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Title

Nitrogen isotopes in intra-crystal coralline aragonites

Authors

Atsuko Yamazaki[1&2], Tsuyoshi Watanabe[1], Naoto Takahata[2], Yuji Sano[2] and Urumu Tsunogai[3]

1. Graduate School of Science, Hokkaido University, N10W8, Kita-ku, Sapporo, 060-0810, Japan
2. Atmosphere and Ocean Research Institute, University of Tokyo, 1-5-1, Kashiwanoha, Kashiwa, Chiba, 277-8564, Japan.
3. Graduate School of Environmental Studies, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, JAPAN

Corresponding author: Atsuko Yamazaki

Mail: zaki@aori.u-tokyo.ac.jp

Phone and fax: +81-4-7136-6065

Abstract (192 words)

To assess the preservation of the nitrogen isotope composition in reef corals, nitrogen isotopes in a well-preserved Pliocene fossil coral (located in the Tartaro formation on Luzon Island, Philippines (14°N, 121°E)) and in a modern coral (Kochi, Japan (32°N, 132°E)) were analysed using stepwise heating methods. The thermal decomposition of aragonite triggered the largest release of nitrogen at 700 °C for the modern coral and 550 °C for the Pliocene coral. The highest rate of nitrogen gas emission occurred at the aragonite collapse temperature, indicating that organic nitrogen was bound within the intra-crystals of coralline aragonites in both corals. After the aragonite collapsed in both corals, the nitrogen isotope ratios increased due to fractionation and then decreased to values similar to those observed in bulk samples of the modern (+10.1%) and Pliocene (+4.4%) corals. These results suggested that fresh organic nitrogen was released due to the decomposition of the internal skeletal structure at higher temperatures (900-1000°C). Nitrogen isotopes in coral skeletons were preserved in intra-crystal aragonite, even in a Pliocene fossil, and stepwise heating methods were shown to be useful for determining the preservation of coralline nitrogen isotopes.

KEYWORDS: coral skeletons, nitrogen isotopes, Pliocene, stepwise heating methods

1. Introduction

The nutrient cycle in surface oceans regulates primary production, which influences the carbon dioxide concentration in the atmosphere. Global warming and cooling have occurred repeatedly over geological time scales and are often accompanied by nutrient concentration changes, especially in the polar and tropical oceans (Sigman and Boyle, 2000). Understanding nutrient circulation changes over geological time scales is useful for predicting future environmental changes. The nitrogen isotope proxy in coral skeletons ($\delta^{15}N_{coral}$) may be applied to fossil corals for the reconstruction of palaeo-nitrogen cycles. Modern and fossil coral skeletons have been used as high-resolution recorders of surface ocean environments at low latitudes (e.g., Gagan et al., 2000, Correge, 2006, Watanabe et al., 2011). The modern $\delta^{15}N_{coral}$ is a record of the high-resolution dynamics of nitrogenous nutrients in surface oceans (Yamazaki et al., 2011a, b). According to the fossil records, corals belonging to the Anthozoa class originated in the Palaeozoic era (Scrutton, 1997), and scleractinian corals appeared in the middle of the Triassic period (~237 Ma) (Stanley and Fautin, 2001). Muscatine et al. (2005) showed that the nitrogen isotope composition in Triassic coral skeletons is similar to that of modern symbiotic coral skeletons. The authors suggested that Triassic corals possessed symbiotic algae and preserved the nitrogen isotope composition in their intra-crystal skeletons. However, few studies have explored the application of nitrogen isotope proxy records in fossil corals over long periods of time because methods that can be used to confirm that organic nitrogen was preserved in intra-crystal coralline aragonites have not yet been developed, even in fossils. In the present study, the preservation of the nitrogen isotope composition in modern and fossil corals was examined using stepwise heating analysis. Stepwise heating methods have been used in the analysis of noble gases and nitrogen in rock samples (e.g., Reynolds et al., 1970; Sano and Pillinger, 1990). This technique has been

applied to nitrogen isotopes of modern coral by Uchida et al. (2008) to detect trace nitrogen in coral skeletons. We divided the heating process into seven steps (200°C to 1000°C) to determine the aragonite collapse temperature and the nitrogen isotope ratios preserved in extra- and intra-crystalline aragonite. We used well-preserved Pliocene fossil corals (3.5-3.8 Ma) collected from Luzon Island, Philippines, which is located in the western Pacific warm pool. In the middle of the Pliocene epoch, the annual mean temperature was 2-4°C warmer than that under preindustrial conditions (Haywood and Valdes, 2004, Haywood et al., 2009, Brierley et al., 2009). Pliocene coral specimens may contain nutrient circulation data in tropical warm pools under global-warming-like conditions.

2. Methods

2.1. Modern coral specimen

To decrypt the $\delta^{15}N_{coral}$ pattern of the Pliocene coral, we compared it with modern $\delta^{15}N_{coral}$ in a living *Porites* coral skeleton from Tatsukushi Bay, Kochi, Japan (32°N, 132°E). We measured modern coral skeletons using the chemical conversion methods described by Yamazaki et al. (2011a) to validate the bulk $\delta^{15}N_{coral}$ values of modern coral skeletons. A rectangular coral skeleton (10×40×7 mm) containing 5 annual bands was cut from the coral colony and powdered using an agate mill to obtain a heterogeneous powder. To remove any organic materials attached to the outer crystals, the coral powder was placed in polypropylene tubes, soaked in NaOH (2 N) and placed inside a dry bath (60°C). Treatment times of 0, 0.5, 1, 2, 3, and 5 hours were tailored to capture intra-crystalline nitrogen. During the treatment process, NaOH and coral powder were mixed once every hour using the tube mixer. At each treatment step, the nitrogen isotope content was determined in 5 samples, and those that exhibited a poor recovery rate during the chemical

79 conversion process were excluded from further analysis. The $\delta^{15}N_{coral}$ and total nitrogen content
80 varied widely, especially when the samples were not cleaned (Fig. 1). After 5 hours of cleaning,
81 the average $\delta^{15}N_{coral}$ value in the modern coral was +9.1±1.5 (2σ)%, and the average total nitrogen
82 content was 63±43 (2σ) ppm. The range (2σ) of $\delta^{15}N_{coral}$ and the total nitrogen values were
83 generally in accordance after 1 hour and in subsequent steps. The heterogeneity of nitrogen
84 released at every cleaning step was attributed to the spatial distribution of nitrogen components,
85 the seasonal variability of $\delta^{15}N_{coral}$ and of the total nitrogen (Yamazaki et al., 2011b) content and
86 differences in the nitrogen recovery rates throughout the chemical conversion process.

87 **2.2. Fossil coral specimen**

88 *Porites* specimens were excavated from the Tartaro formation located on Luzon Island,
89 Philippines (14°N, 121°E). A previous study on the nanofossil assemblages showed that the age of
90 the fossils was approximately 3.5-3.8 million years old (Watanabe et al., 2011). The
91 well-preserved fossil coral was selected by conducting a diagenetic alteration test using X-ray
92 radiography, X-ray diffraction analysis, microanalysis of thin sections via high-energy
93 synchrotron X-ray diffraction, scanning electronic microscopy observations, and optical
94 microscopy observations (Watanabe et al., 2011). Oxygen isotopes ($\delta^{18}O$) were preserved without
95 diagenetic alteration from aragonite to calcite. We used a fossil coral without diagenetic
96 alterations to determine the intra-crystalline nitrogen content using stepwise heating methods.

97 **2.3. Stepwise heating methods**

98 Cubic samples of modern and fossil corals were obtained from each colony, cleaned using milli-Q
99 water in an ultrasonic bath for 20 minutes and dried in an oven (40°C) for 2 days. Rectangular
100 samples 5×3×3 mm in size, which captured approximately 1 year of calcification, were used to
101 adjust the estimated quantity of nitrogen to the mass spectrometer. The sample weights of modern

and fossil corals were 17.89 and 17.93 mg, respectively. Each cubic sample was loaded into a double-walled quartz glass tube and placed overnight in a furnace equipped with a resistance wire under vacuum. A solid cube sample was used to reduce blank effects. Each sample was heated stepwise to temperatures of 200, 450, 550, 700, 800, 900, and 1000 °C over 4 days (2 steps/day). After the sample was heated for 90 minutes at each step, the nitrogen and argon gases released from the samples were directed to a purification vacuum line designed to measure molecular nitrogen at the sub-nanomole level (Takahata et al., 1998). The procedure used for the purification of nitrogen gas is shown in Fig. 2. Carbon dioxide (CO_2) and water (H_2O) were trapped using liquid nitrogen (cold trap 1). Carbon monoxide, hydrocarbons, and hydrogen were then oxidised to CO_2 and H_2O using pure oxygen produced by a copper oxide finger heated to 850°C and a platinum foil catalyst heated to 1000°C. To resorb excess oxygen, the copper oxide furnace was cooled to 600°C and finally to 450°C. In this deoxidised state and in the presence of the platinum foil catalyst, nitrogen oxide gas was deoxidised to nitrogen gas (N_2). CO_2 and H_2O were adsorbed in cold trap 2 during the cooling of the copper oxide finger. A quadrupole mass spectrometer (QMS; HAL201, Hiden Analytical) was used to determine the sample size introduced to the mass spectrometer. The dilution process was repeated to obtain the proper sample volume. To detect the isotopes of trace nitrogen gas at the sub-nanomole level, we used the high-sensitivity static vacuum mass spectrometer (a modified VG3600, VG Micromass Ltd.) at the Atmosphere and Ocean Research Institute at the University of Tokyo. Nitrogen gas calibrated to nitrogen in air ($\delta^{15}N=0\%$) was analysed before and after each sample. Repeated analysis of the standard over the course of 20 days showed that the overall reproducibility was 0.25% (Takahata et al., 1998). After the most recent sample was evaluated at 1000°C, blank analyses were performed at 450°C and 1000°C to estimate the background noise.

125

126 **3. Results and Discussion**

127 During the stepwise heating of the modern coral (Fig. 3a), the nitrogen concentration released
128 from 200 °C to 550 °C was negligible (less than 1 ppm, where ppm is the parts per million of
129 calcium carbonate). The nitrogen concentration was 41 ppm at 700 °C, 27 ppm at 800 °C, 26 ppm
130 at 900 °C, and 5 ppm at 1000 °C. The total nitrogen concentration in the modern coral skeleton
131 was 98 ppm. The $\delta^{15}N_{coral}$ ratios in the modern coral were +5.3% at 700 °C, +11.4% at 800 °C,
132 +15.9% at 900 °C, and +11.4% at 1000 °C. The nitrogen/argon ratios (N_2/Ar) in the isotope
133 analysis of the modern coral were 6980 at 700 °C, 6771 at 800 °C, 7563 at 900 °C, and 1385 at
134 1000 °C, indicating that the effects of air contamination (N_2/Ar: 83) were negligible. The bulk
135 $\delta^{15}N_{coral}$ ratio in the modern coral, which was calculated using the expression shown below, was
136 +10.1±0.9 (2σ)%.

137

138 $\delta^{15}N_{bulk} = \Sigma(fi \cdot \delta^{15}Ni)$

139 where f is the fraction and i is the heating temperature step.

140

141 The modern bulk $\delta^{15}N_{coral}$ and total nitrogen content analysed by stepwise heating corresponded to
142 the values measured by chemical conversion methods (+9.1±1.5%, 63±43 ppm).
143 The nitrogen released from the Pliocene coral skeleton (Fig. 3b) was less than 1 ppm at 200 °C. At
144 the 450 °C step, only a small amount of nitrogen was released (5 ppm). Subsequently, the
145 concentration of nitrogen was 21 ppm at 550 °C, 14 ppm at 700 °C, 9 ppm at 800 °C, 7 ppm at
146 900 °C, and 18 ppm at 1000 °C. The $\delta^{15}N_{coral}$ ratios of the Pliocene coral were -0.5% at 450 °C,
147 -0.7% at 550 °C, +2.9% at 700 °C, +14% at 800 °C, +6.9% at 900 °C, and +5.9% at 1000 °C. The

148 nitrogen/argon ratios (N_2/Ar) in the isotope analysis of the Pliocene coral were 3389 at 550 °C,
149 4554 at 700 °C, 3407 at 800 °C, 7461 at 900 °C, and 4880 at 1000 °C, indicating that the effects of
150 air contamination were negligible. The total nitrogen content in the Pliocene coral (69 ppm) was in
151 agreement with the nitrogen content of modern corals (50-150 ppm: Marion et al., 2005;
152 Yamazaki et al., 2011a, b; this study). The bulk $\delta^{15}N_{coral}$ ratio in the Pliocene coral was +4.4±0.8
153 (2σ)%. The Pliocene $\delta^{15}N_{coral}$ was similar to the modern $\delta^{15}N_{nitrate}$ in the surface water of the
154 tropical western Pacific (+3.6-+4.4%: Liu et al., 1996) and to the modern tropical $\delta^{15}N_{coral}$
155 (+2.5-+6.5%: Muscatine et al., 2005; Marion et al., 2005; Uchida et al., 2008; Yamazaki et al.,
156 2011b). In the present study, the modern $\delta^{15}N_{coral}$ in Tatsukushi Bay was +5% higher than that of
157 the Pliocene corals due to the latitudinal distribution of $\delta^{15}N_{nitrate}$. Yamazaki et al. (2011b)
158 suggested that $\delta^{15}N_{coral}$ values in low latitudes (tropical to subtropical) decreased due to N_2
159 fixation ($\delta^{15}N$; -2-0%).
160 Cuif et al. (2004) reported that the thermal decomposition of coral aragonite begins to accelerate at
161 550 °C, which is supported by the fact that aragonite collapse triggered the maximum nitrogen
162 release from the modern coral at 700 °C and from the Pliocene coral at 550 °C. The fossil coral
163 was more brittle than the modern coral. After the collapse of aragonite, the $\delta^{15}N_{coral}$ values of the
164 modern and Pliocene corals increased during the first three heating steps; however, at higher
165 temperatures (900-1000 °C), the $\delta^{15}N_{coral}$ values suddenly decreased and became similar to the
166 bulk nitrogen isotope values. To examine kinetic fractionation effects during the release of
167 nitrogen, a simple test can be performed. Namely, a plot of $\delta^{15}N_{coral}$ ratios remaining in the sample
168 versus the amount of nitrogen remaining in the sample can be created using Rayleigh's law (Fig.
169 4., e.g., Boyd et al., 1993).
170

171 $\ln R = \ln R_o + (1/\alpha\text{-}1) \ln f,$

172

173 where R is the isotopic ratio ($^{14}N/^{15}N$) of nitrogen remaining in the sample (ln $[^{14}N/^{15}N]_{AIR}$ =
174 5.609), R_o is the original isotopic composition of nitrogen in the sample, *f* is the fraction of
175 nitrogen remaining in the sample, and α is the fractionation factor. The relationship between the
176 $\delta^{15}N_{coral}$ values and the corresponding quantities of released nitrogen was linear. At higher
177 temperatures, the $\delta^{15}N_{coral}$ results departed from the theoretical values expected for a Rayleigh
178 distillation. At the three lower temperatures of aragonite collapse, the isotope fractionation factors
179 (α) were 0.996 (R^2=0.974) for the modern coral and 0.995 (R^2=0.996) for the Pliocene coral. The
180 internal skeletal structure collapsed during the high-temperature steps, which may have released
181 fresh organic nitrogen, thus yielding lower nitrogen isotope ratios.
182 The organic matrix in the coral skeletons may play a major role in the crystal nucleation process
183 and in the micro- and macro-regulation of the crystal morphology in the biomineralisation process
184 (reviewed by Cohen et al., 2003; Allemand et al., 2004; and Tambutte et al., 2011). Early in the
185 calcification process, corals form centres of calcification (COCs). Subsequently, fibre-like
186 aragonite surrounds the COCs (Pratz, 1882, Cuif and Dauphin, 1998). Geochemical analysis of the
187 COCs indicated high concentrations of sulphur-bearing organic molecules (amino acids and
188 sugars) and magnesium, which are more likely to fractionate in COCs than in fibres. Therefore,
189 the organic matrix should be preserved in the inner skeletal structures that collapsed at higher
190 temperature steps (900°C-1000°C). In the Pliocene coral, the nitrogen fraction at the 1000°C step
191 was greater than that at the (900°C) previous step. This result suggests that nitrogen had been
192 firmly preserved in the aragonite skeleton of the *Porites* coral since the Pliocene epoch.

193 Although more data are required, these results suggest that nitrogen in coral skeletons can
194 withstand digenesis over long geological time scales. In future work, the time series of $\delta^{15}N_{coral}$
195 can be used as a proxy for nitrogen dynamics in palaeo-oceans. Our study showed that stepwise
196 heating methods can effectively demonstrate the preservation of $\delta^{15}N_{coral}$ in modern and fossil
197 corals.
198
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204 and the Joint Research Centre in the Atmosphere and Ocean Research Institute, the University of
205 Tokyo.
206
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273 tropical western Pacific: A new proxy for marine nitrate dynamics. Journal of Geophysical
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275

276 **Figure captions**

277 Fig. 1 The (a) $\delta^{15}N_{coral}$ and (b) total nitrogen content in a modern coral analysed by the chemical
278 conversion methods reported by Yamazaki et al. (2011a). To analyse the nitrogen released by
279 intra-crystalline organic matter, the modern coral powder was soaked in hot NaOH (60°C, 2 N)
280 before cleaning (for 0, 0.5, 1, 2, 3, and 5 hours). The number in parentheses is the number of
281 samples at each step. The error bar shows the uncertainty (2σ) at each cleaning time.

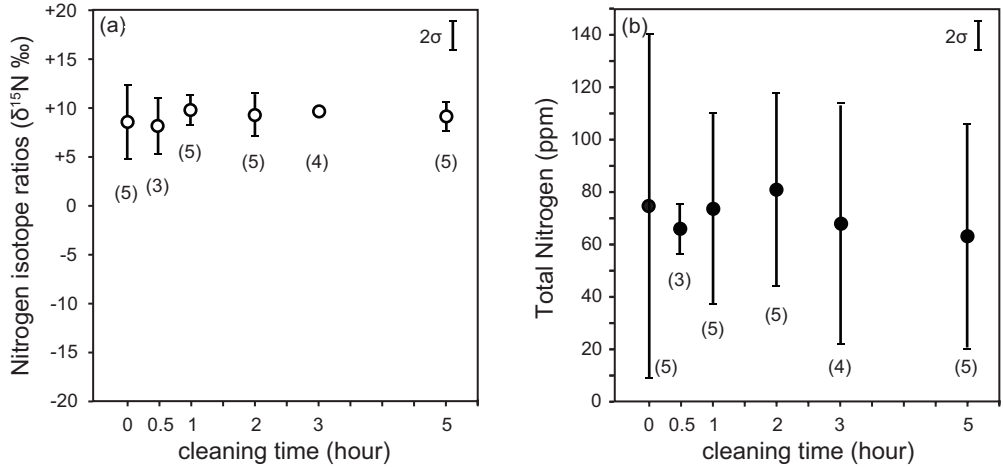
282

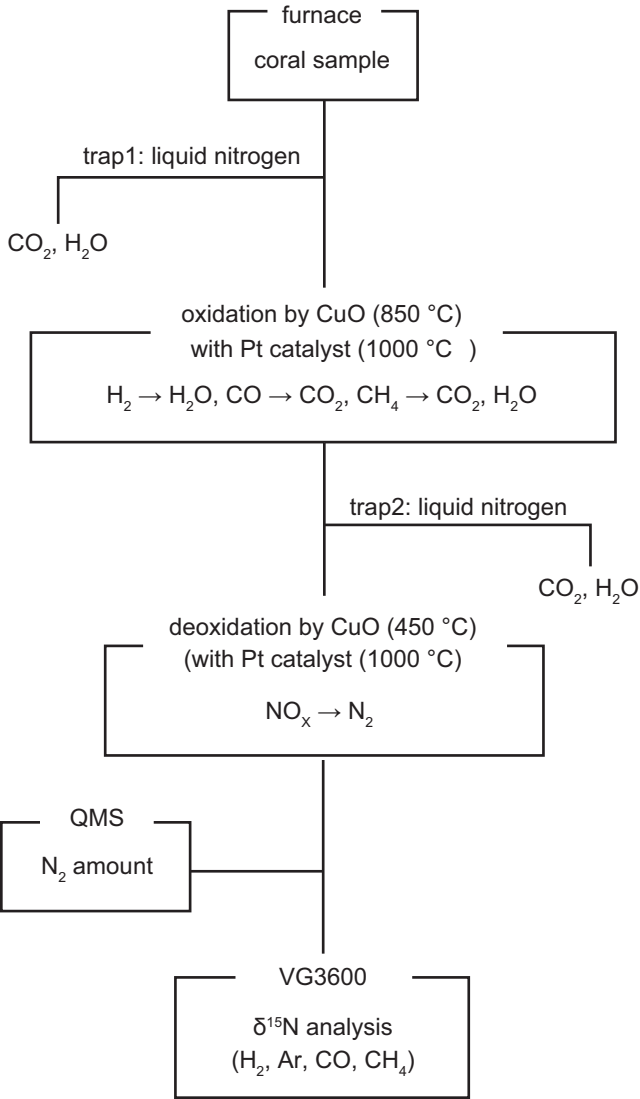
283 Fig. 2 Diagram of the nitrogen purification procedure used for nitrogen isotope analysis, which
284 was performed using a modified noble gas mass spectrometer (VG3600)

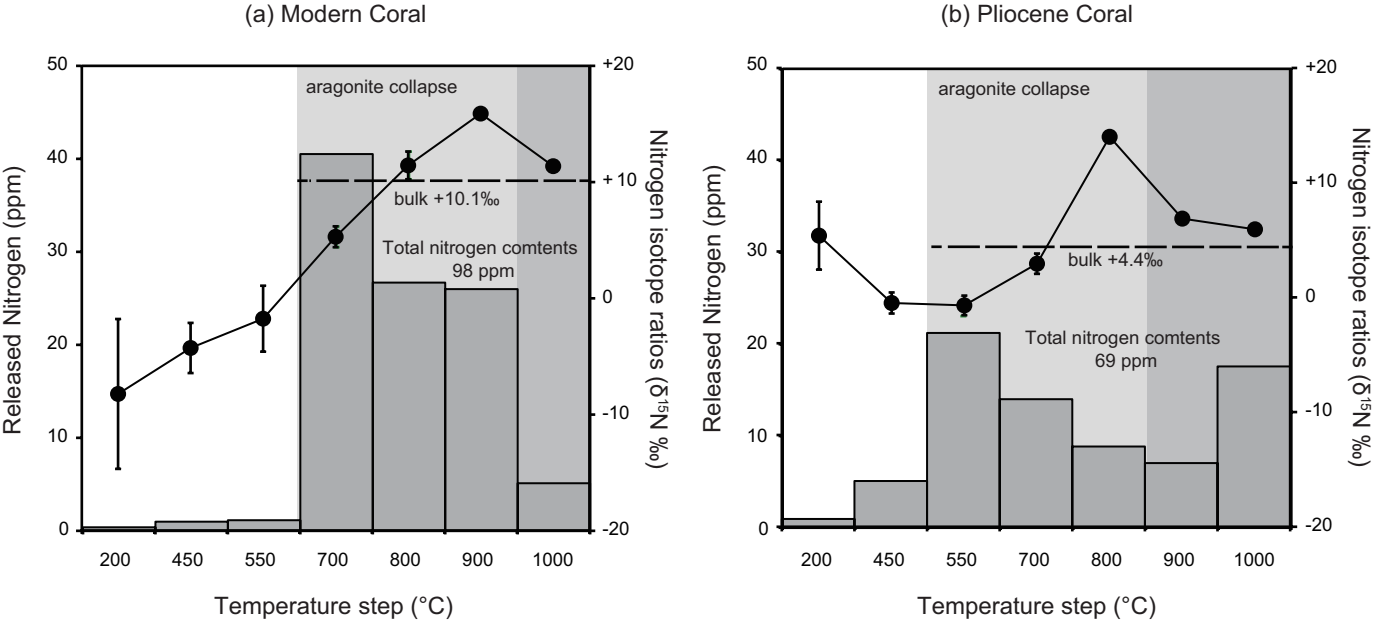
285

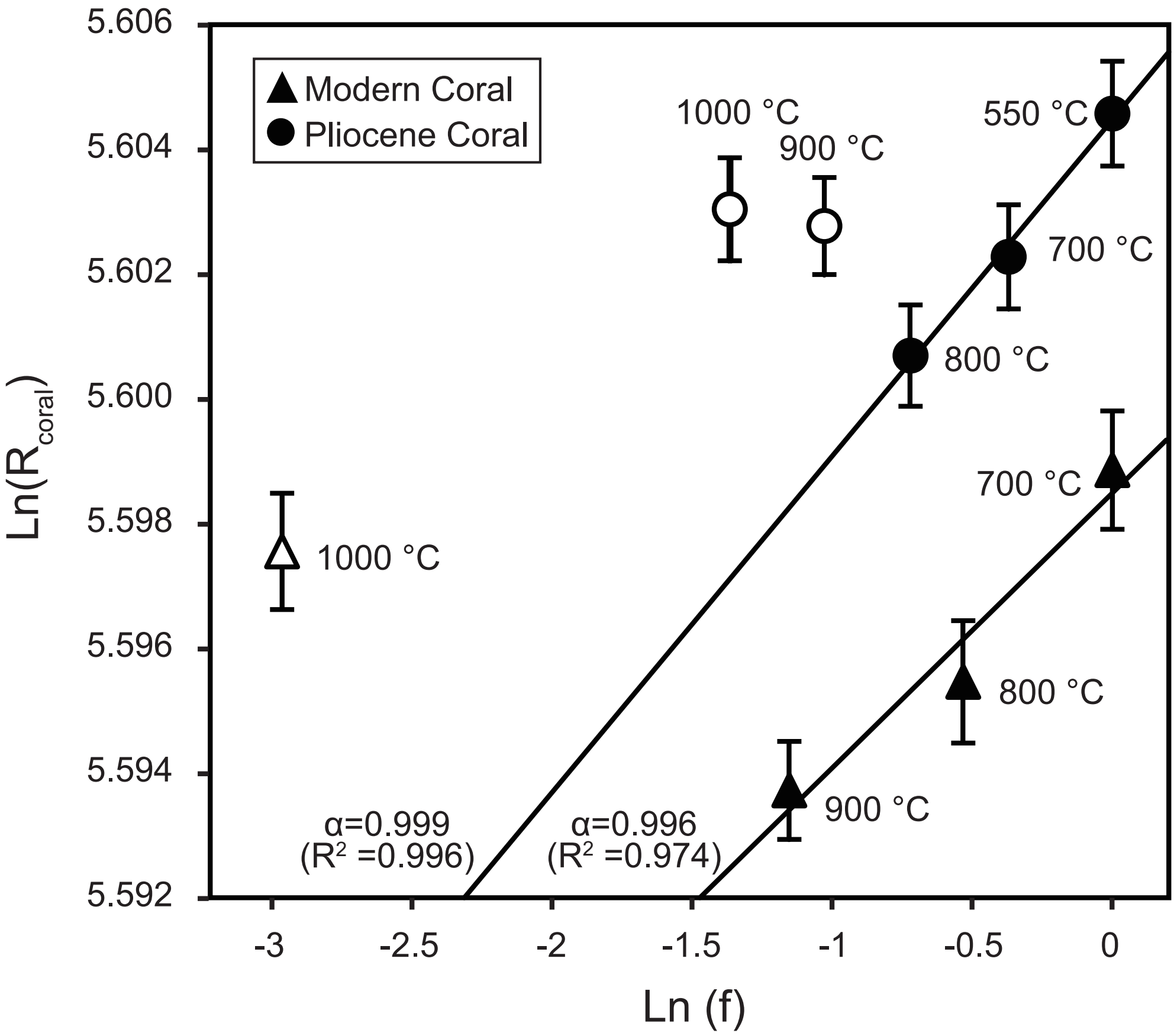
286 Fig. 3 Nitrogen isotopes (line, right axis) and released nitrogen gas (bar, left axis) at each heating
287 step. The nitrogen isotope values (●) and corresponding uncertainty ($\sigma=\sqrt{(\sigma^2_{standard} + \sigma^2_{sample})}$) are

288 plotted. (a) Living modern coral collected from Tatsukushi Bay, Kochi, Japan. (b) Pliocene fossil
289 coral excavated from the Tartaro formation (3.5 Ma) located on Luzon Island, Philippines.
290 Aragonite collapse temperatures (inside grey shading) were determined based on the maximum
291 total nitrogen concentration. The dotted lines indicate the bulk nitrogen isotope values, which were
292 calculated as $\delta^{15}N$ bulk = Σ(fi・δ^{15}Ni) [f: fraction, i: heating temperature step]
293
294 Fig. 4 Calibration lines for the kinetic fractionation of nitrogen isotopes during sample heating. R
295 is the isotopic ratio ($^{14}N/^{15}N$) of nitrogen remaining in the sample (ln $[^{14}N/^{15}N]_{AIR}$ = 5.609), and *f*
296 is the fraction of nitrogen remaining in the sample. The fractionation factors (α) were 0.996
297 (R^2=0.974) for the modern coral (▲) and 0.995 (R^2=0.996) for the Pliocene coral (●) and were
298 calculated from values obtained in the lower three temperatures of aragonite collapse. The
299 fractionation lines of nitrogen isotopes for the modern (△) and fossil (○) corals diverged at high
300 temperatures.


(a)
2σ
Nitrogen isotope ratios (δ15N ‰)
+20
+15
+10
+5
0
-5
-10
-15
-20
(5)
(3)
(5)
(5)
(4)
(5)
0
0.5
1
2
3
5
cleaning time (hour)
(b)
2σ
Total Nitrogen (ppm)
140
120
100
80
60
40
20
0
(5)
(3)
(5)
(5)
(4)
(5)
0
0.5
1
2
3
5
cleaning time (hour)


furnace
coral sample
trap1: liquid nitrogen
CO_2, H_2O
oxidation by CuO (850 °C)
with Pt catalyst (1000 °C)
$H_2 \rightarrow H_2O$, $CO \rightarrow CO_2$, $CH_4 \rightarrow CO_2$, H_2O
trap2: liquid nitrogen
CO_2, H_2O
deoxidation by CuO (450 °C)
(with Pt catalyst (1000 °C)
$NO_x \rightarrow N_2$
QMS
N_2 amount
VG3600
$\delta^{15}N$ analysis
(H_2, Ar, CO, CH_4)


(a) Modern Coral
aragonite collapse
bulk +10.1‰
Total nitrogen comtents
98 ppm
Released Nitrogen (ppm)
Nitrogen isotope ratios (δ15N ‰)
Temperature step (°C)
200
450
550
700
800
900
1000
50
40
30
20
10
0
+20
+10
0
-10
-20
(b) Pliocene Coral
aragonite collapse
bulk +4.4‰
Total nitrogen comtents
69 ppm
Released Nitrogen (ppm)
Nitrogen isotope ratios (δ15N ‰)
Temperature step (°C)


Modern Coral
Pliocene Coral
1000 °C
900 °C
550 °C
700 °C
800 °C
700 °C
1000 °C
800 °C
900 °C
α=0.999
(R² =0.996)
α=0.996
(R² =0.974)
5.606
5.604
5.602
5.600
5.598
5.596
5.594
5.592
Ln(R_coral)
-3
-2.5
-2
-1.5
-1
-0.5
0
Ln (f)