Correlation of boron isotopic composition with ultrastructure in the deep-sea coral *Lophelia pertusa*: Implications for biomineralization and paleo-pH

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[1] Using the CRPG-CNRS Cameca 1270 ion microprobe facility, we have measured boron isotopic compositions (^{11}B/^{10}B) in different ultrastructural components of the deep-sea aragonitic scleractinian coral *Lophelia pertusa*. We observe a systematic difference in B isotopic composition between the Early Mineralization Zone (EMZ) and adjacent fibrous skeleton. In EMZ the measured δ^{11}B values are consistently low. Fibrous aragonite is characterized by systematically higher δ^{11}B values but also displays B isotopic heterogeneity associated with specific growth bands in the calyx wall. The magnitude of the observed B isotopic variations cannot be explained by changes in environmental conditions and is likely caused by biological processes involved in the biomineralization of new skeleton, i.e., "vital" effects. The observed B isotopic variations are opposite to the predictions of geochemical models for vital effects. These models are based on the idea that stable isotopic fractionations (including C and O) in coral skeleton are driven by changes in pH of the fluid from which the skeleton is presumed to precipitate. Our data indicate that pH variations are not responsible for the observed stable isotopic fractionations. Geochemical models therefore do not provide an adequate framework within which to understand coral skeletal formation. Without a better understanding of these processes the use of B isotopic composition to reconstruct paleo-pH variations in the oceans must be considered problematic, at least as far as *Lophelia pertusa* is concerned.

1. Introduction

[2] Deep-sea scleractinian corals and their reef ecosystems have become a topic of increased research interest in recent years [*Malakoff*, 2003] for several reasons. From an ecological perspective, these coral reefs host a highly diverse population of organisms that have adapted to the extreme conditions of the deep ocean [*Bett*, 2001]. However, it has become apparent that deep-sea coral reefs are suffering great damage associated with bottom-trawling techniques of the modern fishing industry [*Grehan et al.*, 2005]. From a global climate perspective, the stable isotope and trace element compositions of deep-sea coral skeletons have been used to study large-scale dynamics of heat transport and circulation patterns of the deep ocean, with implications for long term climatic variability [*Adkins et al.*, 1998; *Schröder-Ritzrau et al.*, 2003]. These efforts require well-determined skeleton ages. Substantial effort is being put into improving dating techniques (e.g., ^{230}Th/U and ^{14}C) for the aragonitic carbonates produced by deep-sea corals [*Druffel et al.*, 1990; *Cheng et al.*, 2000; *Frank et al.*, 2004; *Roark et al.*, 2005]. Furthermore, because the deep ocean is a cold, dark and stable environment, where substantial changes take place on timescales of decades, centuries and millennia, the skeletons of deep-sea corals represent nearly perfect natural "laboratories" for studying "vital effects" in the isotopic and trace element composition of their skeletons. In the near absence of variation in physical (i.e., temperature) or chemical (i.e., salinity or seawater composition) parameters of the ambient environment, substantial variation in chemistry and isotopic composition of the skeleton on micrometer to millimeter length scales can only be ascribed to the effects of biological processes involved in biomineralization of new skeleton. Recently, high spatial resolution sampling techniques, such as micro-mill and Secondary Ion Mass Spectrometry (SIMS), have been employed in the study of C and O isotopic compositions in the skeletons of a variety of deep-sea coral species. These studies have documented dramatic "vital effects" and uncovered a systematic relationship between skeletal ultrastructure and stable isotopic composition [*Blamart et al.*, 2002, 2005; *Adkins et al.*, 2003; *Rollion-Bard et al.*, 2003a, 2003b; *Lutringer et al.*, 2005].

[3] The formation of new skeleton in deep-sea corals follows a universal two-step growth process that is also implied in skeleton formation among shallow-water, zooxanthellate corals [*Cuif and Dauphin*, 1998, 2005; *Cuif et al.*, 2003; *Stolarski and Mazur*, 2005; *Nothdurft and Webb*, 2007]. At the tips of the skeletal structures, the mineralizing cell layer produces centers of calcification (COC) or, equivalently, Early Mineralization Zone (EMZ). These EMZ are subsequently overgrown by fibrous aragonite consisting of cyclically added layers. For a variety of deep-sea corals, including *Lophelia sp.*, it has been demonstrated that the EMZ are characterized by systematically lighter C and O isotopic compositions compared with the fibrous aragonite part of the skeleton. Generally, the δ^{18}O and δ^{13}C values of the EMZ are 4–5‰ and 8–10‰ lower, respectively, than the adjacent fibrous aragonite [*Blamart et al.*, 2002; *Adkins et al.*, 2003; *Rollion-Bard et al.*, 2003b; *Lutringer*, 2005; *Lutringer et al.*, 2005]. Qualitatively and quantitatively similar observations have been made on zooxanthellate corals [*Rollion-Bard et al.*, 2003a; *Meibom et al.*, 2004, 2006] and such stable isotope effects seem to be common to scleractinian corals. Furthermore, the magnitudes of the measured stable isotopic variations are remarkable. Possible changes in environmental conditions, such as seawater temperature and/or salinity, cannot account for the observed stable isotope fractionations.

[4] A number of geochemical models have been proposed, in which this systematic stable isotopic difference between EMZ and fibrous aragonite is ascribed to a biologically induced variation in the pH of a proposed Extra-cytoplasmic Calcifying Fluid (ECF) reservoir that is assumed to exist between the surface of the growing skeleton and the calicoblastic cell-layer of the complex soft-tissue structure, that overlies the skeleton [e.g., *Adkins et al.*, 2003; *McConnaughey*, 2003; *Rollion-Bard et al.*, 2003a]. In these models, relatively high pH conditions during the formation of EMZ result in relatively light C and O isotopic compositions compared with adjacent fibers, which form under generally lower pH conditions. A direct test of such models would be possible if the B isotopic composition of EMZ and adjacent fibrous aragonite could be measured.

[5] In seawater, the total B concentration is relatively constant and B is present in the form of two aqueous species: $B(OH)_3$ and $B(OH)_4^-$. The B isotopic composition is fractionated by about 20‰ between these two aqueous species. Only $B(OH)_4^-$ is incorporated into carbonate minerals [*Hemming and Hanson*, 1992; *Hemming et al.*, 1995]. Because the relative abundances of $B(OH)_3$ and $B(OH)_4^-$ in a solution changes as a function of pH, the B isotopic compositions of each species also change to maintain a constant bulk B isotopic composition of the solution. Therefore, if $B(OH)_4^-$ is incorporated into the carbonate structure during precipitation from a solution, the B isotopic composition of the skeleton should reflect the pH conditions of this solution.

[6] Here we report ion microprobe $\delta^{11}B$ data for EMZ and adjacent fibrous aragonite in *Lophelia pertusa*, a deep-sea coral common in the North-East Atlantic Ocean. The data are discussed in context with existing geochemical models for isotopic vital effects and with the ongoing efforts to use B isotopic compositions of coral skeletons to reconstruct paleo-pCO$_2$ [e.g., *Pearson and Palmer*, 1999; *Reynaud et al.*, 2004; *Hönisch et al.*, 2004; *Hönisch and Hemming*, 2005; *Pagani et al.*, 2005].

2. Sample Description and Analytical Methods

[7] The *Lophelia pertusa* specimen used in this study is from top core MD 01-2454G, collected at 55°31′17″N and 15°39′08″W during the Geomound cruise (2001) at a water depth of 747 meters, where the mean annual bottom water

temperature is 8.5°C ± 1°C. It was selected from a large branch (10 cm length) with multiple living polyps. The *Lophelia* specimen was not coated by Fe and Mn oxide/hydroxides and did not exhibit any signs of diagenetic alteration or bio-erosion. The specimen was cleaned by ultrasonication in doubly distilled water in order to remove sediment particles. It was then dried in an oven at 50°C for 12 hours. Prior to further investigation, standard X-ray diffraction analyses were carried out on powdered fragments of the skeleton to detect any secondary precipitation of calcite; no calcite was observed.

[8] In preparation for ion microprobe analyses, a branch of the specimen was cut perpendicularly to the growth axis at the upper part of the calyx (Figure 1). The surface was polished, and microstructure was revealed by UV fluorescence and secondary electron microscope imaging of lightly etched (with 2% formic acid) surfaces. Prior to ion microprobe analysis, the surface was repolished.

[9] Boron isotopic analyses were carried out with the Cameca 1270 ion microprobe facility at CRPG/CNRS in Nancy, France. A primary beam of $^{16}O^-$ ions with an impact energy of 13 kV and an intensity of 70 nA was focused to a spot of approximately 15-μm wide and 30-μm long using Kohler illumination, which results in an homogeneous beam density and a flat-bottomed sputter-crater. The entrance slit was 250 μm, the field aperture was 5000 μm and the exit slit was 800 μm. Under these instrumental conditions, the mass resolving power (M/ΔM) is about 3000, which is sufficient to resolve any isobaric interference from $^{10}BH^+$ on $^{11}B^+$. During analyses, the energy window was fully opened, to optimize the acceptance of the ions by the spectrometer. Boron isotopes were analyzed in mono-collection mode using the central electron multiplier. Typical acquisition times were 60 cycles of 10 seconds on $^{10}B^+$ and 6 seconds on $^{11}B^+$. The background was measured on mass 9.6 for 2 seconds and found to be completely insignificant. The reference material was an inorganic aragonite crystal with a B concentration of 22 ppm and a $\delta^{11}B$ value of 21‰ relative to the NIST SRM 951 standard. Boron contents of coral skeleton sample were derived from the count rates of the standard corresponding to 22 ppm. Average external reproducibility, estimated from replicate measurements of the aragonite standard, was ±1‰ for boron isotopes. The internal error for $\delta^{11}B$ measurements on the coral was typically about ±0.5‰. Boron isotopic com-
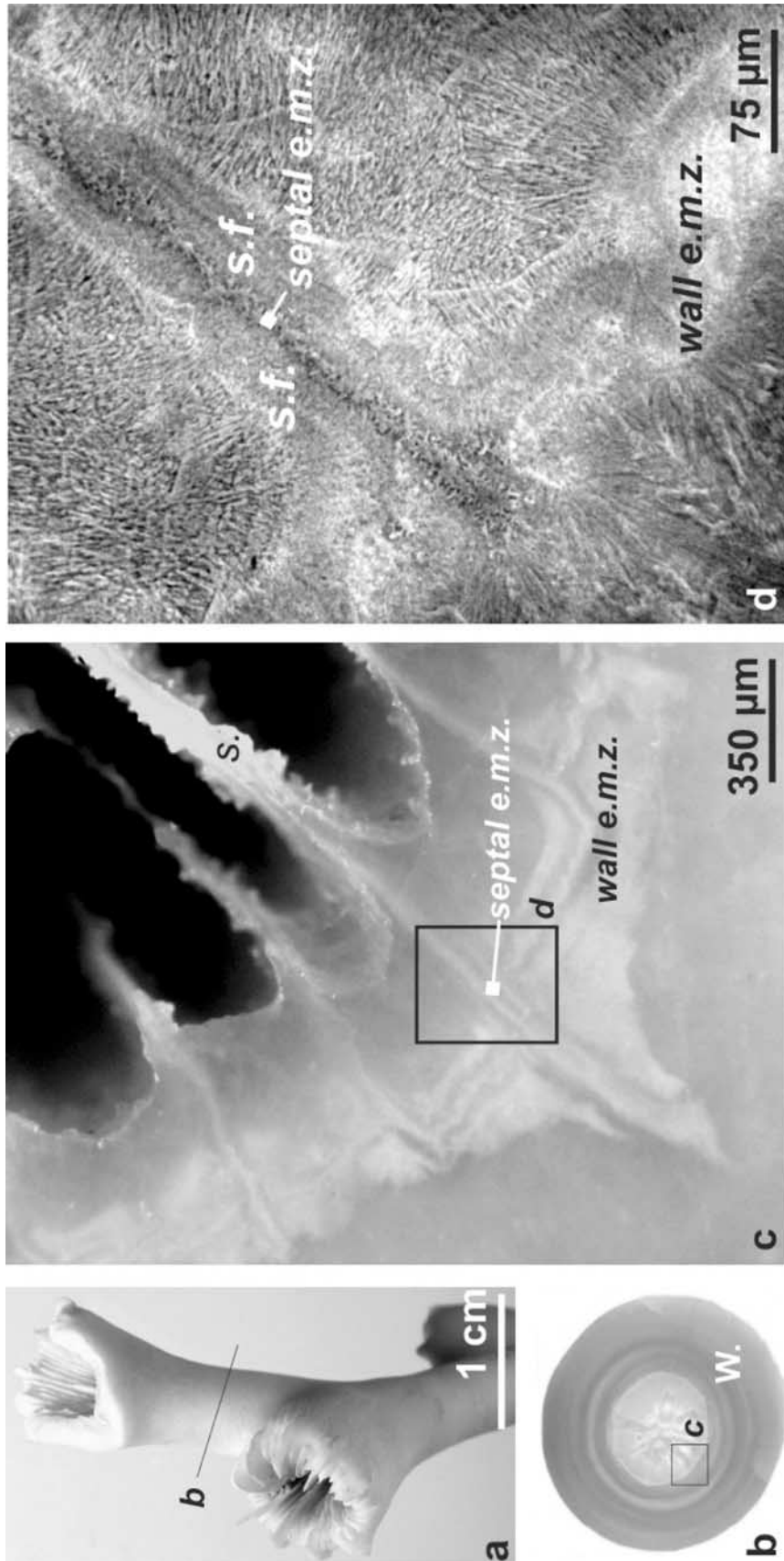
Figure 1. Optical and scanning electron microscope (SEM) images of the *Lophelia pertusa* sample used in this study. (a) Branch of a *Lophelia pertusa* colony. (b) Section perpendicular to growth axis, showing the typical thick walled calyx (w, wall). (c) Partial view (polished surface) of Figure 1b. The zones of early mineralization in the septa (*septal e.m.z.*) and within the wall are clearly visible. (d) SEM image of the intersection between the main septa and the wall. The 25–30 micrometer thick septal e.m.z. is visible, with septal fibers (s.f.) on both sides.

positions are reported in the δ-notation relative to the NIST SRM 951 standard.

$$\delta^{11}B_{sample}(‰) = \left(\left(^{11}B/^{10}B\right)_{sample} \middle/ \left(^{11}B/^{10}B\right)_{standard} - 1\right) \times 10^3$$

3. Results

[10] *Lophelia pertusa* is characterized by a well-organized ultrastructure (Figures 1a–1d). The distribution of EMZ (or COC) defines the macroscopic morphology or architecture of the calyx. The EMZ constitutes the central plane in each septum (Figures 1b, 1c, and 1d) and in the surrounding, circular calyx wall. In the septae, the EMZ is visible as a quasi-linear, or sometimes weakly undulating line of relatively bright aragonite (Figure 1d). The EMZ is surrounded by fibrous aragonite. In the very thick wall, which is typical for *Lophelia*, fibrous aragonite is formed on both sides of the nearly circular line of EMZ and exhibit alternating layers of dense (white) and less dense (grey) growth bands (Figure 1b).

[11] In order to facilitate orientation on the specimen and to create a geometric fix-point, a reference line was defined on the specimen. This reference line follows the essentially circular shape of the EMZ in the calyx wall (Figure 2). Ion microprobe analyses were performed in three profiles relative to this reference line. Two profiles (P1 and P3) start in the vicinity of the reference-line and proceed radially outward toward the outer edge of the wall. A third profile (P2) consists of 7 analyses along the reference line, in the EMZ and in the fibrous aragonite immediately adjacent to the EMZ. The data obtained along these profiles are shown in Figure 3 and listed in Table 1.

[12] From these analyses we make the following important observations:

[13] 1. The overall $\delta^{11}B$ variability recorded in our analyses of *Lophelia pertusa* is about 10‰: from 28‰ to 38‰. Such dramatic isotopic variations are obviously not related to changes in environmental parameters. Indeed the annual temperature and salinity fluctuations at the sampling site are 8.5 ± 1°C and 35.5 ± 0.2‰, respectively [e.g., *Freiwald*, 2002].

[14] 2. All analyses of the EMZ along the reference line are characterized by distinctly lower $\delta^{11}B$ values compared with adjacent fibrous aragonite skeleton. The measured difference in $\delta^{11}B$ between EMZ and fibrous aragonite is as large as 8‰ in P3.

Systematic $\delta^{11}B$ variations take place over length scales on the order of 100 micrometers.

[15] 3. About halfway in the profiles P1 and P3 (~600 μm from the reference line) a distinct dip in $\delta^{11}B$ values are observed in both profiles. This feature mimics the B isotopic composition of the EMZ and is associated with a relatively white band of dense aragonite in the wall. However, no EMZ structures are present in this part of the wall.

4. Discussion

[16] If the coral skeleton is precipitated from a supersaturated solution derived from seawater, one can convert the measured skeletal $\delta^{11}B$ values to pH according the equation [*Dickson*, 1990]:

$$\begin{aligned}pH = pK_B \\ - \log\left(\frac{\delta^{11}B_{sw} - \delta^{11}B_c}{\alpha_{3/4}^{-1}\delta^{11}B_c - \delta^{11}B_{sw} + 1000 \times \left(\alpha_{3/4}^{-1} - 1\right)}\right),\end{aligned}$$
(1)

where $\delta^{11}B_c$ represents the measured boron isotopic composition of the carbonate and $\delta^{11}B_{sw}$ is the boron total isotopic composition of modern seawater, which is assumed to be constant at a value of 39.5‰ relative to the NIST SRM 951 standard. pK_B is the dissociation constant of boric acid, and $\alpha_{3/4}$ is the isotopic fractionation factor between $B(OH)_3$ and $B(OH)_4^-$. Equation (1) assumes that changes in the B isotopic composition of $B(OH)_4^-$ is driven entirely by changes in the pH of the solution, i.e., that no additional (e.g., biologically driven) B isotopic fractionation occurs during coral calcification.

[17] Several values exist in the literature for $\alpha_{3/4}$. *Kakihanna et al.* [1977] determined a value of $\alpha_{3/4}$ = 0.981. Recently, *Oi* [2000] and *Pagani et al.* [2005] have re-evaluated $\alpha_{3/4}$ and propose values of 0.975 and 0.974, respectively. This range in $\alpha_{3/4}$ values introduces only limited uncertainty in the calculated pH values: about 0.2 pH units. The magnitude of the presumed pH variability recorded in the *Lophelia* skeleton according to equation (1) is much larger than this (see below). Therefore the results of applying equation (1) to the measured $\delta^{11}B$ values are insensitive to uncertainty in $\alpha_{3/4}$. However, for consistency, we used only the *Pagani et al.* [2005] value of $\alpha_{3/4}$ = 0.974. A pK_B value of 8.8 was calculated from the *Department of Energy* (*DOE*) [1994] equation, using a salinity of 35.5‰ and a temperature of 8.5°C.
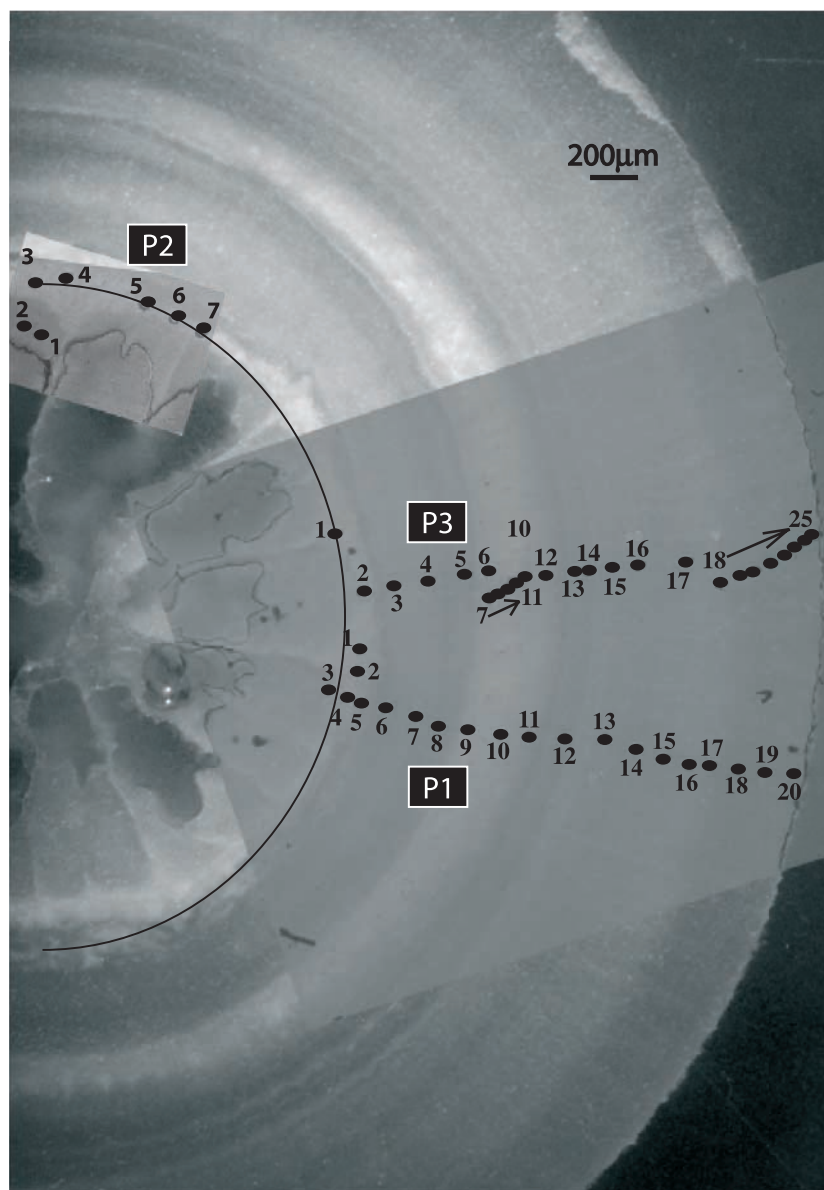
Figure 2. Reflected light microscopy image of the analyzed sample with the three profiles performed. Dark spots are ion microprobe craters, which are organized in three profiles (P1, P2, and P3) that run perpendicular to the reference line or parallel to the nearly circular EMZ in the calyx wall, respectively.

[18] Using these parameters in equation (1), the $\delta^{11}B$ data from *Lophelia* suggest pH variations spanning about 1.3 pH units, within the range 8.9 to 10.2 (Table 1). Taken at face value, the calculated pH during precipitation of the EMZ and the white growth-band in the calyx wall (at about 600 μm from the reference line) is lower, in the range from 8.9 to 9.3, compared with calculated pH values in the range of 9.4 and 10.2 for the adjacent fibrous aragonite (Table 1). These data have strong implications for geochemical modeling of stable isotope vital effects in coral skeleton and for efforts to reconstruct paleo-pH of the oceans.

4.1. Geochemical Models for Stable Isotopic Vital Effects in Coral Skeleton

[19] Existing models based on geochemical data or physiological experiments evoke several processes or mechanisms to depict the vital effects; for recent reviews, see *Swart and Leder* [1996], *Gattuso et al.* [1999], *McConnaughey* [2003] and *Allemand et al.* [2004]. Without exception, these models are based
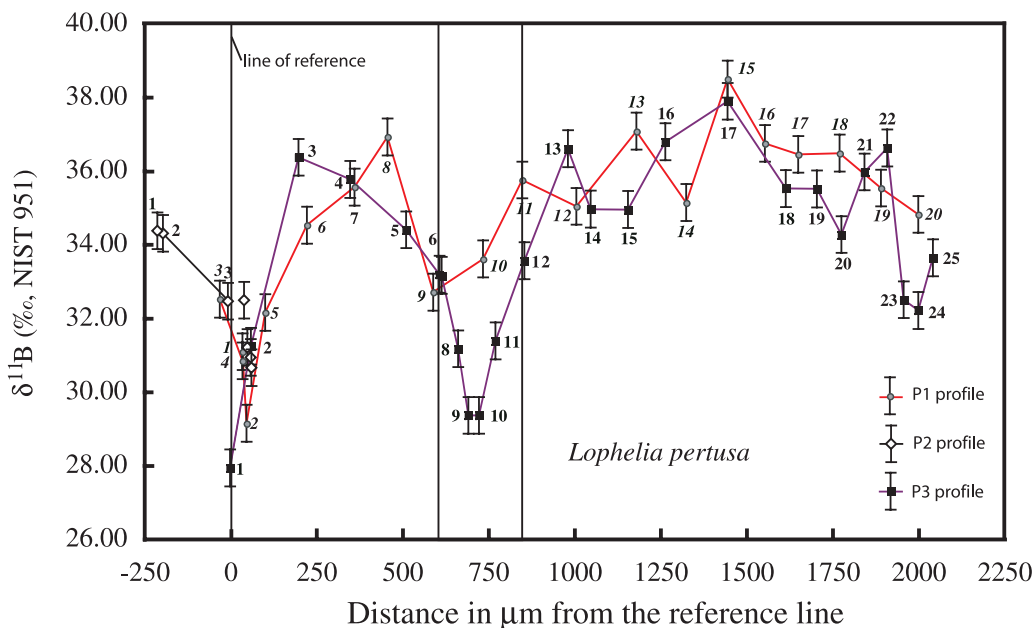
Figure 3. Measured δ^{11}B values plotted against their position relative to the reference line. See text for discussion.

on the concept of the Extra-cytoplasmic Calcifying Fluid (ECF), a hypothetical reservoir thought to exist between the surface of the growing skeleton and the calicoblastic cell-layer. From this ECF, aragonite is thought to form by simple super-saturation. Different processes have been invoked in order to explain the C and O isotopic systematics of coral skeleton. These models can be divided into two types: "kinetic" [*McConnaughey*, 1989] and "carbonate" [*Adkins et al.*, 2003; *Rollion-Bard et al.*, 2003a].

[20] In the "kinetic" model, the isotopic fractionations associated with the hydration of CO_2 are transferred to the skeleton if the precipitation rate is faster than the establishment of equilibrium between CO_2 and H_2O. Increasingly faster precipitation rates result in increasingly light (disequilibrium) C and O isotopic compositions of dissolved inorganic carbonate ions, from which the skeleton forms. High pH enhances carbonate super-saturation and increases the precipitation rate. Isotopically light skeleton is therefore precipitated under relatively high pH conditions.

[21] In the "carbonate" model (which follows a model developed by *Spero et al.* [1997] for foraminifera, based on early experimental observations by *McCrea* [1950]) variations in O isotopic composition result primarily from isotopic fractionation between CO_3^{2-}, and HCO_3^- in equilibrium with H_2O. As the relative proportions of CO_3^{2-} and

HCO_3^- change with pH, so does the O isotopic composition of the skeleton, which reflects the relative proportions of the dissolved carbonate species. Carbon isotopic fractionation is achieved during "membrane crossing" of CO_2 [*Adkins et al.*, 2003]. High pH conditions shift the C and O isotopic composition of the resulting skeleton to lighter values.

[22] These geochemical models have two fundamental aspects in common: (1) they are set within the conceptual framework of a hypothetical ECF, and (2) high pH conditions in the hypothetical ECF reservoir produce skeleton characterized by relatively light C and O isotopic compositions. Because the EMZ is systematically light in C and O isotopic compositions compared with the adjacent fibrous aragonite (inferring high pH), these geochemical models unambiguously predict that EMZ should be characterized by relatively heavy B isotopic compositions (see equation (1)).

[23] The exact opposite is observed (Figure 3). None of the geochemical models predict the observed low δ^{11}B values in EMZ relative to adjacent fibrous aragonite. In fact, this B isotope surprise raises the fundamental question of whether pH variations are indeed the cause of stable isotopic fractionations in coral skeletons, or whether completely different mechanisms are at play. It is tempting to speculate that in fact the observed, positively correlated B, C, and O isotopic fractio-

Table 1. Boron Isotopic Compositions of Carbonates, Distance From the Reference Line, Estimated Boron Concentration, and pH Values[a]

Profile/Point	Distance, μm	$\delta^{11}B$ Carbonates	B, ppm	pH
		Profile 1		
3	−30	32.51	74	9.27
1	36	30.83	71	9.14
4	36	31.08	71	9.16
2	48	29.14	53	9.02
5	102	32.15	77	9.24
6	222	34.52	98	9.46
7	361	35.55	106	9.58
8	457	36.92	102	9.79
9	590	32.70	84	9.28
10	734	33.60	83	9.37
11	849	35.74	109	9.60
12	1006	35.03	106	9.51
13	1180	37.07	106	9.82
14	1325	35.13	102	9.53
15	1445	38.49	100	10.22
16	1554	36.74	96	9.76
17	1650	36.44	95	9.71
18	1771	36.47	95	9.71
19	1891	35.53	95	9.57
20	1999	34.81	96	9.49
		Profile 2		
1	−228	34.30	100	9.43
2	−216	34.24	91	9.43
3	−12	32.39	73	9.26
4	12	31.17	67	9.16
5	24	32.46	74	9.26
6	0	30.85	65	9.14
7	0	30.54	64	9.12
		Profile 3		
1	0	27.92	100	8.94
2	60	31.23	91	9.17
3	198	36.36	73	9.69
4	349	35.76	67	9.61
5	512	34.39	74	9.44
6	608	33.18	77	9.33
7	614	33.15	76	9.32
8	662	31.16	75	9.16
9	692	29.35	77	9.03
10	722	29.35	74	9.03
11	771	31.37	102	9.18
12	855	33.55	108	9.36
13	981	36.59	111	9.73
14	1048	34.95	112	9.51
15	1156	34.94	114	9.50
16	1265	36.78	112	9.76
17	1445	37.88	109	10.01
18	1614	35.51	103	9.57
19	1704	35.50	104	9.57
20	1777	34.26	108	9.43
21	1843	35.96	106	9.63
22	1909	36.61	97	9.73
23	1957	32.49	100	9.27
24	1999	32.20	103	9.24
25	2042	33.63	101	9.37

Notes to Table 1:
   [a] Boron isotopic compositions of carbonates are in ‰ (NIST 951), distance from the reference line is in μm, estimated boron concentration is in ppm, and pH values are calculated from the $\delta^{11}B$ of carbonates with modern $\delta^{11}B$ seawater of 39.5‰, a fractionation factor $\alpha_{3/4}$ of 0.974 [*Pagani et al.*, 2005], and a pK_B value of 8.8 calculated using the *DOE* [1994] equation with a salinity of 35‰ and a temperature of 8.5°C.

nations are the sum of multiple fractionations arising from a variety of factors, amongst them, cellular, or inter-cellular, transport mechanisms. Whatever these processes are, an important constraint is that the associated isotopic fractionations should scale with the observations, which are ~8‰, ~10‰, and ~5‰, for B, C and O isotopes, respectively, between EMZ and fibrous aragonite [*Blamart et al.*, 2002; *Adkins et al.*, 2003; *Rollion-Bard et al.*, 2003b; *Lutringer et al.*, 2005; *Meibom et al.*, 2006]. The "two component" organization of coral skeletons indicated by both microstructural observations, localized chemical measurements and biochemical in situ characterizations provide a framework for explaining these observations [*Cuif and Dauphin*, 2005]. Models based on a fluid-filled space, the ECF, with chemical composition "close to seawater" obviously fail to explain the collective set of observation. The B isotope effects documented here provide additional evidence in support of the existence of two distinctly different ultrastructural domains in the coral skeletons.

4.2. B Isotopic Compositions of Coral Skeleton as Proxy for Paleo-pH Variations?

[24] Facing growing concerns about the rate and consequences of global climate change due to (among many factors) the emission of CO_2 to the atmosphere from fossil fuel burning, the capability to reconstruct the paleo-pH of the oceans, which is related to the partial pressure of CO_2 in the atmosphere, has become an important scientific goal [*Sanyal et al.*, 1995, 1997, 2001; *Pearson and Palmer*, 1999, 2000; *Lemarchand et al.*, 2000, 2002]. Atmospheric variations in pCO_2 can in principle be directly obtained from ice cores. Deep-sea scleractinian corals offer the potential of a complementary data set from the vast reservoir that is the deep ocean. The hope has been that, by combining the U/Th dates with $\delta^{11}B$ values of deep-sea corals it might be possible to derive a seawater pH value and then to reconstruct atmospheric pCO_2 variations with good temporal precision.

[25] However, the data presented in this study raise fundamental concerns about the use of B isotopic composition in coral skeleton as a reliable tracer of paleo-pH. First, as discussed above, it seems questionable to assume that the major control on skeletal $\delta^{11}B$ is the pH of the ambient seawater. Second, even if it is assumed that skeletal $\delta^{11}B$ values are primarily related to the pH conditions of the ambient seawater (e.g., according to equation (1)) our study raises concerns about the accuracy and precision to which skeletal B isotopic compositions can be determined from a skeleton characterized by substantial B isotopic heterogeneity (which is clearly unrelated to changes in environmental conditions). If the observed B isotopic heterogeneity was isolated to specific ultrastructural components, such as the EMZ, while the adjacent fibrous aragonite was essentially homogeneous in $\delta^{11}B$, simply avoiding the EMZ during sampling of the skeleton could potentially yield representative average skeletal B isotopic compositions. However, the B isotopic heterogeneity is more complicated than that (Figure 3). Even regions in the fibrous aragonite part of the skeleton, such as the white and relatively dense aragonite band in the calyx wall (around 700 μm in Figure 2), are characterized by significant B isotopic variation. Such heterogeneity makes it difficult to develop skeletal sampling techniques that might systematically yield representative $\delta^{11}B$ values with accuracy high enough to be useful for paleo-pH reconstructions.

[26] Until these two fundamental problems are resolved, we consider paleo-pH reconstructions based on coral skeletal B isotopic compositions to be problematic.

5. Conclusions

[27] We have measured B isotopic compositions in different ultrastructural components in the deep-sea scleractinian *Lophelia pertusa*. The overall $\delta^{11}B$ variability recorded in our analyses of *Lophelia pertusa* is about 10‰: from 28‰ to 38‰ (relative to the NIST SRM 951 standard). Such dramatic isotopic variations are obviously not related to changes in environmental parameters. We observe a systematic difference (up to 8‰) in the B isotopic composition of Early Mineralization Zone (EMZ) relative to adjacent fibrous aragonite skeleton. Our findings are directly opposite to the predictions of geochemical models for pH-induced "vital" isotope effects in coral skeleton and suggest that stable isotope fractionations for B, C and

O are not primarily driven by changes in pH of the solution from which the skeleton is assumed to form. This B isotope offset is now added to a growing number of biological, biochemical, stable isotopic and trace element studies documenting that EMZ is dramatically different from the fibrous aragonite skeleton [*Tambutté et al.*, 1996, 2007; *Cuif and Dauphin*, 1998; *Blamart et al.*, 2002, 2005; *Adkins et al.*, 2003; *Cuif et al.*, 2003; *Rollion-Bard et al.*, 2003b; *Allison et al.*, 2005; *Lutringer et al.*, 2005; *Meibom et al.*, 2006, 2007]. These observations raise serious doubts about the conceptual framework upon which geochemical models of calcification are based, in particular the concept of the ECF.

[28] It is now evident that an entirely new approach to coral skeletal "vital" effects is required if the underlying biological mechanisms are to be better understood. A systematic study of the B-isotopic variation in various biocarbonates, correlated with observations of skeletal microstructure and histological organization, is needed to increase the understanding of the B isotopic systematics. In absence of such work, the use of B isotopic composition of deep-sea coral skeletons to reconstruct paleo-pH variations of the oceans must be considered with caution.

Acknowledgments

References

Adkins, J. F., H. Cheng, E. A. Boyle, E. R. M. Druffel, and R. L. Edwards (1998), Deep-sea coral evidence for rapid change in ventilation of the deep North Atlantic 15,400 years ago, *Science*, *280*, 725–728.

Adkins, J. F., E. A. Boyle, W. B. Curry, and A. Lutringer (2003), Stable isotopes in deep-sea corals and a new mechanism for "vital effects,", *Geochim. Cosmochim. Acta*, *67*, 1129–1143.

Allemand, D., C. Ferrier-Pages, P. Furla, F. Houlbreque, S. Puverel, S. Reynaud, E. Tambutte, S. Tambutte, and D. Zoccola (2004), Biomineralisation in reef-building corals: From molecular mechanisms to environmental control, *C. R. Palevol.*, *3*(6–7), 453–467.

Allison, N., A. A. Finch, M. Newville, and S. R. Sutton (2005), Strontium in coral aragonite: 3. Sr coordination and geochemistry in relation to skeletal structure, *Geochim. Cosmochim. Acta*, 69, 3801–3811.

Bett, B. (2001), UK Atlantic margin environmental survey: Introduction and overview of bathyal benthic ecology, *Cont. Shelf Res.*, 21, 917–956.

Blamart, D., J.-P. Cuif, and A. Juillet-Leclerc (2002), O-stable isotopes distribution in deep-sea corals from SIMS measurements, Abstract EGS02-A-01713 presented at 27th General Assembly of the European Geophysical Society, Nice, France, 21 –26 April .

Blamart, D., C. Rollion-Bard, J.-P. Cuif, A. Juillet-Leclerc, A. Lutringer, T. C. E. van Weering, and J.-P. Henriet (2005), C and O isotopes in deep-sea coral (*Lophelia pertusa*) related to skeletal microstructure, in *Cold-Water Corals and Ecosystems*, edited by A. Freiwald and J. M. Roberts, pp. 1005–1020, Springer, Berlin.

Cheng, H., J. F. Adkins, R. L. Edwards, and E. A. Boyle (2000), U-Th dating of deep-sea corals, *Geochim. Cosmochim. Acta*, 64, 2401–2416.

Cuif, J. P., and Y. Dauphin (1998), Microstructural and physico-chemical characterisation of centres of calcification in septa of some scleractinian corals, *Paläontol. Z.*, 72, 257–270.

Cuif, J. P., and Y. Dauphin (2005), The environment recording unit in coral skeletons—A synthesis of structural and chemical evidences for a biochemically driven, stepping-growth process in fibres, *Biogeosciences*, 2, 61–73.

Cuif, J. P., Y. Dauphin, J. Doucet, M. Salome, and J. Susini (2003), XANES mapping of organic sulphate in three scleractinian coral skeletons, *Geochim. Cosmochim. Acta*, 67, 75–83.

Department of Energy (DOE) (1994), *Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide system in Seawater, Version 2*, ORNL/CDIAC-74, edited by A. G. Dickson and C. Goyet, Washington, D. C.

Dickson, A. G. (1990), Thermodynamics dissociation of boric acid in synthetic sea water from 273.15 to 298.15 K, *Deep Sea Res., Part A*, 37, 755–766.

Druffel, E. M. R., L. L. King, R. A. Belastock, and K. O. Buesseler (1990), Growth rate of a deep-sea coral using ^{210}Pb and other isotopes, *Geochim. Cosmochim. Acta*, 54, 1493–1500.

Frank, N., M. Paterne, L. K. Ayliffe, T. van Weering, J.-P. Henriet, and D. Blamart (2004), Eastern North Atlantic deep-sea corals: Tracing upper intermediate water Δ^{14}C during the Holocene, *Earth Planet. Sci. Lett.*, 219, 297–309.

Freiwald, A. (2002), Reef-forming cold-water corals, in *Ocean Margin Systems*, edited by G. Wefer et al., pp. 365–385, Springer, Berlin.

Gattuso, J.-P., D. Allemand, and M. Frankignoulle (1999), Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: A review of interactions and control by carbonate chemistry, *Am. Zool.*, 39, 160–183.

Grehan, A. J., V. Unnithan, K. Olu-Le Roy, and J. Opderbecke J (2005), Fishing impacts on Irish deep-water coral reefs: Making the case for coral conservation, in *Proceedings of the Symposium on Effects of Fishing Activities on Benthic Habitats: Linking Geology, Biology, Socioeconomics and Management*, Symp. 41, edited by P. Barnes and J. Thomas, pp. 819–832, Am. Fish. Soc., Bethesda, Md.

Hemming, N. G., and G. N. Hanson (1992), Boron isotopic composition and concentration in modern marine carbonates, *Geochim. Cosmochim. Acta*, 56, 537–543.

Hemming, N. G., R. J. Reeder, and G. N. Hanson (1995), Mineral-fluid partitioning and isotopic fractionation of boron in synthetic calcium carbonate, *Geochim. Cosmochim. Acta*, 59, 371–379.

Hönisch, B., and N. G. Hemming (2005), Surface ocean pH response to variations in pCO_2 through two full glacial cycles, *Earth Planet. Sci. Lett.*, 236, 305–314.

Hönisch, B., N. G. Hemming, A. G. Grottoli, A. Amat, G. N. Hanson, and J. Bijma (2004), Assessing scleractinian corals as recorders for paleo-pH: Empirical calibration and vital effects, *Geochim. Cosmochim. Acta*, 68, 3675–3685.

Kakihanna, H., M. Kotaka, S. Satoh, M. Nomura, and M. Okamoto (1977), Fundamental studies of the ion-exchange separation of boron isotopes, *Bull. Chem. Soc. Jpn.*, 50, 158–163.

Lemarchand, D., J. Gaillardet, É. Lewin, and C. J. Allègre (2000), The influence of rivers on marine boron isotopes and implications for reconstructing past ocean pH, *Nature*, 408, 951–954.

Lemarchand, D., J. Gaillardet, É. Lewin, and C. J. Allègre (2002), Boron isotope systematics in large rivers: Implications for the marine boron budget and paleo-pH reconstruction over the Cenozoic, *Chem. Geol.*, 190, 123–140.

Lutringer, A. (2005), Reconstruction de la variabilité des eaux intermédiaires par l'étude géochimique des coraux profonds, Ph.D. thesis, 280 pp., Univ. Paris-Sud XI, Orsay, France.

Lutringer, A., D. Blamart, N. Frank, and L. Labeyrie (2005), Paleotemperatures from deep-sea corals: Scale effects, in *Cold-Water Corals and Ecosciences*, edited by A. Freiwald and Roberts, pp. 1081–1096, Springer, Berlin.

Malakoff, D. (2003), Deep-sea science—Cool corals become hot topic, *Science*, 299, 195.

McConnaughey, T. (1989), ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: II. In vitro simulations of kinetic isotope effects, *Geochim. Cosmochim. Acta*, 53, 163–171.

McConnaughey, T. (2003), Sub-equilibrium oxygen-18 and carbon-13 levels in biological carbonates: Carbonate and kinetic models, *Coral Reefs*, 22, 316–327.

McCrea, J. M. (1950), On the isotopic chemistry of carbonates and a paleotemperature scale, *J. Chem. Phys.*, 18, 849–857.

Meibom, A., J.-P. Cuif, F. Hillion, B. R. Constantz, A. Juillet-Leclerc, Y. Dauphin, T. Watanabe, and R. B. Dunbar (2004), Distribution of magnesium in coral skeleton, *Geophys. Res. Lett.*, 31, L23306, doi:10.1029/2004GL021313.

Meibom, A., et al. (2006), Vital effects in coral skeletal composition display strict three-dimensional control, *Geophys. Res. Lett.*, 33, L11608, doi:10.1029/2006GL025968.

Meibom, A., S. Mostefaoui, J.-P. Cuif, Y. Dauphin, F. Houlbreque, R. Dunbar, and B. Constantz (2007), Biological forcing controls the chemistry of reef-building coral skeleton, *Geophys. Res. Lett.*, 34, L02601, doi:10.1029/2006GL028657.

Nothdurft, L. D., and G. E. Webb (2007), Microstructure of common reef-building coral genera Acropora, Pocillopora, Goniastrea and Porites: Constraints on spatial resolution in geochemical sampling, *Facies*, 53, 1–26, doi:10.1007/s10347-006-0090-0.

Oi, T. (2000), Ab initio molecular orbital calculations of reduced partition function ratios of polyboric acids and polyborate anions, *Z. Naturforsch. A*, 55, 623–628.

Pagani, M., D. Lemarchand, A. Spivack, and J. Gaillardet (2005), A critical evaluation of the boron isotope-pH proxy: The accuracy of ancient ocean pH estimates, *Geochim. Cosmochim. Acta*, 69, 953–961.

Pearson, P. N., and M. R. Palmer (1999), Middle Eocene seawater pH and atmospheric carbon dioxide concentrations, *Science*, *284*, 1824–1826.

Pearson, P. N., and M. R. Palmer (2000), Atmospheric carbon dioxide concentrations over the past 60 million years, *Nature*, *406*, 695–699.

Reynaud, S., N. G. Hemming, A. Juillet-Leclerc, and J.-P. Gattuso (2004), Effect of pCO_2 and temperature on the boron isotopic composition of the zooxanthellate coral *Acropora* sp., *Coral Reefs*, *23*, 539–546.

Roark, E. B., T. P. Guilderson, S. Flood-Page, R. B. Dunbar, B. L. Ingram, S. J. Fallon, and M. McCulloch (2005), Radiocarbon-based ages and growth rates of bamboo corals from the Gulf of Alaska, *Geophys. Res. Lett.*, *32*, L04606, doi:10.1029/2004GL021919.

Rollion-Bard, C., M. Chaussidon, and C. France-Lanord (2003a), pH control on oxygen isotopic composition of symbiotic corals, *Earth Planet. Sci. Lett.*, *215*, 275–288.

Rollion-Bard, C., D. Blamart, J.-P. Cuif, and A. Juillet-Leclerc (2003b), Microanalysis of C and O of azooxanthellate and zooxanthellate corals by ion microprobe, *Coral Reefs*, *22*, 405–415.

Sanyal, A., N. G. Hemming, G. N. Hanson, and W. S. Broecker (1995), Evidence for a higher pH in the glacial ocean from boron isotopes in foraminifera, *Nature*, *373*, 234–236.

Sanyal, A., N. G. Hemming, and W. S. Broecker (1997), Changes in pH in the eastern equatorial Pacific across stage 5–6 boundary based on boron isotopes in foraminifera, *Global Biogeochem. Cycles*, *11*, 125–133.

Sanyal, A., J. Bijma, H. Spero, and D. W. Lea (2001), Empirical relationship between pH and the boron isotopic composition of *Globigerinoides sacculifer*: Implications for the boron isotope paleo-pH proxy, *Paleoceanography*, *16*, 515–519.

Schröder-Ritzrau, A., A. Mangini, and M. Lomitschka (2003), Deep-sea corals evidence periodic reduced ventilation in the North Atlantic during the LGM/Holocene transition, *Earth Planet. Sci. Lett.*, *216*, 399–410.

Spero, H. J., J. Bijma, D. W. Lea, and B. E. Bemis (1997), Effect of seawater carbonate concentration on planktonic foraminiferal carbon and oxygen isotopes, *Nature*, *390*, 497–500.

Stolarski, J., and M. Mazur (2005), Nanostructure of biogenic versus abiogenic calcium carbonate crystals, *Acta Palaeontol. Polonica*, *50*(4), 847–865.

Swart, P. K., and J. J. Leder (1996), The utility of isotopic signatures in coral skeletons, *Paleontol. Soc. Pap.*, *1*, 249–291.

Tambutté, E., D. Allemand, E. Mueller, and J. Jaubert (1996), A compartmental approach to the mechanism of calcification in hermatypic corals, *J. Exp. Biol.*, *199*, 1029–1041.

Tambutté, E., D. Allemand, D. Zoccola, A. Meibom, S. Lotto, N. Caminiti, and S. Tambutté (2007), Observations of the tissue-skeleton interface in the scleractinian coral *Stylophora pistillata*, *Coral Reefs*, *26*(3), 517–529, doi:10.1007/s00338-007-0263-5.