ABSTRACT

In Yoron Island, cultivation of Sugarcane makes up 52 % of the agriculture on the island, and it comes with extensive use of chemical fertilizers with ammonium sulfate. Moreover, as the main island is geographically formed from karst by the raised coral reef, precipitation smoothly flows to the sea through the underground. In this process, sulfate is brought to the sea as well. It is believed that potential hazards are then caused to the coral reef ecosystem. Considering this background, we aimed to evaluate the influence of sulfate on coral reef by analyzing the stable isotopes ratio of nitrogen ($\delta^{15}$N) and sulfur ($\delta^{34}$S). Since the $\delta^{34}$S of sulfate is 0 $\%$ , the same value would also be shown in coral skeleton if there were significant effect of inflows. Our result reveals that $\delta^{15}$N value in coral skeleton is similar to that of the chemical fertilizer. A contrary, $\delta^{34}$S value obtained did not show similarity with that of the sulfate. However, from the analysis of the $\delta^{34}$S in growth ring, we witness a decrease of 2 $\%$ to 5 $\%$ from 1979 to 2013 and from 1990 to 2013. The conclusive result is that the $\delta^{34}$S value in coral skeleton is not significantly affected by that of the value of sulfate fertilizer. Nonetheless, the consistent decrease of $\delta^{34}$S from 1979 to 2013 may possibly be related to the increase of inflow of land-substance with low $\delta^{34}$S value to the sea.

Key words: Coral reef, growth ring analysis, chemical fertilizer, waters pollution, nitrogen & sulfate isotope

INTRODUCTION

The coral development in reef pool in 2005 and 2006 with 16 examination spots evenly set around the Yoron Island, only one single spot showed the coverage of coral development over 50 % and all other 15 spots were with 5 to 10 % only, which is considered as extremely low (Shimbo 2008). Lately, signs of coral re-growth were noticed, while the overall density is still low, and it is, most probably, stemmed from the fact that the environment is a non-favorable habitat for coral. One of the possible reasons for such a change is the eutrophication of sea water. Chemical fertilizer and livestock excrement flow to the sea as underground water. These substances increased the nitrogen density of the underground water and eventually caused eutrophication (Shimbo 2008). The concentration of nitrate nitrogen in the groundwater in Yoron Island in 1970 was 0.09–0.18 mg L$^{-1}$, whereas the mean concentration in groundwater 8 points sampled in Island in 2006 was 7.0 mg L$^{-1}$, and it was high value (Shimbo 2008). Besides, eutrophication substances (nitrogen, phosphorus) in the 13 points of seawater in the reef are ten times higher than normal seawater in coral reef (Fukami and Shimbo 2007). Afterward, it breeds plankton that blocks out photosynthesis of zooxanthellae, the essential symbiont of coral. In order to prevent the further decrease of coral in the waters of Yoron Island, it is undoubtedly necessary to confront the problems from these water pollutants.

Research in 2001 proved underground water polluted by chemical fertilizers does not only contain nitrate nitrogen but also, under most circumstances, ammonium sulfate (Muramatsu et al. 2010). In Yoron Island, sugar cane cultivation occupies 80 % of the island crop cultivation area, and cultivation type is summer planting and spring planting. The chemical fertilizers used are mainly Ammonium sulfate and BB 538 (N: 15 %, P: 13 %, K: 8 %), and the timing of fertilization is early in the planting. On the influence of chemical fertilizer application on groundwater quality, Nakanishi and Nomura (2016) examined SO$_4^{2-}$ in groundwater at Kikai Island from 2001 to 2010. Kikai Island, Kagoshima prefecture consists of the raised limestone as with Yoron Island, moreover, sugarcane has been widely cultivated as a primary crop in the lowland island. The results revealed the influence as the SO$_4^{2-}$ concentration at the usual period was approximately 4 mg L$^{-1}$ while that of sugarcane fertilization period was 8–10 mg L$^{-1}$.

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Moreover, the coefficients of permeability of red soils and Ryukyu limestones of Miyako Island and Kikai Island, which has similar geological structures of Yoron Island, were examined and found to be about $10^{-2}$ to $10^{-3} \text{ms}^{-1}$ and $10^{-3}$ to $10^{-2} \text{ms}^{-1}$, it means water permeation is very fast in these islands (Nakanishi 2001). In addition, it is presumed that the distance of the groundwater flow channel under low islands is at most 20 km, hence, the short time lag until it discharges to adjacent seas (Nakanishi 2017). For these reasons, we considered that the outflow of Ammonium sulfate might cause eutrophication to the adjacent ocean in Yoron Island.

In recent years, the research has been conducted using stable isotope as a tracers of substances transfer in the environment. It is commonly understood that nitrogen in normal air should be lower than when chemical fertilizers are in use, and in this δ^{15}N value can also be pictured (Muramatsu et al. 2010). Sulfur in sea water mainly exists as a dissolve of SO_4^{2-} with a constant concentration of 2.75 g L^{-1} and δ^{34}S value as 21.0 %. Approximately 0.6 wt% of SO_4^{2-} is captured in coral skeleton formed from CaCO_3 (Tanaka and Ohde 2007). Any changes in the δ^{34}S value of sulfur in sea water as in the form of sulfuric acid may also be reflected in those values in coral. Hence, our hypothesis is, if excessive chemical fertilizers, especially the ones with sulfate, flow to the sea as polluted underground water, influences on isotope ratio should be recorded and shown in δ^{15}N and δ^{34}S in the coral skeleton, which is essential to regulate nitrogen and sulfur in sea water.

Marion et al. (2005) has studied and analyzed comparatively δ^{15}N in coral skeleton in waters with and without nitrogen inflows from agricultural activities. This study illustrated that low index was found in water with nitrogen pollution. Yet, researches and proofs of the correlation of chemical fertilizers and δ^{15}N in coral skeleton are still inadequate, and up to this moment, there are no reports about the tracers between δ^{34}S in fertilizers with sulfur (the main component of sulfate) and δ^{15}N in coral skeleton.

In the past few years, there is a considerable number of researches with chemical analysis of the development direction of the growth rings of hermatypic coral skeleton process, which aimed to give back the sea environment in hundreds of year ago (Gagan et al. 2000; Yamazaki et al. 2011). Taking the details in the above researches as a reference, we analyze the annual changes in δ^{15}N and δ^{34}S in the growth ring of coral in shallow waters nearby Yoron Island. And, we also examined δ^{34}S of land-substances (such as soil and chemical fertilizers, etc.) that flow from the island to the sea through underground water with the comparison to the δ^{34}S in coral, to visualize the influences of these substances on coral along the timeline since 1979.

**STUDY SITES AND METHODS**

**Study sites**

This research is conducted in Yoron Island in Kagoshima prefecture. The island is located at 27°02’ North and 128°25’00” East, 20.08 km² in area and with a circumference of 23.5 km (Fig. 1).

As for the breakdown of agriculture and the livestock industry, sugarcane 50 %, livestock 41 %, other vegetables 9 % (Yoron city 2017). The wastewater treatment has a 33.5 % penetration rate of population in 2004, the drainage of undeveloped households is considered to be drained into the environment. While the wastewater treatment, the agricultural settlement drainage facility covers 48 %, additionally, 52 % are covered by the household wastewater treatment tank in areas where is not maintained (Shimbo 2008). The main island is made up of Ryukyu limestone and thus relatively flat the whole of the island. The underground foundation is Shimajiri mudstone with low water penetration so that any precipitation would flow to nearby waters within an extremely short period of time. Considering the factor that fast-developing agriculture on the island brings a rapidly increasing amount of displacement every year, significant influences on coral reef can be expected. The width of the reef pool is about 1 km at the maximum in the eastern part. _Gracilaria_ and _Ulva_ have seen as the main plants in the reef pool, meanwhile, _Sargassum_, which is a large algae, has grown abundantly in the 1980s, but it does not confirm in 2004 (Noro and Ikoma 2004). In this research, we did not consider the isotope influence to coral skeleton from the other plants, because of low plant density in this reef pool derived from oligotrophication.

**Sampling**

Samples were taken in the reef pool along the Shinaha beach and Seamans beach by scuba diving. Eight _Genus Porites_ with a diameter of 10 to 60 cm were taken in total. Immediately after being taken out from the sea, samples were washed with pure water on the ship. It was followed by wind-drying for 24 hours, frozen in a plastic case filled with ethanol, and sent to the laboratory. Samples used for δ^{15}N analysis were fixed by electric saw into 10 mm thick
slices. We extracted the natural soil for 3 samples in an undeveloped agricultural area, which sample was excepted surface part of the soil. Cattle manure gathered and sampled in the stables of livestock raiser which was piled up in open-air place. Soil (sugarcane) collected 6 samples in the furrow and one field of sugarcane which was also excepted surface part.

Isotope ratio measurements

As for the $\delta^{34}$S analysis, samples with diameter over 40 cm were drilled by a core boring machine into a columnar shape with diameter 40 to 60 cm long. They were then fixed into plates with a width of 70 mm and thickness of 10 mm, and dry frozen for 24 hours. $\delta^{15}$N analysis was conducted as Yamazaki et al. (2011) stated- processed sample segments were cleaned by ultrasonic waves under 25°C for 20 minutes in order to remove the powder substances on the surface. Plus, a 2 M NaOH solution cleaning under 80°C for 25 minutes for the removal of any excessive organic remains such as the organic tissues of algae and other animal cells.

After that, they were cut along their growth rings by micro drills under UV light, and 0.5 g of skeleton powders were taken up for one-year’s ring. Next, 3 M hydrochloric acid was sufficiently added to the sample vial with the skeleton powder until no more carbon dioxide foaming, it was to clear out all calcium carbonate in the sample. The next step is a 3-hour heat up to over 400°C for completely burning out all organic substances. The sample was then first filtrated by Advantec’s glass filter GA-100, second filtrated with pure water and the washing-off of all hydrochloric acid till the washing remains became neutral. The final filtrates were then dried under 60°C before the analysis.

As for $\delta^{34}$S analysis, sample segments were cut along their growth rings by dental drills and 0.7 g of skeleton powders were taken up for one-year’s ring. It was then completely dissolved in 10 mL of 6 NHCl with 300 μL drops of 1 M BaCl solution and sulfur was precipitated in the form of BaSO4. The sample was then filtrated by a 0.7
μm GF/F glass filter. Next, the filter with precipitates was put in a 40°C drying machine for 24 hours. It was then sealed in a tin container with pre-gasification processing equipment and acidified under pyrolysis. After that, it was converted into sulfur dioxide in a sealed tube with reduced copper and finally, the $\delta^{34}S$ value was measured by an isotope meter.

The isotope measuring meter used in both $\delta^{15}N$ and $\delta^{34}S$ analysis is "Delta V Advantage" made by Thermo Fisher Scientific, which is connected with the pre-gasification equipment "Flash EA" by the Thermo Fisher Scientific. Both $\delta^{15}N$ and $\delta^{34}S$ values are expressed as "per mille" of the standard substance.

$$\delta (\%) = \frac{Rx}{Rs - 1} \times 1000$$

Here, Rx and Rs refer to the isotope value of the sample and that of standard substance, respectively. $^{15}N/^{14}N$ ratio is the nitrogen in earth’s atmosphere, and Barium Sulfate Working Standard was applied as standard substance for $^{34}S/^{32}S$ ratio. Measurement accuracies for nitrogen isotope ($\delta^{15}N$) and sulfur isotope ($\delta^{34}S$) are $\pm 0.15\%$ and $\pm 0.3\%$, respectively.

Moreover, as for the comparison of $\delta^{34}S$ isotope ratio, soil on the island, Saccharum officinarum soil, sulfate, BB538 (N:15%, P:13%, K:8%) and cattle manure from Yoron Island were also taken as samples. These samples were all dissolved in extra-pure water, precipitated as copper and finally, the $\delta^{34}S$ value was measured by acidified under pyrolysis. After that, it was sealed in a tin container with pre-gasification processing. The isotope measuring meter used in both $\delta^{15}N$ and $\delta^{34}S$ analysis is "Delta V Advantage" made by Thermo Fisher Scientific, which is connected with the pre-gasification equipment "Flash EA" by the Thermo Fisher Scientific. Both $\delta^{15}N$ and $\delta^{34}S$ values are expressed as "per mille" of the standard substance.

RESULTS AND DISCUSSION

The results of $\delta^{15}N$ analysis of the coral skeleton from Shinaha coast is shown in Table 1. The lowest value obtained is $-2.7\%$ from spot 4 outside the reef pool, and the highest is $4.5\%$ from spot 3, which is inside the pool. Besides, the values of all samples reached the peak in 2011’s one except that from spot 1 which is inside the reef pool. The $\delta^{15}N$ values from the 3 samples inside the pool were obtained as 2.6$-$3.7$\%$ and that outside the pool is 1.5$-$3.6$\%$, with no significant differences.

Fig. 2 shows the transition of $\delta^{15}N$ of coral skeleton in Shinaha beach in reef and out reef from 2010 to 2013. The most significant decrease trend (about 7$\%$) was seen at point 4 of out reef. On the other hand, at point 2 of in reef, a slightly increasing trend of about 1$\%$ was confirmed. The value of all samples has fluctuated, it is presumed that the $\delta^{15}N$ of coral skeleton records the annual change of nitrogen in the sea area.

Fig. 3 shows the major $\delta^{15}N$ supplied from the land area and $\delta^{15}N$ in the coral skeleton. $\delta^{15}N$ is a constant value that subject to its supplying source (Heaton 1986). As shown in Fig. 3, Muramatsu et al. (2010) reported the $\delta^{15}N$ of each fertilizer and feed which has the value with Ammonium phosphate ($-4.9\%$), Ammonium sulfate (4.8%), Organic formula feed (4.9%). The $\delta^{15}N$ value of the coral skeleton collected nearby the outlets in Todoroki River of the Shiraho coast in Ishigaki Island is 3.0$\%$ to 8.6$\%$ (Yamazaki et al. 2011). The water in Todoroki River is considered with high amount of inflow from both domestic wastewater and from chemical fertilizers (Kinjo et al. 2006). When we understand it from the $\delta^{15}N$ value of domestic wastewater and treated sewage water has a high value of 7$\%$ or more (Owada et al. 2003), therefore, considering the $\delta^{15}N$ value of Yamazaki et al. (2011), the Todoroki River water and coral skeleton should be more affected by domestic wastewater rather than by chemical fertilizers. Nonetheless, the 4-year coral skeleton of $\delta^{15}N$ values obtained from both inside and outside of the reef in the 6 samples range from $-2.7\%$ to 4.5$\%$, which was different range from Todoroki River coral, and they show variation along with the value range of chemical fertilizer.

| Year | $\delta^{15}N$ (%) Shinaha in reef | $\delta^{15}N$ (%) Shinaha out reef |
|------|---------------------------------|-----------------------------------|
|      | 1 | 2 | 3 | 4 | 5 | 6 |
| 2010 | 1.7 | 0.8 | 2.9 | 2.6 | 3.2 | 3.4 |
| 2011 | 2.9 | 4.1 | 4.5 | 4.4 | 3.7 | 4.3 |
| 2012 | 3.3 | 3.0 | NA | 1.6 | 2.8 | 3.5 |
| 2013 | 3.5 | NA | NA | $-2.7\%$ | NA | 3.3 |
| Mean | 2.9 | 2.6 | 3.7 | 1.5 | 3.2 | 3.6 |
These results are suggested that coral reef is more likely to be affected by chemical fertilizer than the influence of domestic wastewater or sewage.

Fig. 4 shows the value of $\delta^{34}$S from 1990 to 2013 of coral inside the reef pool in the eastern island (Seamans beach), and Fig. 5 shows those from other samples obtained on the island. In Fig. 4, the highest value recorded was 23.2 $\%$ in 1992 and the lowest one was 21.6 $\%$ in 2013. $\delta^{34}$S value of sea water should remain constant at 21.0 $\%$ (Seal et al. 2000). Yet, the values in our research vary from 21.6 $\%$ to 23.2 $\%$, which obviously differ from normal sea water.

One of the factors for this isotope effect, is possibly...
caused by the evaporation of sea water in the reef pool. The action of the evaporation from the sea surface is to remove a "lightweight" water molecule from seawater selectively, therefore the isotopic ratio of the sea water in high evaporation area shows high value (Kohzu et al. 2005; Hiyama et al. 2008; Tsunoda et al. 2006). The coral reef nearby the coastline of Yoron Island is well-developed as a lagoon that a separated reef pool can be formed. Furthermore, in the shallow water area of Yoron Island, sea surface water temperature is higher in the reef pond than in the open ocean (Kishimoto 2006). Evaporation increases along with water surface temperature (Takahashi et al. 2015). The higher δ³⁴S value obtained in the coral is possibly caused by the high evaporation rate inside the reef pool, as in the process, heavy δ³⁴S selectively remains in the sea water. Further consideration will be needed to yield any findings of the comparison of the sea water temperature and δ³⁴S values inside the pool and those in the outer sea.

As for the control samples, the value of sulfate was −0.6‰, BB538 (2.7‰), sugarcane soil (5.7‰), cattle manure (11.4‰) and natural soil (19.7‰) as indicated in Fig. 5. This Fig. also reveals that the δ³⁴S value of the coral in Seamans beach ranges 21.6‰ to 23.2‰. It is independent to that of chemical fertilizers (sulfate), which is −0.6‰. As a result, the δ³⁴S in the coral skeleton did not show a specific value of chemical fertilizer (−0.6‰ to 2.7‰), further, a direct influence from sulfate to coral is not successfully proved here. Normally, δ³⁴S value of sea water should be rather constant (Seal et al. 2000). Yet, a trend of decrease is noted from 1990 to 2013. A possible speculation is the increasing inflow of substances with lower δ³⁴S value than that of sea water as shown in Fig. 5, such as chemical fertilizers (sulfate and BB538), farm soil and cattle manure, and that is recorded in the growth ring of
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coral. In other words, the influence of the inflow of land-substance on the ecosystem of coral is considerable. In order to disclose their correlations, data of the increasing amounts of chemical fertilizer and cattle manure since the past up to the present time should be revealed and compared with the $\delta^{34}S$ value in the growth ring of coral.

Fig. 6 shows the $\delta^{34}S$ values of the coral in reef pool on the northern island from 1979 to 2013 and the annual precipitation from the same period as measured by the rain gauge of Japan Meteorological Agency. The $\delta^{34}S$ in coral skeleton range from 20.1% to 25.0%, it shows the largest fluctuation range in this study. Similar to the index of coral skeleton in Seamans beach, the $\delta^{34}S$ value here is not as the 0.6% of ammonium sulfate.

The main reason for the increase of 1.6% from 1986 to 1987 (*1 in Fig. 6) could be related to the small amount of precipitation in 1986 (1086 mm year$^{-1}$). With less precipitation, the inflow of land-substance with low $\delta^{34}S$ value might decrease and in turns caused an increase of the $\delta^{34}S$ value in sea water. Likewise, the increase of 1.9% from 1993 to 1994 (*2 in Fig. 6) can be related to the low precipitation in 1993 (1245 mm year$^{-1}$). On the contrary, the decrease of 2.2% from 2012 to 2013 (*3 in Fig. 6) is due to the plenty of rain fall in 2012 (2522 mm year$^{-1}$), so inflow of farm soil with low $\delta^{34}S$ value increased. Despite the high precipitation of 1817 mm in 1998, $\delta^{34}S$ in coral did not show the corresponding variation. And, in 1994 and 1995 with just average precipitations (1580 mm year$^{-1}$), there occurred sharp decreases of 2.5%. In subtropical islands zone, substance inflow to the sea increases with precipitation (Muraoka and Asai 2013). This means depending on changes in precipitation, the inflow of low $\delta^{34}S$ value from land-substance also might be changed, which could in turns affect the $\delta^{34}S$ value in coral. A statistical analysis with Pearson correlation coefficient of the annual precipitation in Yoron Island and $\delta^{34}S$ value in coral is implemented as mirror correlation (Table 2).

The index between the sample from Shinaha reef pool and the annual precipitation is $-0.18$, which is invalid to prove the correlation. On the other hand, the correlation with the coral in the reef pool in Seamans beach is $-0.29$, which is a negative coefficient with precipitation. That means, in Island East, the mechanism of correlation may be like when precipitation increases, land-substance with low $\delta^{34}S$ increases, which could be recorded in decreased values in the growth ring of the coral. As shown in the two coeffi-
coefficients between the precipitation and the δ⁴⁴S values in two coral samples, the relationship of increased precipitation and inflow of land-substance should be reexamined using δ⁴⁴S value in coral. We need to increase the number of sample spots and to obtain annual inflow amount of land-substance to sea, and it is necessary to conduct statistical analysis between inflow amount and coral skeleton.

**CONCLUSION**

The δ¹⁵N value of the 4-years coral skeleton obtained in Shinaha coastline ranged from −2.7 ‰ to 4.5 ‰, which is closer to the value of −5.2 ‰ to 4.2 ‰ of chemical fertilizers than to sewage (above 7 ‰). Thus, we concluded that supply of nitrogen for coral eutrophication from chemical fertilizer was much considerable than those from sewage. The δ⁴⁴S value of coral in shallow waters (inside the reef) nearby Yoron Island differs from the δ⁴⁴S value of ammonium sulfate (0.6 ‰). From our analysis of the δ⁴⁴S value in coral skeleton, the recovery of coral habitat was difficult due to the inflow of fertilizers with sulfate. The δ⁴⁴S value of the coral sample from Seaman’s beach was positively related to the annual precipitation. There are coincident of decreases in both the values in Seaman’s beach and in Shinaha beach from 1990 to 2013 and from 1979 to 2013 that may be resulted from the continuous inflow of low δ⁴⁴S land-substances to the sea.

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