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The build up of the isotopic signal in skeletons of the stony coral *Porites lutea*

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Abstract

The build up of the isotopic signal in corals was followed by sampling the newly formed skeleton at a monthly resolution for a period of two years in order to establish the interrelations between the calcification processes and the skeletal isotopic composition. We deployed two underwater sampling schemes, which provide a monitor of the changes in water temperature and $\delta^{18}O$ and in the corresponding newly accreted skeleton of undisturbed *Porites lutea* colonies under natural conditions and four transplanted colonies, which maintained the genetic identity throughout the experiment. The results indicate that $\delta^{18}O$ of the newly accreted skeleton does not correlate with ambient temperature although the seasonal temperature variability at the site (winter to summer) is in the order of 6 °C and $\delta^{18}O$ of seawater is constant throughout the year. In contrast to the newly formed surface skeleton, the isotopic compositions of the deep and older parts of the skeleton show the predicted annual isotopic pattern with highly significant correlation between $\delta^{18}O_s$ and SST. The transformation between temperature-independent to temperature-dependent isotopic signal occurs several months after the skeleton was formed at the surface. The position of the skeleton in relation to the open sea may generate the difference between $\delta^{18}O_s$ of the surface skeleton and that of the skeleton previously accreted further down the tissue layer. Our data support the general model of a multi-step skeletogenesis process, where the temperature independent skeleton is entails the first step, the production of skeletal scaffold, and the environmental temperature signature is captured by the next two other steps: the thickening and the periodic abrupt uplift occurring at the depth of the tissue layer. However, re-examination and development of the current isotopic models for coral calcification are required in order to explain the observed different temperature dependency during the growth's sequence.

1. INTRODUCTION

Isotopic compositions and trace element contents of aragonite skeletons of tropical scleractinian corals are determined in a complex way by seawater characteristics at the time of deposition. Hence, banded corals provide high resolution archives which store past climatic information and are extensively used to reconstruct past environmental conditions in the tropics, a major component of the earth's climate system that exerts a strong influence on climate variability worldwide. In particular, oxygen isotopic composition ($\delta^{18}O$) of the banded skeleton tracks temperature and salinity histories of both modern (Cole et al., 1993; Dunbar et al., 1994; Quinn et al., 1998; Felis et al., 2009) and ancient (Fairbanks and Matthews, 1978; Beck et al., 1997; Gagan et al., 1998; Hendy et al., 2002; Cobb et al., 2003; Correge et al., 2004; Ault et al., 2009; Wu and Grottoli, 2010) oceans due to the observation that skeletal $\delta^{18}O$ varies as a function of sea surface temperatures (SST) and the isotopic composition of seawater. However, calibration studies usually fail to produce the full range of

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the local environmental variables; different slopes, relating $\delta^{18}O$ to SST, are assigned for different regions of the ocean for the same coral species (Leder et al., 1996; Gagan et al., 1998; Guilderson and Schrag, 1999; Stephans et al., 2004). These discrepancies and the reduced sensitivity of the coral thermometer are commonly attributed to the "vital effect" (Urey et al., 1951) that results from kinetic fractionation and differences in metabolism (McConnaughey, 1989a,b) or in pH and carbonate ion concentration at the deposition site (Spero et al., 1997; Zeebe, 1999; Adkins et al., 2003; Suzuki et al., 2005). Local heterogeneity in water characteristics at the sampling sites and the large isotopic variability within the ultra-structural features of the skeleton (Rollion-Bard et al., 2003; Meibom et al., 2006) may also contribute to mask the signal.

In scleractinian corals, morphological and functional models of calcification, usually describe the presence of an extracellular skeleton located at the base of the tissue. In non-perforate corals this morphology includes an oral epidermal layer that is juxtaposed to seawater and aboral calicobalstic tissue layer (of ectodermal origin) apposed to and secreting the calcium carbonate skeleton. In perforate corals, such as *Porites*, the calicoblastic tissue penetrates the massive skeleton down to a depth of several millimeters (Barnes and Lough, 1992). Thus the skeletal elements are believed to provide a good recording of both environmental and physiological signals (Barnes and Lough 1993; Swart et al., 2010). Indeed, Barnes and Lough (1993) suggested that the density band formation of *Porites* spp. depends on extrinsic and intrinsic processes, with three growth phases. The first growth phase occurs when a scaffold is formed upon which there is further calcification. In the second phase, the skeletal scaffold created at the outer surface is thickened throughout its depth by the tissues that are apposed to this newly formed skeleton (i.e. formed over the last 4–13 months). In the third phase, the density pattern can be modified by periodic and abrupt uplifting of the lower margin of the tissue layer, a process that occurs about every 30 days. Taylor et al. (1993, 1995) estimated that each part of the first two steps of growth, the scaffolding and thickening, contribute 50% each to the total skeleton. It is important to note that calcification processes that add new $CaCO_3$ crystals on the old crystals are not limited to a two-dimension field on the coral surface, but take place in a three-dimensional space and throughout the depth of the living tissue layer. Consequently, skeletal material that is produced in the chemical physical and biological conditions of the deep tissue layer may be accreted onto a portion of the skeleton that was created earlier, under a different set of conditions at the surface of the colony.

We followed the build up of the isotopic signal in order to establish the interrelations of the calcification processes with the isotopic composition by sampling the newly formed skeleton at a monthly resolution. We deployed two underwater sampling schemes, which provided a monitor of the ambient seawater temperature, its $\delta^{18}O$ and the corresponding isotopic composition of the skeletal material over a period of two years. The first part involves sampling of the newly accreted skeleton of undisturbed coral colonies under natural conditions. The second part involves transplanting and splitting four *Porites lutea* colonies, retrieving a sample for examination each month. This enabled us to assess genetically identical colonies along the time period of this study in order to maintain the genetic identity throughout the experiment. The combination of the two experiments gives a full representation of the isotopic signal build up in the surface of the coral.

2. MATERIALS AND METHODS

The study site is located at the northern tip of the Gulf of Aqaba (Eilat-29°30.05′N, 34°55′E). The fringing reefs in the region are highly diverse (Loya, 1972) and are situated near the northern limit of reef building by stony (scleractinian) corals. The Gulf of Aqaba is surrounded by desert, with negligible precipitation and runoff. The mean net evaporation (~350 cm/year) greatly exceeds precipitation (~3 cm/year; Genin et al., 1995) and is the main driving force of the Gulf circulation. The excess of evaporation over precipitation causes a northward surface inflow from the Red-Sea through the shallow Straits of Tiran and a southward deep outflow back to the Red Sea. Two seasons characterize the Gulf of Aqaba, winter (December–April) and summer (May–November). The mean seasonal SST amplitude is 6 °C at the study site, with a minimal value during February (21 °C) and a maximal value in August (27 °C) (Paldor and Anati, 1979). The vertical stratification is weak and is driven mainly by temperature rather than salinity variations (Berman et al., 2000). The heating of surface water mass during summer causes stratification of the water column with the main thermocline at a depth of about 200 m. Winter cooling of the surface water mass results in deep water mixing that can reach up to 800 m (Genin et al., 1995).

The sampling scheme employed in this study consisted of two phases during which local water temperature, $\delta^{18}O$ of seawater and $\delta^{13}C$ of DIC were measured concurrently with measurements of $\delta^{18}O$ and $\delta^{13}C$ of the newly accreted skeleton.

During one year, between June 2001 and June 2002, we retrieved coral fragments from the surface of arbitrarily chosen *in situ Porites lutea* colonies, from 6 and 40 m depths. Four fragments of *P. lutea* colonies were collected every month using chisel and hammer. The same colony was not revisited in ensuing months in order to avoid sampling of stressed corals. These samples were used to obtain the surface skeleton, the most recent skeleton that had been produced by the coral during the preceding month.

The second phase was designed to compare between and within genetically identical colonies, as some variance of the previous results might have been affected by inter-colony isotope diversity. On January 2005 four colonies of the coral *P. lutea* were collected by SCUBA from a depth of 6 m from the northern tip of the Gulf of Eilat. Prior to collection, each colony was stained *in situ* with a 20 ppm solution of Alizarin red-S for 24 h. This allowed us to ascertain the skeletal layer above which there was newly accreted skeleton. Each colony was then cored using underwater hydraulic drill (Ingersoll-Rand 7803R) into 30–40 plugs, 4 cm in diameter and 5 cm in height. The coral plugs were glued

onto marble bases and placed for acclimation on a platform at 6 m depth for a period of 3 months. Every 1–3 month, from March 2005 to December 2006, one plug from each of the four genotypes was retrieved randomly for isotopic measurements.

2.1. Environmental measurements

Temperature loggers (StowAway TidbiT), deployed at 6 m and 40 m depths at the study site, provided a continuous record of *in situ* temperature at 1 h intervals. The accuracy of measurements is ±0.2 °C. Water samples for $\delta^{18}O$ analysis were collected by SCUBA at monthly or bi-monthly intervals in borosilicate bottles, sealed and kept in a refrigerator at 4 °C until measurement. Seawater samples for $\delta^{13}C$ of dissolved inorganic carbon (DIC) were collected by SCUBA at the same time intervals in borosilicate-glass bottles, and 1 ml of saturated $HgCl_2$ was introduced to 100 ml of seawater in order to stop all biological activity. The bottles were kept in a refrigerator at 4 °C until measurements were carried out. All water samples were collected in duplicate.

Seawater $\delta^{18}O$ measurements are based on the procedure of equilibration of water with carbon dioxide (Epstein and Mayeda, 1953) modified to continuous flow isotope ratio mass spectrometry (Nelson, 2000). Water sample of 0.5 ml was slightly acidified using phosphoric acid. Vials were flushed with a mixture of 0.5% carbon dioxide in helium, reaching full equilibration at constant temperature. The CO_2 gas was analyzed for $\delta^{18}O$ using a Finnigan MAT 252 mass spectrometer connected online to a GasBench II. Data are reported in per-mill units relative to VSMOW. The external precision, laboratory water standards in different runs, of replicated analysis is better than 0.06‰.

Measurements of $\delta^{13}C$ of DIC were performed on $HgCl_2$ poisoned 1 ml water sample, using GasBench II. The samples were acidified and the evolved CO_2 was introduced to the mass-spectrometer using He-flow. Precision of replicate analyses is better than 0.05‰.

2.2. Skeletal sampling

The coral's tissue was removed using an airbrush with filtered seawater. The exposed skeleton fragments were soaked in hydrogen peroxide (H_2O_2; 30%) for 12 h in order to remove remaining organic matter (Boiseau and Juillet-Leclerc, 1997). The skeletons were then washed several times with deionized water and were left over night to dry at room temperature.

Sampling of the skeleton's surface was designed to obtain the $CaCO_3$ which represents the last month's growth. Hence, the coral's extension rate determined the depth from the coral surface that was drilled. During phase one of the experiment, where natural colonies were sampled, the average long-term extension rate of corals growing at the 6 m depth was 12 mm/year, approximately threefold the extension rate of 4 mm/year measured for the 40 m corals (Felis et al., 2003; Rosenfeld et al., 2003). To account for the difference in growth rates between depths, surface skeleton was sampled to a depth of 1 mm within the skeleton of 6 m corals and to 0.3 mm in the 40 m corals, using a low speed dental drill. This sampling scheme generated samples that roughly correspond to one month of growth at both depths. During the second phase, surface skeleton was sampled to a depth of 0.25 mm in each plug, since the corals reduced their extension rates after being cored from their mother colony (Fig. 1a).

The isotope record for the years 2001–2006 and the horizontal drilling along the deep summer and winter bands were performed on a 5 mm thick skeleton slice. This slice was cut from the colony along the coral's major axis of growth and the annual growth bands were revealed by X-ray (Fig. 1b). Sampling was performed using a 0.5 mm diameter dental drill at 0.5 mm increments. Consequently, between 10 and 12 samples per annual band were collected and analyzed to construct the age model (Analyseries; Linsley et al., 1999).

Skeletal $CaCO_3$ samples, weighing between 150 and 250 μg, were reacted with 100% orthophosphoric acid to produce CO_2 for mass spectrometric analyses on a GasBench II connected on-line to a Finnigan MAT 252. Isotopic measurements of $\delta^{13}C$ and $\delta^{18}O$ are reported in per-mill units (‰) relative to the international Vienna-Peedee Belemnite Limestone Standard (VPDB). Calibration was maintained by routine analyses of internal and international standards. The reproducibility of replicated analysis of our internal laboratory standard is 0.06‰ and 0.08‰ for carbon and oxygen, respectively.

Statistical analysis of the measured data was generated using Statistica 9.0. All variables are normally distributed and p-levels $\leqslant 0.05$ were considered significant.

3. RESULTS

3.1. Phase I: natural colonies

Mean monthly temperatures, measured at 6 m and 40 m depth from June 2001 to June 2002, exhibited a typical annual cycle. At 6 m depth, temperature reached a maximum of 27.0 °C during August and a minimum of 21.5 °C during March. At 40 m depth, maximal summer temperature during August was 1.5 °C lower than at 6 m and was only 25.5 °C. However, winter temperatures were the same at both depths (Fig. 2a). The amplitude of the annual SST cycle was 5.5 °C at 6 m and 4 °C at 40 m. $\delta^{18}O_{sw}$ did not exhibit seasonal cycle at both depth environments with no significant difference between the two (t-test, $p > 0.05$; Fig. 2a). The average $\delta^{18}O_{sw}$ at the study site was 1.73‰ relative to VSMOW and ranges between 1.56‰ and 1.86‰.

$\delta^{18}O$ compositions of newly formed skeleton (top skeletal layer), sampled throughout the year, did not exhibit any apparent annual periodicity (Fig. 2b). The correlation between $\delta^{18}O_s$ composition of newly formed skeleton and measured SST was low, both at 6 m and 40 m depth habitats (Fig. 6a; $r^2 = 0.254$ and 0.003, respectively; $p = 0.17$ and 0.71, respectively). The average annual $\delta^{18}O_s$ composition at 40 m was -1.96 ± 0.34‰, significantly different from that of 6 m, which was -2.40 ± 0.38‰. The significant difference detected between depths is

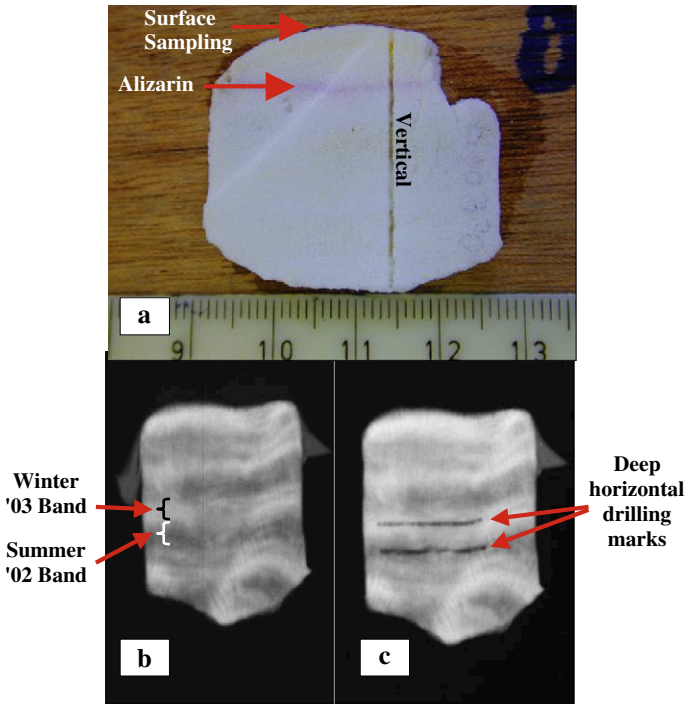

Fig. 1. Photograph and X-ray radiograph of a single plug, sampled from the coral *Porites lutea*, demonstrating the 3 sampling strategies applied in this study. (a) Surface sampling, obtained by scraping the surface of the coral. Vertical sampling along the maximum growth axis represented by the vertical line, which is the result of drilling. The pink is the Alizarin stain. All the material above the Alizarin was added during the experiment. (b) X-ray radiograph showing the annual density bands with corresponding winter and summer bands. (c) X-ray radiograph after horizontal drilling within single summer and winter band.

pronounced during summer months in comparison to the winter months, where it became indistinct.

3.2. Phase II: split colonies of genetic identity

3.2.1. Environmental measurements

Monthly seawater temperatures exhibited an annual cycle reaching a maximum of 27.0 °C during August 2005 and a minimum of 21.2 °C during February 2006 (Fig. 3a). The amplitude of the annual SST cycle was 5.8 °C. Monthly $\delta^{18}O_{sw}$ did not exhibit seasonal or annual patterns and no significant differences were observed among the $\delta^{18}O_{sw}$ values. (Fig. 3b; One way ANOVA; $n = 10$; $p > 0.05$). The average $\delta^{18}O_{sw}$ at the study site was $1.76 \pm 0.03‰$ and ranged between 1.71‰ and 1.81‰. Also $\delta^{13}C_{DIC}$ did not exhibit seasonal or annual patterns (Fig. 3b). The average $\delta^{13}C_{DIC}$ at the study site was $1.11 \pm 0.09‰$ relative to VPDB and ranged between 0.94‰ and 1.20‰.

3.2.2. $\delta^{18}O$ and $\delta^{13}C$ of the newly formed skeleton ($\delta^{18}O_s$ and $\delta^{13}C_s$)

Average $\delta^{18}O_s$ and $\delta^{13}C_s$ values of the newly formed skeleton, sampled from the four colonies each month, did not exhibit clear seasonal or annual patterns between March 2005 and December 2006 (Fig. 4). The average $\delta^{18}O_s$ was $-2.22 \pm 0.32‰$, ranging between −1.73‰ and −2.74‰ and the average $\delta^{13}C_s$ was $-1.99 \pm 0.32‰$, ranging from −1.51 to −2.69‰. No significant correlation was found between average $\delta^{18}O_s$ composition of newly formed skeleton and SST (Fig. 6b; $r^2 = 0.258$; $p > 0.05$). The absence of seasonal pattern, as well as no significant correlation between $\delta^{18}O_s$ and temperature (r^2 of 0.47; 0.30; 0.22; 0.01), is also apparent when each genotype is considered separately.

3.2.3. $\delta^{18}O_s$ composition of deep skeleton, 2001–2006

Vertical drilling, along the main growth axis, was conducted on three individual coral plugs in order to compare the $\delta^{18}O_s$ of newly formed skeleton with the deeper parts of the skeleton (Fig. 5). A clear annual cycle in $\delta^{18}O_s$ was observed prior to the staining and coring from the mother colony (i.e. before January 2005). After January 2005, $\delta^{18}O_s$ continued its regular annual cycle although with a small isotopic enrichment. Similar results were observed for the 3 corals that were vertically drilled. Correlation analysis of vertically drilled $\delta^{18}O_s$ versus seawater temperatures from January 2005 to October 2006 shows a significant correlation ($r^2 = 0.709$; $p < 0.0001$; Fig. 6c). Moreover, performing the same analysis on the full vertical samples (from September 2002 till October 2006) resulted with high correlation coefficient and linear trend ($r^2 = 0.583$; $p < 0.0001$). The stability of the isotopic signal within a single density band was tested by choosing one winter and one summer band (as portrayed in the X-rays) in two plugs: 'May 2006 B' and 'December 2006 B'. Fig. 1b shows the X-rays of coral 'May 2006 B' before and after drilling. The wide dark bands are the summer bands and the narrow white bands are the winter bands. Average

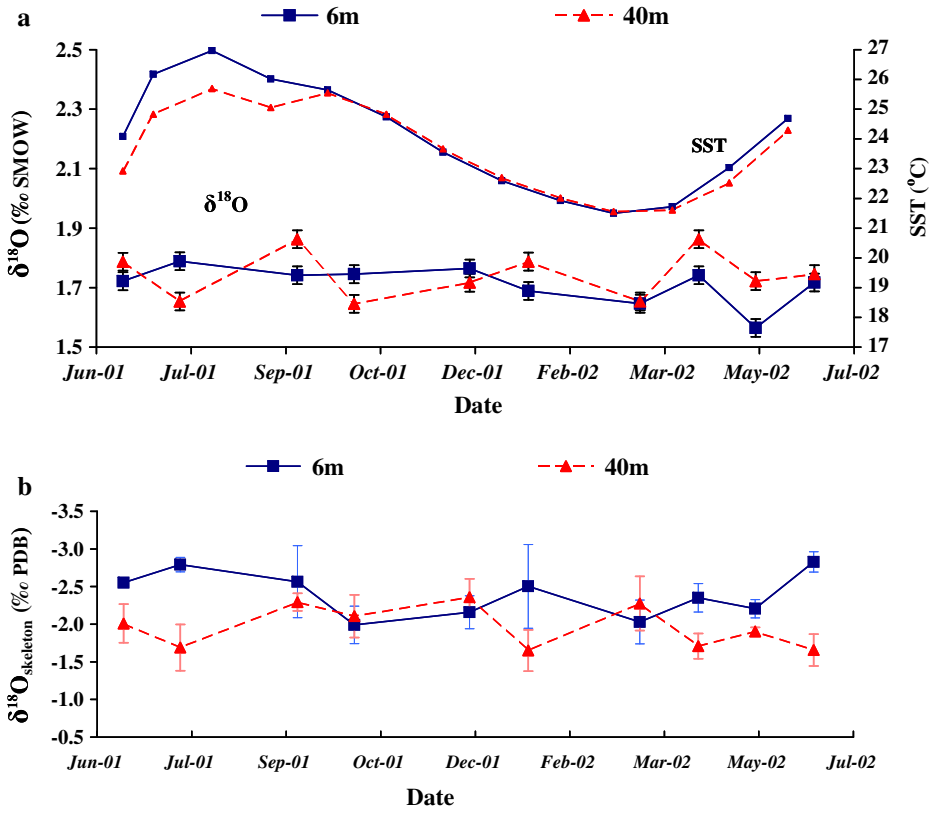

Fig. 2. (a) Sea surface temperature and seawater $\delta^{18}O$ at depths of 6 and 40 m for the period June 2001 and June 2002. SST shows the regular annual cycle and $\delta^{18}O$ is identical between depths and practically constant over the year. (b) Average $\delta^{18}O$ of the newly formed skeletal aragonite of 4 different natural colonies at each month and at each depth. No annual pattern is observed.

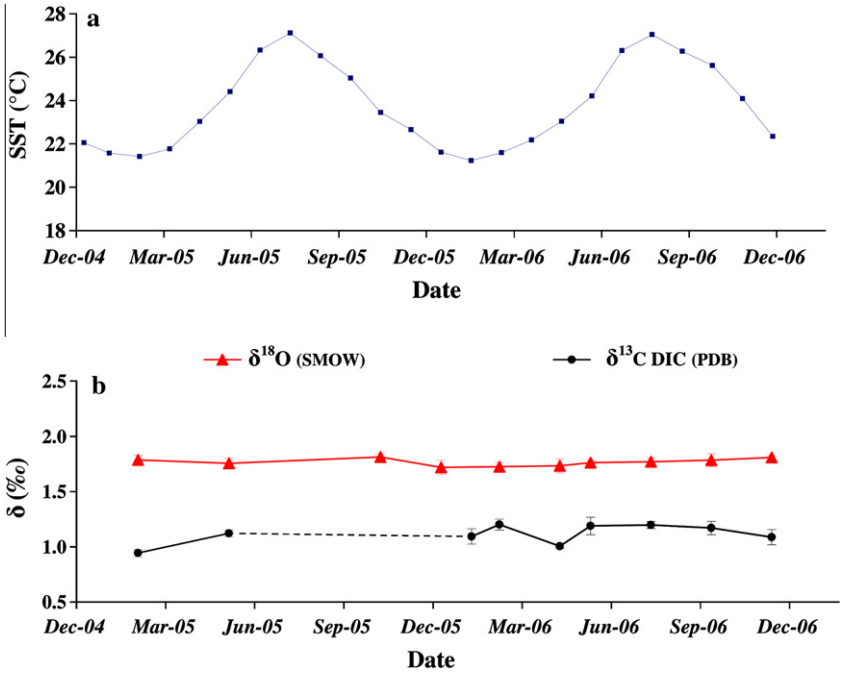

Fig. 3. (a) Average monthly SST for the period December 2004 to December 2006 at the study site of the transplanted and split colonies at depth of 6 m. (b) Seawater $\delta^{18}O$ and $\delta^{13}C$ of dissolved inorganic carbon at the same site for the same period. Both parameters are almost constant with time.

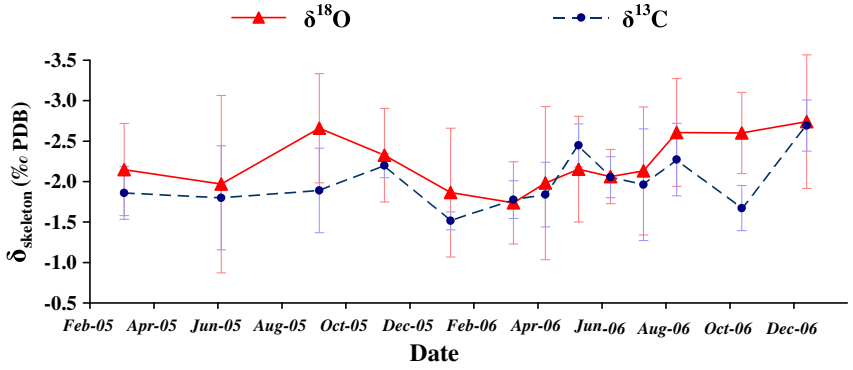

Fig. 4. Average $\delta^{18}O$ and $\delta^{13}C$ of the newly formed skeletal aragonite of the transplanted and split colonies. Each data point is the average of the four different colonies and the standard deviation reflects the inter-colony variability. The analytical uncertainty is much smaller for individual determination.

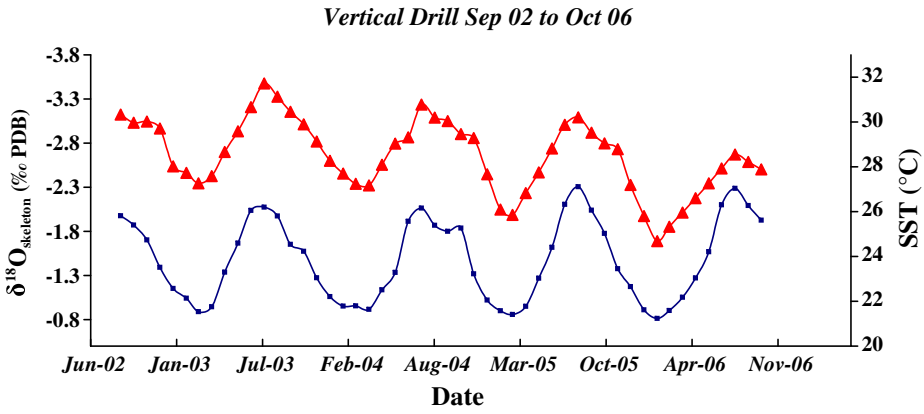

Fig. 5. Skeletal $\delta^{18}O$ (triangles) and measured SST obtained from the vertical drilling along the maximum growth axis of the coral plug harvested at October 2006. Age model for the years 2002–2006 was constructed using AnalySeries program. The clear annual cycles in skeletal $\delta^{18}O$ tracks the seasonal temperature variability in the study site. Alizarin stain at January 2005.

$\delta^{18}O_s$ composition of summer and winter bands for coral 'May 2006 B' (Fig. 7) were -3.00 ± 0.08 ($n = 35$) and -2.59 ± 0.11 ($n = 36$), respectively. Average of summer and winter bands for coral 'December 2006 B' were -3.06 ± 0.13 ($n = 31$) and -2.51 ± 0.14 ($n = 39$), respectively. Winter and summer band average values were significantly different (t-test for independent samples, $p < 0.001$) for both corals and the standard deviation of 31–39 independent analyses within each band is close to two standard deviations of a single determination of $CaCO_3$ powder.

4. DISCUSSION

The invariable $\delta^{18}O_{sw}$ seawater composition measured throughout the year and at two depths, 6 m and 40 m, suggest that the water column at the study site is well mixed and $\delta^{18}O_{sw}$ gradients do not develop. Turbulence and mixing processes mask the effect of evaporation at this depth range while the inflows of rain and fresh water from terrigenous sources are negligible. This result affirms assumptions of earlier work that the main factor forcing $\delta^{18}O_s$ composition of corals growing at this site is temperature rather than $\delta^{18}O_{sw}$ composition of seawater (Klein et al., 1993; Felis et al., 2000; Rosenfeld et al., 2003).

The isotopic composition of skeleton samples, taken from the surface of the colonies, shows no clear seasonal pattern from both phases of our work consisting of monthly sampling over two years (Figs. 2b and 4). Taking into account that fractionation models are based on temperature dependence of $\delta^{18}O_s$ (McConnaughey, 1989a,b; Adkins et al., 2003) and the fact that monthly SST measurements exhibit an annual temperature cycle with an amplitude of 5.8 °C (Fig. 2a), it was expected that the skeleton would exhibit a clear annual cycle between summer and winter, with heavier $\delta^{18}O_s$ values during winter. The lack of correlation between $\delta^{18}O$ compositions of surface skeletons and measured SST (Fig. 6) implies that $\delta^{18}O$ of the newly formed skeletal aragonite, deposited at the coral surface, is independent of the ambient temperature. In contrast to the newly formed surface skeleton, the isotopic compositions of the deep and older parts of the skeleton show the predicted annual isotopic pattern (Fig. 5) with highly significant correlation between $\delta^{18}O_s$ and SST (Fig. 6c).

The transformation from a temperature independent isotope signal to a temperature dependent signal can be accounted for by two possible mechanisms. The first is to invoke an extensive process of isotope exchange which occurs post skeleton deposition. This is the less probable

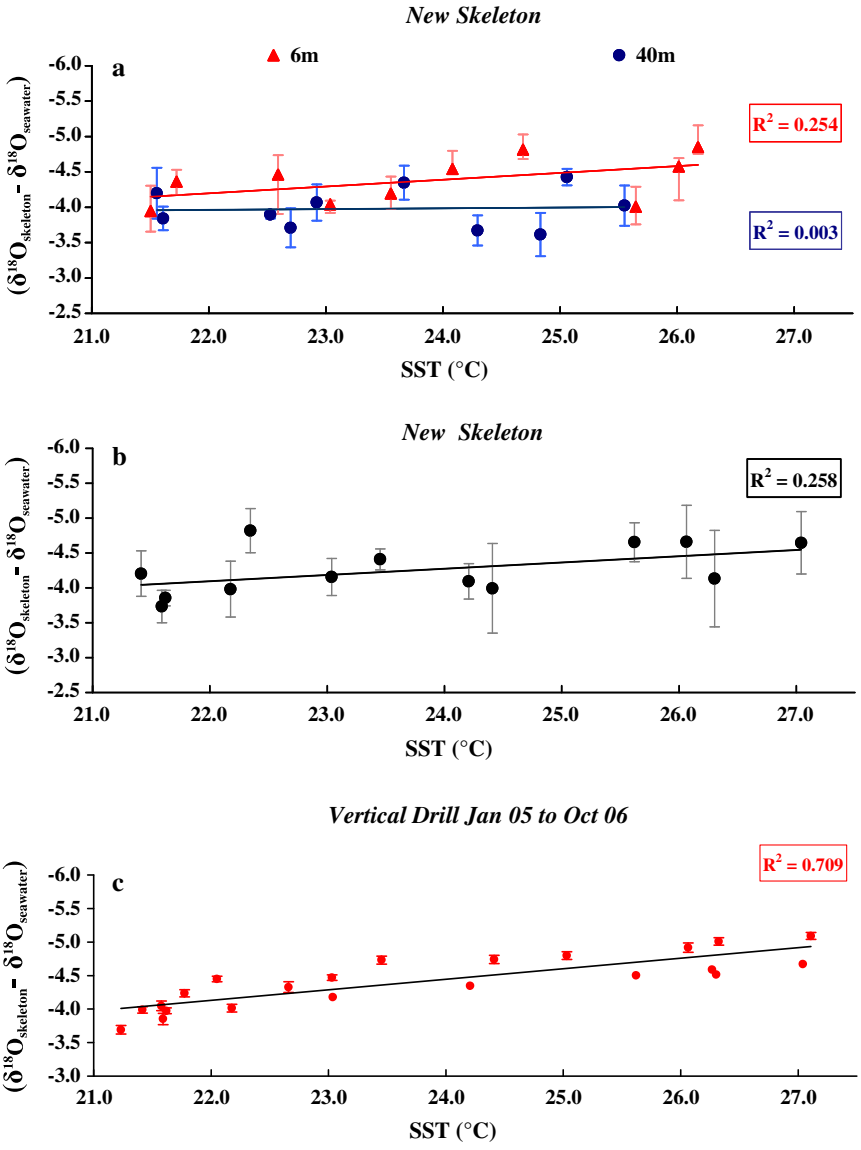

Fig. 6. The relation between the newly formed skeletal $\delta^{18}O_s$–$\delta^{18}O_{sw}$ and measured SST for (a) natural colonies at depth of 6 and 40 m during 2001; (b) transplanted and split colonies for the period 2005–2006 and (c) the vertical drilling representing January 2005 to October 2006, the post alizarin staining, in a plug whose surface was used to determine newly formed skeleton. We note that the regression coefficient for the period 2002 to 2006 ($r^2 = 0.583$) is higher than the regressions of the newly formed skeletons. The measured seawater $\delta^{18}O$ was used for the subtraction.

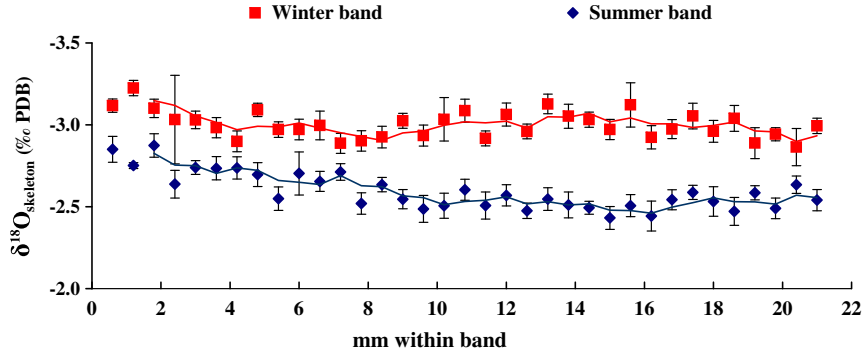

Fig. 7. Aragonite $\delta^{18}O$ of a single summer band and a single winter band obtained from horizontal drilling of 20 mm within the same density band, showing the stability of the isotope signal within the band and the statistically different $\delta^{18}O$ between summer and winter.

mechanism as dissolution-precipitation features of aragonite crystals were not reported in the ultra-structure of the few upper millimeters of the corals' skeletons and the relatively low seawater temperatures do not allow efficient isotope exchange on a monthly time scale. The second is to attribute the formation of the temperature dependent signal to the crystals which are produced further down the tissue layer during the continuous process of skeletogenesis. These crystals are deposited on top overlying the crystals produced at the surface of the colony and are responsible for tracking the environmental variation.

Several studies have recognized the multi-step nature of the skeletogenesis process and its implication on the structure and geochemistry on different spatial and temporal scales. For long time scales (month to a year) Barnes and Lough (1993) suggested that three growth processes contribute to density band formation of *Porites* spp. First, the colony's skeleton extends by growth at its outermost surface. Second, the skeletal scaffold created at the outer surface is thickened throughout the depth of the tissue layer (tissue usually occupies skeleton formed over the last 4–13 months). Third, the density pattern is then modified by periodic and abrupt uplifting of the lower margin of the tissue layer, a process which occurs about every 30 days. Taylor et al. (1993, 1995) calculated and estimated that each part of the two steps of growth, the scaffolding and the thickening, contribute 50% each to the total skeleton. Cuif and Dauphin (2005) showed that crystallization of coral fibers is permanently controlled by the polyp's basal calicoblastic epidermis through a cyclic two-step process. The first step occurs at the tips of growing structures (early mineralization zones) and is characterized by a microgranular mineralization with randomly oriented grains. The second step in skeletogenesis is the thickening stage which produces fibers through a cyclic process permanently driven by biochemical secretions. On short time scales (day-night) Cohen et al. (2001) distinguished an area on the colony of *Porites lutea* which was accreted during day time, with a four times stronger correlation coefficient between Sr/Ca and SST compared to an area accreted during nighttime. Our sampling technique collected aragonite that was deposited on an average of about a month. Therefore, we cannot attribute isotopic compositions to preferential sampling of centers of calcification over aragonite fibers or vise versa (Adkins et al., 2003). Hence, the transition between temperature-independent to temperature dependent isotopic composition is suggested to be correlated to the different phases of skeletal deposition, on time scales of months. Our observation that the surface of the coral is isotopically different from the deep skeleton is further supported by the comparison between $\delta^{18}O$ of surface skeleton of *P. lobata* and *Pavona clavus* colonies to $\delta^{18}O$ of the deep skeletons along the axes of rapid growth of the same colonies (McConnaughey, 1989a,b). It was shown that the surface variations between individual colonies were greater than vertical variations between colonies and far larger than anything likely to arise from probable combinations of temperature and isotopic composition of seawater. This was attributed to the general effect of "disequilibrium" (McConnaughey, 1989a,b) but can also be interpreted as resulting from surface skeletogenesis being independent of temperature. Rodrigues and Grottoli (2006) tested $\delta^{18}O$ of 0.25–0.5 mm of the surface of *P. compressa* and *Montipora capitata* following an elevation of 3 °C during a bleaching experiment. The surface $\delta^{18}O_s$ of both species failed to record the warm temperature after 1 month, strengthening the hypothesis that this is a temperature independent process.

The general model for coral calcification describes a tissue layer, which is separated from the skeleton by a thin alkaline solution between the surface of the forming skeleton and the calicoblastic cell-layer. This fluid reservoir, often referred to as the extracellular calcifying fluid (ECF), is close in composition to seawater and skeletal calcium carbonate is formed as a result of super-saturation (Adkins et al., 2003; McConnaughey, 2003; Gaetani and Cohen, 2006). This general model cannot account for our observation regarding the two different temperature dependencies of the coral aragonite $\delta^{18}O$. It was also challenged by the nano-scale distribution of Sr within the forming skeleton (Houlbreque et al., 2009). A new model, which invokes two calcification processes, is required. We suggest that the position of the tissues in relation to the open sea generates the difference between $\delta^{18}O_s$ of the surface skeleton and of the skeleton accreted further down the tissue layer. The surface skeleton is only four tissue layers away from the seawater. In contrast, the skeleton accreted further down the tissue layer, which is several mm thick, is more secluded and might be formed at a greater distance of up to two orders of magnitude away from the open sea, behind a labyrinth of tissue and skeleton. Apparently, diffusion related fractionation processes and reservoir exploitation, between the environment (open sea) and the calcification sites, have lower impact on the isotopic composition of the surface skeleton. Moreover, in this well mixed microenvironment, variations in calcification rates might not be expressed as variations in isotopic composition of the skeleton. On the other hand, the isotopic composition of skeleton formed further down the tissue layer is more responsive to diffusion related fractionation processes, reservoir exploitation and changes in local pH and calcification rate; hence it does reflect temporal changes of the environment and contains a clear temperature dependency.

The presence of temperature dependent and temperature independent isotopic compositions in the same skeleton has several implications for the reconstruction of past environments. The first is that the full amplitude of the sea surface temperature will be dampened when stored in the coral. The exact magnitude of the dampening depends on the mass ratio between the two types of crystals. It is probable that the need for "local calibration curves" and the large variations in the observed slopes (Leder et al., 1996; Stephans et al., 2004), as well as the relatively large variation among colonies at the same site in different ocean basins, are the manifestation of this mixing. The second implication is that corals may fail to record short thermal events. The temperature dependent signal is build few months after the accretion of the surface skeleton. Hence, the coral will track only part of a short-term thermal event in the correct position of the density band that is correlated to this time. A priori, this

$\delta^{18}O$ signal will be dampened. This was well demonstrated in the study of the 1998 warming in the Indian Ocean as reflected by the Seychelles corals (Levy et al., 2006). The same applies to long acclimation time that is needed in order to track the coral response to environmental changes. We speculate that at least part of the "isotope vital effects" that are associated with growth rates and attributed to kinetic effects (Heikoop et al., 2000; Suzuki et al., 2005) might result from this mix of environmental dependent and independent signals which is probably different for each species and perhaps even for each colony.

5. SUMMARY

The build up of the isotopic signal of *Porites lutea* was followed by sampling the newly formed skeleton at a monthly resolution under natural conditions and on transplanted colonies. The results indicate that $\delta^{18}O$ skeleton signal transformed from a temperature independent signal to a temperature dependent signal as the coral grows. This transformation occurs several months after the skeleton is formed at the coral's surface. The position of the skeleton in relation to the open sea probably generates the difference in $\delta^{18}O_s$ between the surface skeleton and skeleton accreted deeper in the tissue layer. We suggest that the mixing between the temperature dependent and the temperature independent crystals is responsible for dampening the temperature amplitude of SST and the long acclimation time which is required in order to capture environmental parameters. The mixing also contributes to the general phenomenon of "isotopic disequilibrium" in coral and kinetic effects, which are related to growth rate. An important implication of these observations is that *Porites*-like corals are limited in their ability to capture short-term warming events and a priori, the isotopic manifestation of these events will be dampened.

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