# Nitrogen isotopes of organic nitrogen in reef coral skeletons as a proxy of tropical nutrient dynamics

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[1] Understanding tropical nutrient dynamics is essential for quantifying marine productivity. In tropical and subtropical oceans, however, the spatial and continuous observation of nutrients has been scarce because of low nutrient concentration. The nitrogen isotopes of organic nitrogen in coral skeletons ($\delta^{15}N_{coral}$) could be used to record nitrogenous nutrient origins at oceanic surfaces. Here, we show the intra- and inter-reef variations of $\delta^{15}N_{coral}$ in the western Pacific. The zonal distribution of $\delta^{15}N_{coral}$ was found inside a coral reef corresponding with $\delta^{15}N$ of seawater nitrate ($\delta^{15}N_{nitrate}$). The extended analysis of $\delta^{15}N_{coral}$ among various coral reefs also shows a latitudinal gradient from tropical to temperate in the western Pacific. The $\delta^{15}N_{coral}$ records high-resolution dynamics of nitrogenous nutrients through the geologic time scale. **Citation:** Yamazaki, A., T. Watanabe, and U. Tsunogai (2011), Nitrogen isotopes of organic nitrogen in reef coral skeletons as a proxy of tropical nutrient dynamics, *Geophys. Res. Lett.*, *38*, L19605, doi:10.1029/2011GL049053.

## 1. Introduction

[2] Nutrient availability at the ocean surface defines the primary productivity, which influences climate processes and biogeochemical cycles [*Behrenfeld et al.*, 2006, *Falkowski et al.*, 1998]. Nutrients are supplied to the ocean surface by physical ocean circulation, mixed-layer dynamics, upwelling, and atmospheric deposition [*Behrenfeld et al.*, 2006]. Understanding nutrient dynamics in oceans is essential for the estimation of primary productivity. Although oligotrophic open oceans compose more than 75% of the world's oceans, the spatial and continuous observation of nutrients has been scarce. Coral reefs widely distributed oligotrophic oceans (auxiliary material, Figure S1).[1] Reef corals have been used as high-resolution recorders of paleo-environments at low latitudes [*Barnes and Lough*, 1996; *Druffel*, 1997; *Gagan et al.*, 2000]. Geochemical proxies in coral skeletons may enable us to detect the origins of nutrients and their historical changes. The nitrogen isotope of organic matter in coral skeletons ($\delta^{15}N_{coral}$) can vary with that of nitrogenous sources [*Marion et al.*, 2005; *Uchida et al.*, 2008]. The 30-year record of $\delta^{15}N_{coral}$ in Bali, for instance, captured the historical increase in fertilizer use through agricultural runoff into the ocean as a decline in $\delta^{15}N_{coral}$ [*Marion et al.*, 2005]. Nitrogen in symbiotic corals mainly originates from zooxanthellae metabolism, and $\delta^{15}N$ is well preserved in coral skeletons [*Muscatine*, 2005]. The $\delta^{15}N_{coral}$ in symbiotic corals (+4.09 ± 1.51‰) is substantially lower than that in non-symbiotic corals (+12.25 ± 1.81‰) because the former reflects both phototrophic and heterotrophic nutritional lifestyles. In addition, the mean $^{15}N$ values in skeletal organic matrixes in symbiotic corals are similar to those in algae and animal tissue even in Triassic fossil coral skeletons [*Muscatine*, 2005]. However, it is not well understood which nutrients within the nitrogenous are recorded in $\delta^{15}N_{coral}$. Reef corals take up various nitrogen compounds, including 1) dissolved inorganic nitrogen (DIN) assimilated by zooxanthellae, 2) particle and dissolved organic nitrogen (PON, DON) consumed by coral, and 3) nitrogen fixation of symbiotic cyanobacteria [*Rahav et al.*, 1989; *Heikoop et al.*, 1998; *Lesser*, 2004; *Lesser et al.*, 2007].

[3] Here, we compared the $\delta^{15}N_{coral}$ and $\delta^{15}N$ of nitrate in seawater ($\delta^{15}N_{nitrate}$). We developed an analytical method for $\delta^{15}N_{coral}$ using mutual Continuous-Flow Isotope Ratio Mass-Spectrometry (CF-IRMS) system with $\delta^{15}N_{nitrate}$ analysis. In this method, 28 mg of carbonate powder can be applied to determine seasonal variations of $\delta^{15}N_{coral}$ in coral cores. In addition, we examined the inter-reef variation of $\delta^{15}N_{coral}$ in the western Pacific. We found both the intra-reef zonal variation and the inter reef latitudinal gradient in $\delta^{15}N_{coral}$.

## 2. Materials and Methods

### 2.1. Study Site

[4] To examine the intra-reef $\delta^{15}N_{coral}$ variation, coral specimens and water samples were corrected from the Shiraho Coral Reef, Ishigaki Island, Japan, which is known to be typical of coral reef topography [*Nakamura and Nakamori*, 2007]. Ishigaki Island, southwest of the Ryukyu Islands, Japan (24°21′–31′N, 124°4′–16′E). This site is in a subtropical climate (Figure S2). The annual average temperature is 24 °C, and the humidity is 78% (statistics from 1971 to 2000; Ishigaki-jima Meteorological Observatory). The average annual precipitation is approximately 2000 mm, 60% of which falls in the rainy season from May to June or is caused by a typhoon in August and September. The Shiraho Coral Reef is an 800-m-wide fringing reef composed of a shallow lagoon between a reef flat and a well-developed reef crest. The Todoroki River flows into the Shiraho Coral Reef at the southeast of Ishigaki Island, and sugarcane, pasture, and paddy fields are distributed in the drainage basin. In heavy rain, red soil floods from the fields into the coral reef through the Todoroki River. The Todoroki River is one of the major nitrate sources for the study sites. Another DIN, ammonium, is mainly emitted from reef sediments of sea grass beds, not from the Todoroki River [*Blanco et al.*, 2008; *Miyajima et al.*, 2001].

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### 2.2. Sampling

[5] On August 26 and 29, 2009, we drilled live *Porites* coral colonies at the mouth of the Todoroki River in the Shiraho Coral Reef. The five *Porites* coral colonies (C1–C5) were 50–80 cm in diameter and living at a depth of less than 1 m from near the river mouth to the reef crest. The coral drilling was performed using an air-drill [*Adachi and Abe*, 2003]. In this study, we created a 1 in (2.5 cm) diameter core tube, and we drilled 30 cm cores from the tops of the coral colonies. Seawater samples were collected at 50 m intervals along a 750 m line from the river mouth to the reef crest. The sampling was performed 4 times: 6:00 am and 12:00 pm on August 23, 18:00 pm on August 24, and 0:00 am on August 25, 2009. River water at the Todoroki River mouth was sampled on March 25, 2009, and August 25, 2009. One-liter HDPE bottles were used for the water sampling. All water samples were filtered through precombusted 2.5 cm Whatman GF/F filters and refrigerated with 60 ml PPCO bottles until analysis.

### 2.3. Subsampling and Pretreatment of Coral Skeletons

[6] Figure S3 shows the overall preparation sequence of the nitrogen isotope analysis of the coral skeletons. Each coral core was cut to make a 5 mm thick slab. The annual bands of each coral skeleton were observed in X-radiographs. We cut skeletal pieces from a part of 2006 to 2008 in each slab while avoiding contamination of the organic tissue at the tops of the coral cores. The coral specimens were rectangles that were 7 mm wide, 3 cm long, and 5 mm thick. The coral specimens were cleaned with ultrasonic water at 25°C for 20 minutes to remove powder from the skeletal structure. The coral skeletal pieces were treated with NaOH (2 M, 80°C, 15 min) to remove any residual extrinsic organic material, such as animal tissue and endolithic organisms [*Muscatine*, 2005]. The pieces were then rinsed in DIW-distilled water through a Milli-Q Gradient (Millipore, Billerica, MA, USA) with an ultraviolet photooxidation system (UV at 254 and 185 nm) and then dried at 40°C in an oven for three days. The dried samples were reduced to a fine powder in an agate mill.

### 2.4. Nitrogen Isotope Analysis for Coral Skeletons

[7] Nitrogen isotope values of organic nitrogen in coral skeletons were analyzed using the method developed by *Tsunogai et al.* [2008]. This method involves oxidation/reduction methods such as the oxidation of organic nitrogen to nitrate using persulfate [*Knapp et al.*, 2005; *Tsunogai et al.*, 2008, 2010], reduction of nitrate to nitrite using spongy cadmium, and further reduction of nitrite to nitrous oxide using sodium azide.

[8] First, organic nitrogen in the coral skeletons was oxidized to $NO_3^-$ using persulfate under an alkaline condition. The coral skeletal powder (28 mg) was decalcified by 1 N HCl 0.6 ml in 30 ml Teflon bottles for 2 hours. Then, 0.4 ml DIW and 50 $\mu$l oxidizing reagent (peroxodisulfate) [*Tsunogai et al.*, 2008] were added. The Teflon bottles were capped tightly with Teflon screw caps and autoclaved for 1 h at 121°C. After the samples were cooled for 8 hours, needle crystals of $CaSO_4$ were deposited. A 1 ml volume of the sample solution, except the $CaSO_4$ crystals, and 9 ml DIW were pipetted into 10 ml vials with butyl rubber caps. For two coral samples, we prepared internal standards, including L-alanine ($\delta^{15}N$ = +1.78 ± 0.06%AIR), L-histidine ($\delta^{15}N$ = −7.96 ± 0.05% AIR), and tuna flakes ($\delta^{15}N$ = +12.55 ± 0.06% AIR). The diluted organic nitrogen standards with DIW (400 $\mu$M-N) were oxidized into $NO_3^-$ by the same methods. The internal standard samples compounded the organic material of the coral skeletons (1 ml), the 400 $\mu$M -N standard (1 ml), and the 8 ml DIW in 10 ml vials. Next, $NO_3^-$ was reduced to $NO_2^-$ using spongy cadmium, of which, 0.5 g was added to each vial, followed by 0.3 g of NaCl and 0.1 ml of a 1 M $NaHCO_3$ solution which resulted in a pH of approximately 8.5. The samples were then shaken for 5 hours on a horizontal shaker at a rate of 2 cycles/second. Subsequently, a reduction of $NO_2^-$ to $N_2O$ was performed using sodium azide. Then, 10 ml of the samples was decanted into clean 20 ml vials and then capped tightly with butyl rubber caps. After purging by helium to evacuate the air from the headspace and the sample solution for 2 min, 0.4 ml of azide/acetic acid buffer was added to each vial via a syringe, and the mixture was shaken. After 2 hours, the solution was made basic by adding 0.2 ml of 8 M NaOH with a syringe and shaking to prevent residual $HN_3$ from escaping into the laboratory during the subsequent isotopic analysis.

[9] The stable isotopic compositions of $N_2O$ were determined using our Continuous-Flow Isotope Ratio Mass-Spectrometry (CF-IRMS) system [*Tsunogai et al.*, 2008; *Konno et al.*, 2010; *Hirota et al.*, 2010] which consists of an original helium purge and trap line, a gas chromatograph (Agilent 6890) and a Finnigan MAT 252 (Thermo Fisher Scientific, Waltham, MA, USA) with a modified Combustion III interface. The standard deviation of the coral sample measurements was less than 0.2‰ ($\sigma$). This method is expected to be applicable to other carbonate skeletons, such as bivalves and foraminifera.

### 2.5. Analysis of Nitrate in Water Samples

[10] The concentrations of $NO_3^-$ and $NO_2^-$ in the seawater and river water samples were measured by ultraviolet high-performance liquid chromatography (UV-HPLC) using 1 ml of the seawater samples. The $NO_2^-$ concentrations were under the detection limits in every sample. For the nitrogen isotope analysis, the sample $NO_3^-$ was chemically converted to $N_2O$. 10 ml aliquots of the samples were decanted into clean 10 ml vials, and 0.5 g of spongy cadmium and 20 $\mu$l of a 1 M $NaHCO_3$ buffer (pH 8.5) were added at the $NO_3^-$ to $NO_2^-$ reduction step. The samples were then shaken for 20 hours on a horizontal shaker at a rate of 2 cycles/second. The methods after the reduction of $NO_2^-$ to $N_2O$ are referred to as "2.4. nitrogen isotope analysis for coral skeletons."

## 3. Results and Discussions

[11] The $\delta^{15}N_{coral}$ of the five coral colonies decreased from the Todoroki River mouth to the reef's edge [Figure 1; +8.6‰ (C1), +4.3‰ (C2), +5.7‰ (C3), +3.8‰ (C4), and +3.0‰ (C5)]. Dissolved nitrate was detected at 50 m, 100 m, and 150 m points from the shore (Table 1). At low tide (12:00 and 0:00), the nitrate concentrations were higher than at high tides. The largest nitrate concentration was 16.4 $\mu$M at the 100 m point at 12:00. The nitrate concentrations were under the detection limit (<1 $\mu$M) from the 200 m to 500 m points, which suggests that reef habitats such as corals and algae consumed the nitrate. We took water samples from 600 m to 750 m points at 12:00, and nitrate contents less than 1.0 $\mu$M were detected at the 600 m, 650 m, and 750 m points

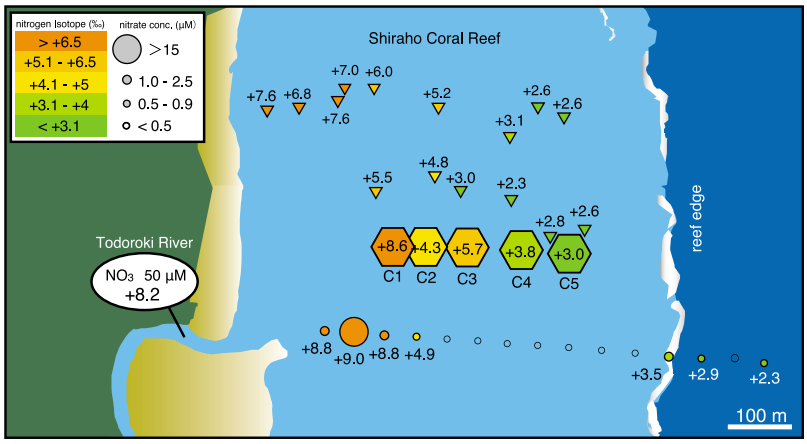


**Figure 1.** Distribution of $\delta^{15}N_{coral}$ (C1–C5) and $\delta^{15}N_{nitrate}$ with concentration of $NO_3^-$ (closed circle) on the map of Shiraho Coral Reef. Seawater samples were collected at 50 m intervals along a sampling line (Figure S2, SW line) crossing the coral reef from the mouth of the Todoroki River. The $\delta^{15}N$ values of the coral corresponded with that of macro algae (solid triangle) [*Umezawa et al.*, 2002a].

(Table 1). The $\delta^{15}N_{nitrate}$ of the Todoroki River mouth was +8.3‰ in spring and +8.2‰ in summer. The $\delta^{15}N_{nitrate}$ of the seawater samples was +7.6 to +8.9‰ at 50 m, +6.1 to +9.0‰ at 100 m, and +4.3 to +8.8‰ at 150 m. The $\delta^{15}N_{nitrate}$ values outside the reef edge were lower by 4–6‰ than those around the river mouth, e.g., +3.5‰ (600 m point), +2.9‰ (650 m point), and +2.3‰ (750 m point) at 12:00.

[12] The zonal distribution of $\delta^{15}N_{coral}$ agreed well with $\delta^{15}N_{nitrate}$. The nitrate must have been supplied into the Shiraho Coral Reef from two sources: $^{15}N$-enriched nitrate from the river water and $^{15}N$-depleted nitrate from the open ocean. The variation of the nitrate concentration depending on the tides supports that the Todoroki River was the major source of nitrate for the reef and that the countercurrent of the seawater to the river interrupts the draining of nitrate during high tides. The Todoroki River emitted nitrate was probably supplied from the agricultural watershed irrigated sugarcane fields and pasture [*Umezawa et al.*, 2002a; *Blanco et al.*, 2008]. $\delta^{15}N_{nitrate}$ in Todoroki River watershed was analyzed (+5.1–+6.5‰ for ground water under fields and 10.4‰ for well water at livestock barn, respectively). This suggests that the higher $\delta^{15}N_{nitrate}$ of river water originated from the mixture of agricultural waste. The $\delta^{15}N_{nitrate}$ values in the offshore water (+2.3 to +3.5‰) agreed with those in the Kuroshio water (+3.3 ± 1.0‰) *Liu et al.* [1996]. The Kuroshio surface water northeast of Taiwan (~130 km from Ishigaki Island) is a mixture of original nitrates in the intermediate waters showing $\delta^{15}N_{nitrate}$ values of +5 to +6‰ and reproduced nitrates, some of which originated from nitrogen fixation [*Liu et al.*, 1996]. In the reef flat, the $\delta^{15}N_{coral}$ showed intermediate values (C2–C4; +3.8 to +5.7‰) between the river water and the open ocean. The distribution of $\delta^{15}N_{coral}$ was comparable with that of the $\delta^{15}N_{algae}$ reported by *Umezawa et al.* [2002b] (Figure 1). The $\delta^{15}N_{algae}$ gradually decreased from +7.6‰ inshore to +2.6‰ near the reef edge, which agreed with the $\delta^{15}N_{coral}$. This result indicates that the $\delta^{15}N_{coral}$ recorded $\delta^{15}N_{nitrate}$ values even though there was a nitrate deficiency. However, the $^{15}N$ discrimination associated with the nitrate assimilation by zooxanthellae may change the $\delta^{15}N_{coral}$ values [*Muscatine and Kaplan*, 1994; *Heikoop et al.*, 1998]. Such fractionation is expected to be minimal because nitrate is rapidly incorporated into internal coral pools typical of N-limited coral reef waters [*Heikoop et al.*, 2000; *Marion et al.*, 2005].

[13] We also examined the distribution of $\delta^{15}N_{coral}$ in the western Pacific, including the tropical open ocean (Okinotori Island), the subtropical coral reef (Ishigaki Island), and the temperate zone (Koshiki Island, Tatsukushi, Kushimoto)

**Table 1.** Nitrate Concentrations and $\delta^{15}N_{nitrate}$ of Seawater Samples Corrected at Six Hour Intervals on SW Line[a]

| Distance From the River Mouth (m) | Sampling Time | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 6:00 | | 12:00 | | 18:00 | | 0:00 | |
| | $NO_3^-$ conc. ($\mu$M) | $\delta^{15}N$ (‰) | $NO_3^-$ conc. ($\mu$M) | $\delta^{15}N$ (‰) | $NO_3^-$ conc. ($\mu$M) | $\delta^{15}N$ (‰) | $NO_3^-$ conc. ($\mu$M) | $\delta^{15}N$ (‰) |
| 50 | 0.5 | +8.0 | 1.4 | +8.8 | 0.5 | +8.9 | 1.8 | +7.6 |
| 100 | <0.5 | - | 16.4 | +9.0 | 0.9 | +6.1 | 9.0 | +8.7 |
| 150 | <0.5 | - | 2.3 | +8.8 | 1.2 | +4.3 | 1.3 | +7.4 |
| 200–550[b] | - | - | - | - | - | - | - | - |
| 600 | NS[c] | NS | 1.0 | +3.5 | NS | NS | NS | NS |
| 650 | NS | NS | 0.4 | +2.9 | NS | NS | NS | NS |
| 700 | NS | NS | <0.5 | - | NS | NS | NS | NS |
| 750 | NS | NS | 0.6 | +2.3 | NS | NS | NS | NS |

[a]Nitrate concentrations were high during low tide (12:00 and 0:00), which indicates that the river was the dominant source of nitrate for the coral reef.
[b]$NO_3^-$ conc. were too low <0.5.
[c]NS means no sample.

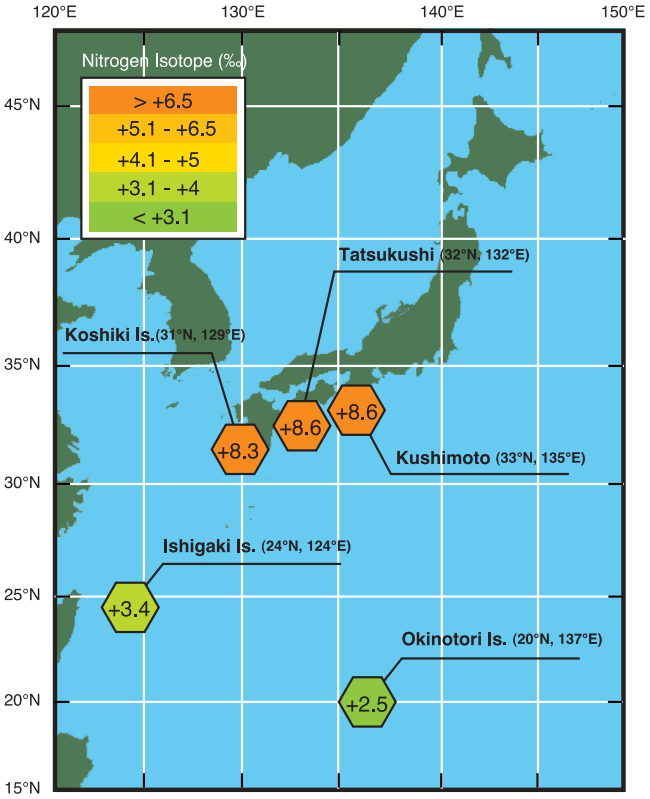


**Figure 2.** Latitudinal distribution of $\delta^{15}N_{coral}$ in the western Pacific. The coral specimens collected from Okinotori Island (20°25′N, 136°05′E), Ishigaki Island (24°23′N, 124°15′E), Koshiki Island (31°47′N, 129°47′E), Tatsukushi (32°56′N, 132°42′E), and Kushimoto (33°27′N, 135°45′E).

(Figure 2). We collected coral colonies from five sites and analyzed the nitrogen isotopes in bulk skeletal samples. The $\delta^{15}N_{coral}$ increased with latitude (Figure 2), while the $\delta^{15}N_{coral}$ in the tropical open ocean was +2.5‰, that in the subtropical coral reef was +3.4‰, and that in temperate zone was +8.3 to +8.6‰. In tropical and subtropical oceans, nitrogen compounds from $N_2$ fixation ($\delta^{15}N$ −2~0‰) [*Wada and Hattori*, 1976] decreased $\delta^{15}N_{nitrate}$ in ocean surfaces due to nutrient deficiency. However, the high values of $\delta^{15}N_{nitrate}$ originated from nitrate without the effect of nitrogen fixation at higher latitudes. The latitudinal distribution of $\delta^{15}N_{coral}$ showed regional variations in the $\delta^{15}N_{nitrate}$, which represents nutrient sources.

[14] We conclude that the nitrogen isotopes in coral skeletons can be used to reconstruct past $\delta^{15}N_{nitrate}$ distributions in coral reefs. $\delta^{15}N_{coral}$ can also be applied to fossil corals to understand past nitrate dynamics and utilizable nitrogen sources for reef corals. In addition, the time series of $\delta^{15}N_{coral}$ through coral cores could record seasonal to annual changes of nitrate dynamics in low latitudes for hundreds of years.

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