

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for branching corals in the Berau Marine Conservation Area in East Kalimantan, Indonesia

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Abstract. Carbon and nitrogen isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) values in coral tissue and zooxanthellae of the coral reef species *Porites sp.*, *Seriatophora sp.* and *Stylophora sp.* from the Berau Marine Conservation Area, East Kalimantan, Indonesia were measured. Coral samples were collected during both the dry season (August 2006) and the rainy season (November 2007). Most of the $\delta^{15}\text{N}$ values of the coral tissue during the dry season were heavier than those of the zooxanthellae. This result indicates that the zooxanthellae must be the main nutrient source for the coral tissue. However, the majority of $\delta^{15}\text{N}$ values for the coral tissues during the rainy season had similar or even lighter values than those of the zooxanthellae, suggesting that during the rainy season corals also utilize particulate organic matter (POM) as a food source.

Key words: $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, coral tissue, zooxanthellae, particulate organic matter (POM)

Introduction

The Berau Marine Conservation Area (MCA), East Kalimantan, Indonesia has the third highest coral diversity (507 species) in the world (Turak 2003). This area, which consists of 31 islands, is an important coral habitat and a part of the Coral Triangle, the center of tropical marine fish and invertebrate biodiversity. The Berau MCA is also known as the largest rookery for the green turtle in Southeast Asia and a manta ray habitat, as well as including the unique Kakaban Lake with its stingless jellyfish (Wiryanan et al. 2005).

Currently, coral reefs in the Berau MCA are under stress from the combined impact of human exploitation, physical disturbance, pollution, and increased sediment (Turak 2003; Wiryanan et al. 2005). Conversion of mangrove forest to shrimp ponds in the estuary areas, coal mining and loading along the Berau river, and conversion of land for agricultural use and logging in the highlands have been occurring for a long time. All of these human activities could lead to higher sediment loads in the Berau river and ultimately affect the condition of the reefs, especially those located nearest the river mouth.

Data from the EU's Berau Forest Management Project (BFMP, Obidzinski and Andrianto 2005) noted that during 3 years (1997-2000), Berau lost approximately 127,500 ha of forest. In the following six years, from 2000 to 2006, Berau lost 41.1% of protected forest (353,775 ha in 2000 to 208,374 ha in 2006); while fixed forest production decreased from 758,049 ha to 589,567 ha, a reduction of 22.2%

(Anonymous 2007). All this deforestation, from both legal and illegal logging activities, has a tremendous big impact on the condition of soil and water resources (Obidzinski and Andrianto, 2005).

The average river discharge is approximately 1,200 m³/s into the coastal waters. The sediment concentration in the river water was 50 mg/l on average and Buschman (2007) estimated that for every year approximately 2 metric tons of sediment are exported to the Berau coastal area.

The main objective of this study is to understand the effects of the sediment discharge on the condition of the coral reef using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. Analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have been successfully used to trace the input of organic matter, assuming that the $\delta^{13}\text{C}$ of the consumer is correlated with the $\delta^{13}\text{C}$ of their diet and that the $\delta^{15}\text{N}$ value of the consumer is heavier than the $\delta^{15}\text{N}$ value of their diet (Peterson and Fry, 1987, Yamamuro and Kayanne, 1995)

All reef-building hard corals contain zooxanthellae which live symbiotically within the tissue of the corals and provide energy and nutrients. Generally, coral animals obtain most of their nutrients from the zooxanthellae, causing the $\delta^{15}\text{N}$ value of the coral to be heavier than the zooxanthellae. However, in turbid water conditions, the zooxanthellae may fail to provide enough nutrients due to a turbidity dependent reduction in products of photosynthesis, resulting in a change in the relative $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the coral and zooxanthellae. In this study, we compared isotopic values of zooxanthellae and

coral tissue collected from high sedimentation coastal areas and clear water offshore areas at two different seasons and depths.

Materials and Methods

Coral samples were collected both during the dry season, in August 2006, and the rainy season, in November 2007, from three sites, designated Locality-1 (nearest the Berau River mouth at the Rabu-rabu and Panjang Islands), Locality-2 (an intermediate distance from the river mouth, at Derawan Island), and Locality-3 (farthest from the river mouth, at the Semama and Sangalaki Islands) (Fig.1-A and B). Samples were collected at two different depths, 3 meters and 10 meters.

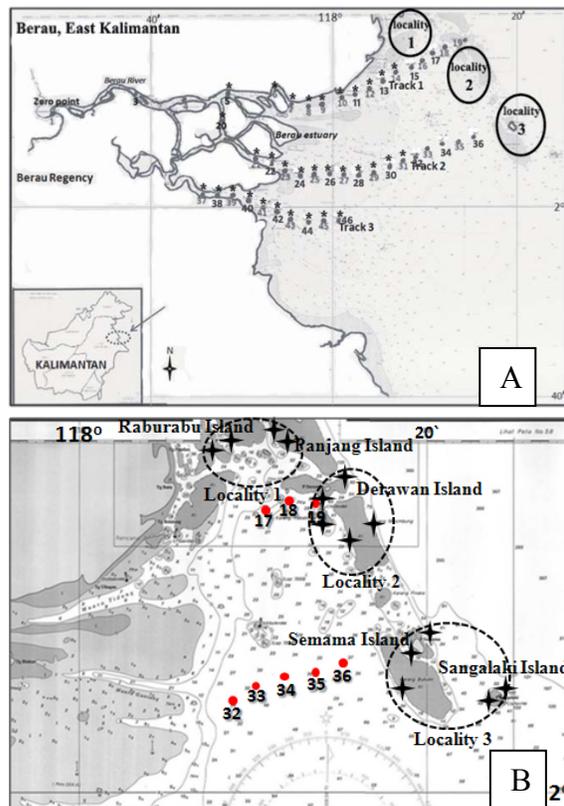


Figure 1. (A) Map of the study area showing the sampling points for sediment and plankton along track 1, track 2, and track 3. (B) Map of coral reef sampling areas at Locality 1, Locality 2, and Locality 3. ★ indicates a dive point.

Zooxanthellae were separated using a standard ethanol and sonication method. Coral samples were treated with an ultrasonic generator for 10 min and centrifuged at 3000 rpm for 10 min (Piniak and Lipschultz 2004). The resulting pellet of zooxanthella was decalcified using HCl to remove any carbonates and then rinsed twice with DDW. Coral tissue was obtained by decalcification method. Sample was ground into coarse fragments and placed in a cellulose

tube. The tube was placed in a beaker containing 0.2 N HCl on a stirrer (Horai et al. 1989). Decalcified zooxanthellae and the tissue were then centrifuged and freeze-dried overnight.

In addition, water parameters such as salinity, temperature, turbidity, pH, and dissolved oxygen (DO) were monitored using a Water Quality Checker (Horiba) along three track lines, track 1, track 2, and track 3 representing the northern, middle, and southern parts of the river effluent, respectively. Water parameters were also measured near the coral reefs (Fig.1). Bulk Particulate Organic Matter (POM) samples were collected from surface water using plankton nets with 100 μm and 310 μm mesh sizes. POM was centrifuged at 2500 rpm. The resulting pellet was decalcified by HCl treatment, rinsed twice, and freeze-dried overnight. Zooplanktons were disaggregated from the POM samples using a binocular. Aliquots (0.8 ± 0.05 mg) of all samples were placed in tin capsules for isotopic measurements.

A total of 132 coral reef and 160 POM samples were measured using an ANCA mass spectrometer. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are expressed in ‰ as defined by the following equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. Measurement errors were 0.1‰ for $\delta^{13}\text{C}$ and 0.3‰ for $\delta^{15}\text{N}$ and data are reported relative to Pee Dee Belemnite (PDB) and Air standards, respectively.

Results and Discussion

Water quality tests

The water salinity ranged from $32.5 \pm 1.4\text{‰}$ in the rainy season to $35.8 \pm 0.8\text{‰}$ in the dry season. The average salinity at the bottom of the water column was slightly higher than the surface water salinity in both seasons. Turbidity values (Table 1) measured in Locality 1 were higher than all others.

Table 1. Average water parameters at the reef areas.

Locality	Dry season (Aug 2006)		Rainy season (Nov 2007)	
	surface	bottom	surface	bottom
Turbidity (NTUs)				
Locality 1	0.8 ± 1.0	2.5 ± 2.5	1.5 ± 1.3	2.5 ± 2.6
Locality 2	0	0.3 ± 0.5	0	0.5 ± 1.0
Locality 3	0	0	n.d	n.d
pH				
Locality 1	8.4 ± 0.1	8.4 ± 0.0	8.0 ± 0.1	7.9 ± 0.0
Locality 2	8.4 ± 0.1	8.4 ± 0.1	8.0 ± 0.0	8.0 ± 0.0
Locality 3	8.4 ± 0.1	8.4 ± 0.1	n.d	n.d
DO (mg/l)				
Locality 1	6.3 ± 0.2	6.4 ± 0.1	5.5 ± 0.0	5.5 ± 0.0
Locality 2	6.7 ± 0.9	6.8 ± 0.9	5.7 ± 0.1	5.6 ± 0.1
Locality 3	6.8 ± 1.2	6.8 ± 1.1	n.d	n.d

Turbidity at Locality 1 was slightly higher during the rainy season than in the dry season, which may have

been the result of heavy rainfall and higher river discharge, and, consequently, pH and DO values were lower in the rainy season.

These results correspond to the difference between the average rainfall rate during the dry season of August 2006 (83.7 mm) and the rainy season of November 2007 (235.8 mm) (Anonymous 2008).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of coral tissue and POM

The range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of coral tissue and zooxanthellae from three branching coral reefs are shown in Fig. 2. The average $\delta^{13}\text{C}$ values of coral tissue and zooxanthellae of the *Porites sp.* were less negative than those of *Stylophora sp.* and *Seriotopora sp.*. The average $\delta^{15}\text{N}$ values for coral tissue in all the branching corals was always heavier than those of the zooxanthellae. However, analysis of variance revealed that there were no significant differences in $\delta^{15}\text{N}$ value among species ($p > 0.05$).

The average $\delta^{13}\text{C}$ value of POM near the coral reefs was $-18.5 \pm 1.3\text{‰}$. The average $\delta^{13}\text{C}$ value of POM during the dry season ($-18.6 \pm 0.8\text{‰}$) was slightly lighter than during the rainy season ($-18.4 \pm 1.6\text{‰}$). Almost all $\delta^{13}\text{C}$ values of POM depend on the marine planktonic organic carbon (-19.5‰ ; Chisholm and Koike, 1996) rather than the coral ecosystem (-12 to -13‰ ; Muscatine et al. 1989).

$\delta^{15}\text{N}$ values of POM ranged from 0.9‰ to 6.3‰ with an average of $4.2 \pm 1.6\text{‰}$. The average $\delta^{15}\text{N}$ value during the dry season ($5.5 \pm 0.9\text{‰}$) was heavier than during the rainy season ($3.3 \pm 1.4\text{‰}$). The shift in $\delta^{15}\text{N}$ values of POM at each locality is shown in Fig. 2. The decrease in $\delta^{15}\text{N}$ value of POM during the rainy season may be caused by denitrification processes which can reduce $\delta^{15}\text{N}$ values by 20-30% (Montoya 2007).

As shown in Fig. 2, the average $\delta^{15}\text{N}$ values of POM during the rainy season at each locality more depleted than those collected during the dry season. Hence, $\delta^{15}\text{N}$ values of POM during dry season were heavier than $\delta^{15}\text{N}$ values of coral tissue. In contrast, $\delta^{15}\text{N}$ values of POM during the rainy season were lighter than $\delta^{15}\text{N}$ values of the coral tissue, which may reflect an increase in primary production

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values differ with depth

Muscatine and Kaplan (1994) noted that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of zooxanthellae and coral tissue generally become more depleted in most species with increasing depth. Our data (Fig. 3) exhibited a similar trend between samples collected at a depth of 3 m or 10 m, but there were no significant differences in isotopic values.

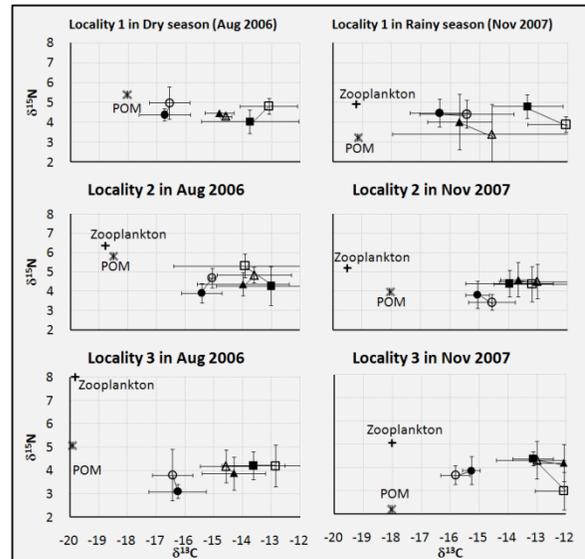


Figure 2. Average values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of coral tissue (open symbols), zooxanthellae (filled symbols), and POM and zooplankton at each locality during the dry season (August 2006) and the rainy season (November 2007). \square *Stylophora sp.*, \circ *Seriotopora sp.*, \square *Porites sp.*

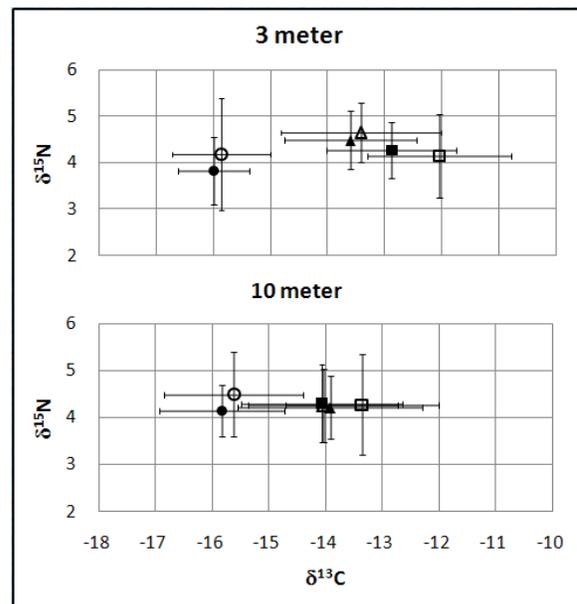


Figure 3. Average values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for coral tissue (open symbol) and zooxanthellae (filled symbol) at a depth of 3 meters and 10 meters. \square *Stylophora sp.*, \circ *Seriotopora sp.*, \square *Porites sp.*

$\delta^{15}\text{N}$ differences between coral tissue and zooxanthellae

During the dry season, the average $\delta^{15}\text{N}$ value for coral tissues was $4.8 \pm 0.7\text{‰}$ and that for zooxanthellae was $4.2 \pm 0.6\text{‰}$. During the rainy season, the average $\delta^{15}\text{N}$ values of coral tissues and zooxanthellae were $4.1 \pm 1.0\text{‰}$ and $4.4 \pm 0.7\text{‰}$, respectively.

The $\delta^{15}\text{N}$ values of consumer tissues are known to correlate with their diet. The $\delta^{15}\text{N}$ values are observed to increase with increasing trophic level. The $\delta^{15}\text{N}$ difference between coral tissue and zooxanthellae indicates that the zooxanthellae play a role in providing nutrients to the coral. During the dry season, most of the coral tissue samples had $\delta^{15}\text{N}$ values that were heavier than those of the zooxanthellae. This result indicates that the zooxanthellae must be the main source of nutrients for the coral. However, measurement of samples collected during the rainy season revealed that the $\delta^{15}\text{N}$ values of the corals were not always heavier than those of the zooxanthellae. The $\delta^{15}\text{N}$ values had a tendency to be similar to, or even lighter than, those of the zooxanthellae.

Fig. 4 shows the mean differences in $\delta^{15}\text{N}$ ($\Delta\delta^{15}\text{N}$) between coral tissue and zooxanthellae at each locality. During the dry season, the mean differences in $\delta^{15}\text{N}$ were always positive, while during the rainy season the differences were negative. The $\Delta\delta^{15}\text{N}$ is statistically different between the dry season and the rainy season at the 95% confidence interval (ANOVA, $p < 0.000$). This result suggests that during the rainy season the corals utilize food sources other than the zooxanthellae. Since November is the beginning of the rainy season and algal blooms, the corals are likely to be using POM as their main source of organic nutrient compounds

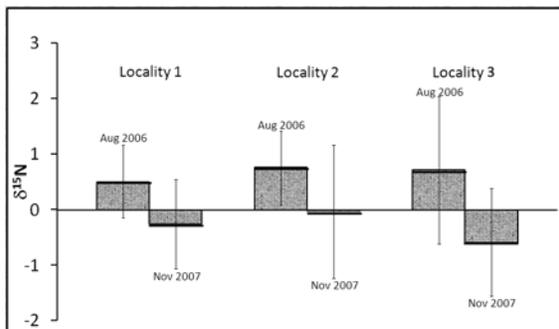


Figure 4. Average values of $\delta^{15}\text{N}$ differ seasonally between coral tissue and zooxanthellae at each of the three localities. During the dry season, the $\delta^{15}\text{N}$ values of the coral were heavier than those of the zooxanthellae, as indicated by the positive values, while during the rainy season, coral $\delta^{15}\text{N}$ values were lighter than zooxanthellae values.

Another possibility is that corals mainly consume zooplankton during the rainy season. However, during the dry season, the $\delta^{15}\text{N}$ value of the zooplankton was $7.1 \pm 1.2\text{‰}$, while during the rainy season it was $5.1 \pm 0.1\text{‰}$. These results show that the $\delta^{15}\text{N}$ values of marine zooplankton during both seasons were heavier than coral values. The heavier $\delta^{15}\text{N}$ values of the zooplankton imply that these branching coral reefs do not depend on zooplankton as their main food source.

Conclusion

This study documents the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from three branching coral species; *Stylophora sp.*, *Seriatopora sp.*, and *Porites sp.*, collected in two different seasons and at different turbidity levels. Different trends for the $\delta^{15}\text{N}$ values of coral tissue and the zooxanthellae were detected for each season. The majority of the coral tissue samples collected during the dry season had $\delta^{15}\text{N}$ values lighter than the zooxanthellae, but during the rainy season the opposite trend was observed.

Sedimentation rates only slightly affected the $\delta^{15}\text{N}$ values at the three localities. In spite of the fact that the coral polyps depend on zooxanthellae as a source of nutrients, our results suggest that the polyps also feed on POM from the surrounding waters which is probably produced during algal blooms (Muscatine and Kaplan, 1994). Moreover, coral tissues have previously been reported to have adapted their feeding habits from autotrophic to heterotrophic (Anthony and Fabricius, 2000). They are able to use POM as a food source.

Through direct observation and based on the results Suhendra (2006), we recognized that there is a difference in turbidity levels between Locality 1 and Locality 3. The sediment fraction at Locality 1 was dominated by silt which may come from the Berau River. The sedimentation rate for Locality 1 was also higher than the other localities (Suhendra 2006). However, this study did not detect isotopic differences between coral tissue and zooxanthellae in turbid water versus clear water.

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References

- Anonymous (2008) Berau in Figures 2007. Central Board of Statistic of Berau Regency, East Kalimantan, Indonesia.
- Anthony KRN., Fabricius KE (2000) Shifting role heterotrophy and autotrophy in coral energetics under varying turbidity. *J Exp Mar Biol Ecol* 252: 221-253
- Buschman F (2007) Can the reefs off the coast of the Berau region survive the changing land use inland? *Forest Sci News* 3 (8): 3-4
- Chisholm B, Koike H (1996) Reconstruction prehistoric Japanese diet using stable isotopic analysis. In: *Interdisciplinary Perspectives on the Origins of the Japanese. International Symposium*. Int Res Cent Japan Stud 199-222
- De Niro, M.J., S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42: 495-506

- Horai S, Hayasaka K, Murayama K, Wate N, Koike H, Nakai N, (1989) DNA amplification from ancient human skeletal remains and their sequence analysis. *Proc Japan Acad* 65 (B) No. 10: 229-233
- Montoya JP (2007) Natural abundance of ^{15}N in marine planktonic ecosystem. In "Michener, R. and K. Lajtha. 2007. *Stable Isotopes in Ecology and Environmental Science*". Blackwell Publishing, Singapore. 176-201
- Muscantine L, Kaplan IR (1994) Resource partitioning by reef corals as determined from stable isotope composition. I. $\delta^{15}\text{N}$ of zooxanthellae and animal tissue vs. depth. *Pac Sci* 48: 304-312.
- Muscantine L, Porter JW, Kaplan IR 1989. Resource partitioning by reef corals as determined from stable isotope composition. I. $\delta^{13}\text{C}$ of zooxanthellae and animal tissue vs. depth. *Pac Sci* 48: 304-312
- Obidzinski K, Andrianto A (2005) Illegal Forestry Activities in Berau and East Kutai Districts, East Kalimantan: Impacts on Economy, Environment and Society. Forest Governance Program, Center Int For Res (CIFOR). Bogor, Indonesia.
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Ann Rev Ecol Syst* 18: 293-320
- Piniak GA, Lipschultz F (2004) Effects of nutritional history on nitrogen assimilation in congeneric temperate and tropical scleractinian corals. *Mar Biol* 145:1085-1096.
- Suhendra D (2006) Effect of Sediment on Scleractinian Corals Community in Derawan Islands, East Kalimantan. Master Thesis unpublished. Bogor Agricultural University. Bogor, Indonesia.
- Swart PK, Saied A, Lamb K (2005) Temporal and spatial variation in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of coral tissue and zooxanthellae in *Montastrea faveolata* collected from the Florida reef tract. *Limnol Oceanogr* 50(4): 1049-1058.
- Turak E (2003) Derawan REA, 2003: Coral Biodiversity and Reef Status. The Nature Conservancy, East Kalimantan. Indonesia.
- Yamamuro M, Kayanne H (1995) Carbon and nitrogen stable isotopes of primary producers in coral reef ecosystems. *Limnol Oceanogr*. 40(3): 617-621
- Wiryanan B, Khazali M, Knight M (2005) Toward Berau Marine Protected Area, Environmental Status and Processes. Cooperation Program between Mitra Pesisir (CRMP II), The Nature Conservancy, and WWF Indonesia. Jakarta.