AGU PUBLICATIONS

Geochemistry, Geophysics, Geosystems

RESEARCH ARTICLE
10.1002/2014GC005322

Key Points:
- Stable isotopic composition of *Acropora* polyps at four temperatures is examined
- Oxygen isotopic composition was significantly correlated with temperature
- Calcification flux is examined to evaluate the growth-rate effect on stable isotopes

Supporting Information:
- Readme
- Tables S1 and S2

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Citation:
Nishida, K., K. Ishikawa, A. Iguchi, Y. Tanaka, M. Sato, T. Ishimura, M. Inoue, T. Nakamura, K. Sakai, and A. Suzuki (2014), Skeletal oxygen and carbon isotope compositions of *Acropora* coral primary polyps experimentally cultured at different temperatures, *Geochem. Geophys. Geosyst.*, *15*, 2840–2849, doi:10.1002/2014GC005322.

Received 28 FEB 2014
Accepted 14 JUN 2014
Accepted article online 18 JUN 2014
Published online 14 JUL 2014

Skeletal oxygen and carbon isotope compositions of *Acropora* coral primary polyps experimentally cultured at different temperatures

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Abstract We investigated temperature and growth-rate dependency of skeletal oxygen and carbon isotopes in primary polyps of *Acropora digitifera* (Scleractinia: Acroporidae) by culturing them at 20, 23, 27, or 31°C. Calcification was most rapid at 27 and 31°C. We obtained a δ^{18}O-temperature relationship ($-0.18‰$ °C^{-1}) consistent with reported ranges for *Porites*, indicating that juvenile *Acropora* polyps can be used for temperature reconstruction. A growth-rate dependency of skeletal isotopes was detected in the experimental polyps cultured at lower water temperatures, when the skeletal growth rate of these polyps was also low. The estimated upper calcification flux limit for a kinetic isotope effect to be observed in the δ^{18}O-growth rate relationship (~0.4–0.7 g $CaCO_3$ cm^{-2} yr^{-1}) was similar to the calcification flux in *Porites* corresponding to a linear extension rate of 5 mm yr^{-1}, the maximum rate at which the kinetic isotope effect is evident. This result suggests that the calcification flux can be used as a measure of growth rate-related isotope fractionation, that is, the kinetic isotope effect, in corals of different genera and at different growth stages.

1. Introduction

Coral skeletons such as those of massive *Porites* colonies have annual density bands visible on X-ray images that allow particular years and seasons to be identified. The stable oxygen isotope (δ^{18}O) composition of corals is a long-term and high-resolution record of climate [e.g., *Gagan et al*., 2000]. Skeletal δ^{18}O depends on both the precipitation temperature and the oxygen isotope composition of the ambient seawater, which in turn reflects sea surface salinity. The δ^{18}O record of *Porites* corals, for example, has been used to reconstruct El Niño-Southern Oscillation events in the tropical Pacific in both the near and the distant past [*Cobb et al*., 2003; *Watanabe et al*., 2011].

Although skeletal δ^{18}O of *Porites* corals has been of great utility in many climate studies, skeletal δ^{18}O in other coral genera has been less studied. In many reefs in the Indo-Pacific region, *Acropora*, which includes many temperate species, is the most abundant coral genus [*Wallace and Willis*, 1994]. For this study, we selected primary polyps of *Acropora digitifera* (Scleractinia: Acroporidae), a dominant species in Okinawan reefs [*Nakajima et al*., 2009], for a temperature-controlled experiment. Ecological and environmental studies of post-settlement juvenile corals [*Babcock et al*., 2003; *Edmunds*, 2007; *Putnam et al*., 2008] have been reported recently. Temperature proxies (e.g., δ^{18}O) of the polyp skeleton can be useful for identifying the period of settlement of the polyps and for reconstructing the skeletal growth record of the juvenile corals to reveal their life history. In addition, as in adult corals, skeletal chemistry of coral primary polyps is useful as environmental proxies [*Cohen et al*., 2009; *Inoue et al*., 2011].

Previous studies have reported that skeletal isotopic compositions are related to the growth rate [*McConnaughey*, 1989a, 1989b; *Allison et al*., 1996; *Cohen and McConnaughey*, 2003; *Hayashi et al*., 2013], and isotopic disequilibria detected in coral skeletal δ^{18}O and δ^{13}C have been attributed to metabolic and kinetic isotope effects. *McConnaughey et al*. [1997] observed that "kinetic fractionations originate from slower hydration and hydroxylation of CO_2 by molecules bearing the heavy isotopes ^{13}C and ^{18}O," and called this

Table 1. Temperature, Light Intensity, Salinity, and Water Flow Rate in Each Treatment of the Temperature-Controlled Culturing Experiment

Temperature (°C)	Light (μmol m^{-2} s^{-1})	Salinity	Water Flow Rate (mL min^{-1})
Temperature Experiment			
31.3 ± 0.7	129.6 ± 2.3	34.8 ± 0.2	155
27.0 ± 0.2	128.6 ± 2.2	34.8 ± 0.2	145
23.3 ± 1.2	130.6 ± 3.3	35.0 ± 0.0	153
20.0 ± 1.2	131.1 ± 2.5	35.0 ± 0.0	151
Before the Experiment			
27.5 ± 1.3	<34.2 ± 6.9	34.2 ± 0.2	0

phenomenon a kinetic isotope effect. Further, kinetic isotope effects in coral skeletons associated with the skeletal extension rate have occasionally been reported [*McConnaughey*, 1989a, 1989b; *Allison et al.*, 1996; *Cohen and McConnaughey*, 2003]. No study, however, has examined whether the skeletal isotopic compositions of primary polyps exhibit a growth-rate dependency.

We cultured primary polyps of *A. digitifera* at four temperature settings to investigate the relationship between δ^{18}O of the skeletal aragonite and temperature, and to assess the potential use of this species as a paleotemperature proxy. We also compared the δ^{18}O and δ^{13}C of the experimental specimens with values expected under inorganic equilibrium, and examined the influence of the skeletal growth rate on the stable oxygen and carbon isotope compositions of the primary polyps. *Acropora digitifera* is particularly suitable for investigating relationships between skeletal isotopic compositions and the skeletal growth rate of individual polyps because *A. digitifera* planulae can be induced to metamorphose by administering a peptide [*Suwa et al*., 2010; *Ohki et al*., 2013]. Thus, genetically and developmentally homogeneous primary polyps can be obtained for experimental use. Unfortunately, metamorphosis cannot be similarly induced in *Porites*.

2. Materials and Methods

We cultured primary polyps of *A. digitifera* at Sesoko Station, University of the Ryukyus, Motobu, Okinawa, Japan. We collected several *A. digitifera* colonies from a fringing reef of Sesoko Island and obtained gametes spawned by these colonies on 1 June 2013. We cultured *A. digitifera* planulae at approximately 27°C for 10 days and then prepared primary polyps following *Inoue et al*. [2012] and *Tanaka et al*. [2013]. Ten six-well culture plates containing settled primary polyps were prepared on 12 June 2013. To prepare symbiotic primary polyps, we infected primary polyps of *A. digitifera* with zooxanthellae (*Symbiodinium*, clade A) from the giant clam *Tridacna crocea*, because this type of zooxanthellae easily infects coral primary polyps [*Hirose et al*., 2008; *Suwa et al*., 2010]. The primary polyps were then cultured at approximately 27°C for 4 more days until the infection was recognizable.

On 16 June, we removed two plates and used the polyps on them to determine the initial masses, areas, and skeletal chemistry of the polyps. Then, also on 16 June, we transferred two culture plates containing settled primary polyps to each of four 12 L aquariums, which contained seawater that had been filtered through a 1 μm mesh. Each aquarium was maintained at a nominal treatment temperature of 20, 23, 27, or 31°C (Table 1), and the water temperature of each aquarium was recorded every 10 min. Before the experiment, the specimens were cultured in the laboratory under shaded natural light conditions, but during the temperature experiment, the aquariums were illuminated with metal-halide lamps for 12 h each day (08:00–20:00); the average irradiance was approximately 130 μmol m^{-2} s^{-1} (Table 1).

The total experimental duration of each temperature treatment was 10 days. Ten polyps in each culture plate were marked individually by using a permanent marker to write their numbers on the plate bottom. On day 1 (16 June) and day 8 (23 June), 80 marked polyps cultured in the four temperature treatments (20 per treatment) were photographed under a light microscope (Figure 1) and their areas were measured by using ImageJ/NIH version 1.45 image analysis software (available as freeware at http://rsbweb.nih.gov/ij/). We used JMP statistical software (SAS Institute Inc.) for all statistical analyses, and compared dry weight and areas among the experimental temperature treatments by one-way factorial ANOVA followed by Tukey's HSD test. Significance (type I error level) was set at $\alpha = 0.05$. All data were assessed for normality and homogeneity. At the end of the treatments (26 June), polyp tissues were first removed from each individual with a water-pik and then each six-well plate with polyps was cleaned in an ultrasonic bath to remove any residual tissue. Almost complete removal of tissue was confirmed by visual inspection of the polyps under a stereomicroscope. We used the flat end of a microspatula to carefully remove each polyp from the six-well plates. Among the polyps cultured at 20°C, nine specimens fractured when we removed them from the

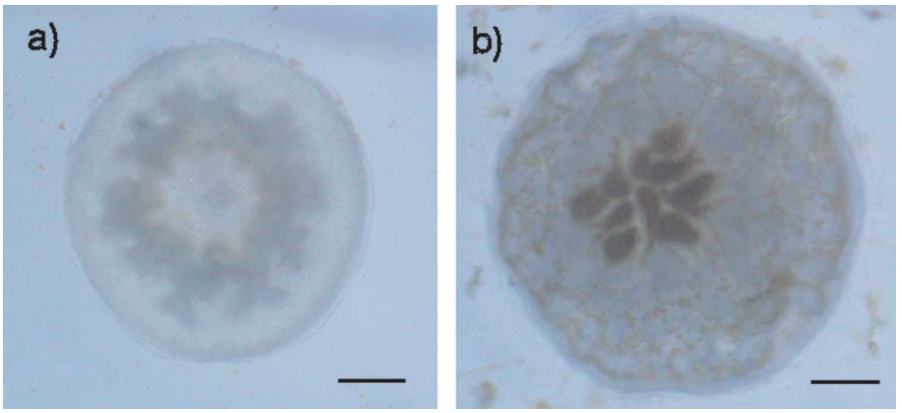

Figure 1. A living polyp cultured at 27°C (specimen 4–1) photographed under a light microscope. The polyp was photographed on (a) 16 June and (b) 23 June (day 1 and day 8 of the experiment, respectively). Scale bar, 200 μm.

plate because of their small size. Thus, we could not collect all of the marked polyps (see Table S1). The removed samples of the cultured and initial polyps were dried and weighed on a microbalance.

Skeletal carbonates of a total of 30 polyps (see Table S2) were individually analyzed for oxygen and carbon isotopes. The samples were treated with 104% H_3PO_4 at 25°C in a custom-made carbonate preparation device [*Ishimura et al*., 2004], and the isotopic ratios were determined with a Micromass Isoprime mass spectrometer. Here we report the oxygen and carbon isotope ratios of the coral skeletons ($\delta^{18}O_c$ and $\delta^{13}C_c$) relative to Vienna Pee Dee Belemnite (V-PDB), adopting the consensus values of $-2.20‰$ and $+1.95‰$, respectively, for the NBS 19 international reference standard relative to V-PDB. For the skeletal samples, the measurement precision was better than $0.1‰$ and $0.2‰$ (1SD) for $\delta^{18}O$ and $\delta^{13}C$, respectively. On 6, 13, 21, and 24 June, we collected water samples from the treatment tanks for $\delta^{18}O$ measurement of the seawater ($\delta^{18}O_w$) and for $\delta^{13}C$ measurement of dissolved inorganic carbon (DIC) in the seawater ($\delta^{13}C_{DIC}$). These analyses were carried out with a Thermo-Fisher Scientific DELTA plus XL mass spectrometer at SI Science Co., Japan. The precision was better than $0.1‰$ and $0.1‰$ (1SD) for $\delta^{18}O_w$ and $\delta^{13}C_{DIC}$, respectively. All $\delta^{18}O_w$ values were calculated relative to Vienna Standard Mean Ocean Water (V-SMOW), and $\delta^{13}C_{DIC}$ was calculated relative to V-PDB.

3. Results and Discussion

3.1. Skeletal Growth of Primary Polyps at the Four Different Temperatures

Temperature, light intensity, salinity, and the water flow rate of each treatment in the experimental tanks are shown in Table 1. Among the marked polyps ($N = 80$), only two (one polyp each in the 27°C and 23°C treatments) died during the experiment. The polyps cultured at higher temperature calcified much more than those cultured at lower temperatures (Figure 2), as indicated by significant differences in the skeletal dry weight of primary polyps between the 27°C and 31°C treatments and the other treatments. The skeletal weight of the primary polyps cultured at 20°C did not differ significantly from that of the initial polyps (Figure 2), indicating insignificant growth at this temperature. The area of the primary polyps increased significantly with water temperature (Figure 2), but there was no significant difference in area among each temperature treatment.

Inoue et al. [2012] conducted a temperature-controlled experiment with polyps of *A. digitifera* using methods almost identical to those of the present study in which they employed four temperature settings, 27, 29, 31, and 33°C (i.e., a narrower range of temperature settings than that used in the present study). They reported that polyps cultivated at 27 and 31°C showed similar growth rates with no significant difference, although they suggested that the optimum growth temperature was between 29 and 31°C. Therefore, our experimental result of skeletal growth of primary polyps cultured at 20–31°C is consistent with the previous result of skeletal growth of polyps cultured at 27–33°C [*Inoue et al*., 2012]. Future ocean water temperature increases due to global warming [*IPCC*, 2013] are expected to result in severe damage to coral reefs [*Hoegh-Guldberg*, 1999; *Wilkinson and Souter*, 2008]. In adult *Acropora* corals, short-term but extreme thermal stress

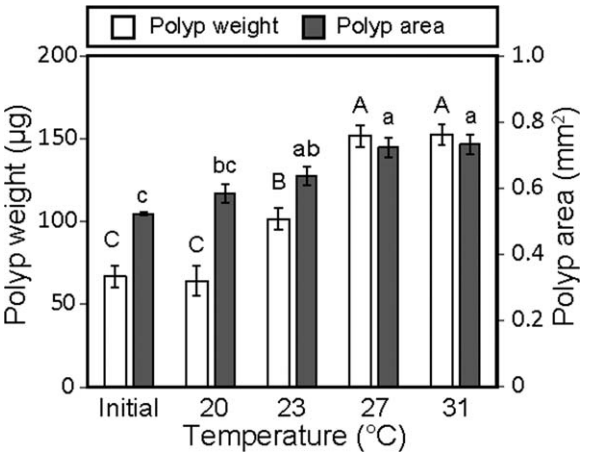

Figure 2. Weight ($N = 98$) and skeletal area ($N = 80$) of the experimental polyps. Different letters above the bars indicate statistically significant differences (ANOVA followed by Tukey's HSD test, $\alpha = 0.05$). Error bars represent the standard error (± 1 SE).

(the threshold temperature: 30–31°C) can cause bleaching and reduced coral cover of adult *Acropora* corals in Okinawa, as was shown by a global coral bleaching event that occurred in 1997–1998 [*Loya et al*., 2001; *van Woesik et al*., 2004]. Moreover, it has been reported that corals in the reproduction and development life stages are often less able to tolerate high water temperature than adult corals [*Cossins and Bowler*, 1987; *Johnston and Bennett*, 1996]. Our experimental data, however, indicated that polyps calcified rapidly at 31°C, and that the survival rate of primary polyps at 31°C was the same as that of polyps cultured at lower temperatures. *Loya et al*. [2001] reported that, in 1998 during the global mass bleaching event, juvenile *Acropora* colonies in Okinawa survived in the intertidal zone despite being exposed to high irradiance and desiccation during summer midday low tides while adult *Acropora* colonies were severely damaged at the temperature of 30–31°C. They hypothesized that small colonies, which are often flat for up to 2 years, before they begin to branch and form three-dimensional structures, should have higher mass transfer than large three-dimensional *Acropora* colonies, and hence preferentially survive over larger colonies under environmentally stressful conditions. Thus, they suggested that the surviving juvenile *Acropora* colonies in Okinawa may allow the recovery of local *Acropora* populations. The relationship between water flow and coral survival under high temperatures was also examined experimentally and theoretically in *Nakamura and van Woesik* [2001]. Thus, our experimental results might indicate a higher thermal tolerance of primary polyps of *A. digitifera* due to a flat structure in the early post-settlement stage. In Okinawa, *A. digitifera* spawns each year from May to June, at which time the ambient temperature of Okinawan waters is 27°C [*Negri et al*., 2007]. Fast growth in summer may be advantageous for the survival in early postsettlement stage around Okinawa where short-term fluctuations of water temperature are often observed. However, a temperature experiment of longer duration conducted with primary polyps of *A. digitifera* is needed to investigate the long-term effects of exposure to high temperatures.

3.2. Use of $\delta^{18}O$ of Primary Polyps as a Paleotemperature Proxy

The isotope values of the skeletal carbonate of the individual specimens ranged from $-5.5‰$ to $-2.9‰$ ($\delta^{18}O_c$) and from $-8.1‰$ to $-3.8‰$ ($\delta^{13}C_c$), respectively (Table S2); whereas, $\delta^{18}O_w$ and $\delta^{13}C_{DIC}$ were $+0.2 \pm 0.0‰$ ($N = 4$) and $+0.5 \pm 0.1‰$ ($N = 4$), respectively. As the skeletal $\delta^{18}O_c$ is affected by both water temperature and $\delta^{18}O$ of ambient seawater, we subtracted the $\delta^{18}O_w$ from the $\delta^{18}O_c$ of polyps to remove the effect of $\delta^{18}O_w$ from the $\delta^{18}O_c$ (Figure 3a). The difference between skeletal $\delta^{18}O_c$ and $\delta^{18}O_w$ correlated strongly with water temperature ($R = 0.91$, $P < 0.001$; Figure 3a), whereas skeletal $\delta^{13}C_c$ did not (Figure 3b). Each polyp skeleton consisted of aragonite deposited during the pretreatment period (before the culture plates were transferred to the different water temperature treatments) as well as aragonite deposited during the treatments. Thus, we estimated the $\delta^{18}O_c$ and $\delta^{13}C_c$ values of skeletal carbonate deposited during each treatment by a mass balance calculation. We calculated $\delta^{18}O_p$ and $\delta^{13}C_p$ (treatment deposition) from $\delta^{18}O_i$ and $\delta^{13}C_i$ (pretreatment deposition) and $\delta^{18}O_c$ and $\delta^{13}C_c$ (measured values) as follows (Figures 3c and 3d):

$$\delta^{18}O_p = \delta^{18}O_c[W_c/(W_c - w_i)] - \delta^{18}O_i(w_i/W_c)\ [W_c/(W_c - w_i)],$$
$$\delta^{13}C_p = \delta^{13}C_c[W_c/(W_c - w_i)]\ - \delta^{13}C_i(w_i/W_c)\ [W_c/(W_c - w_i)],$$

where W_c is the weight of each polyp measured after the temperature treatment and w_i is the mean initial polyp weight (58.5 ± 4.5 μg, excluding two outliers); $\delta^{18}O_i$ and $\delta^{13}C_i$ are the mean $\delta^{18}O$ and $\delta^{13}C$ values of

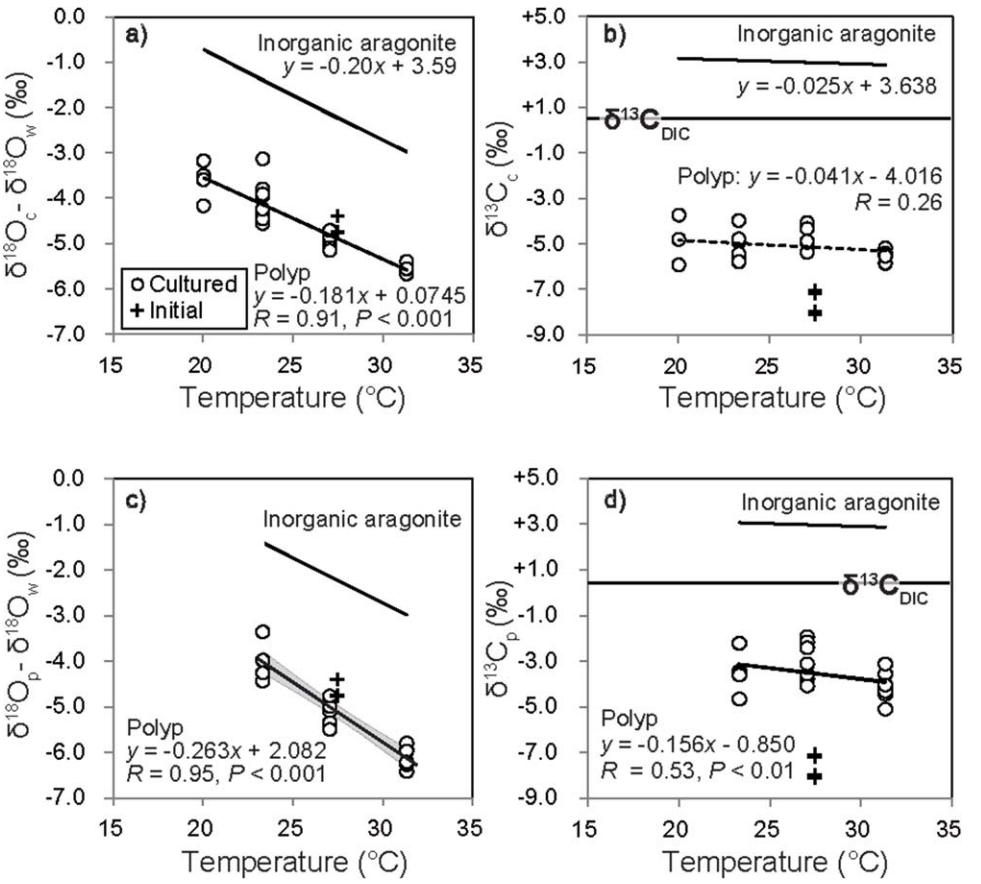

Figure 3. $\delta^{18}O$ and $\delta^{13}C$ in primary polyps cultivated at the four temperatures. (a) Relationship between the difference $\delta^{18}O_c - \delta^{18}O_w$ of the whole polyp skeleton and water temperature. $\delta^{18}O_w$ was $+0.2 \pm 0.0‰$ (mean, ± 1 standard deviation) during the experiment ($N = 4$). We also show the equilibrium curve of inorganic aragonite calculated by the method of *Kim et al*. [2007] using our temperature data and $\delta^{18}O_w$ values. (b) Relationship between $\delta^{13}C_c$ of the whole polyp skeleton and water temperature. $\delta^{13}C_{DIC}$ was $+0.5 \pm 0.1‰$ (mean ± 1 standard deviation) during the experiment ($N = 4$). We also show the equilibrium curve of inorganic aragonite calculated by the method of *Romanek et al*. [1992] and *Zhang et al*. [1995] using our temperature data and $\delta^{13}C_{DIC}$ values. (c) Relationship between the difference $\delta^{18}O_p - \delta^{18}O_w$ and water temperature, and (d) the relationship between $\delta^{13}C_p$ and water temperature. We estimated $\delta^{18}O_p$ and $\delta^{13}C_p$ of the skeleton deposited at each temperature treatment by a mass balance calculation to remove the effect of the $\delta^{18}O_c$ of aragonite deposited during the pretreatment period. Open circles show the skeletal $\delta^{18}O$ values of the polyps cultivated in the four temperature treatments, and crosses show those of the initial polyps (pretreatment). Solid lines and dashed lines show statistically significant and insignificant correlations, respectively. The gray shading in Figure 3c shows the 95% confidence interval of $\delta^{18}O_p$.

the initial polyps; and $\delta^{18}O_p$ and $\delta^{13}C_p$ are the $\delta^{18}O$ and $\delta^{13}C$ values of the polyp skeleton deposited during the treatment. The variation of $\delta^{18}O_p$ due to the initial variation of polyp weight (± 4.5 μg) was within 0.3‰ (at 31°C), 0.3‰ (27°C), 0.2‰ (23°C), and 0.4‰ (20°C); and the variation of $\delta^{13}C_p$ due to the initial variation of polyp weight was within 0.2‰ (at 31°C), 0.2‰ (27°C), 0.9‰ (23°C), and 2.1‰ (20°C). To calculate the $\delta^{18}O$-temperature relationship, we eliminated data for the polyps cultured at 20°C, and we used only data from polyps weighing more than 75 μg, in order to eliminate $\delta^{18}O$ values including a large contribution from the initial polyps. Using the $\delta^{18}O_p$ and $\delta^{18}O_w$ values, we obtained the following relationship between water temperature and aragonite-water isotope fractionation:

$$\delta^{18}O_p - \delta^{18}O_w = -0.263T + 2.082,$$

where T is the water temperature (°C; Figure 3c). The difference between skeletal $\delta^{18}O_p$ and seawater $\delta^{18}O_w$ also correlated strongly with water temperature ($R = 0.95$, $P < 0.001$; Figure 3c). By comparison, $\delta^{18}O$ temperature sensitivity values in *Porites* range from $-0.08‰\ °C^{-1}$ to $-0.22‰\ °C^{-1}$ [*Gagan et al*., 2012]; other reported values include $-0.21‰\ °C^{-1}$ in *Pavona* [*McConnaughey*, 1989a] and $-0.22‰\ °C^{-1}$ in *Acropora* [*Juillet-Leclerc et al*., 1997]. The $\delta^{18}O$ temperature sensitivity of primary polyps of *A. digitifera* in this study ($-0.263‰\ °C^{-1}$) is slightly higher than these published values, but it is close enough to these published values for the relationship to be considered a temperature proxy. Therefore, in this species, $\delta^{18}O$ of individuals

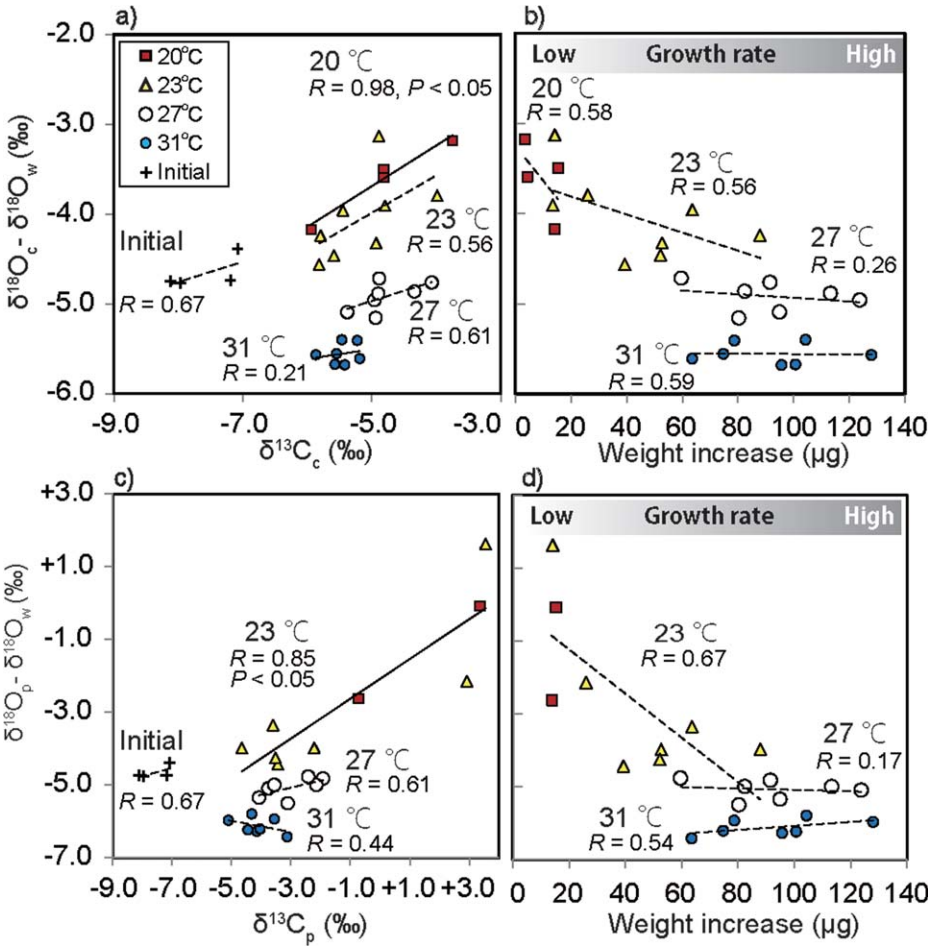

Figure 4. Relationships between (a) the difference $\delta^{18}O_c - \delta^{18}O_w$ and $\delta^{13}C$ of the whole polyp skeleton; (b) the difference $\delta^{18}O_c - \delta^{18}O_w$ and the weight increase during the temperature experiment, indicating the skeletal growth-rate dependency of $\delta^{18}O$; (c) the difference $\delta^{18}O_p - \delta^{18}O_w$ and $\delta^{13}C_p$ of the polyp skeleton cultured in the temperature treatments, where $\delta^{18}O_p$ and $\delta^{13}C_p$ were calculated by using a mass balance equation; and (d) the difference $\delta^{18}O_p - \delta^{18}O_w$ and the weight increase. We used $\delta^{18}O_p$ and $\delta^{13}C_p$ from polyps weighing more than 75 μg, in order to eliminate skeletal isotopic values including a large contribution from the initial polyps. Open circles indicate skeletal $\delta^{18}O$ values of the polyps cultivated in the four temperature treatments, and crosses indicated those of the initial polyps (pretreatment). Solid and dashed lines show statistically significant and insignificant correlations, respectively.

in the early stage of juvenile coral growth (i.e., primary polyps) can be used to reconstruct water temperatures within the range of 23–31°C.

We also calculated the equilibrium curve of $\delta^{18}O$ in inorganic aragonite by the method of *Kim et al*. [2007], using our temperature data and $\delta^{18}O_w$ values (Figures 3a and 3c); and we calculated the equilibrium curve of $\delta^{13}C$ of inorganic aragonite by the method of *Romanek et al*. [1992] and *Zhang et al*. [1995], using our temperature data and $\delta^{13}C_{DIC}$ values (Figures 3b and 3d). The gap between the curves for the experimental polyps and the equilibrium curves for inorganic aragonite was 2.0–3.3‰ for $\delta^{18}O_p$ and 4.8–8.0‰ for $\delta^{13}C_p$, with the primary polyp values being lower than the equilibrium values for inorganic aragonite (Figures 3c and 3d). This difference is called the "vital effect," and it is often observed in corals [*McConnaughey*, 1989a]. The vital effect offset reported by *McConnaughey* [1989a] ranges from 0 to 6‰ ($\delta^{18}O$) and from 0 to 12‰ ($\delta^{13}C$), and *Rollion-Bard et al*. [2003] reported offsets of 2–5‰ ($\delta^{18}O$). Thus, the vital effect offsets of *A. digitifera* primary polyps are within the ranges reported for other coral species.

3.3. Growth-Rate Control on Skeletal $\delta^{18}O$ and $\delta^{13}C$

The correlations between $\delta^{18}O_c$ and $\delta^{18}O_p$ of the experimental polyps and the skeletal dry weight increase (indicating the growth rate; Figures 4b and 4d) were negative at 20 and 23°C, and the slope of the relationship was less steep at 23°C than at 20°C. In each temperature treatment, $\delta^{13}C_c$ and $\delta^{13}C_p$ of the

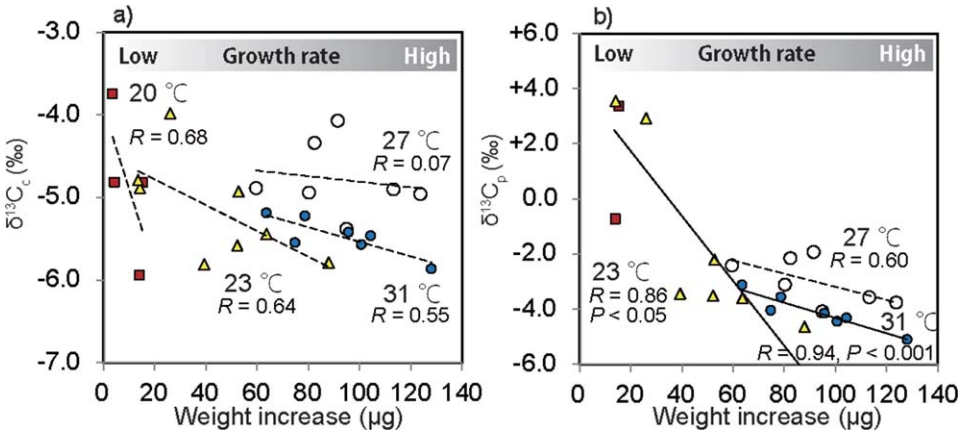

Figure 5. Relationships between (a) $\delta^{13}C_c$ of the whole polyp skeleton and the weight increase during the temperature experiment, and (b) $\delta^{13}C_p$ of the polyp skeleton cultured in the temperature treatments, calculated by using a mass balance equation, and the weight increase during the temperature experiment. We used $\delta^{13}C_p$ from polyps weighing more than 75 μg, in order to eliminate $\delta^{13}C$ values including a large contribution from the initial polyps. Solid and dashed lines show statistically significant and insignificant correlations, respectively.

experimental polyps were also negatively correlated with the skeletal dry weight increase (Figure 5), and the slope of the relationship was less steep at higher water temperatures. Thus, the overall trends of both $\delta^{18}O_p$ and $\delta^{13}C_p$ clearly showed a growth-rate dependency at low temperature (Figures 4b, 4c, and 5). In addition, both $\delta^{18}O$ and $\delta^{13}C$ of polyps varied over a wider range at lower water temperatures (Figures 3a and 3c). The growth-rate dependency of skeletal isotopic fractionation suggests that polyps showing the slow skeletal growth at lower water temperatures are sensitive to isotopic fractionation due to the kinetic isotope effect. Therefore, specimens showing rapid skeletal growth indicate smaller errors in the $\delta^{18}O_c$ and $\delta^{13}C_c$. Both growth rate [*McConnaughey*, 1989a; *Felis et al*., 2003] and light conditions [*Hayashi et al*., 2013] have been proposed to suppress the kinetic isotope effect in coral skeletons. *Hayashi et al*. [2013] compared the dependency of $\delta^{18}O_c$ on growth-rate variations in cultured *Porites* corals with that reported by *McConnaughey* [1989a]. *McConnaughey* [1989a] found that in *Pavona clavus*, more slowly growing portions of colonies (lateral surfaces) are less depleted in ^{18}O and ^{13}C than rapidly growing portions (upper surfaces), and both $\delta^{18}O_c$ and $\delta^{13}C_c$ approach their calculated equilibrium values at growth rates below about 2 mm yr^{-1}. In contrast, *Hayashi et al*. [2013] reported that in a long-term outdoor culture experiment with *Porites australiensis*, skeletal growth rate had a little influence on $\delta^{18}O_c$ variation, and it affected $\delta^{18}O_c$ and $\delta^{13}C_c$ differently. Because light intensity differed between their study and the study by *McConnaughey* [1989a], *Hayashi et al*. [2013] hypothesized that strong light conditions may suppress the kinetic isotope effect. In our experimental results for *Acropora* polyps, both the $\delta^{13}C$ and $\delta^{18}O$ data indicated depletion of heavier isotopes at faster growth rates, which might support the kinetic trend model of *McConnaughey* [1989a, 2003]. In this study, the growth-rate dependency of skeletal isotopic fluctuations disappeared at high temperatures (27 and 31°C), although the light level in this study (~130 μmol m^{-2} s^{-1}) was the same in each temperature treatment and lower than that in the experiment of *Hayashi et al*. [2013], who cultured *Porites*

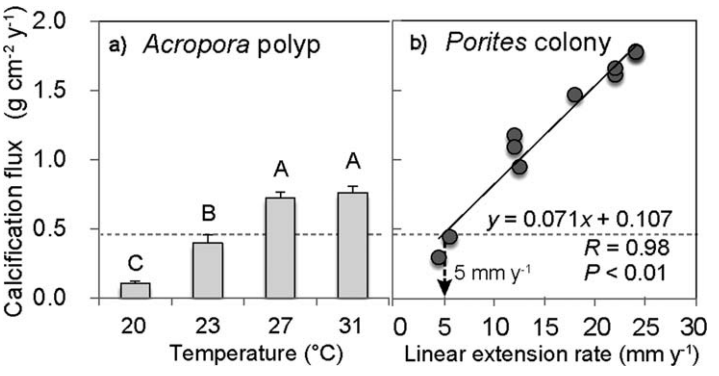

Figure 6. Calcification fluxes of the *Acropora* polyps and in a *Porites* colony. Error bars represent standard errors (±1 SE). (a) The calcification flux of *Acropora* polyps in relation to water temperature. Different letters above the bars indicate statistically significant differences (ANOVA followed by Tukey's HSD test, $\alpha = 0.05$). Error bars represent the standard error (±1 SE). (b) The calcification flux of *Porites* in relation to the linear extension rate (based on the data of *Gagan et al*. [2012]). The estimated upper limit of the calcification flux for detection of a kinetic isotope effect in the primary polyps (~0.4–0.7 g $CaCO_3$ cm^{-2} yr^{-1}) is similar to that observed in *Porites* colonies at the linear extension rate of 5 mm yr^{-1}.

australiensis outdoors, and also lower than the normal light level experienced by adult *A. digitifera* colonies, which generally grow just below the low-tide level.

3.4. Comparison of the Calcification Flux Between Aragonite From a *Porites* Colony and From *Acropora* Polyps

So that we could compare the δ^{18}O-growth rate relationship of *A. digitifera* polyps with *Porites* data that had been obtained from coral at a different growth stage, we calculated the calcification flux of the *A. digitifera* polyps and of a *Porites* colony.

We estimated the calcification flux (g $CaCO_3$ cm^{-2} yr^{-1}) of the *A. digitifera* polyps for which we had analyzed the δ^{18}O and δ^{13}C values from their measured weights and areas:

$$\text{Calcification flux} = (W_c - wi)/[S(d/365)],$$

where S is the polyp area and d is the culture period (number of days). The calcification flux averaged 0.10 ± 0.02, 0.40 ± 0.06, 0.72 ± 0.04, and 0.76 ± 0.05 g $CaCO_3$ cm^{-2} yr^{-1} (mean $\pm$ 1SD) at 20, 23, 27, and 31°C, respectively (Figure 6a). We calculated the calcification flux of a *Porites* colony (Figure 6b) from skeletal bulk density (g $CaCO_3$ cm^{-2}) values and linear extension rates (mm yr^{-1}) reported by *Gagan et al*. [2012, Table 2]. A growth rate-related isotopic fractionation (i.e., the kinetic isotope effect) is known to be evident in slow growing coral colonies with linear extension rates of $<$5 mm yr^{-1}) [*McConnaughey*, 1989a; *Gagan et al*., 2012], although *Allison et al*. [1996] reported that isotopic fluctuation was evident in *Porites* with a linear extension rate of $<$20 mm yr^{-1}. The results of our temperature-controlled experiment suggest that the maximum calcification flux at which a kinetic isotope effect could be detected in the primary polyps was $\sim$0.4–0.7 g $CaCO_3$ cm^{-2} yr^{-1} (Figure 6a). This flux is similar to the calcification flux corresponding to a linear extension rate of 5 mm yr^{-1}, the maximum rate at which the kinetic isotope effect is evident in *Porites* (Figure 6b). Therefore, we propose that the calcification flux may be a useful new measure for determining a notable kinetic isotope effect in corals of different genera and at different growth stages. The calcification flux limit was similar between *Acropora* polyps and adult *Porites*, even though the former were cultured at a low light level ($\sim$130 μmol m^{-2} s^{-1}) and the latter were grown at the normal light level of shallow water. Both higher linear extension rates and higher light levels have been proposed to suppress the appearance of a growth-rate dependency in skeletal isotope ratios, indicating a kinetic isotope effect on the coral skeleton isotopic composition. In addition, we suggest the importance of functioning of symbiotic algae to support calcification flux, which is another measure of the appearance of growth-rate dependency of skeletal isotope ratios.

3.5. Skeletal δ^{13}C of *A. digitifera* and Symbiosis

In the primary polyps cultured in our temperature experiment, $\delta^{13}C_c$ and $\delta^{13}C_p$ ranged from $-5.9‰$ to $-3.8‰$ and from $-5.0‰$ to $-0.8‰$, respectively (Figures 3b and 3d). The $\delta^{13}C_{DIC}$ values remained the same from the planulae stage to the end of the temperature experiment, whereas the $\delta^{13}C_c$ values measured in specimens before the temperature experiment were 2.4–6.7‰ lower than the average $\delta^{13}C_c$ value of the cultured specimens in the experiment. Light conditions before the experiment were notably different from those during the experiment (Table 1); the polyps were cultured under higher light levels during the experiment than before the experiment. Thus, the isotopic composition of the DIC in the coral calcifying space, which is used for skeletal calcification, might have been affected by the level of photosynthetic activity. *Swart* [1983] suggested that increased fixation of ^{12}C by zooxanthellae during periods of elevated photosynthesis rates increases the ^{13}C concentration in DIC in the calcifying space; as a result, the skeletal δ^{13}C would be higher when photosynthetic activity is higher. *Reynaud-Vaganay et al*. [2001] cultured zooxanthellate scleractinian corals, *Acropora* sp. and *Stylophora pistillata*, at two different light intensities and reported that the skeletal δ^{13}C of these two species was heavier at higher light levels than at lower light levels. A photosynthetic effect on the skeletal δ^{13}C of planktonic foraminifera has also been reported [*Spero and Parker*, 1985; *Spero and Lea*, 1993], and planktonic foraminifera show the same relationship between photosynthetic activity and carbon isotopic fractionation as that observed in coral skeletons. Because $\delta^{13}C_{DIC}$ was approximately constant during our temperature experiment with *Acropora* polyps, we suggest that the carbon isotopic fluctuations found in this study may reflect a strong biological control, driven by the level of photosynthetic activity of the zooxanthellae, which differed before and during the experiment because of

the difference in light levels. More experimental studies about the interacting effects of light and temperature are also needed to understand these effects on the calcification flux and skeletal isotope compositions.

4. Conclusion

By performing a culture experiment at four different temperature settings, we confirmed that the relationship between skeletal $\delta^{18}O$ of primary polyps of *A. digitifera* can be used as a temperature proxy. From the calculated $\delta^{18}O$ of the skeletal material deposited during each temperature treatment, we obtained a $\delta^{18}O$-temperature relationship of $-0.28‰\ °C^{-1}$; thus, juvenile specimens of this species (primary polyps) can be used for temperature reconstruction. A growth-rate dependency of skeletal isotopes was apparent in slow growing polyps cultured at the lower water temperatures. So that we could compare the $\delta^{18}O$-growth rate relationship between corals of different genera and at different stages of growth, we calculated the calcification flux for the *A. digitifera* primary polyps and a *Porites* colony. We found that the upper limit of the flux at which the $\delta^{18}O$-growth rate relationship suggested a kinetic isotope effect in the primary polyps was comparable to that in the *Porites* colony. Therefore, we propose that the calcification flux can be used as a measure of substantial growth rate-related isotope fractionation (the kinetic isotope effect) in corals belonging to different genera.

Acknowledgments

We thank Yumiko Yoshinaga at AIST for stable isotopic analysis and the members of the University of the Ryukyus for suggestions on culturing methods and comments. We also thank Nicky Allison and an anonymous reviewer for their assistance in evaluating this paper. This study was supported by a Sasagawa Scientific Research grant from The Japan Science Society; KAKENHI grants 21340166, 24244090, 22224009, and 26220102 funded by the Japan Society for the Promotion of Science; and a research fund from The Canon Foundation and AIST.

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