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Variation of geochemical signals in coral skeletons: Environmental changes or biological processes?

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Abstract. Corals are widely distributed throughout a long stretch of geological time and can provide high-resolution histories of climatic variabilities in the tropics, which play a key role in understanding the Earth's climate system. Geochemical approaches to corals have been widely used for reconstructing palaeoclimates because the geochemistry of the skeleton is believed to vary as a function of several environmental conditions. However, large variations that cannot be ascribed to a single environmental factor have been observed among and/or within calibrations of coral-based proxies. Two main unsolved factors could lead to these large discrepancies: unexpected environmental changes in reefs and unknown biological processes occurring at coral biomineralization sites. In this review, we show the recent progress in dealing with this question by application of coral culture technique and micro analytical methods to skeletal geochemistry in corals and discuss on how the degree of geochemical variation could be affected by environmental changes and how by biological processes during the skeleton's calcification. The next challenge will be to perform high-resolution analysis on cultured corals growing under controlled and/or constant environmental conditions. Such efforts hold the promise of yielding important new insights into the various biomineralization processes that may affect the chemical and isotopic composition of the skeletons, with the goal of understanding how environmental changes express themselves in geochemical variability.

Key words: corals, culture technique, biomineralization, oxygen isotopes, trace elements, paleoclimates

Introduction

Corals have long drawn great interests due to their geological importance and usefulness. Reef-building corals are present today in the tropical and subtropical oceans and can be found in a significant fraction of the geologic record, with a range extending back to the middle Triassic. Their skeletons continuously grow up to one of the largest biological architectures, which supports a wealth of diverse life in nutrient-poor regions. Moreover, the phenomenon of global warming has given corals additional importance as powerful tools for high-resolution paleoclimatic reconstructions. Since the beginning of the industrial revolution, human activities have contributed to climatic variability. Knowledge of the causes and magnitude of past climate change is critical for assessment of the anthropogenic impact and its likely long-term effects. Reliable proxy records of palaeoclimate parameters have been identified: ice cores (Raynaud *et al.*, 1993), tree rings (Cook, 1995), seasonal snow (Thompson *et al.*, 1995), foraminifera (Spero and Williams, 1988; Bemis and Spero, 1998). There are, however, very few suitable proxy records for tropical seawater. Hermatypic scleractinian corals have great potential in this respect as they: (1) precipitate a calcium carbonate skeleton with growth bands that can be used as a chronological clock (Barnes and Lough, 1996), (2) are benthic organisms sometimes harboring a limited bathymetric distribution (Sheppard, 1982) and (3) are found back to 240 millions years ago (Chadwick-Furman, 1996). These characteristics explain why corals have been extensively used. However, such proxies need to be calibrated before their use. The great majority of calibrations have been carried out in the field. For example, paleothermometers have been calibrated by comparing temperature records with $\delta^{18}O$ (e.g., Weil *et al.*, 1981; Leder *et al.*, 1996; Wellington *et al.*, 1996), or

trace element ratios such as the Sr/Ca (e.g., McCulloch *et al.*, 1994; de Villiers *et al.*, 1995), Mg/Ca (e.g., Mitsugushi *et al.*, 1996) and U/Ca (e.g., Min *et al.*, 1995) ratios in Recent coral skeletons. The temperature signal inferred from each proxy is sometimes consistent (Beck *et al.*, 1992; McCulloch *et al.*, 1996), but there remains some degree of inconsistency in other cases (Cardinal, 1996; Boiseau and Juillet-Leclerc, 1997). This lack of uniformity among calibrations (Figure 1) was found even at the same locality where water chemistry is almost the same (Gagan *et al.*, 2000; Marshall and McCulloch, 2002; Watanabe *et al.*, 2002, 2003) and even among the same coral species growing under the same conditions, calibrations can show discrepancies among colonies (Allison, 1996; Wellington *et al.*, 1996; Linsley *et al.*, 1999; Watanabe *et al.*, 2003). These discrepancies can be partly explained by sampling resolution and contamination by different skeletal elements which have various geochemical signals (Figure 2). Recent advances of microanalytical methods and sampling techniques have overcome these problems, which attenuated geochemical signals in corals due to lower sampling resolution. However, two main unsolved problems still remain, unexpected environmental heterogeneities in reef environments (e.g., light intensity, photoperiod, nutrients, salinity, currents) and unknown biological processes involved in the mechanism of biomineralization. In order to establish accurate coral proxies for reconstructing palaeoclimates, it is crucial to understanding the exact factors controlling the geochemical signals in their skeletons. In this review, we show the recent progress made in application of coral culture technique and microanalytical methods to skeletal geochemistry in corals and discuss how the degree of geochemical variation could be affected by environmental changes and by biological processes during calcification.

Coral culture experiment

The calibrations for coral proxies are usually established by comparing coral geochemical signals with environmental changes in a study area. Recent studies of water chemistry in coral reefs reveal more complex and unexpected changes at local sites of coral growth (SST, Winter *et al.*, 1991; $\delta^{18}O$ in seawater, Juillet-Leclerc and Schmidt, 2001; Sr/Ca ratio in seawater, de Villiers, 1999; Stoll and Schrag, 1998).

Calibrations carried out in the laboratory under controlled conditions may be necessary to decipher the effect of each environmental factor used, either separately or in combination. Such an approach has been used successfully with foraminifera (e.g., Spero and Lea, 1993; Spero *et al.*, 1997) but difficulties encountered with culture techniques have precluded the development of experimental calibrations of proxy records in corals. We report here an experimental technique enabling the culture of corals under controlled conditions which allows accurate sampling of the skeleton deposited.

The experiment was conducted in the laboratory using colonies of branching zooxanthellate scleractinian corals, *Stylophora pistillata* and *Acropora* sp. (belonging, respectively, to the families Pocilloporidae and Acroporidae) and collected in the Gulf of Aqaba. Apexes (2 cm) of these parent colonies were cut and glued on glass slides using underwater epoxy (Devcon®), as described by Reynaud-Vaganay *et al.* (1999). The tanks were supplied with heated Mediterranean seawater pumped from a depth of 50 m. The seawater renewal rate was 5 times per day and the seawater was continuously mixed with a pump (6 l min^{-1}). Light was provided by metal halide lamps (400 W) on a 12:12 photoperiod. The temperature was controlled to within $\pm 0.1°C$.

At the end of the incubation period, the ring skeleton deposited on the glass slide was removed with a scalpel, the powder was dried overnight at room temperature and stored in glass containers pending analysis. Each sample was weighed and then ultrasonicated for one minute to reduce it to a fine powder. Following the treatment described by Boiseau and Juillet-Leclerc (1997), the skeletal powder was soaked in hydrogen peroxide for 12 h to eliminate the organic matter, filtered on Nucleopore polycarbonate membranes (0.4 μm), and dried at 40°C for 4 h.

For isotopic measurements, a subsample of 100 μg of aragonite powder was dissolved in 95% H_3PO_4 at 90°C (Craig, 1957). The CO_2 gas evolved was analyzed using a VG Optima mass spectrometer with a common acid bath. The data are expressed in the conventional delta notation relative to the Vienna Peedee Belemnite (V-PDB), through measurements of the NBS-19 standards.

Advantages of this technique

Coral nubbins (small pieces of branching colonies) are being increasingly used for measurements of physiological parameters, both in the field and in the laboratory (see Davies, 1995). Such biological material offers many advantages including the small size of the specimens and the possibility of studying several replicates of identical genetic signature (i.e., clones) from a single parent colony. Moreover, nubbins are free of encrusting and boring organisms. Cultured corals have been, however, seldom used to investigate the re-

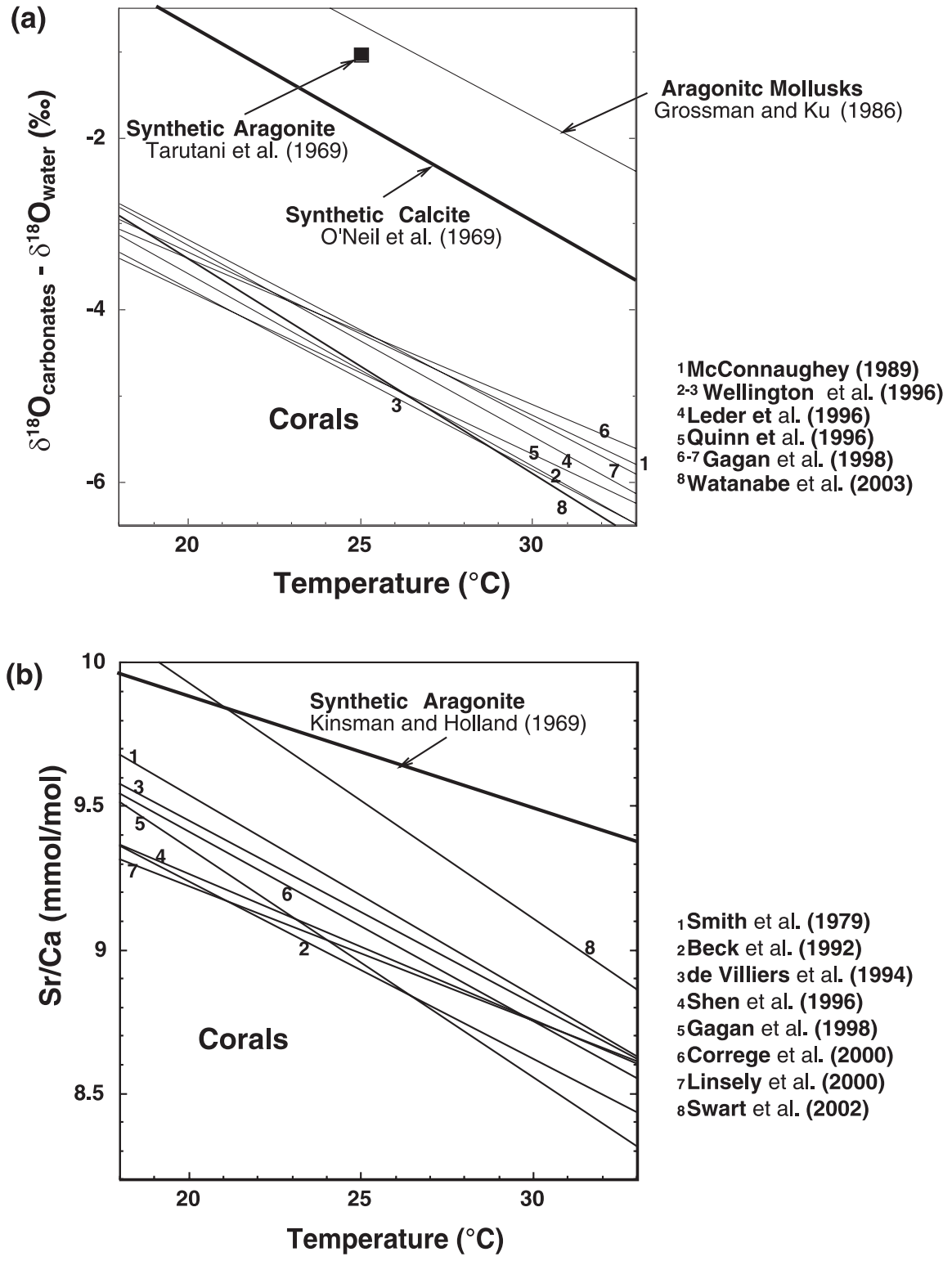

Figure 1. Diagrams showing the disagreement between different calibrations of SST vs. (a) the oxygen isotopic composition in *Porites lutea* and (b) the Sr/Ca ratio in corals. Each calibration has been calculated by comparing the geochemical measurement with the SST variability. Also shown are calibrations for synthetic aragonites ($\delta^{18}O$, Tarutani *et al.*, 1969; Sr/Ca ratio, Kinsman and Holland, 1969 and aragonitic molluscs by Grossman and Ku, 1986).

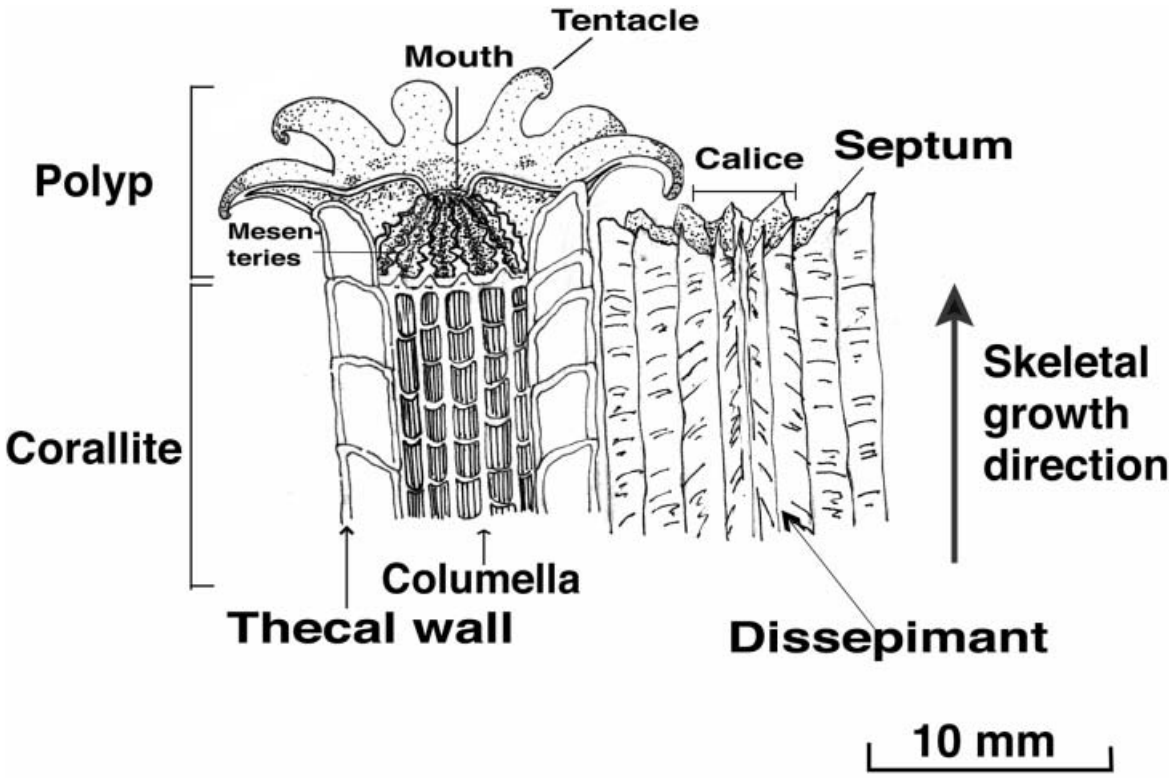

Figure 2. Schematic diagram of coral skeletons.

sponse of skeletal stable isotopic composition and trace element concentrations to changes in environmental parameters. The study of samples cultured under controlled conditions is the only way to investigate the effect of a single environmental parameter on the physiology and skeletal composition of scleractinian corals.

The coral culture technique on glass slides offers several advantages. Firstly, the samples are small (about 2 cm), which makes their manipulation easy and a large number of replicates available. Secondly, the experiments can be short (6–8 weeks) due to the rapid horizontal growth on the slide. Thirdly, sampling of the skeleton deposited during a known time interval is achieved with great accuracy (without any mixing with skeleton deposited previously). Fourthly, the sampled nubbins are not sacrificed and can be used again. This technique provides an unique opportunity to investigate the effect of environmental factors on the isotopic composition and trace elements concentration in coral skeleton and, therefore, to calibrate the proxies used to derive information on past tropical oceanic climate.

This culture technique has been used to investigate the effect of temperature, light, and nutrients on the stable isotopes composition and trace-elements concentration of coral skeleton (Reynaud-Vaganay *et al.*, 1999, 2001; Reynaud *et al.*, 2002).

Temperature

It has been 40 years since Keith and Weber (1965) documented the range of carbon and oxygen isotopic compositions in corals. By correlating the oxygen isotopic composition of bulk skeletal samples with the mean annual seawater temperature, Weber and Woodhead (1972) established that $\delta^{18}O$ in corals was a function of sea surface temperature. In order to calibrate the relationship between temperature and $\delta^{18}O$, we have cultured nubbins of coral at 5 temperatures (21, 23, 25, 27 and 29°C), and analyzed the skeleton deposited on the slide at each temperature (Reynaud-Vaganay *et al.*, 1999). The calibration equation between skeletal $\delta^{18}O$ and temperature obtained for *Acropora* sp. was $\delta^{18}O_{coral} = -0.27\ T°C + 5.35$ ($r^2 = 0.89$, N = 17, t-test; $p < 0.0005$) and that for *S. pistillata* was $\delta^{18}O_{coral} = -0.13\ T°C + 2.10$ ($r^2 = 0.22$, N = 43, t-test; $p = 0.002$) (Figure 3).

The slope obtained for *Acropora* sp. was very close to the one reported by Weber and Woodhead (1972; −0.28‰ $°C^{-1}$). Cornu (1995) investigated samples of

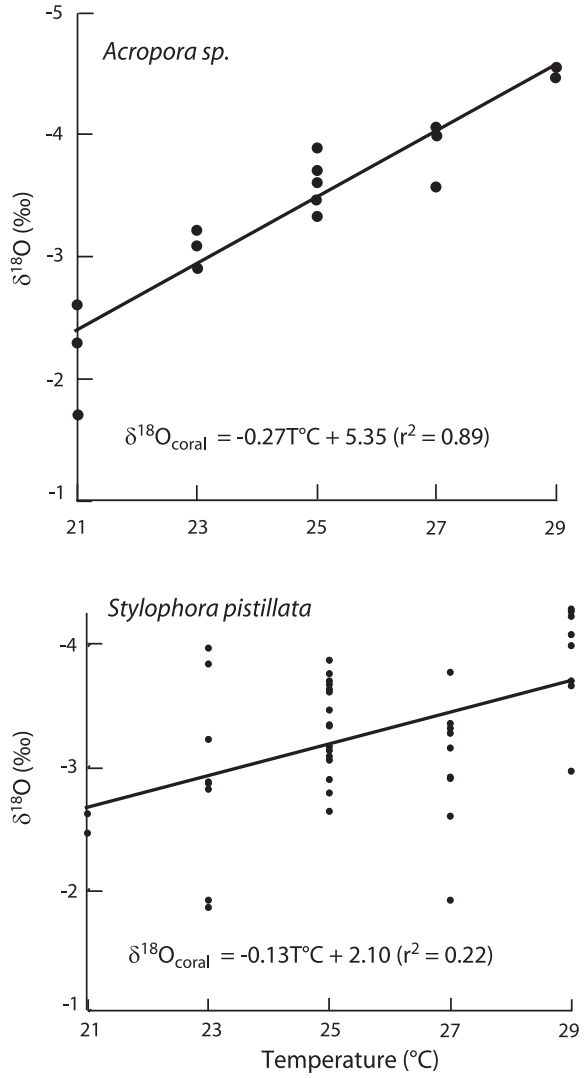

Figure 3. Skeletal $\delta^{18}O$ vs. temperature in coral samples of *Acropora* sp. and *S. pistillata*.

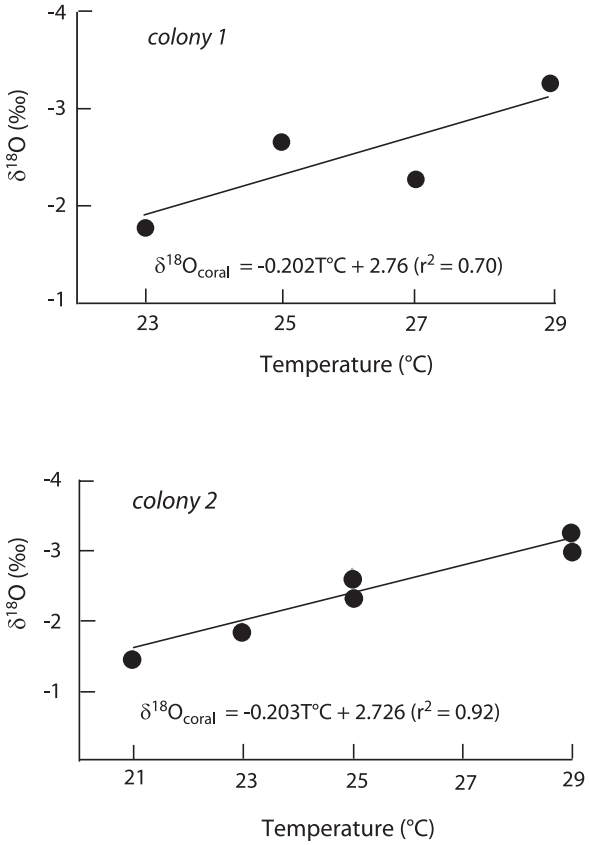

Figure 4. Skeletal $\delta^{18}O$ vs. temperature in nubbins of two different parent colonies of *S. pistillata*.

Acropora nobilis from two colonies in Mayotte (Indian Ocean) and reported slopes of −0.19 and −0.27‰ $°C^{-1}$. Juillet-Leclerc *et al.* (1997) have reported a slope of −0.15‰ $°C^{-1}$ for *Acropora formosa* (Great Barrier Reef, Australia). These authors suggested that such a low slope is the result of secondary aragonite deposition, which reduces the annual isotopic amplitude corresponding to the annual temperature difference.

The slope obtained with *S. pistillata* (−0.13‰ $°C^{-1}$) is low compared to the one reported by Weber and Woodhead (1972; −0.22‰ $°C^{-1}$), although the correlation coefficient is weak ($r^2 = 0.22$). It must be pointed out that several parent colonies have been used in this experiment, which could explain the large variability of the results. If we plot isotopic results only from a single parent colony, the slope is −0.20‰ $°C^{-1}$ (Figure 4).

Very recently, Suzuki *et al.* (2005) conducted a laboratory experiment in which they grew *Porites* sp. colonies in thermostated seawater between 21 and 29°C. They observed that oxygen isotope ratios displayed a large intercolony variability (1‰) for each culture temperature.

In our study, we found that $\delta^{13}C$ was also controlled by changes in seawater temperature ($\delta^{13}C = -0.17 \times T + 1.88$), which is similar to a result obtained for molluscs (Grossman and Ku, 1986; $\delta^{13}C_{mollusk-DIC} = -0.131\ T°C = 2.40$). The study of Bemis *et al.* (2000) on foraminifera also showed a correlation between these two parameters. On the other hand, Suzuki *et al.* (2005) did not find any correlation between coral skeletal $\delta^{13}C$ and temperature.

Light

It has been shown that skeletal $\delta^{13}C$ of corals can be affected by several parameters: light (e.g., Fairbanks

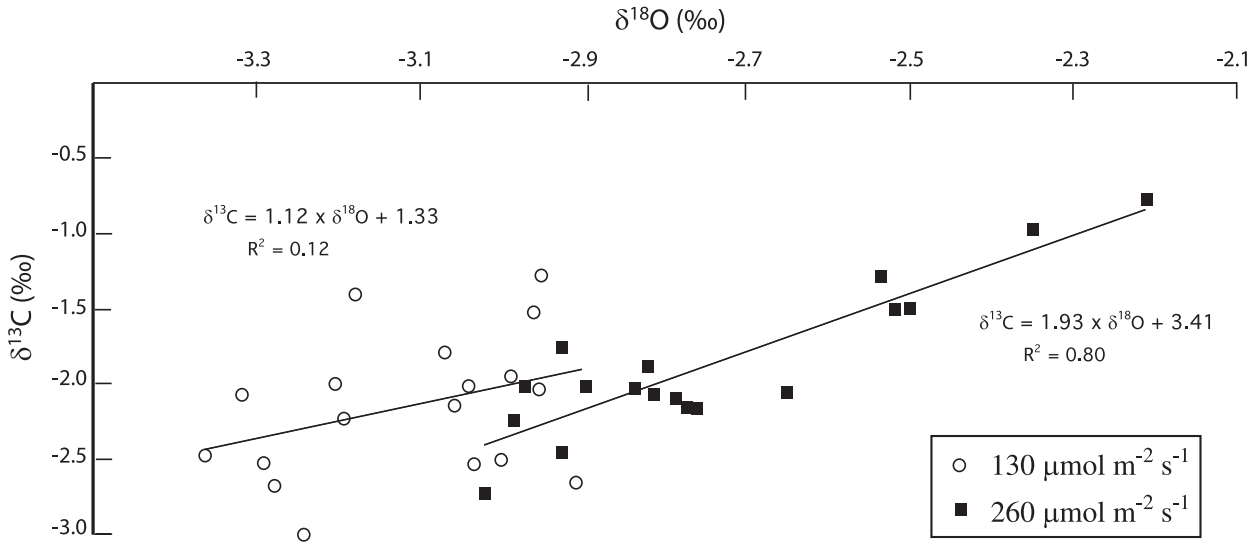

Figure 5. Skeletal $\delta^{13}C$ and $\delta^{18}O$ vs. light in samples of *Acropora* sp.

and Dodge, 1979; Juillet-Leclerc *et al*., 1997; Swart *et al*., 1996), seawater $\delta^{13}C_{DIC}$ (Nozaki *et al*., 1978; Swart *et al*., 1996), nutrition (Felis *et al*., 1998; Grottoli and Wellington, 1999), respiration (Swart *et al*., 1996), and spawning (Kramer *et al*., 1993; Gagan *et al*., 1994, 1996). To decipher the unique role of light on skeletal $\delta^{13}C$, we cultured corals nubbins under two different light levels: a light intensity of 130 and 260 μmol m^{-2} s^{-1} (referred to as low and high light, respectively), other environmental parameters remaining constant (Reynaud-Vaganay *et al.*, 2001).

The average skeletal $\delta^{13}C$ of *Acropora* sp. was lower under low light than under high light (−3.0 vs −2.7‰, ANOVA, $p = 0.04$), and was significantly correlated with the rate of calcification in both light treatments (Figure 5). Weil *et al.* (1981) investigated the effect of light on skeletal $\delta^{13}C$ in controlled conditions; they measured skeletal $\delta^{13}C$ values between −5.2 and −3.1‰ for *Pocillopora damicornis*, and between −3.2 and −0.4‰ in *Montipora verrucosa*. Only *Montipora verrucosa* showed a positive relationship between these two parameters.

The increase of skeletal $\delta^{13}C$ with increasing light observed in this study seems to support the model of Goreau (1977). There is an increased fixation of $^{12}CO_2$ by zooxanthellae during periods of high photosynthesis, leading to an increased concentration of $^{13}CO_2$ in the carbon pool which supplies dissolved inorganic carbon (DIC) for calcification. Hence, the skeleton deposited is ^{13}C enriched. This general model needs a revision to accommodate the finding that calcification and photosynthesis actually draw carbon from two reservoirs (seawater and metabolic DIC), and that respiratory CO_2 is suggested as the major source of DIC for calcification (Erez, 1978; Tanaka *et al.*, 1986; Furla *et al.*, 2000). Since photosynthesis is a rapid process, the diffusional pathway of HCO_3^- does not provide enough carbon to sustain photosynthesis (only 15%). Zooxanthellae must actively pump bicarbonate, leading to isotopic fractionation. It has been suggested that zooxanthallae preferentially fix ^{12}C-DIC in low light; the organic matter produced is therefore ''isotopically light''. Under high light conditions, zooxanthellar photosynthesis uses both ^{12}C- and ^{13}C-DIC, and the photosynthetic products catabolized by the coral are therefore heavier. $CaCO_3$ precipitation uses two different sources of carbon: coelenteric bicarbonate and metabolic CO_2. The diffusional pathway is unaffected by light variations, but this pathway represents only 30% of the total carbon into the skeleton (Furla *et al.*, 2000). 70% of the DIC used for calcification is metabolic CO_2 (Furla *et al.*, 2000), so, the skeleton deposited under high light is ''isotopically heavier''. On the other hand, in low light, the organic matter is respired and CO_2 released, so the $CaCO_3$ deposited is ^{13}C depleted.

Another result of this study is that skeletal $\delta^{13}C$ correlates with daily calcification rate: $\delta^{13}C$ increases when the calcification rate increases. Previous workers reached an opposite conclusion: rapid skeletal growth appeared to be associated with lower skeletal $\delta^{13}C$ values (e.g., Land *et al.*, 1975; McConnaughey, 1989). On the other hand, Erez (1978) and Swart *et al.* (1996) did not observe any correlation between skeletal $\delta^{13}C$

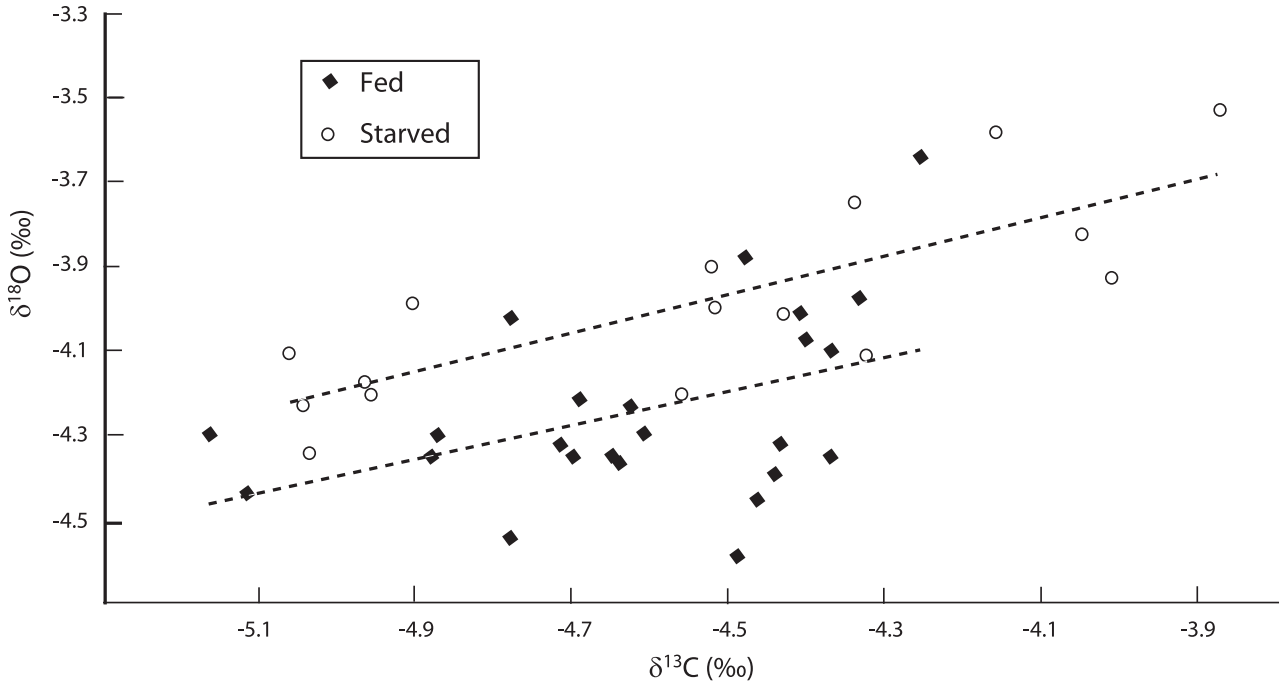

Figure 6. Skeletal $\delta^{13}C$ and $\delta^{18}O$ in fed and starved nubbins of *S. pistillata*.

and calcification rate. In our study, no correlation was found between skeletal $\delta^{13}C$ and other physiological parameters: net and gross photosynthesis (P_n and P_g, respectively), respiration (R), and the P_g/R ratio. In a previous study, Swart *et al.*, (1996) noted an inverse relationship between $\delta^{13}C$ of the coral skeletons and the P/R ratio. This correlation arises because of a slight positive association between $\delta^{13}C$ and respiration rate. But these authors did not observe any correlation between photosynthesis, calcification or extension and skeletal $\delta^{13}C$.

The fate of oxygen during metabolic and photosynthetic processes has received considerably less attention than that of carbon despite the fact that $\delta^{18}O$ is widely used for paleotemperature reconstruction. However, we have found that the average skeletal $\delta^{18}O$ of *Acropora* sp. was significantly lower under low light than under high light (−4.2 vs −3.8‰, ANOVA, $p < 0.0001$).

Feeding

Corals are known to flourish in oligotrophic tropical water, and can be considered as ''mixotrophic'' (both autotrophic and heterotrophic) organisms. They are able to fix inorganic carbon through the photosynthetic activity of their dinoflagellate symbionts, the zooxanthellae (Muscatine, 1990). They may also derive a fraction of their energy either through the predation of bacterioplankton (Sorokin, 1991; Ferrier-Pagès *et al.*, 1998) and zooplankton (Sorokin, 1991; Sebens *et al.*, 1996), or through the use of dissolved organic matter (Sorokin, 1973; Al-Moghrabi *et al.*, 1993). Such heterotrophic nutrition was suggested to be predominant in deep waters, where rates of photosynthesis are low (Muscatine *et al.*, 1989; Anthony and Fabricius, 2000).

Several processes have been shown to affect the skeletal $\delta^{13}C$: nutrition (Felis *et al.*, 1998; Grottoli and Wellington, 1999; Grottoli, 2000), respiration (Swart *et al.*, 1996) and coral spawning (Kramer *et al.*, 1993; Gagan *et al.*, 1994, 1996). It has also been suggested that foraminiferal $\delta^{13}C$ can be affected by the seawater pH (Spero *et al.*, 1997) and temperature (Bemis *et al.*, 2000). In our experiment, light, seawater temperature and pH, $\delta^{13}C_{DIC}$, and also the rate of respiration remained constant. No spawning event has been observed. Therefore, the skeletal $\delta^{13}C$ signal could only be altered by feeding (Reynaud *et al.*, 2002). ''Nubbins'' of *Stylophora pistillata* were separated into two sets. One was fed 3 times a week with *Artemia salina* nauplii, mixed with an Ultra Turrax, 3 times wk^{-1} for 12 wk. The other was maintained without any food (starved colonies).

In this study, no difference was measured in skeletal $\delta^{13}C$ between fed and starved colonies (average = −4.6‰, t-test, $p = 0.5$, $df = 37$) (Figure 6).

Previously, Grottoli and Wellington (1999) found that a reduction of zooplankton induced an increase in skeletal $\delta^{13}C$ of at least 0.5‰. This opposite result can be explained by the use of different coral species (*Pavona* sp. in Grottoli and Wellington, 1999), but this remains to be tested. Another explanation is that the $\delta^{13}C$ value of the *Artemia* prey (−12‰) was high compared to values measured in natural zooplankton (−20‰ in Land *et al.*, 1975 or −22‰ in Spero, 1992). The change induced by feeding might therefore have been too small to be detectable. Moreover, in our experiment, feeding also increases the calcification rate, which itself increases skeletal $\delta^{13}C$ (Reynaud-Vaganay *et al.*, 2001). Therefore, the effect of feeding on carbon fractionation may have been overwhelmed by the opposite effect of the calcification rate. Grottoli (2000) concluded that changes in light accounted for almost 80% of the variation in $\delta^{13}C$ in the coral skeleton. In the present experiment, light was constant, which could explain the lack of difference between fed and starved corals.

Only one study has investigated the relationship between skeletal $\delta^{18}O$ composition and nutrition (Grottoli and Wellington, 1999). These authors did not find any change in skeletal $\delta^{18}O$ with feeding, which disagrees with the results of our study, since the skeletal $\delta^{18}O$ of *Stylophora pistillata* was significantly lower in fed than in starved colonies (−4.24 vs. −4.05‰ respectively. t-test. $p < 0.001$). This difference may, however, partly result from an indirect effect of the calcification rate.

Biomineralization in corals

Even if the effect of environmental factors on coral geochemical signals were clear, another important possibility that could lead to a different calibration in the effect of different biological processes. To clearly understand the various aspects of coral biomineralization and the related properties of coral structures with respect to environment recording capabilities, we review here the skeletogenetic process.

Patterns of coral biomineralization, from overall scale to nanometer scale

In a study of the early calcification stages that occur after settlement of *Pocillopora damicornis* larvae, Vandermeulen and Watabe (1973) observed that early mineralization occurs on the whole outside surface of the basal ectoderm of the young polyps. The resulting subcircular mineral plate is made of nanograins, and 36 hours after larval settlement the mineralizing surface is reduced to radiating lines that build the embryonic septa. These very early septa are made only of ''small crystals or granules'' (Vandermeulen and Watabe, 1973). Then, a second mineralizing process is developed on both sides of the growing granular septa, reinforcing this initial framework. This second step produces fibres. According to Vandermeulen and Watabe (1973), the first indication of this additional fibrous skeleton occurs 72 hours after larval settlement.

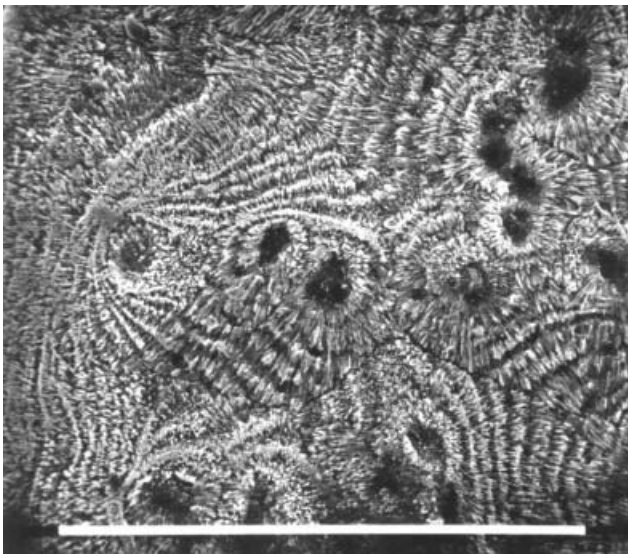

Figure 7. Microstructure of coral skeleton of *Porites* showing different sizes and shapes of the early mineralization zones (EMZ) and fibres. (Scale bar 100 μm)

It is now well known that the skeleton of every coral polyp is actually built by these two mineralizing domains: the early mineralization zones (EMZ) and the fibrous zone that persists throughout the life of the individual (Figure 7). From a historical standpoint the fibres that build the main part of coral skeletons were discovered by Pratz (1882) and the ''centres of calcification'' were initially defined by M, Ogilvie-Gordon as ''the points from which fibres diverge'' (Ogilvie, 1896). But the existence of centres of calcification as true biological domains has long remained doubtful. For instance Wells (1956) always used the quotation marks around the term. In the same way, when Gladfelder (1982) described the ''tiny crystals'' at the growing tips of the corallite of *Acropora cervicornis*, she never used the expression centres of calcification. Only recently has attention been drawn to the specific properties of these domains. In size, shape and chemical properties, they exhibit visible differences with respect to surrounding fibres (Cuif and Dauphin, 1998).

On adult corallites, centres of calcification observed at the tip of the septal structures are disposed in a

great variety of ways, and it has long been hypothesized that they could be potentially useful for high rank taxonomy and phylogenetical investigations. Results of a parallel study of molecular phylogeny and microstructural analysis carried out on more than 40 species (Cuif *et al.*, 2003) brought full support to this hypothesis. A striking correspondence has been established between the three-dimensional arrangement of these ''centres of calcification'' and the molecular phylogeny that results from 28S rRNA comparisons.

This suggests that, after formation of the continuous radial line of microgranular material in the larval stage, a first step in the reduction of the early mineralization zone (EMZ), this evolutionary process has continued. Evolutionary processes have broken the continuous radial lines into variously arranged clusters of mineralizing spots, whose arrangements reflect the major phylogenetic lineages. There is thus an absolute continuity between the granular mineralization that occurs a few hours after the larval settlement and the biomineralization mechanisms that produce the tiny granular crystals at the growing tips of adult corallites. Clearly, the inadequately defined and long disputed concept of ''centre of calcification'' should be replaced by the biologically grounded term of ''Early Mineralization Zones'' (EMZ).

The biochemically driven crystallization process and the stepping growth of fibres

In addition to the Vandermeulen and Watabe observations of granular and fibrous crystals as the two basic units of all coral skeletons, the fibrous tissues exhibit a very characteristic growth mode. By using various etching solutions on polished surfaces of coral skeletons, differences in sensitivity to etching within fibres are easily revealed. In addition, it appears that these zones of high and low solubility are clearly coordinated between fibres from a given corallite (Cuif and Dauphin, 1998). Obviously, growth of fibres is not a free-running process simply directed by crystal growth competition (Barnes, 1970), but a biologically driven process.

Explanation of the biological control on skeleton growth can probably be found by in-depth study of the relationship between the mineral and organic components of the coral skeletons. Presence of an organic matrix within the skeletons has long been demonstrated (e.g., Mitterer, 1978; Constantz and Weiner, 1988), but as pointed out by Johnston (1980), models that aim at explaining its role in skeletogenesis have been made in ''a complete ignorance'' of its localization with respect to the spatial arrangements of the skeleton units. In addition to the stepping growth of fibres demonstrated by chemical etching, mapping of organic compounds has been made on the same surfaces, demonstrating a striking correspondence between the two layered patterns (Cuif *et al.*, 2003). Clearly, the relationships between organic and mineral components have to be examined at the submicronic scale, because the few-microns-thick fibre growth units of fibres show an exact correspondance between mineral and organic distributions.

Biochemical mappings have also supported former observations (e.g., Goreau, 1959) that had provided the first evidences for a possible role of glucidic macromolecules in the coral biomineralization process. Owing to the extremely fine X-ray tuning and submicronic resolution of the synchrotron beam, it was possible to obtains *in situ* characterization of the status of sulfur. It was thus shown that sulfur was included in sulfated polysaccharides and not in combination with mineral ions (Cuif *et al.*, 2003). In addition, alcyan blue staining of isolated organic matrices after a two-dimensional electrophoresis also demonstrates the presence of both highly acidic and very heavy glucidic molecules within coral skeletons.

Additionally, thermogravimetric measurements including infrared study of the emitted gases during heating have shown the presence of a much higher organic content than previously suspected. Usually, organic material is admitted in the proportion of 0.1% of weight for coral skeletons. ATG measurements result in a good support of an estimate proposed by Cohen and McConnaughey (2003) ''in the range of 1% weight''. These ATG measurements allow a more precise understanding of the overall composition of the ''nonmineral part'' in skeletons of both symbiotic and deep-sea corals. In all cases, organic components appear to be hydrated. Of the 2.5% to 3% weight of the compounds that are decayed before the thermal degradation of aragonite, about 1% weight (of the total skeleton) is water. ATG diagrams clearly show that this water is structurally linked to organic compounds, because its appearance at a temperature of about 300°C is associated with the first degradation of organic material leading to CO_2 peaks (Cuif *et al.*, 2004)

With respect to the difference between EMZ and fibrous tissue, *in situ* mapping also emphasizes the structural and biochemical specificity previously observed. High concentration of organic matrices (both proteins and acidic polysaccharides) is always visible by both synchrotron radiation mapping and more conventional chemical staining (Cuif and Dauphin, 2005a). Obviously, a well differentiated biomineralization process occurs at the growing tips of the coral skeletons.

Evidence for a close relationships between organic

and mineral components at the submicrometer scale is now given by Atomic Force Microscopy (AFM). From the first pictures it was clearly visible that coral skeleton growth units are not purely mineral layers separated by organic membranes. To date, all skeletons have shown very comparable arrangements of nanograins embedded in a very interactive material (very high contrast in phase imaging; Figure 8). This is clearly linked to global properties of the interactive material such as elasticity, viscosity, etc., suggests that the weakly interactive nanograins are the mineral component whereas the interactive component is the ''nonmineral'', i.e., the hydrated proteoglycan assemblage. This hypothesis is also supported by the quantitative observation of the respective proportion and volumes occupied by grains and matrix, respectively.

Thus, far from being built of crystals freely developing in a fluid with a composition ''close to sea water'', as commonly claimed, the coral skeleton mineralization is a permanently controlled process, with the crystallization mechanisms involving a high proportion of specific macromolecules under the form of hydrated and sulfated acid proteoglycans that interplay with mineral ions at the submicronic scale.

Geochemical signals in coral microstructures

Geochemical features within coral microstructures are little known mainly because of the lack of resolution of analytical instruments, chemical detection being especially difficult within coral crystal units consisting of early mineralization zones (EMZ) (sub micrometer scale) and fibres (nanometer scale). Most recently, the development of microanalytical techniques such as the ion microprobe has enabled the detection of chemical variation in coral microstructures on the micro- to submicron scale. We discuss here geochemical variations corresponding with microstructures in coral skeletons from millimeter to nanometer scales.

Since the late 1990's, microanalytical methods such as LA ICP-MS (laser ablation inductively coupled plasma mass spectrometry) and SIMS (secondary ion mass spectrometry) have been applied to measurement of coral skeletons (Allison, 1996; Hart and Cohen, 1996; Sinclair *et al.*, 1998; Fallon *et al.*, 1999). The aim of these studies was to reconstruct paleoclimate with ultrahigh resolution. However, chemical profiles commonly showed large scatter. These large variations on the small spatial scale of coral skeletons were believed to be an analytical error caused by unsteady instrumental conditions. Using X-ray absorption near-edge structure spectroscopy (XANES) and extended x-ray absorption fine structure spectroscopy (EXAFS), Greegor *et al.*, (1997) observed that about 40% of the strontium existed as strontianite ($SrCO_3$). After this finding, the presence of strontianite domains was discussed as one of the possible causes for discrepancies among Sr/Ca thermometers. Recent works (Allison *et al.*, 2001; Finch *et al.*, 2003; Finch and Allison, 2003) reexamined this experiment of Greegor *et al.*, (1997) taking coral species commonly used for paleoclimatic studies. The results suggested that the occurrence of strontium as strontianite in coral skeletons is much smaller than that as a replacement for calcium in aragonite. Until now it has been thought that such chemical heterogeneity in coral skeletons was derived from skeletal microstructures and/or biological processes. With secondary ion mass spectrometry (SIMS), Cohen *et al.* (2001) analyzed the Sr/Ca ratio of both EMZ and fibres of *Porites* under the assumption that EMZ formed during the nighttime and fibres during the daytime. The results showed that the slope of the linear relationship between the Sr/Ca ratio in EMZ and temperature was close to that deduced from inorganic aragonite. In contrast, the calibration line between the Sr/Ca ratio in fibres and temperature was far from that for inorganic aragonite. They concluded that the Sr/Ca ratio was influenced by the biological activity of symbiotic algae during the daytime. Cohen *et al.* (2002) compared the Sr/Ca ratio between symbiotic and asymbiotic coral colonies of *Astrangia poculata*. The results also showed that symbiotic activity influences the accuracy of the Sr/Ca thermometer. Meibom *et al.* (2003) conducted the micro-scale Sr/Ca profiles along theca walls of *Porites* using an ion microprobe and detected an unexpected large variation corresponding to a temperature change of 14–16°C if coral Sr/Ca ratios are assumed to depend only on temperature (Figure 9). This variation could not be explained by local temperature and they proposed that it is due to metabolic changes synchronous with the lunar cycle.

A similar large unexpected variation was also found in coral $\delta^{18}O$ using SIMS measurements (Rollion-Bard *et al.*, 2003a, b). Their results showed that the variation of $\delta^{18}O$ of 50 μm spot analysis with SIMS was up to ten times larger than that for millimeter interval measurements with conventional acid digestion and isotope ratio mass spectrometry (Figure 9). The millimeter scale amplitude of $\delta^{18}O$ was equivalent to that expected from local temperature change. Since such a large $\delta^{18}O$ variation was also observed in that of the non-symbiont-bearing deep coral *Lophelia*, they proposed that the cause of this large variation was the rapid changes of pH in calcification site rather than the activity of symbiotic algae. To confirm this

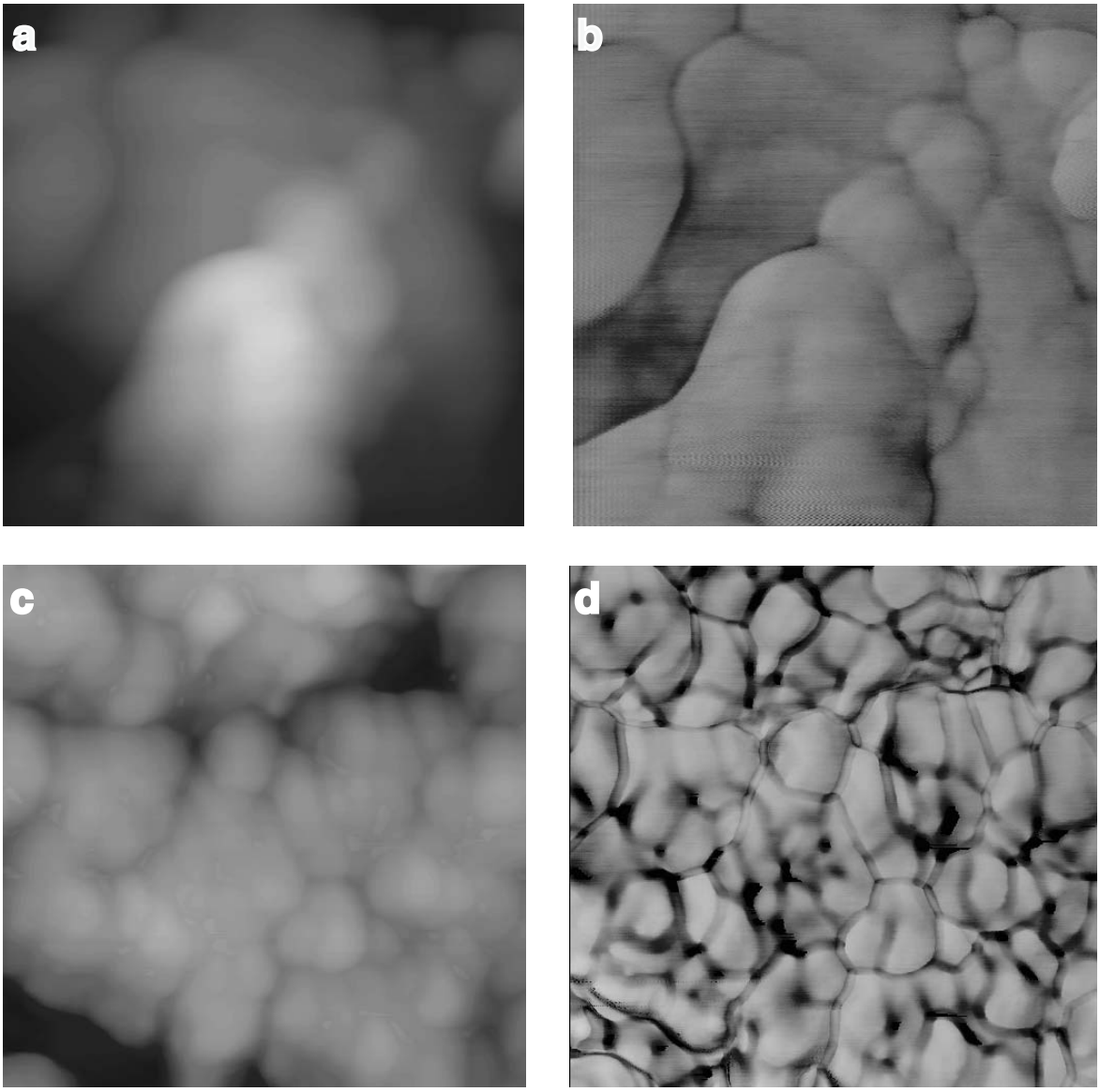

Figure 8. Nano scale microstructures of coral skeletons of *Porites australiensis* (a–b) and *Favia stelligera* (c–d) imaged with Atomic Force Microscopy (AFM). Pictures in the left column (a, c) represent topography and those in the right column (b, d) phase contrast, respectively (field view 500 nm).

hypothesis, they measured boron isotopes which reflected pH changes during coral aragonite precipitation and explained that the full range of $\delta^{18}O$ variation was modeled by taking into account the rate of O isotopic equilibrium between dissolved carbonate species (H_2CO_3, HCO_3^-, CO_3^{2-}). Based on this SIMS result for boron isotopes, they calculated that the range of pH changes during calcification was between 7.1 and 9.0 and that calcification took one to twelve hours.

How are geochemical signals controlled by environmental conditions and biological processes?

The important aim of these efforts introduced above is to better understand the isotopic and elemental fractionation mechanisms in order to improve the calibration between geochemical signals and environmental changes. The coral culture technique enables direct comparison between controlled environmental factors and chemical composition of coral skeletons. The results of culture experiments suggest that the de-

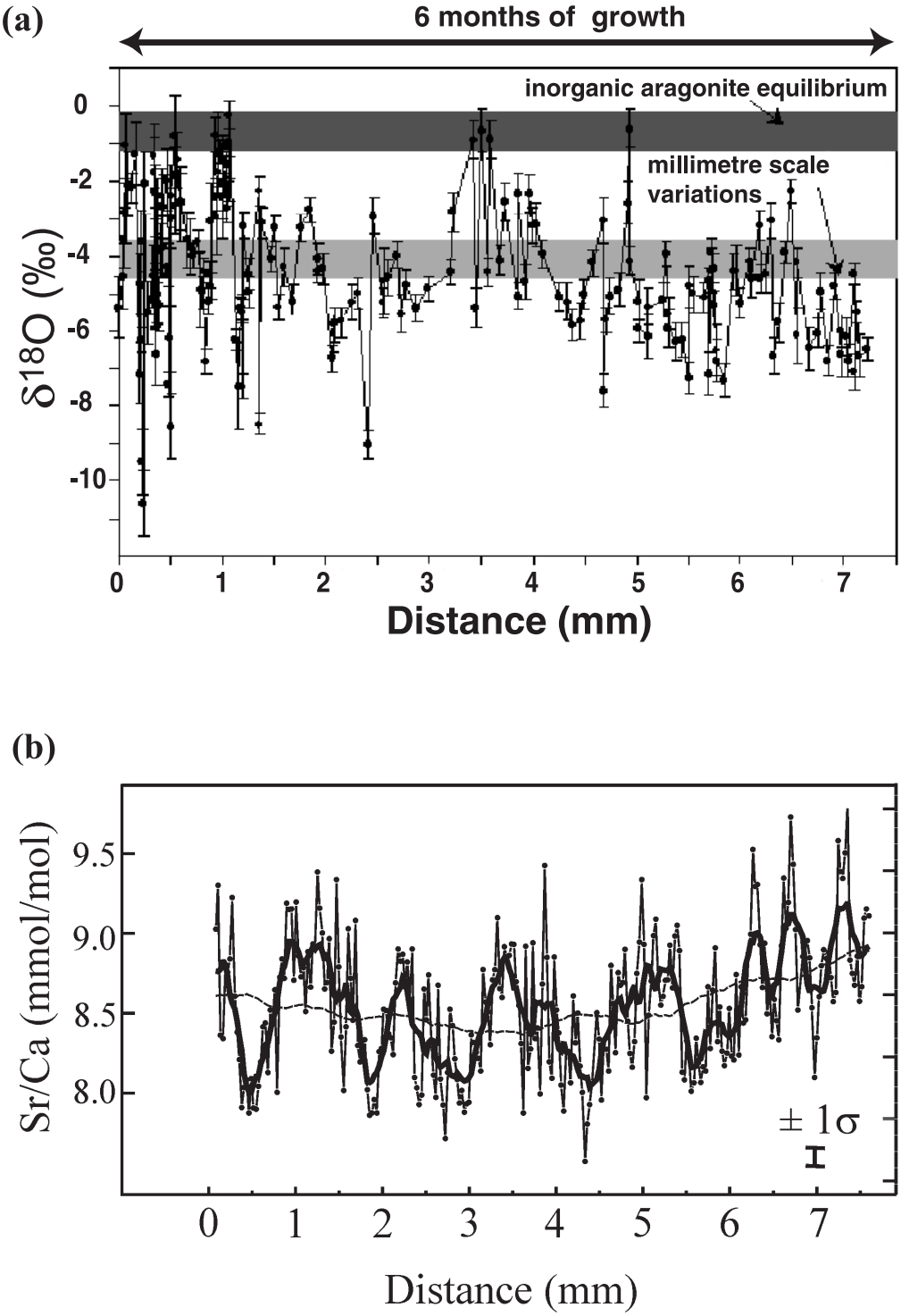

Figure 9. Micro-scale heterogeneity observed in the coral *Porites* of $\delta^{18}O$ (Rollion-Bard *et al.*, 2003a) and of Sr/Ca (Meibom *et al.*, 2003), measured by ion microprobe.

pendencies of geochemical signals on environmental changes are significant. In particular, temperature proxies such as $\delta^{18}O$ and Sr/Ca in corals could be the most faithful, although there still remains a large scatter among the calibrations even at controlled temperatures. They may be affected by additional factors such as the effect of light intensity on skeletal $\delta^{18}O$ (Reynaud-Vaganay *et al.*, 2001), the effect of Ca^{2+} concentration in seawater on Sr/Ca ratio (Ferrier-Pages *et al.*, 2002), and biological processes.

High-resolution sampling for chemical and isotopic measurements, mostly using secondary ion mass spectrometers, has now well established that chemical and isotopic fractionations in EMZ are significantly different from the equivalent properties in fibres (Cohen *et al.*, 2001; Adkins *et al.*, 2003; Rollion-Bard *et al.*, 2003a). Without a doubt two different crystallization mechanisms are running in EMZ and fibres, different enough to create such strong compositional differences that they cannot be interpreted by some global biological influence (i.e., photosynthesis).

The finding of nanometer-size grains in coral skeletal elements observed by AFM (Cuif and Dauphin, 2005a) suggests that we need higher-resolution analysis for full understanding of the coral biomineralization processes. At the moment, relevant experimental efforts are still somewhat scattered and limited in scope, but the development of new microanalytical techniques, such as the NanoSIMS, enables precise measurement of the recording process in the stepping growth layers and has opened the field of biomineralization to a new generation of studies. Future application of this newly constructed NanoSIMS ion microprobe on cultured coral specimens growing under controlled and/or constant environmental conditions will have the unique capability to measure high-precision oxygen isotopic compositions and other geochemical variations in coral skeletons at the scale of subnanometer to one micrometer, corresponding with the basic building blocks in the calcification unit. Such a microanalytical approach with combination of coral culture technique will provide important new information about the biomineralization process and help constrain the degree to which environmental and/or biological processes affect the geochemical signals in corals (as well as in biogenetic carbonates formed by other marine organisms).

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