A novel culture technique for scleractinian corals: application to investigate changes in skeletal $\delta^{18}O$ as a function of temperature

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ABSTRACT: A novel experimental protocol is described that assists investigations of the effect of environmental parameters on records from the carbonate skeletons of scleractinian corals. It involves the culture of coral colonies on glass slides so as to time the skeletal deposition and environmental records precisely. The value of the technique is demonstrated via calibration of the relationship between skeletal $\delta^{18}O$ and seawater temperature in 2 species of coral obtained from the Gulf of Aqaba. Colonies were grown at 5 temperatures between 21 and 29°C. For *Acropora* sp. this relationship gave a slope of $-0.27‰ °C^{-1}$, a value close to previous estimates. The $\delta^{18}O$ signature of *Stylophora pistillata* displayed a high variability between colonies and gave an average slope much lower than previous estimates ($-0.13‰ °C^{-1}$). These data may indicate a taxonomic difference and the need to re-examine the systematics of this genus. Nevertheless, such variability in colonies of a single species or of a set of closely related species may have implications for the use of coral skeleton as proxy records.

KEY WORDS: Corals · *Acropora* sp. · *Stylophora pistillata* · Skeletal $\delta^{18}O$ · Culture · Temperature

INTRODUCTION

Since the beginning of the industrial revolution, human activities have contributed to climate variability. Knowledge of the causes and magnitude of past climate change is critical for the assessment of the anthropogenic impacts and their likely long term effects. Reliable proxy records of paleoclimate parameters have been identified. For example, the study of various isotopic and other chemical compounds present in ice cores has been used extensively to investigate short term climatic changes (Raynaud et al. 1993). In order to study climatic variability over recent centuries, temperature proxy records displaying high precision (less than 0.5°C) and resolution (better than

6 mo) are required. Tree ring investigations (Cook 1995) or isotopic analyses of seasonal snow (Thompson et al. 1995) have provided such records for continental climatic reconstruction. There are, however, very few suitable proxy records for tropical seawater. Hermatypic scleractinian corals have a 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 & Lough 1996), (2) are benthic organisms sometimes showing a limited bathymetric distribution (Sheppard 1982) and (3) can be dated back to 240 million years ago (Chadwick-Furman 1996). These characteristics explain why corals have been extensively used to reconstruct, for example, sea surface temperature (Dunbar & Wellington 1981, de Villiers et al. 1995).

Such proxies need to be calibrated in order to be used. The great majority of calibrations have been car-

ried out in the field. For example, paleothermometers have been calibrated by comparing temperature records with $\delta^{18}O$ (e.g. Leder et al. 1996, Wellington et al. 1996), or trace elements such as the Sr/Ca (e.g McCulloch et al. 1994, de Villiers et al. 1995), Mg/Ca (e.g. Mitsuguchi et al. 1996) and U/Ca (e.g. Min et al. 1995) ratios, in the skeletons of recent corals. 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 et al. 1997). This may be due to confounding effects of other environmental factors (e.g. light intensity, photoperiod, nutrients, salinity, currents).

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 & Lea 1993, Spero et al. 1997) but difficulties encountered with culture techniques have precluded the development of experimental calibrations of proxy records in corals. One notable exception is the pioneer study of Weil et al. (1981) that successfully used cultured corals to investigate the effect of temperature and irradiance on the skeletal $\delta^{18}O$ of the corals *Montipora verrucosa* and *Pocillopora damicornis*.

We report here an experimental technique enabling the culture of corals under controlled conditions in order to accurately sample the skeleton deposited. This technique has been used to derive a calibration curve of $\delta^{18}O$ versus temperature for *Acropora* sp. and *Stylophora pistillata*.

MATERIAL AND METHODS

Biological material. The experiment was conducted in the laboratory using colonies of the branching zooxanthellate scleractinian corals, *Stylophora pistillata* (Esper 1797) and *Acropora* sp. belonging, respectively, to the families Pocilloporidae and Acroporidae. These colonies were propagated from fragments of parent colonies collected in the Gulf of Aqaba. At least 2 branch tips were sampled from 10 parent colonies of *S. pistillata* (N = 26) and 6 tips were sampled from a single parent colony of *Acropora* sp. The parent colonies had been grown for several months in different aquaria. The specimens were glued on ground glass slides (3 × 6 × 0.2 cm) using underwater epoxy (Devcon®, Fig. 1A) and evenly distributed in 2 aquaria (15 l). The tanks were supplied with heated Mediterranean seawater pumped from a depth of 50 m. The seawater renewal rate was approximately 5 times d^{-1} and the seawater was continuously mixed with a
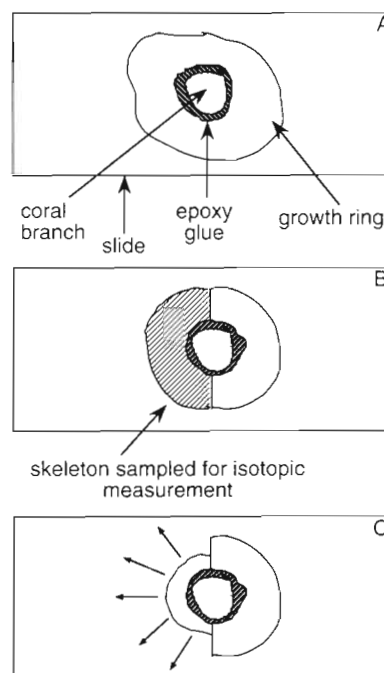

Fig. 1. Culture of corals on glass slides and sampling procedure. (A) Top view of a glass slide after 2 to 3 mo in the aquarium, (B) sampling of the skeleton for isotopic determinations, (C) recolonization of the slide by newly deposited skeleton

Rena® pump (6 l min^{-1}). Light (260 µmol m^{-2} s^{-1}) was provided by metal halide lamps (Philips HPIT, 400 W) on a 12:12 h photoperiod. Seawater was continuously aerated with outside air. The temperature was controlled to within ±0.1°C using a temperature controller (EW, PC 902/T).

All colonies were initially cultured for 54 d at 25°C; half of the ring skeleton deposited on the glass slide was then removed with a scalpel (Fig. 1B), dried overnight at room temperature and stored in glass containers pending determination of $\delta^{18}O$. Thereafter, colonies in one aquarium were grown first at 27°C for 50 d and then at 29°C for 92 d. Colonies in the other aquarium were grown successively at 23°C for 70 d and 21°C for a period of 113 d. Skeletal samples were collected as described above at the completion of each period for determination of isotopic composition.

Each sample was weighed and then ultrasonicated for 1 min to reduce it to a fine powder. Following the treatment described by Boiseau & Juillet-Leclerc (1997), the skeletal powder was soaked in hydrogen peroxide (30%, v:v) for 12 h to eliminate the organic matter, filtered on Nucleopore polycarbonate membranes (0.4 µm), and dried at 40°C for 4 h.

The rate of $CaCO_3$ dissolution was measured on 3 additional colonies of *Stylophora pistillata* and *Acropora* sp. These colonies were bleached in order to

remove all organic matter, rinsed thoroughly and dried at room temperature for several weeks. The skeleton deposited on the slide was sampled, ground, weighed and left in H_2O_2 30% (v:v) for 12 h. The samples were then filtered on Nucleopore membranes (0.4 μm) of known weight. The filters were rinsed with distilled water, dried (40°C for 7 h) and weighed again.

Oxygen isotopic measurement. 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 a standard, which is PDB for carbonates:

$$\delta^{18}O = \left[\frac{(^{18}O/^{16}O)_{sample}}{(^{18}O/^{16}O)_{standard}} - 1 \right] \times 10^3$$

The external precision, estimated using an internal standard, is 0.16‰ (2 × standard deviation, SD). The reproducibility of isotopic measurements calculated from replicate coral samples is 0.16‰ (2 × SD) for *Acropora* sp. and 0.24‰ (2 × SD) for *Stylophora pistillata*.

The oxygen isotope composition of seawater samples was measured on samples collected twice a month using a Finnigan MAT 252. The reproducibility of oxygen isotope measurements was ±0.10‰ (2 × SD). The skeletal $\delta^{18}O$ was corrected from changes in seawater $\delta^{18}O$ (Hut 1987).

Environmental parameter measurements. Temperature (precision: ±0.05°C) was logged at 10 min intervals using a Seamon® temperature recorder (Table 1). Dissolved oxygen concentration was measured using a polarographic electrode (Ponselle) calibrated daily against air-saturated seawater and a saturated solution of sodium sulfite (zero oxygen). pH was measured using a combined Ross pH electrode (Orion 8102SC) calibrated daily against NBS (National Bureau of Standards) buffers (pH 4.006 and pH 7.413 at 25°C). Determination of total alkalinity was carried out potentiometrically according to the method described by Gran (1952). Salinity was measured with a conductivity meter (Meter LF 196) (Table 2). Irradiance was measured using a 4 π quantum sensor (Li-Cor, LI-193SA).

Growth rate. Each colony was photographed before skeleton sampling. The perimeter length and the surface area of the skeleton deposited at each temperature were estimated using Sigma-Scan image analysis software. The linear growth rate was estimated by normalizing the surface area by the average perimeter length over time (mm d^{-1}).

Skeletal structure. The morphology and microstructure of the skeletal calcareous fibro-crystals were

Table 1. Parameters relating to the temperature in each culture aquarium

| | Aquarium 1 | | | Aquarium 2 | | |
	25°C	27°C	29°C	25°C	23°C	21°C
Average	24.88	27.12	29.18	24.87	23.13	21.18
SE	0.015	0.003	0.001	0.013	0.003	0.010
N	4736	6822	12597	5134	10128	14023
Min.	15.90	26.58	28.725	15.83	22.55	20.89
Max.	28.18	28.28	29.525	27.23	24.15	21.40

Table 2. Chemical characteristics of the seawater used in the culture tanks. Mean ± SE; the sample size is shown in parentheses

Seawater $\delta^{18}O$ (‰ versus SMOW)	1.29 ± 0.01 (39)
pH (seawater scale)	8.143 ± 0.001 (384)
Total alkalinity (meq kg^{-1})	2.676 ± 0.005 (5)
Dissolved O_2 (μmol kg^{-1})	189.0 ± 0.3 (292)
CO_2 partial pressure (μatm)	506 ± 9 (6)
Dissolved inorganic carbon (mmol kg^{-1})	2.346 ± 0.004 (5)
Salinity	37.9 ± 0.1 (12)

examined using scanning electron microscopy (Philips, 505). These features were compared in cultured colonies (both the skeleton deposited on the glass slide and on the branches were examined) as well as in colonies grown in the field (hereafter referred to as 'wild' colonies). The wild colonies of *Stylophora pistillata* and *Acropora* sp. were collected, respectively, in the Gulf of Aqaba (1 m depth) and Mururoa, French Polynesia (2 m depth).

Diamond polished surfaces were etched using formic acid (1% w:w) containing 2% (w:w) glutaraldehyde for 40 s in order to improve observation of both the primary aragonite of calcification centers and growth laminations of secondary fibrous tissue.

Statistical analysis. The relationship between $\delta^{18}O$ and temperature was investigated using a predictive regression technique. All statistical analyses were carried out using the statistical package SyStat (5.2, SAS Institute, Inc.). Results are reported as mean ± standard error of the mean (SE).

RESULTS

Growth rate

Skeletal extension and deposition occurred vertically along the normal growth axis and horizontally on the glass slide (Fig. 1). The growth of coral tissues on the rim of the epoxy glue started a few days after the

preparation of the colonies. The deposition of calcium carbonate began when the tissue layer was 2 to 3 mm wide and occurred on the glass slide within approximately 10 d.

There was no significant effect of the species on the linear growth rate (ANOVA, p = 0.14, F = 2.23, df = 73). Temperature had a statistically significant effect on the linear growth rate (p = 0.03, F = 4.72) and this effect was the same for both *Stylophora pistillata* and *Acropora* sp. as shown by the lack of interaction between temperature and species (p = 0.1, F = 2.78). The maximum linear growth rate occurred at 27°C and was similar for *S. pistillata* and *Acropora* sp. (0.13 mm d^{-1}; Fig. 2). The linear growth rate of both species was not significantly different at 21, 23, 25 and 27°C but was significantly greater in *Acropora* sp. than *S. pistillata* at 29°C (0.11 versus 0.05 mm d^{-1}).

The amount of $CaCO_3$ sampled ranged from 14 to 107 mg and was always greater for *Acropora* sp. samples than for *Stylophora pistillata*.

Skeletal structure

The corallites of both *Stylophora pistillata* and *Acropora* sp. deposited on the glass slides typically had 6 primary septa fused with the axial columella and were surrounded by short vertical spinules (Fig. 3a,b). Round-shaped microcrystalline units of the calcification centers occurred at the distal ends of the pillars (Fig. 3c,d). Microstructural observations did not reveal any difference in the shape, size and global arrangement of the fibrous units in cultivated and wild colonies of *S. pistillata* (Fig. 3e,g). The microstructural organisation of the skeleton of cultivated *Acropora* sp. (Fig. 3f) exhibited the typical scaly appearance of acroporid corals (Fig. 3h). Scales are the growing ends of sclerodermites several 100s of μm long, growing side by side but maintaining crystallographic characteristics all along their sinuous growth line.

Organic matter removal

An average 91 ± 2% of the skeletal powder was lost after a 12 h peroxide treatment and subsequent filtration in *Stylophora pistillata*. The total loss of sample
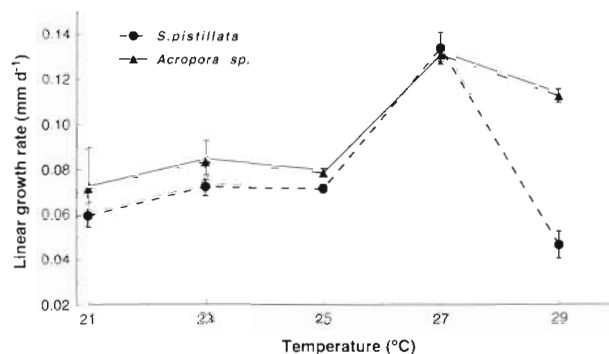

Fig. 2. *Stylophora pistillata* and *Acropora* sp. horizontal linear growth rates (mm d^{-1}) of colonies as a function of temperature

was less important (59 ± 6%) for samples of *Acropora* sp. This difference is statistically significant (ANOVA, p < 0.0001). The rate of $CaCO_3$ dissolution was 7.6 ± 2.8% for *S. pistillata* and 4.8 ± 0.7% for *Acropora* sp. (N = 3 for each species).

The efficiency of organic matter extraction was tested for several samples of *Acropora* sp. No significant changes in the skeletal δ^{18}O were found when the H_2O_2 treatment was longer than 12 h (Fig. 4). There was no difference in efficiency for samples collected from colonies grown at different temperatures (data not shown).

Oxygen stable isotope

The seawater δ^{18}O remained relatively constant both during a dial cycle (1.28‰ vs SMOW ± 0.01, N = 3) and during the course of the year (1.29‰ ± 0.01 vs SMOW, N = 39).

Skeletal δ^{18}O is shown as a function of temperature in Fig. 5. Samples of *Stylophora pistillata* displayed a variability much greater than that of *Acropora* sp. For example, the range of δ^{18}O of *S. pistillata* and *Acropora* sp. at 25°C was, respectively, 1.24 and 0.56‰. Such variability does not seem to be related to differences in the culture tanks since the average δ^{18}O was not significantly different in colonies of *S. pistillata* cultured at 25°C in 2 different aquaria (−2.47 and −2.72‰; t-test, p = 0.24). The equation of *Acropora* sp. calibration curve is: $y = -0.27x + 5.35$ (r^2 = 0.89, N = 17). For *S.*

Fig. 3. Skeletal morphology and microstructure of (a to f) cultivated and (g,h) 'wild' colonies of *Stylophora pistillata* and *Acropora* sp. (a,b) Morphology of typical corallites of *S. pistillata* (a; 72×) and *Acropora* sp. (b; 85×). (c,d) Calcification centers at the distal ends of the spinules of *S. pistillata* (c; 850×) and *Acropora* sp. (d; 475×). (e,g) Fibrous microstructure of cultivated (e; 1650×) and 'wild' (g; 1650×) specimens of *S. pistillata*. (f,h) Microstructure of cultivated (f; 950×) and 'wild' (h; 950×) specimens of *Acropora* sp.
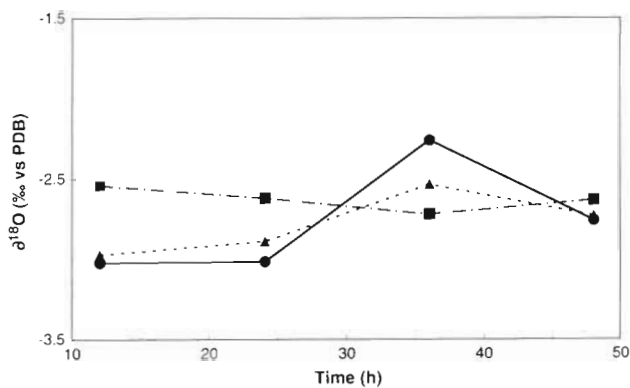
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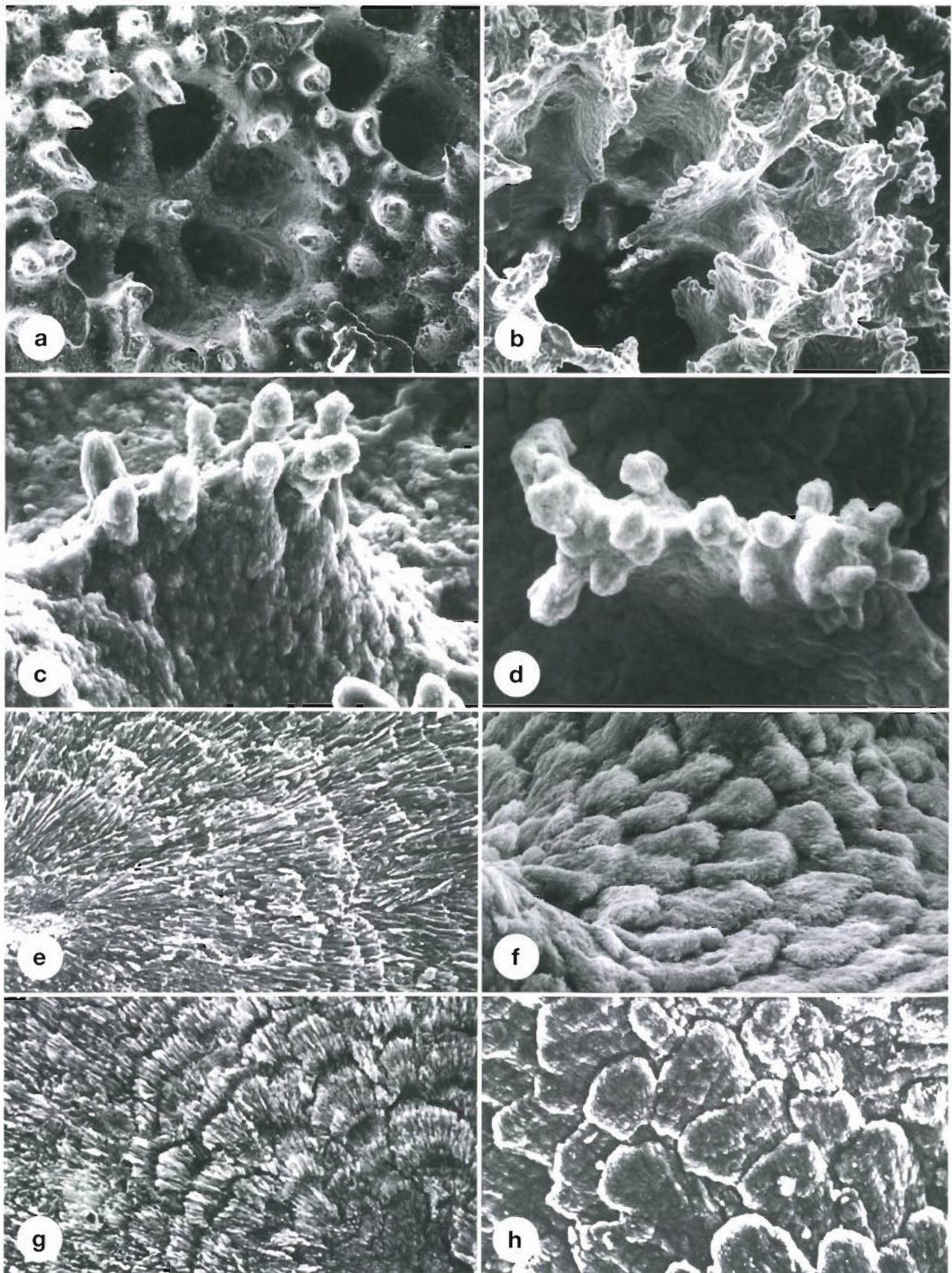
Fig. 4. Skeletal $\delta^{18}O$ of 3 different samples of *Acropora* sp. cultured at 27°C as a function of duration of the H_2O_2 treatment

pistillata, it is: $y = -0.13x + 2.10$ ($r^2 = 0.22$, N = 43). r is significantly different from 0 for both *S. pistillata* (*t*-test, p = 0.002) and *Acropora* sp. (*t*-test, p < 0.0005).

DISCUSSION

Coral nubbins (small pieces of branching colonies) and explants (fragments of massive colonies) are being increasingly used for measurements of physiological parameters, both in the field and in the laboratory (see Davies 1995). Several types of nubbins have been described: cemented on bricks (e.g. Edmonson 1929), glued on PVC pipes (e.g. Davies 1995) or suspended on

Fig. 5. Skeletal $\delta^{18}O$ versus temperature in samples of (A) *Acropora* sp. and (B) *Stylophora pistillata*

nylon wire (Al-Moghrabi et al. 1993). 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 (Davies 1995). Moreover, nubbins are free of encrusting and boring organisms. Cultured corals have been, however, seldom used to investigate the response of skeletal stable isotopic composition and trace element concentrations to changes in environmental parameters. The respective role of environmental and physiological controls on the isotopic composition of reef coral skeletons is a matter of debate (Swart 1983). The study of samples cultured under controlled conditions is the only way to investigate the effect of environmental parameters on the physiology and skeletal composition of scleractinian corals. The parameters of interest (e.g. temperature, light, salinity, concentrations of nutrients) can be changed one at a time or simultaneously. The physiological attributes can then be investigated and the skeleton deposited, recovered and analyzed. The information recovered using such a technique would be extremely valuable to analyze the environmental and physiological control of the skeletal composition but one needs to be able to precisely sample the $CaCO_3$ precipitated during the experiment without any interference from $CaCO_3$ precipitated prior to the experiment.

Weil et al. (1981) studied the effect of light and temperature on the $\delta^{18}O$ and $\delta^{13}C$ skeletal isotopic composition of the corals *Montipora verrucosa* and *Pocillopora damicornis* grown in aquaria. Their method presented several disadvantages mostly related to the sampling of skeletal material. The specimens were stained with Alizarine Red-S at the beginning of each experiment, which was used as a chronological marker. At the end of the experiments, the corals were sacrificed, the tissues were removed and the skeleton deposited during the experiment (i.e. past the skeletal marker) was clipped. Alizarine staining was previously shown to be toxic for corals and to result in a decrease in calcification by ca 25% (Dodge et al. 1984). It is therefore possible that the composition of the skeleton precipitated after staining is altered.

The coral culture technique on glass slides described in the present paper offers several advantages. Firstly, the samples are small (about 1 cm), which makes their manipulation easy and a large number of replicates readily available. Secondly, the experiments can be of relatively short duration due to the rapid horizontal growth on the slide. Linear growth varied between 0.04 and 0.13 mm d^{-1} and was highest at 27°C, a value consistent with data from the literature (see Buddemeier & Kinzie 1976 for a review). Thirdly, and more importantly, sampling of the skeleton and tissues
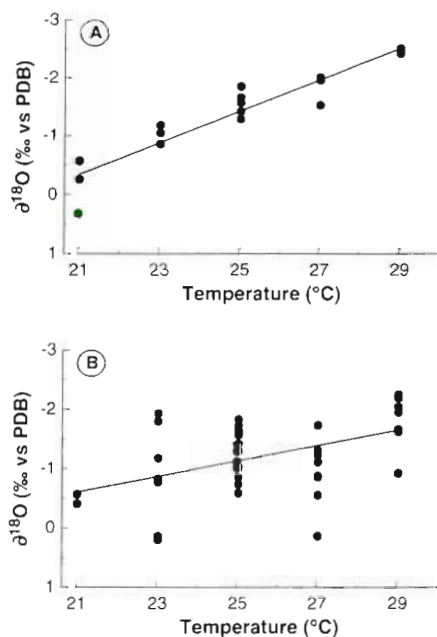
deposited during a known time interval is achieved with great accuracy which suppresses any mixing with skeleton deposited during the previous experiment. Fourthly, the sampled colonies are not sacrificed and can be subsequently used, enabling repeated-measures experimental plans.

It has been shown that the skeletal isotopic composition can vary as a function of the skeletal structure (Land et al. 1975). A potential drawback of the culture technique described in the present paper relates to the possible differences between the structure of the skeleton sampled (deposited horizontally on the glass slide) and the structure of the skeleton deposited on the branch. There was, however, no significant difference in the skeletal microstructure of colonies collected in the field and cultured under controlled conditions, according to the microscopic observations. It is interesting to note that there is no axial polyp on the horizontal part of the skeleton of cultured colonies of *Acropora* sp.; their initiation only begins when the skeleton reaches the edge of the slide.

Another limitation is that branching coral species were used in the present study whereas massive species are used in most paleoreconstruction investigations. Branching species were chosen because their growth rate is higher than that of massive species. They were therefore better candidates to validate the use of the new culture technique. We do not anticipate, however, any problem to apply this procedure to massive species.

The presence of organic matter of algal or animal origin in skeletal samples can significantly affect the stable isotopic signature of fossil corals (Boiseau & Juillet-Leclerc 1997). This problem is likely to be much greater in recent corals in which the amount of organic matter is considerably higher. A wide range of treatments have been used so far: vacuum roasting at 250 to 300°C (McConnaughey 1989), hydrogen peroxide (H_2O_2) soaking (Weil et al. 1981), ultrasonic cleaning (Aharon 1991), low temperature oxygen plasma furnace (Goreau 1977, Erez 1978) and 5% solution of sodium hypochlorite (Weber & Woodhead 1970, 1972, Land et al. 1975). In some cases, no pretreatment of the carbonate was carried out prior to analysis (McConnaughey 1989, Leder et al. 1996).

A loss of 59 to 91% of the raw samples occurred during the H_2O_2 treatment. Such treatment induces not only an oxidation of organic matter, but also a loss of $CaCO_3$ due to 2 processes (Boiseau & Juillet-Leclerc 1997). A small proportion (less than 8%) is lost as a result of skeletal dissolution because H_2O_2 is acidic (pH 4.2). The second, and most important, loss occurs during the subsequent step, when the mixture of H_2O_2 and skeletal powder is filtered. Some powder remains adhered to the glassware. Additionally, some particles

of aragonite are trapped within the fibers of the filter. It is not possible to recover these particles without collecting some fibers as well, which would contaminate the sample and lead to spurious isotopic data. The larger size of the skeletal particles in *Acropora* sp. enables a better recovery on the filter and explains the lower loss of aragonite in these samples. We found that the superficial dissolution of aragonite that takes place during the H_2O_2 treatment removes the organic matter efficiently, including the material intimately attached to the skeleton. A 12 h incubation with H_2O_2 was sufficient for the total removal of the organic matter.

The experimental set-up enables the investigation of the variability within and between colonies, as well as between a genus belonging to 2 different families. *Stylophora pistillata* does not display a significant variability within a single colony since the skeletal $\delta^{18}O$ was similar (between 0.2 to 0.6‰ at 25°C) in clones sampled from the same parent colony. The same result was obtained for clones sampled from a single colony of *Acropora* sp. The variability between clones sampled from different parent colonies is important in *S. pistillata* with differences in $\delta^{18}O$ of up to 2‰ (at 23°C) but cannot be assessed in *Acropora* sp. since all samples were collected from the same parent colony. Such variability may result from the geographic origin of the parent colonies, which were collected in several localities in the Red Sea. It is, however, difficult to understand how colonies of the same species could retain physiological differences when they are cultured under identical experimental conditions. This may indicate a taxonomic difference. All parent colonies were identified as *S. pistillata* according to the present taxonomic knowledge of the genus *Stylophora* (Veron & Pichon 1976, Kühlmann 1983). It has however been demonstrated that the taxonomic status of that genus is still unclear in the Red Sea (Gattuso et al. 1991). Moreover, samples retained different color and shape when they were grown in the same experimental conditions. Marchioretti et al. (1995) have also found a significant difference in the pigment content and growth rate of colonies identified as *S. pistillata* and grown in the same aquarium. The variability of the $\delta^{18}O$ signal in colonies of a single or a set of closely related species has implications for the interpretation of the isotopic data of recent and fossil corals. Firstly, fossil corals used for reconstructing the temperature record are often sampled from cores. The small sample size (a few cm) seldom enables identification at the species level. It is therefore possible that a pool of species of the same genus is used, each having a different response of $\delta^{18}O$ with respect to temperature. Secondly, calibration curves are often obtained on skeletal samples collected from a single colony of recent corals (Leder et al. 1996). Our data suggest that different cal-

ibration curves may be obtained with other colonies. The magnitude of these differences and their significance when estimating paleotemperatures from the fossil record remain to be evaluated. Weber & Woodhead (1972) overcame this problem by deriving calibration curves for 44 coral genera using a large number of samples collected from several colonies and at different localities. Additionally, the sample weight was several g and the isotopic ^{18}O signal probably represented the average annual temperature.

The slopes of the relationship between skeletal $\delta^{18}O$ and temperature obtained with *Stylophora pistillata* and *Acropora* sp. using a predictive regression (Model I) are expressed in ‰ $°C^{-1}$ and compared with data from the literature in Table 3. Most authors have used a standard regression technique to investigate the relationship between temperature (Y) and $\delta^{18}O$ (X). This is statistically inappropriate when none of the variables are controlled, as is the case for calibration carried out with colonies collected in the field. Geometric regression technique (Model II) should be used in this case. Temperature is controlled when calibrations are carried out under experimental conditions (present study) and is therefore used as the X variable, providing slopes in ‰ $°C^{-1}$ as opposed to units of $°C$ ‰$^{-1}$ found in other studies. The data provided in the literature do not enable derivation of the slope expressed in ‰ $°C^{-1}$ from the slope reported in $°C$ ‰$^{-1}$. The slopes obtained in previous papers are reported in Table 3 as the inverse of the slope in $°C$ ‰$^{-1}$. It must be pointed out that this is an approximate procedure. In general, slopes of the calibration curve obtained for the corals range between -0.14‰ $°C^{-1}$ (*Diploria labyrinthi-*

formis; Cardinal 1996) and -0.29‰ $°C^{-1}$ (*Montipora* sp.; Weber & Woodhead 1972). The slope obtained for *Acropora* sp., -0.27‰ $°C^{-1}$ (or $-3.28 \pm 0.28°C$ ‰$^{-1}$ when temperature is the Y-axis), is close to the one reported by Weber & Woodhead (1972; -0.28‰ $°C^{-1}$ or $-3.57°C$ ‰$^{-1}$). Cornu (1995) investigated samples of *Acropora nobilis* from 2 colonies recovered in Mayotte (Indian Ocean) and reported slopes of -0.19 and -0.27‰ $°C^{-1}$. Juillet-Leclerc et al. (1997a) have reported a slope of -0.15‰ $°C^{-1}$ for *Acropora formosa* (Great Barrier Reef, Australia). They suggested that such a low slope was a result of secondary aragonite deposition which reduces the annual isotopic amplitude corresponding to the annual temperature difference (Juillet-Leclerc et al. 1997b).

The slope obtained with *Stylophora pistillata* (-0.13‰ $°C^{-1}$) is low compared to the one reported by Weber & Woodhead (1972; -0.22‰ $°C^{-1}$) and the correlation coefficient is weak. As mentioned above, the taxonomic status of the samples of *S. pistillata* used is not clear at the species level. The large variability of the results and the low correlation coefficient may result from a high variability within the genus as reported previously (Weber & Woodhead 1970).

The new experimental coral culture technique described in the present paper enables easy and accurate sampling of calcium carbonate deposited under various controlled environmental conditions. This technique provides a unique opportunity (1) to investigate the effect of environmental factors on the isotopic composition and trace element concentration in coral skeleton and, therefore, (2) to calibrate the proxies used to derive information on past tropical oceanic cli-

Table 3. Slope of the relationship between $\delta^{18}O$ and temperature (‰ $°C^{-1}$). The inverse value of the slope expressed in $°C$ ‰$^{-1}$ is given. Only 4 of the 44 calibrations of Weber & Woodhead (1972) are shown. *b* is the slope of the regression line

Source	Type and species	Locations	N	b	SE	r^2
Weber & Woodhead (1972)	*Pocillopora*	24 locations	224	−0.26		−
	Montipora	20 locations	20	−0.29		−
	Acropora	27 locations	835	−0.28		−
	Stylophora	15 locations	102	−0.22		−
Weil et al. (1981)	*Montipora verrucosa*	Aquarium culture	17	−0.22	0.03	0.74
	Pocillopora damicornis	Aquarium culture	25	−0.23	0.02	0.80
Cornu (1995)	*Acropora formosa*	Mayotte	3	−0.22	0	1
	A. nobilis	Mayotte	7	−0.27	0.03	0.93
	A. nobilis	Mayotte	5	−0.19	0.01	0.99
Juillet-Leclerc (1997)	*Acropora formosa*	Great Barrier Reef, Australia	21	−0.15	0.02	0.73
This study	*Acropora* sp.	Aquarium culture	17	−0.27	0.02	0.89
	S. pistillata	Aquarium culture	43	−0.13	0.02	0.22

mate. The skeletal microstructure is similar in corals grown in aquaria and *in situ*.

The slope of the relationship between skeletal $\delta^{18}O$ and temperature in *Acropora* sp. is close to the *in situ* calibration previously reported in the literature. The large variability of the skeletal $\delta^{18}O$ obtained in pieces of *Stylophora* sampled from different parent colonies provides indirect evidence that within-genus variability may scramble the information recorded in skeletal carbonate. It is suggested that the taxonomic status of the colonies used should be carefully considered prior to using a particular calibration curve to make inferences on past temperature. This culture technique is presently being used to investigate the effect of environmental parameters other than temperature, such as salinity, light and CO_2 partial pressure on the stable isotope composition and trace element concentration of coral skeleton.

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Erratum

A novel culture technique for scleractinian corals: application to investigate changes in skeletal $\delta^{18}O$ as a function of temperature

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The $\delta^{18}O$ data which appeared in Fig. 5 of the above-mentioned paper are incorrect. Values of seawater $\delta^{18}O$ were mistakenly added instead of subtracted from aragonite $\delta^{18}O$. The correct data are 2.04‰ more negative than shown in the original figure. The corrected figure is presented below.

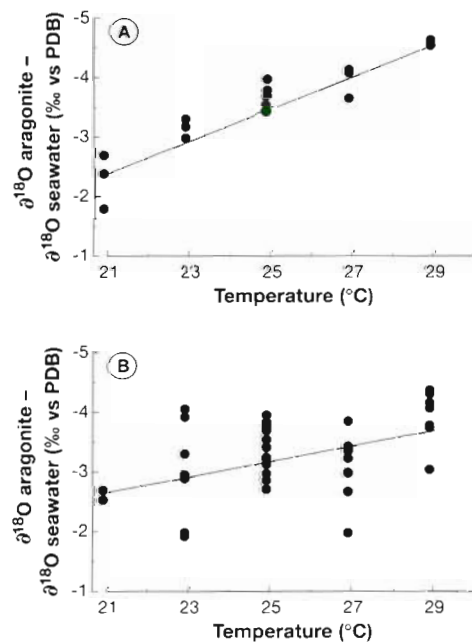This change makes no difference to the conclusions of the paper.

Fig. 5. Skeletal $\delta^{18}O$ versus temperature in samples of (A) *Acropora* sp. and (B) *Stylophora pistillata*