, at intermediate pH it is HCO_3^-, and at high pH CO_3^{2-} is dominant. McCrea (1950) [9] noted that the $\delta^{18}O$ of the carbonate seems to be related to the proportion of HCO_3^- and CO_3^{2-} in the solution. This was later confirmed experimentally [28] and described theoretically by Udowski and Hoefs (1993) [10]. The $\delta^{18}O$ of carbonate is pH dependent since the $\delta^{18}O$

values of the different dissolved carbonate species at equilibrium with H_2O present a large difference with $\delta^{18}O^{CO_2}_{SMOW} = 41.2‰$ [15], $\delta^{18}O^{HCO_3^-}_{SMOW} = 34.3‰$ [29], $\delta^{18}O^{CO_3^{2-}}_{SMOW} = 18.4‰$ [28] and $\delta^{18}O^{OH^-}_{SMOW} = -41.1‰$ [10] (note that $\delta^{18}O_{PDB} = 0.97002 \times \delta^{18}O_{SMOW} - 29.98$) and because the $\delta^{18}O$ of carbonate reflects the proportion of the three dissolved carbonate species.

Thus the large O isotopic disequilibrium observed in the coral skeleton may indicate large pH variations in the ECF. However, using the equation of Udowski and Hoefs (1993) [10], the ion probe data would indicate a pH variation between 8.2 and 13.4 ± 0.3. Such very high pHs are not compatible with coral biology and are in disagreement with the pH range calculated from the $\delta^{11}B$ data (between 7.1 and 9.0). Thus the large range of micrometre scale $\delta^{18}O$ values cannot be only explained by pH variations in the ECF.

5.3. *Modelling of O isotopes fractionation during coral growth*

Large $\delta^{18}O$ variation in coral aragonite could be predicted if $CaCO_3$ precipitation occurs faster than O isotopic equilibration in the carbonate species-water system. While HCO_3^-–CO_3^{2-} couples have a quasi-instantaneous kinetic of isotopic equilibration, the exchange is slow between HCO_3^- (or CO_3^{2-}) and H_2O [6], constituting a limiting step. The two following reactions must be considered in order to calculate the rate of this isotopic equilibration:

$$CO_2 + H_2O \Leftrightarrow HCO_3^- + H^+ \quad \text{hydration} \qquad (1)$$

$$CO_2 + OH^- \Leftrightarrow HCO_3^- \quad \text{hydroxylation} \qquad (2)$$

For Eq. 1, the equilibrium constant is $k_{+1} = 0.037$ s^{-1} [30] and for the backward reaction, $k_{-1} = 2.66 \times 10^4$ kg/mol/s [29]. For Eq. 2, the equilibrium constant is $k_{+2} = 4.05 \times 10^3$ kg/mol/s [30] and $k_{-2} = 1.76 \times 10^{-4}$ s^{-1} [29]. All the equilibrium constants are calculated for a temperature of 25°C and seawater salinity of 35‰. As mentioned above, pH controls among others (i) the relative fractions of the three dissolved carbonate species (H_2CO_3, HCO_3^-, CO_3^{2-}) and also (ii) the respective roles of CO_2 hydration and CO_2 hydroxylation in the formation of HCO_3^- [30]. Isotopic mass balance implies that HCO_3^- is produced by hydration with an initial $\delta^{18}O$ of 27.5‰ and by hydroxylation with a $\delta^{18}O$ of 13.8‰ (Fig. 9), before reaching the oxygen isotopic equilibrium value at 34.3‰. In the pH range indicated by the $\delta^{11}B$ variations, the fractions of HCO_3^- produced by hydration and by hydroxylation are 95% and 5%, respectively, at pH 7 and 26% and 74% at pH 9. The time required for HCO_3^- to reach oxygen isotopic equilibration with H_2O (i.e. $\delta^{18}O_{HCO_3^-} = 34.3‰$) was calculated according to the approach previously described [6] and using the above mentioned equilibrium constants for

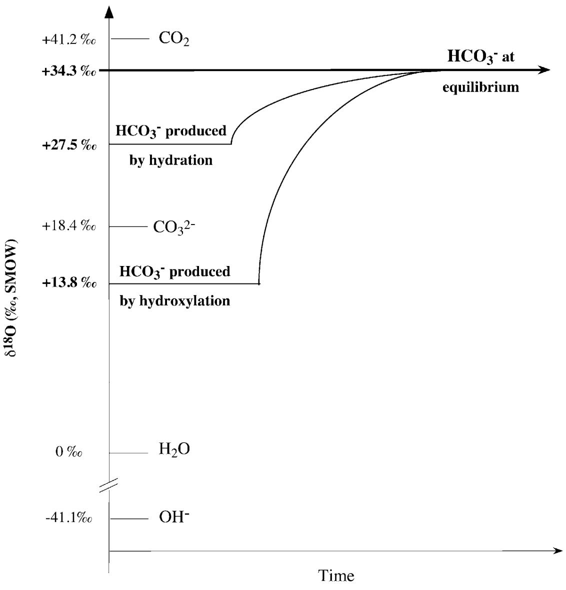

Fig. 9. Theoretical evolution of the oxygen isotopic composition of HCO_3^- in solution versus time. The $\delta^{18}O$ values (‰, SMOW) at equilibrium with H_2O ($\delta^{18}O = 0‰$) are shown for the different species contributing to the formation of HCO_3^- (at 19°C, except for CO_2 at 25°C). HCO_3^- is formed by hydration with a $\delta^{18}O$ of +27.5‰ (2/3 oxygen coming from CO_2 with $\delta^{18}O = 41.2‰$ [15] and 1/3 coming from H_2O with $\delta^{18}O = 0‰$) and by hydroxylation (2/3 oxygen coming from CO_2 with $\delta^{18}O = 41.2‰$ [15] and 1/3 coming from OH^- with $\delta^{18}O = -41.1‰$ [10]). The HCO_3^- formed by these two reactions is initially not at isotopic equilibrium with seawater and reaches its $\delta^{18}O$ equilibrium value (+34.3‰, [29]) after some time shown with no units (see text for detailed kinetics calculation).

reactions (1) and (2). Because the kinetics of hydration and hydroxylation are different, the rate of O isotopic equilibration of HCO_3^- with water is lower at high pH. The $\delta^{18}O$ of the carbonate was calculated for different times and different pH values from the relative proportions of HCO_3^- and CO_3^{2-} in the solution and their $\delta^{18}O$ assuming that they are in isotopic equilibrium, as their isotopic equilibration is quasi-instantaneous (reaction of deprotonation). This approach predicts the $\delta^{18}O$ and $\delta^{11}B$ values of coral carbonate for a given pH and a given residence time of dissolved carbonate in the calcifying fluid before being precipitated as $CaCO_3$. O isotopic equilibrium between carbonate and water [16] is reached for residence times higher than 12 h at pH 9 and 1 h at pH 7. All the present micrometre scale $\delta^{18}O$ values can thus be explained by this pH dependent rate of equilibration if the time required for calcification is ≈3 h for synapticulae and less than 12 h for trabeculae (Fig. 10). The occurrence of nearly instantaneous calcification is suggested by three data points (Fig. 10). Because C isotopic equilibration in solution occurs on the order of minutes [31], no significant micrometre scale $\delta^{13}C$ variations due to such a process are expected to accompany the $\delta^{18}O$ variations, in agreement with the present observations (Fig. 8b).

5.4. *Origin of vital effect*

The in situ $\delta^{11}B$ and $\delta^{18}O$ systematics in *Porites* coral demonstrate that the 'vital effect' on O isotopes is in fact mostly due to variations of the pH in the coral calcifying fluid and to different residence times of the dissolved carbonate species in the ECF before calcification. These pH variations control (i) the relative fractions of dissolved

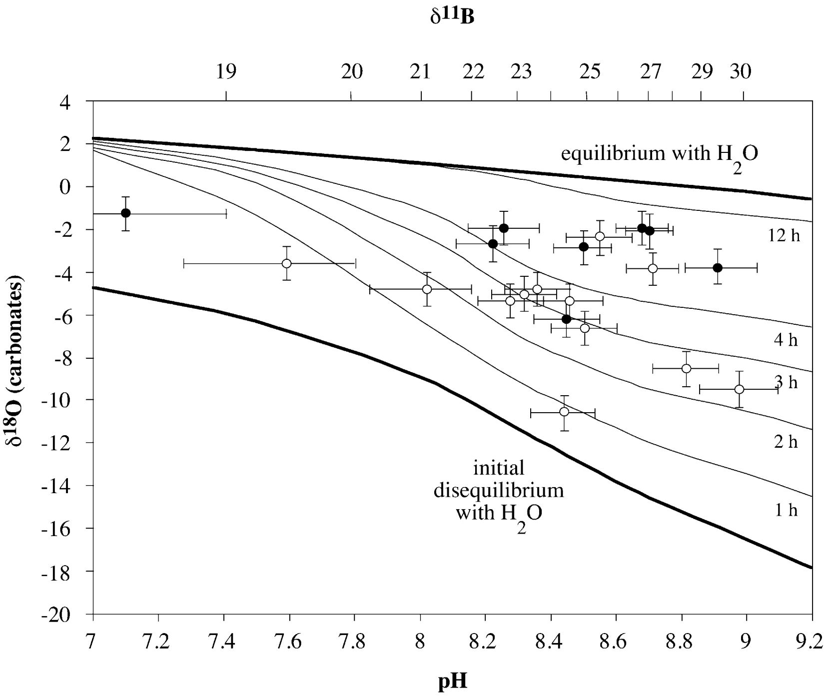

Fig. 10. $\delta^{18}O$ versus $\delta^{11}B$ micrometre scale variations for trabeculae (solid dots) and synapticulae (open dots) in *Porites* coral. The lines represent the evolution of $\delta^{18}O$ values versus pH, calculated at 25°C for O isotopic equilibration of dissolved carbonate species (HCO_3^- and CO_3^{2-}) with H_2O. The strong O isotopic disequilibrium, which is more salient for synapticulae and is primarily responsible for the so-called 'vital effect', can be explained if calcification occurs over time scales of ≈3 h. The calibration used to calculate pH from $\delta^{11}B$ values is from [23]. Using another calibration [24], it is calculated that calcification must occur over 4 h to reproduce the O isotopic disequilibrium observed in synapticulae. In both cases, calcification of trabeculae occurs in less than 12 h.

HCO_3^- and CO_3^{2-} in the ECF and (ii) the kinetics of their isotopic equilibration with water before carbonate precipitation. Thus variations in the photosynthetic activity of the symbiotic zooxanthellae, e.g. between day and night, are able to induce changes both in pH and in calcification rate, resulting in variable and out of equilibrium $\delta^{18}O$ values for coral aragonite. Trabeculae having, on average, higher $\delta^{18}O$ values than synapticulae, their growth rate must be slower. Such an interpretation is not in contradiction with the rather constant $\delta^{13}C$ values measured at the millimetre and micrometre scales. In fact these $\delta^{13}C$ values reflect the different proportions of dissolved inorganic carbon coming directly from seawater (25–30%) and of metabolic CO_2 coming from coral respiration (70–75%), these relative proportions being independent of conditions of illuminations [32]. The regulation of pH is maintained by the activity of Ca-ATPase, which controls the H^+ concentration in the ECF via exchange with Ca^{2+}. The role of the Ca-ATPase on the pH and on the $\delta^{18}O$ of the skeleton coral was also proposed by Adkins et al. (2003) [33] to explain the large range of oxygen isotopic variation (about 6‰) in deep-sea corals.

This oxygen isotopic systematic in corals is reminiscent of the observation of large micrometre scale variations in Sr/Ca ratios in zooxanthellate corals, which were not attributed to temperature changes of seawater, but rather to disequilibrium processes related to the symbiont activity of the algae, the disequilibrium being reduced at night [34]. This was also previously postulated to explain the ranges in C and O isotopic compositions found within single skeletal elements, e.g. theca, septum, columella and dissepiments [35]. Finally, parts of the coral skeleton with $\delta^{11}B$ values lower than ≈ 20‰ correspond to regions where calcification occurs at pHs below ≈ 8 and where O isotopic equilibrium between dissolved carbonate and H_2O is reached more rapidly. In these regions, the amplitude of carbonate oxygen isotopic disequilibrium is at a maximum of 8‰ whereas in regions with $\delta^{11}B$ values higher than ≈ 24‰ the amplitude of carbonate oxygen isotopic disequilibrium can theoretically reach 20‰ (Fig. 10). The present results show that the $\delta^{18}O$ micrometre scale variability is reduced to ±2‰, when regions of calcification occurring at pHs below 8 are chosen. Significant improvements in the precision of 'vital effect' calibrations and O-based palaeotemperature reconstructions are thus anticipated if these zones are analysed preferentially in the different coral species.

Finally, it is important to stress that the large oxygen isotopic variations observed at the micrometre scale disappear at larger scales. The oxygen isotopic variability at the millimetre scale represents an average of the micrometre scale variations modulated by other processes following lower frequencies. This is demonstrated by the monthly cycles in Fig. 5 or the temperature seasonal cycles in Fig. 3.

6. Conclusion

Ion microprobe technique offers the possibility of micrometre scale combined measurements of oxygen, carbon and boron isotopes. This study has shown the extreme variability at such a scale of coral $\delta^{18}O$ and $\delta^{11}B$ values. At the micrometre scale the coral $\delta^{11}B$ variations are proxies of pH variations at the sites of calcification. This pH range is from 7.1 to 9.0, in agreement with direct microsensors measurements [25–27]. Oxygen isotopes also respond to pH changes through the relative fractions of dissolved carbonate species in the calcifying fluid and through the kinetics of their isotopic equilibration with water before carbonate precipitation. Thus, pH variations at the sites of calcification are responsible for the 'vital effect' observed for O isotopic composition.

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