Korean J. Limnol. 38 (Special issue): 8~11 (2005)

Carbon Stable Isotope Ratios of Phytoplankton and Benthic Diatoms in Lake Katanuma with Reference to Those of Other Lakes

Doi, Hideyuki*, Eisuke Kikuchi[1], Shigeto Takagi and Shuichi Shikano[1]

(Graduate School of Life Sciences, Tohoku Univ., Katahira, Aoba-ku, Sendai 980-8577, Japan
[1]Center for Northeast Asian Studies, Tohoku Univ., Kawauchi, Aoba-ku, Sendai 980-8576, Japan)

Carbon stable isotope ratios of producers varied in lake ecosystems. In the present study, we tried to estimate the seasonal variations of carbon isotope ratios of phytoplankton and benthic diatoms in a strongly acidic lake ecosystem. Lake Katanuma is a volcanic, strongly acidic lake (average pH of 2.2), located in Miyagi, Japan. Only two algal species dominate in Lake Katanuma; *Pinnularia acidojaponica* as a benthic diatom, and *Chlamydomonas acidophila* as a green alga. Carbon isotope values of *P. acidojaponica* varied seasonally, while those of particulate organic matter, which were mainly composed of *C. acidophila* remained fairly stable. The differences suggested that CO_2 gas was more frequently limited for *P. acidojaponica* than *C. acidophila*, since high density patches of benthic diatoms were sometimes observed on the lake sediment. Generally, carbon concentration mechanisms (CCMs) of microalgae can fix bicarbonate in lakes, and affect the carbon isotope values of microalgae. While, in Lake Katanuma, CCMs of the microalgae may scarcely function because of high CO_2 gas concentration and low pH. This is the reason for low seasonal amplitude of carbon isotope values of phytoplankton relative to those in other lakes.

Key words : isotope fractionation, CO_2 gas, algal mat, acidic lake, carbon concentration mechanisms

INTRODUCTION

Several studies have been reported that carbon isotope ratios ($\delta^{13}C$, unit; ‰) of plankton are frequently changed seasonally in freshwater ecosystems (Zohary *et al*., 1994). Comparing the carbon isotope data for microalgae in many ecosystems, France (1995) reported that benthic microalgae tend to be more enriched in $\delta^{13}C$ than phytoplankton because of boundary layer effects. However, few studies have examined the differences in the $\delta^{13}C$ of benthic diatoms and phytoplankton in the same natural environment. Commonly, the phytoplankton and benthic algal communities in lake ecosystems are composed of many species. The complexity of the species composition may make it difficult to analyze the relationships between the carbon isotope ratios of algae and their physiological and environmental conditions *in situ*, since the isotope ratios of microalgae are mainly measured for an entire algal community (Yoshioka *et al*., 1989; Takahashi *et al*., 1990b). The seasonal variation in the $\delta^{13}C$ of planktonic and benthic microalgal communities is inevitably influenced by changes in species composition. A few studies have reported seasonal variation in the carbon isotope ratio at the species level (Zohary *et al*., 1994).

Lake Katanuma is a volcanic, strongly acidic

* Corresponding Author: Tel: +81-22-217-7561, Fax: +81-22-217-7560, E-mail: h-doi@cneas.tohoku.ac.jp

lake (average pH of 2.2). Only a few algal species including a green alga *Chlamydomonas acidophila*, and a diatom *Pinnularia acidojaponica* are exclusively dominant in Lake Katanuma because of its strong acidity. This study examined the seasonal changes in the δ^{13}C of phytoplankton and benthic diatoms in a strongly acidic lake, and compared the difference in the δ^{13}C between the two algal species. In Lake Katanuma, because of its limited phytoplankton and benthic algal species, we could accurately analyze the relationships between the carbon isotope ratios of the phytoplankton and benthic diatoms and physiological and environmental factors. Moreover, we could determine the differences of the δ^{13}C of phytoplankton and benthic diatoms in same natural environment.

MATERIALS AND METHODS

Lake Katanuma is located at 38° 44′N, 140° 43′E, with a lake surface area of 0.14 km^2, and maximum depth of 20 m. Lake Katanuma is strongly acidic, with an average pH of 2.2. Hydrogen sulfide and heat are supplied from the lake bottom. Lake Katanuma is basically a dimictic lake, stratification period is from April to August, and circulation is observed between September and December. Between January and mid−March the lake is almost covered with ice. Zooplankton and nekton have not been observed in Lake Katanuma (Doi *et al*., 2001).

To measure the δ^{13}C of particulate organic matter (POM; mainly *Chlamydomonas acidophila*), bottom waters from just above the sediment surface were collected from four different stations at 1, 2, 4, and 10 m depths with a Van−Dorn water sampler (3−L volume) from April to December in 2000 (see Fig. 1 in Doi *et al*., 2003). We collected POM from just above the lake bottom to compare the δ^{13}C of POM with those of benthic diatoms in same natural environment. The bottom waters were filtered through Whatman GF/F glass filters (precombusted at 500°C for two hours) in order to collect POM samples. Sediment samples for *Pinnularia acidojaponica* separation were collected from three stations at 1 and 4 m depths with an Ekman−Birge grab from November in 2001 to December in 2002 (except January to March 2001; ice covered period), since *P. acidojaponica* was rarely found in sediments

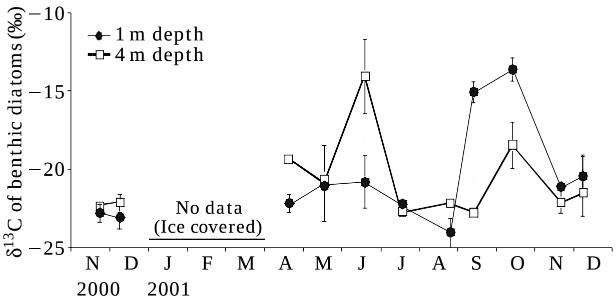

Fig. 1. The seasonal variation in the δ^{13}C of benthic diatoms from November 2000 to December 2001. mean ± 1 S.D. (n = 3)

at 10 m depth because of light limitation. Sediments were collected from the upper 0.5 cm and used for diatom separation. *P. acidojaponica* cells were separated from the sediment using its phototactic movement (Doi *et al*., 2003). The samples of carbon isotope ratios were measured with mass spectrometer (DELTA plus, Finnigan Mat). Results are reported in the delta notation (δ^{13}C, unit: ‰). Analysis errors were within ±0.2‰ for δ^{13}C.

RESULTS

In Lake Katanuma, the δ^{13}C of POM varied within a fairly narrow range from −26.4 to −23.7 ‰, and the seasonal amplitude of the value were only 2.7‰ (Table 1). Previous studies reported larger seasonal amplitudes of the δ^{13}C of phytoplankton ranged from 5.3 to 20 in the other lakes (Table 1). In Lake Katanuma, the δ^{13}C of *P. acidojaponica* varied seasonally and vertically over a range from −24.6 to −14.0‰ (Fig. 1). At 1 and 4 m, there was significant seasonal difference in the δ^{13}C of the diatoms (ANOVA, $p < 0.01$, n = 27). The mean δ^{13}C of *P. acidojaponica* (−20.3 ± 2.7 ‰) was significantly higher than that of POM (−25.0 ± 0.7‰) (Student t−test: $p < 0.001$, n = 27), although both appear to assimilate the same carbon source.

DISCUSSION

The δ^{13}C of POM (mainly *C. acidophila*) in Lake Katanuma had smaller seasonal fluctuations than those of phytoplankton in other lakes

Table 1. The seasonal variations (range and amplitude) in $\delta^{13}C$ (‰) of microalgal species and phytoplankton (POM) in lakes.

Lakes	References	Microalgal species	Range	Amplitude
L. Katanuma	This study	*Chlamydomonas acidophila*	−26.4 to −23.7	2.7
L. Kizaki	Yoshioka *et al*., 1989	Phytoplankton	−35 to −15	20
L. Fukami-ike	Takahashi *et al*., 1990a	*Microcystis aeruginosa*	−29.0 to −16.7	12.3
L. Suwa	Takahashi *et al*., 1990b	POM	−29.2 to −18.0	11.2
L. Kinneret	Stiller, 1977	Plankton > 63 μm	−33 to −18	15
L. Kinneret	Zohary *et al*., 1994	*Peridinium gatunense*	−23.2 to −17.9	5.3
		Melosira granulata	−32.0 to −23.8	8.4
		Microcystis aeruginosa	−28.3 to −20.2	8.1
		Nanoplankton	−27.4 to −19.0	8.4
L. Greifen	Hollander and McKenzie, 1991	POM	−39 to −28	11

(Table 1). The primary reason for the small seasonal variation in the $\delta^{13}C$ of *C. acidophila* in Lake Katanuma seems to be the maintenance of the high CO_2 concentration (0.040 to 10.5 mmol L^{-1}), despite the extremely low pH (Doi *et al*., 2003). A difference in dissolved inorganic carbon (DIC) species generally produces considerable variation in the fractionation factor of phytoplankton in a lake (Yoshioka, 1997). With a low CO_2 concentration, phytoplankton transport HCO_3^- into the cell, where it is converted into CO_2 by intracellular carbonic anhydrase (CA) in carbon concentration mechanisms (CCMs) (Lucas and Berry, 1985). At the CA step, the carbon isotope fractionation was 10, which is higher than that with passive CO_2 diffusion (Paneth and O'Leary, 1985). In Lake Katanuma, however, DIC was composed only of CO_2 gas; consequently, DIC assimilation by *C. acidophila* only occurred via passive CO_2 diffusion. This suggests that the relatively constant high CO_2 concentration caused the small seasonal fluctuation in isotope fractionation by *C. acidophila*. Moreover, the fluctuations in photosynthetic activity influenced the $\delta^{13}C$ of phytoplankton (Yoshioka *et al*., 1989). In Lake Katanuma the photosynthetic activity of *C. acidophila* might be reduced by the acidity of the lake water, since the capacity for photosynthesis is low at low pH (Bukaveckas, 1993). The low photosynthetic activity of *C. acidophila* at low pH (pH 2.2) in Lake Katanuma might maintain the lower growth rate in the lake compared with other lakes.

The $\delta^{13}C$ of *P. acidojaponica* in Lake Katanuma varied seasonally, especially at 1 and 4 m depth. Benthic algae have diffusive boundary layers over 1 mm thick (Riber and Wetzel, 1987). In Lake Katanuma, *P. acidojaponica* often forms high-density patches (algal mats) on the sediment surface in the photic zone. These *P. acidojaponica* mats were sometimes 0.5～1.0 mm thick (Satake and Saijo, 1978). Therefore, *P. acidojaponica* might promote ^{13}C-enrichment because its supply of DIC is limited by its patch thickness (France, 1995). In fact, the $\delta^{13}C$ of *P. acidojaponica* was higher when algal biomass was high (Doi *et al*., 2003).

The mean $\delta^{13}C$ of phytoplankton (*C. acidophila*) were lower and more stable than those of benthic diatom (*P. acidojaponica*). The difference in mean $\delta^{13}C$ values between phytoplankton and benthic diatoms may be explained by the boundary layer effects and higher variability of $\delta^{13}C$ values of the benthic diatoms by the heterogeneity of diatom density. *P. acidojaponica* often form high density patch (algal mat) in Lake Katanuma, and the $\delta^{13}C$ of the diatoms in the algal mat probably became much higher values (^{13}C-enrichment) due to the limitation of CO_2 supply.

ACKNOWLEDGEMENTS

We thank Dr. Itoh, K., Department of Agriculture, Tohoku University, for her assistance in the stable isotope analytical facilities, as well as to Dr. Hino, S., and Mr. Itoh, T. Department of Materials and Biological Chemistry, Faculty of Science, Yamagata University, for measurement the DIC data.

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(Manuscript received 20 December 2004,
Revision accepted 25 February 2005)