Growth-rate influences on coral climate proxies tested by a multiple colony culture experiment

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ARTICLE INFO

ABSTRACT

As application of coral-based climate reconstruction has become more frequent at tropical sites, increased attention is being paid to the potential ambiguities of coral thermometers that are intrinsic to the biomineralisation process, including the so-called vital effect, the growth-rate-related kinetic effect, and the $[CO_3^{2-}]$ effect. Here we studied how the growth rate influenced the skeletal oxygen and carbon isotope ratios ($\delta^{18}O$ and $\delta^{13}C$) and the Sr/Ca ratio in a common-garden experiment involving the long-term culture of *Porites australiensis* clone colonies. Comparison of the seasonal minimum $\delta^{18}O$ values during summer showed a negligible influence of the large intercolony variation in growth rate (2–10 mm yr^{-1}) on $\delta^{18}O$ variation, but $\delta^{18}O$ was relatively sensitive to temporary intracolony growth-rate changes related to colony health. In contrast, the Sr/Ca ratio was robust against both inter- and intracolony growth-rate variation. We found a positive shift in $\delta^{13}C$ in slower growing corals, which we attributed to the kinetic behaviour of the calcification reaction. The seasonal fluctuation in $\delta^{13}C$ corresponded not to changes in light intensity nor to $\delta^{13}C$ of dissolved inorganic carbon in seawater, but to photosynthetic efficiency as measured by pulse-amplitude photometry. These findings support the inference that coral skeletal Sr/Ca and $\delta^{18}O$ in a long-lived colony can function as a palaeoclimate archive by recording signals of clonal growth. We also propose practical guidelines for the proper interpretation of coral records.

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1. Introduction

Corals are rich archives of climatic changes in tropical seas during the recent and distant past (Felis et al., 2004; Gagan et al., 1998, 2000; Urban et al., 2000; Watanabe et al., 2011). In particular, application of the dual proxy method (Gagan et al., 2000), which involves simultaneous analyses of skeletal oxygen isotope ratios ($\delta^{18}O$) and Sr/Ca ratios, allows temperature and salinity variations to be distinguished and thus has great potential for exploration of the precise dynamics of climate change. Although coral skeletal climatology has already made a remarkable contribution to our understanding of climate system variability, including phenomena such as El Niño-Southern Oscillation, increased attention is being paid to the potential ambiguities of coral thermometers that are intrinsic to the biomineralisation process, including the so-called vital effect (Urey et al., 1951; Erez, 1978) and

the growth-rate-related kinetic effect (McConnaughey 1989; Cohen and McConnaughey 2003). Moreover, a $[CO_3^{2-}]$ effect on skeletal $\delta^{18}O$ values has also been proposed, but only in foraminifera and deep-sea non-symbiotic corals (Spero et al., 1997; Adkins et al., 2003). In particular, coral growth rates may affect $\delta^{18}O$ and the carbon isotope ratio ($\delta^{13}C$) (McConnaughey 1989; Cohen and McConnaughey 2003; de Villiers et al., 1995; Felis et al., 2003; Maier et al., 2004; Suzuki et al., 2005), as well as elemental ratios such as Sr/Ca (de Villiers et al., 1995), Mg/Ca (Inoue et al., 2007), and U/Ca. As a practical way of addressing these ambiguities, some level of replication (ideally, in three separate corals) of geochemical tracers at each site is recommended to enhance the reliability of the palaeoclimate record (Lough, 2004), but such replication is both costly and time-consuming.

In this study, we examined the magnitude of growth-rate influences on coral $\delta^{18}O$, $\delta^{13}C$, and Sr/Ca ratios in a long-term common-garden culture experiment of *Porites australiensis* corals. *Porites* is the coral genus most commonly used for palaeoclimate studies in Indo-Pacific regions (Gagan et al., 2000). We designed an experiment for examining the influence of growth rate on skeletal composition at multiple levels of the biological hierarchy,

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including both intracolony and interclone growth-rate variability. This experiment takes advantage of the fact that coral clone colonies can be easily established by dividing a parental colony (Davies, 1995).

2. Materials and methods

2.1. Coral cultivation details

Five colonies of *P. australiensis* (colonies 1–5, Figs. A.1c–l, in the Supplementary data) were collected from a fringing reef near Sesoko Island, Okinawa (26 °N, 128 °E, Fig. A.1a), between 18 November 2003 and 21 January 2004. Colonies growing at least 40 m apart were chosen to ensure that they were genetically distinct (Fig. A.1b). The colonies were cut into 1.5 cm × 1.5 cm × 1.5 cm cubes at Sesoko Station, Tropical Biosphere Research Centre, the University of the Ryukyus. The clone cube colonies were first allowed to heal and then further acclimated in an outdoor tank under moderate light intensity, controlled by partial shading with a sun-screen mesh (reduced by ∼24% compared with the ambient light intensity). Before the beginning of culture (16 March, 2004), all colonies were stained with Alizarin red S (15 mg L^{-1}, 24 h with aeration). We labelled each colony with a number (1–5), indicating the parental colony, and an uppercase letter (A–D), indicating the clone colony. To test the reproducibility of coral growth variation of clone colonies from the same parental colony, the clone colonies were cultured together in a single water tank. The number of clone colonies from each parental colony was different because some clone colonies were harvested before the end of the culture period for other purposes. At the end of the experiment in 2010, parental colonies 1–3 were each represented by four clone colonies (A–D), parental colony 4 by two (A, B) clone colonies, and parental colony 5 by a single clone colony (A). All of these clone colonies were cultured for 75 months from 16 April 2004 to 23 June 2010 in a flow-through outdoor tank, to which seawater was supplied continuously (turnover time: ∼2 h). The seawater was continuously aerated. Light intensity, which was controlled by partial shading during the experiment, appeared to be similar to its intensity in a shallow coral reef environment at a water depth of ∼3 m.

Temperature, salinity, and irradiance were continuously recorded by data loggers at 30-min intervals (temperature and salinity, Compact-CTW, Alec Electronics Co. Ltd., Kobe, Japan; irradiance, Hobo, Onset, Pocasset, MA, USA). The oxygen isotope ratio of water ($\delta^{18}O_w$) and the carbon isotope ratio of dissolved inorganic carbon ($\delta^{13}C_{DIC}$), together with salinity, of discretely collected water samples were also measured. The discrete water samples were collected at frequencies ranging from 2–3 times a week to once a month during the culture period. All oxygen and carbon isotope values of the samples are represented in the common δ notation as the per mil (‰) deviation from a standard:

$$\delta^{18}O(‰) = [(^{18}O/^{16}O)_{sample}/(^{18}O/^{16}O)_{standard}-1] \times 1000 \qquad (1)$$

$$\delta^{13}C(‰) = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard}-1] \times 1000. \qquad (2)$$

The $\delta^{18}O_w$ values are reported relative to V-SMOW (Vienna Standard Mean Ocean Water), and the $\delta^{13}C_{DIC}$ values are reported relative to V-PDB (Vienna Peedee Belemnite). All water properties were measured by previously described procedures (Ushie et al., 2010). The chlorophyll fluorescence of all colonies was assessed non-invasively by using a pulse-amplitude modulation (PAM) fluorometer weekly beginning in August 2007. At the end of the experiment, corals were sampled and processed for skeletal isotopic measurements and sex identification (see Section 2.3 for details of the PAM measurement procedure).

2.2. Isotopic and elemental compositions of coral skeletons

We examined the skeletal composition of a single clone colony from each of the five parental colonies. Because our aim in this study was not to examine inter-clone (among clones of a single parental colony) variation of skeletal composition, we assumed that the skeletal composition of clone A of each parental colony was representative of that parental colony for the purpose of evaluating intra-specific (intercolony) variation of skeletal composition in *P. australiensis*.

Coral colonies were sectioned into 6-mm-thick slabs and cleaned with Milli-Q water. No chemical treatment of the samples was carried out prior to isotopic analysis. Coral specimens were microsampled vertically along the major growth axis, as shown in X-ray photographs (positive image) of the coral slabs taken by a "soft" (i.e., relatively long wavelength) X-ray camera (Sofron Type STA-1005, SOFRON Co. Ltd., Tokyo, Japan). Samples were extracted for isotopic microprofiling as described elsewhere (Gagan et al., 1998; Watanabe et al., 2011; Suzuki et al., 2003, 2005; Omata et al., 2005, 2008). Microsamples were obtained at 200-μm intervals along the major growth axis of all colonies, except one slow-growing colony (Colony 2A) was subsampled at 100-μm intervals (see Table A.1). The average temporal sampling resolution was finer than 1 week, but the resolution gradually became lower as the colony growth-rate decreased. Microsamples of the five coral colonies, each weighing approximately 70–110 μg, were reacted with 104% H_3PO_4 at 25 °C in a custom-made carbonate preparation device (Ishimura et al., 2008), and isotopic ratios were determined with a Micromass Isoprime mass spectrometer (Isoprime Limited, Cheadle, UK). Oxygen and carbon isotope ratios of corals are reported relative to V-PDB by adopting consensus values of −2.2‰ and 1.95‰, respectively, for the NBS 19 international reference standards relative to V-PDB. The precision was better than 0.1‰ and 0.05‰ (1σ) for the oxygen and carbon isotope ratios, respectively. Isotopic microprofiling was performed on more closely spaced microsamples around the seasonal extremes to ensure full recovery of the seasonal amplitude fluctuation. Colonies 2A and 5A, however, were not microsampled over the interval covering the period from summer 2003 to winter 2005, because their growth rate was very slow, and isotope ratios were not measured.

In addition, we measured Sr/Ca ratios with an inductively coupled plasma atomic emission spectrophotometer (IRIS Advantage, Thermo Electron Co., Ltd), as described by Mishima et al. (2009). About 100 μg of skeletal material, a sample size similar to that used for the isotopic measurements, was used for Sr/Ca analysis. The reference material JCp-1 prepared by the Geological Survey of Japan was used for the standard material (Okai et al., 2002). Sr/Ca measurements were made at closer intervals than the $\delta^{18}O$ measurements, especially in the intervals of some colonies characterised by slow skeletal growth. For skeletal material formed after 2006, both the isotopic and elemental measurements were made on aliquots from the same microsamples in most cases. All sample analyses were carried out at the Geological Survey of Japan, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan.

2.3. PAM measurement

To assess the photosystem II (PS II) efficiency of the *in hospite* symbiotic algae, we applied a chlorophyll fluorescence technique (Schreiber et al., 1986) using a Diving-PAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany) (Nakamura et al., 2005). The PAM method is non-invasive and enables PS II efficiency to be assessed without causing any damage to the corals. Minimum fluorescence (F_0) was determined by using 3-μs pulses from a