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EXPRESS LETTER

Skeletal isotopic responses of the Scleractinian coral *Isopora palifera* to experimentally controlled water temperatures

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We cultivated *Isopora palifera* clone colonies at six different temperatures for testing the utility of its skeletal $\delta^{18}O$ as a paleotemperature proxy. Specimens cultivated at higher temperatures exhibited lower calcification rates, especially at 31.0°C, than those cultured at lower temperatures. The skeletal $\delta^{18}O$ of the specimens cultured at 21.1–29.5°C correlated strongly with water temperature, and $\delta^{18}O$–temperature sensitivity was –0.15‰ $°C^{-1}$. When we removed the thermal factor from the skeletal $\delta^{18}O$ and $\delta^{13}C$ trends at 21.1–29.5°C by subtracting the estimated equilibrium values of inorganic aragonite from the analyzed $\delta^{18}O$ and $\delta^{13}C$ values, we found that the calcification rate variation has little influence on the isotopic compositions of the specimens examined. Thus, the skeletal $\delta^{18}O$ of *I. palifera* corals without severe bleaching can be a good temperature proxy at temperatures below ~30°C.

Keywords: stable isotopes, coral, calcification, bleaching, temperature experiment

INTRODUCTION

Scleractinian corals are major builders of coral reefs, which are one of the most diverse ecosystems on Earth (Connell, 1978). Throughout the Indo–West Pacific, Acroporidae (Scleractinia: Astrocoeniidae) is a major framework builder of shallow-water coral reef communities (Done, 1982; Veron and Wallace, 1984; Wallace and Willis, 1994), and members of this family play ecologically, geographically, and geologically important roles in tropic and subtropic coral reefs. *Isopora palifera* which belongs to Acroporidae, the model species used in this study, is the type species of genus *Isopora* (Wallace *et al*., 2007). Fossil *I. palifera* corals were dominant in the interval corresponding to the Last Glacial Maximum in a drill core from the Great Barrier Reef obtained by the Integrated Ocean Drilling Program Expedition 325 (Webster *et al*., 2011; Yokoyama *et al*., 2011), and this species is recognized as a key species of Holocene reefs in the northwest Pacific (Hongo and Kayanne, 2011; Hongo, 2012). In addition, fossil *Isopora* corals dominate Quaternary coral reef terraces in the Kikai Islands of the northern Ryukyu archipelago, Japan (Webster *et al*., 1998).

Massive *Porites* corals are important reef builders, and the suitability of the *Porites* skeleton as a paleotemperature proxy has been well-studied at present (e.g., Gagan *et al*., 2000). However, it is difficult to collect fossil core samples of massive corals such as *Porites* species in the reef area compared to *Isopora* species which forms encrusting plates covering wide area of reef flat (Veron, 2000). On the other hand, it has not been reported whether the *Isopora* skeleton can be used for paleoclimate reconstruction.

In this study, we analyzed the stable oxygen and carbon isotope compositions ($\delta^{18}O$ and $\delta^{13}C$) of specimens of *I. palifera* cultured at six temperatures covering the temperature range of Okinawan reefs. We then examined the skeletal $\delta^{18}O$–temperature relationship as a potential paleotemperature proxy, and we also assessed the influence of calcification and bleaching rates on isotopic fluctuations of $\delta^{18}O$ and $\delta^{13}C$.

MATERIALS AND METHODS

We cultured *I. palifera* at Sesoko Station, University of the Ryukyus, Motobu, Okinawa, Japan. One colony of

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Table 1. Skeletal $\delta^{18}O$ and $\delta^{13}C$, and calcification and bleaching rates in each experimental specimens of I. palifera

Temperature (°C)	Calcification rate (%)	Bleaching rate (%)	$\delta^{13}C_c$ (vs. VPDB)	$\delta^{18}O_c$ (vs. VPDB)
21.1	6.2	0	–4.1	–3.5
21.1	3.3	22.2	–3.8	–3.5
21.1	3.4	21.2	–3.4	–3.3
21.1	4.3	—	–3.2	–3.7
21.1	1.6	19.3	—	—
23.4	3.8	21.4	–4.4	–3.7
23.4	7.6	0.6	–3.6	–3.8
23.4	3.2	13.6	–4.1	–4
23.4	3.4	—	–3.6	–3.8
23.4	3.8	15.8	—	—
25.4	6.3	7.4	–4.1	–4.2
25.4	4.0	11.9	–3.8	–4.1
25.4	2.2	21.3	–3.4	–3.7
25.4	2.7	22.1	–3.7	–3.8
25.4	0.7	—	—	—
27.5	4.7	20.3	–3.8	–4.4
27.5	1.8	5.2	–3.6	–4.2
27.5	8.1	14.7	–3.7	–4.4
27.5	7.7	18.8	–4	–4.5
27.5	1.7	—	–4.3	–4.3
29.5	3.3	6.6	–4.5	–4.8
29.5	1.7	17.6	–4.1	–4.8
29.5	1.8	25.1	–4.3	–4.6
29.5	1.7	—	–4.3	–4.7
29.5	3.3	22.2	—	—
31.0	1.0	23.3	–2.1	–3.6
31.0	0.9	25.2	–2.3	–3.5
31.0	0.7	31.4	–1.9	–2.6
31.0	0.7	31.4	–1.8	–3.1
31.0	–1.1	17.9	—	—

I. palifera was collected from a fringing reef of Bise, Okinawa, Japan, and maintained in a running-seawater tank under natural light conditions until use. Similarly sized cubes, hereafter referred to as nubbins (each face approximately 4 cm^2), were cut from the parent colony and attached to acrylic plates with superglue on 23 November 2010. The plates were then placed in a running-seawater tank and cultured under natural light conditions for approximately two months, until coral tissues started to spread over the surface of the acrylic plates. The monthly temperatures of the running-seawater tank (mean ± standard deviation) were 21.6 ± 1.3, 19.8 ± 0.8 and 20.5 ± 0.9°C from December 2010 to February 2011.

On 3 February 2011, five nubbins were placed in each of six 12-L aquariums (thus, a total of 30 nubbins, five per treatment, were used in the experiment) containing seawater that had been filtered through a 1-μm mesh. Each aquarium was maintained at a nominal treatment temperature of 21.1 ± 0.2, 23.4 ± 0.3, 25.4 ± 0.5, 27.5 ± 0.1, 29.5 ± 0.6, or 31.0 ± 0.4°C, shown as mean ± standard deviation, with a 12:12 light:dark photoperiod (190–200 μmol m^{-2} s^{-1}) under metal-halide lamps (Funnel2 150W, Kamihata, Japan). The water temperature of each aquarium was recorded every 30 min by data loggers (Water Temp Pro, Onset, MA). The salinity is almost constant (approximately 34.8) during the experiment.

The temperature experiment was performed for 29 days. On 3 February and 3 March 2011 (the first and last days of the experiment), coral skeletal weight was measured as buoyant weight, which directly reflects skeletal weight (Anthony *et al.*, 2008). The calcification rates (growth and daily calcification rates) were calculated as the percentage increment in buoyant weight relative to the initial weight during the experiment, and as the increase of buoyant weight per day, respectively. We quantified the degree of bleaching of *Isopora* nubbins by using the procedure of Anthony *et al.* (2008). Briefly, we used ImageJ 1.44 software (http://imagej.nih.gov/ij) to measure luminance, which represents chlorophyll concentration of symbiont, and defined the bleaching rate as the reduction of luminance of each coral nubbin relative to the luminance of the skeletal area with maximum lumi-

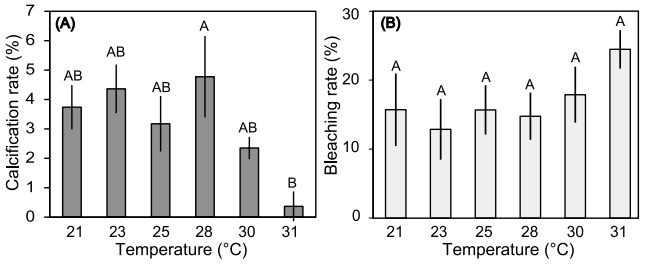

Fig. 1. Calcification and bleaching rates of coral nubbins in the six temperature treatments. (A) Calcification rate expressed as the percentage (%) change in skeletal weight relative to the initial weight. N = 30. (B) Bleaching rates (decrease in luminance relative to maximum luminance) over the experimental period. N = 24. Different letters above the error bars indicate significant differences. Error bars show ±SE.

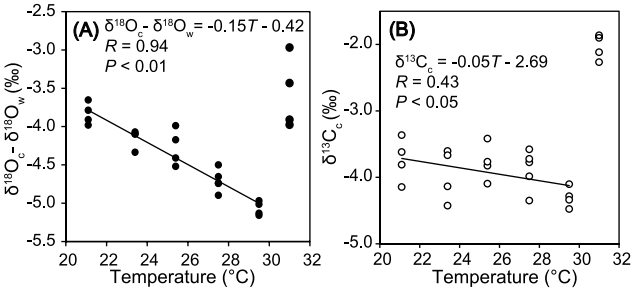

Fig. 2. $\delta^{18}O$ and $\delta^{13}C$ in I. palifera cultivated at the six temperatures. (A) Relationship between ($\delta^{18}O_c$–$\delta^{18}O_w$) and water temperature. $\delta^{18}O_w$ was calculated from the measured salinity data to be +0.3‰ by using the equation of Hayashi et al. (2013). (B) Relationship between $\delta^{13}C_c$ and water temperature. The nubbins of the 31.0°C treatment were excluded when the correlation curves of $\delta^{18}O$ and $\delta^{13}C$ were calculated.

nance.

We compared calcification and bleaching rates among the experimental temperature treatments by ANOVA followed by Tukey's HSD test. Significance was set at α = 0.05. We used JMP statistical software (SAS Institute Inc.) for all statistical analyses. At the end of the treatment (3 March), polyp tissues were completely removed from each individual with a water pick.

We used a dental drill to microsample the area of skeletal growth over acrylic plates during the experiment. Microsamples from 25 nubbins, each weighing approximately 70–100 μg, were reacted 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 Isoprime mass spectrometer (Micromass). 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 Peedee 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. The precision of $\delta^{18}O_c$ and $\delta^{13}C_c$ was better than 0.1‰ (1 SD). We calculated $\delta^{18}O$ of the seawater ($\delta^{18}O_w$) from the measured salinity data by using the equation of Hayashi *et al*. (2013):

$$\delta^{18}O_w = 0.24S - 8.03$$

where S denotes salinity. The calculated $\delta^{18}O_w$ was +0.3‰ relative to Vienna Standard Mean Ocean Water (V-SMOW).

Results and Discussion

Calcification and bleaching of I. palifera in the six temperature treatments

Calcification and bleaching rates of the experimental nubbins of *I. palifera* are shown in Fig. 1 and Table 1. The calcification rate of the experimental nubbins cultured at higher temperature tended to be lower than the rate of those cultured at lower temperatures, and those of the nubbins cultured at 31.0°C were particularly low (Fig. 1A). We detected significant differences in the growth rate expressed as a percentage (%) between nubbins grown at 27.5°C and 31.0°C (Fig. 1A). The average values of bleaching rate at temperature between 21.1 and 27.5°C were comparable, although the average values seem to increase from 27.5 to 31.0°C (Fig. 1B).

Mass coral bleaching events attributed to extreme thermal stress occurred globally during 1997–1998 (Loya *et al*., 2001; van Woesik *et al*., 2004). Marshall and Baird (2000) reported that during this event among the most bleached corals on the Great Barrier Reef, where the temperature exceeded 30°C, were those of the Scleractinian family Acroporidae, including *Isopora*; *I. palifera*, in particular, was severely damaged. Thermal bleaching of corals results from the accumulation of oxidative stress in the symbiont, at extreme water temperatures (Brown, 1997; Baird *et al*., 2009; Nesa and Hidaka, 2009). It is also reported that extreme thermal stresses cause the decrease of calcification (Anthony *et al*., 2008; Cantin *et al*., 2010; Inoue *et al*., 2012). Suzuki *et al*. (2003) reported abrupt reductions in skeletal growth immediately after the bleaching based on the skeletal isotope microprofiles of *Porites* corals during the 1997–1998 mass bleaching event. In addition, Inoue *et al*. (2012) showed in an experimental study that the bleaching rate of *Acropora* polyps cultured above 31°C is significantly higher compared with that of polyps cultured at 27°C or 29°C. Our study also showed the increased bleaching rate of the *I. palifera* nubbins at 31°C associated with a decreased calcification rate, therefore, this trends observed

Table 2. $\delta^{18}O$–temperature (T) sensitivities of biogenic and inorganic aragonite. Effective temperature ranges are shown.

Reference	$\delta^{18}O$–T sensitivity (‰ °C^{-1})	Source of carbonate	Range of temperature
This study	–0.15	Coral (*Isopora*)	21–30°C
Juillet-Leclerc *et al.* (1997)	–0.22	Coral (*Acropora*)	26–31°C
McConnaughey (1989)	–0.21	Coral (*Pavona*)	20–29°C
Dunbar and Wellington (1981)	–0.28	Coral (*Pacillopora*)	22–29°C
Gagan *et al.* (2012)	–0.22 to –0.08	Coral (*Porites*)	20–31°C
Hayashi *et al.* (2013)	–0.23 to –0.18	Coral (*Porites*)	19–30°C
Leder *et al.* (1996)	–0.22	Coral (*Montastraea*)	21–30°C
Watanabe and Oba (1999)	–0.26	Bivalve	21–31°C
Böhm *et al.* (2000)	–0.20	Coralline sponge	3–28°C
Böhm *et al.* (2000)	–0.23	Coralline sponge, mollusc, and foraminifera	3–28°C
Grossman and Ku (1986)	–0.23	Mollusc and foraminifera	3–22°C
Kim *et al.* (2007)	–0.21	Inorganic aragonite	0–40°C

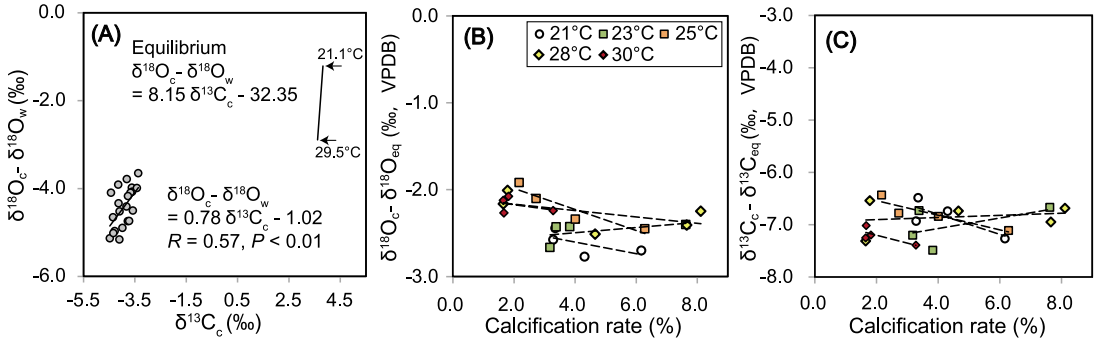

Fig. 3. Relationships between (A) ($\delta^{18}O_c$–$\delta^{18}O_w$) and $\delta^{13}C$ in I. palifera, (B) ($\delta^{18}O_c$–$\delta^{18}O_{eq}$) and calcification rate, (C) ($\delta^{13}C_c$–$\delta^{13}C_{eq}$) and calcification rate, for each nubbin. The equilibrium curve of inorganic aragonite was calculated by the method of Romanek et al. (1992), Zhang et al. (1995), and Kim et al. (2007), and the calculated temperature range is 21.1–29.5 °C. We subtracted the equilibrium values ($\delta^{18}O_{eq}$ and $\delta^{13}C_{eq}$) of inorganic aragonite from the analyzed $\delta^{18}O_c$ and $\delta^{13}C_c$ of I. palifera to remove the thermal factor from the skeletal $\delta^{18}O$ and $\delta^{13}C$ values. The isotopic values of the experimental nubbins of the 31.0 °C treatment were excluded because of poor growth.

in *I. palifera* might indicate thermal bleaching effect on skeletal calcification.

Skeletal $\delta^{18}O$ of I. palifera as a paleotemperature proxy

Table 1 shows the $\delta^{18}O_c$–$\delta^{18}O_w$ and $\delta^{13}C_c$ values of *I. palifera*. As the skeletal $\delta^{18}O_c$ is affected by both water temperature and $\delta^{18}O$ of ambient seawater, we subtracted the $\delta^{18}O_w$ (+0.3‰ in each aquariums) from the $\delta^{18}O_c$ of *I. palifera* to remove the effect of $\delta^{18}O_w$ from the $\delta^{18}O_c$. The $\delta^{18}O_c$–$\delta^{18}O_w$ and $\delta^{13}C_c$ values of *I. palifera* ranged from –5.1‰ to –3.0‰ and from –4.5‰ to –1.8‰, respectively (Fig. 2). The nubbins cultured at 31.0°C showed significantly lower values of $\delta^{18}O_c$ and $\delta^{13}C_c$ than the nubbins at the other temperatures. Those specimens in general grew poorly (Fig. 1A); thus, it suggests that the analyzed $\delta^{18}O_c$ and $\delta^{13}C_c$ represent the value before the experiment. Hence, we discuss further only the results for the specimens cultured at 21.1–29.5°C. We used the measured $\delta^{18}O_c$ values and the calculated $\delta^{18}O_w$ values (excluding the data from the 31.0°C treatment) to obtain the following relationship between water temperature and aragonite–water isotope fractionation:

$$\delta^{18}O_c - \delta^{18}O_w = -0.15T - 0.42 \ (R = 0.94, P < 0.01),$$

where T is the water temperature (°C). Skeletal $\delta^{18}O_c$ of *I. palifera* correlated strongly with water temperature (Fig. 2A), which suggests that it can be used for paleotemperature reconstruction.

Compared with reported $\delta^{18}O$–temperature sensitivities of inorganic aragonite and other biogenic aragonites, including those in other corals and bivalves (Table 2), the $\delta^{18}O$ temperature sensitivity of *I. palifera* (–0.15‰ °C^{-1}) is slightly lower than other coral genera (Table 2).

However, the reported $\delta^{18}O$ temperature sensitivity of *Porites*, the genus for which the most data are available, showed some variation ranged from –0.08‰ °C^{-1} to –0.22‰ °C^{-1} (Gagan *et al*., 2012). There might also be intercolony variations of $\delta^{18}O$ temperature sensitivity in *I. palifera*, a possibility that should be investigated further.

Effects of growth rate on skeletal $\delta^{18}O$ and $\delta^{13}C$

We calculated the equilibrium values of inorganic aragonite within the temperature range of this experiment to evaluate the effect of growth rate on $\delta^{18}O_c$ and $\delta^{13}C_c$ (Fig. 3A). We calculated the equilibrium curve of $\delta^{18}O$ in inorganic aragonite ($\delta^{18}O_{eq}$) by the method of Kim *et al*. (2007), using our temperature data and calculated $\delta^{18}O_w$ values (Fig. 3A). We also calculated the equilibrium curve of $\delta^{13}C$ of inorganic aragonite ($\delta^{13}C_{eq}$) by the method of Romanek *et al*. (1992) and Zhang *et al*. (1995) (Fig. 3A). We used our temperature data and the yearly average value of $\delta^{13}C$ of dissolved inorganic carbon (DIC) in seawater ($\delta^{13}C_{DIC}$) reported by Hayashi *et al*. (2013) (+1.0‰ ± 0.2‰ V-PDB, mean ± SD, n = 30) to calculated $\delta^{13}C_{eq}$. Then, we subtracted the $\delta^{18}O_{eq}$ and $\delta^{13}C_{eq}$ values of inorganic aragonite from the $\delta^{18}O_c$ and $\delta^{13}C_c$ values of *I. palifera* to remove the thermal factor from the coral carbonate $\delta^{18}O_c$ and $\delta^{13}C_c$ results (Figs. 3B and C). The difference between observation and equilibrium calculation ranged from 0‰ to 2.9‰ for $\delta^{18}O$ and from 4.7‰ to 7.5‰ for $\delta^{13}C$, with the skeletal values of *I. palifera* being lower than the equilibrium values of inorganic aragonite. This difference is called the "vital effect," and it is often observed in corals (McConnaughey, 1989). When we excluded the data of the 31°C treatment, the differences ranged from –7.5‰ to –6.4‰ for $\delta^{13}C$ and from –2.8‰ to –1.9‰ for $\delta^{18}O$ (Figs. 3B and C). In general, kinetic isotope effects means isotope effects originated from the disequilibrium reaction. In the case of coral skeletal $\delta^{18}O$ and $\delta^{13}C$, McConnaughey *et al*. (1997) defined kinetic isotope effect as follow: "Kinetic fractionations originate from slower hydration and hydroxylation of CO_2 by molecules bearing the heavy isotopes ^{13}C and ^{18}O." Further, kinetic isotope effects of coral skeletons are occasionally associated with the skeletal extension rate. The kinetic isotope effect has been reported previously in slow-growing coral colonies (linear extension rate < 5 mm y^{-1}) (McConnaughey, 1989; Gagan *et al*., 2012). In contrast, Hayashi *et al*. (2013) reported that in a out-door tank experiment with *Porites*, linear extension rate (2–15 mm yr^{-1}) had little influence on $\delta^{18}O$ variation, and suggested that light conditions have been proposed to suppress the kinetic isotope effect in coral skeletons. We found that the growth rate of each nubbin grown at 21.1–29.5°C showed poor correlation with $\delta^{13}C_c$ and $\delta^{18}O_c$ (Figs. 3B and C). The calcification rate might not affect the $\delta^{13}C_c$ and $\delta^{18}O_c$ of *I. palifera* nubbins showing no severe bleaching; thus, the $\delta^{18}O_c$ can be a good temperature proxy at temperatures below ~30°C.

Conclusions

We confirmed by cultivating *I. palifera* specimens at six different temperatures that skeletal $\delta^{18}O$ of this species can be used as a paleotemperature proxy. Skeletal $\delta^{18}O$ of the specimens cultured at 21.1–29.5°C correlated strongly with water temperature, and its $\delta^{18}O$–temperature sensitivity was –0.15‰ °C^{-1}. We also examined the effect of calcification rate, and found that the factor might have little influence on $\delta^{18}O$ variation at 21.1–29.5°C for the specimens without severe bleaching. Hence, the skeletal $\delta^{18}O$ of *I. palifera* can be of great utility for paleotemperature reconstruction of modern and fossil *Isopora* corals at temperatures below ~30°C.

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