

# Seasonal dynamics of carbon stable isotope ratios of particulate organic matter and benthic diatoms in strongly acidic Lake Katanuma

Hideyuki Doi<sup>1,\*</sup>, Eisuke Kikuchi<sup>2</sup>, Shuji Hino<sup>3</sup>, Takeru Itoh<sup>3</sup>, Shigeto Takagi<sup>1</sup>,  
Shuichi Shikano<sup>2</sup>

<sup>1</sup>Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, Aoba-ku Sendai 980-8577, Japan

<sup>2</sup>Center for Northeast Asian Studies, Tohoku University, Kawauchi, Aoba-ku Sendai 980-8576, Japan

<sup>3</sup>Department of Materials and Biological Chemistry, Faculty of Science, Yamagata University, Yamagata 990-8560, Japan

**ABSTRACT:** Lake Katanuma is a strongly acidic volcanic lake (average pH 2.2) located in NE Japan in which only 2 algal species are found: *Pinnularia acidojaponica*, a benthic diatom, and *Chlamydomonas acidophila*, a phytoplankton species. Although the  $\delta^{13}\text{C}$  of phytoplankton generally varies seasonally in lake ecosystems, in Lake Katanuma the mean  $\delta^{13}\text{C}$  of particulate organic matter (POM, mainly phytoplankton) is constrained to a narrow range from  $-26.4$  to  $-23.7\%$ . A major reason for this is the continuous supply of dissolved  $\text{CO}_2$  gas available to *C. acidophila* from fumaroles at the lake bottom. The  $\delta^{13}\text{C}$  of *P. acidojaponica* in Lake Katanuma varied seasonally and was positively correlated with *P. acidojaponica* abundance at 1 and 4 m depths. This suggests that the higher *P. acidojaponica* biomass at 1 and 4 m produced the  $^{13}\text{C}$ -enrichment in the high-density algal mats because of the limited dissolved  $\text{CO}_2$  gas. The mean  $\delta^{13}\text{C}$  of benthic diatoms was higher than that of phytoplankton in Lake Katanuma, although the diatoms seemed to assimilate the same carbon source ( $\text{CO}_2$  gas) in the lake water.

**KEY WORDS:** Carbon stable isotope · POM · Phytoplankton · Benthic diatoms · Inorganic carbon · Seasonal variation · Acidic conditions

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## INTRODUCTION

The carbon isotope ratios ( $\delta^{13}\text{C}$ ) of phytoplankton vary widely over seasons and among species, especially those in freshwater ecosystems (Calder & Parker 1973, Zohary et al. 1994, France 1995). Seasonal variation in the  $\delta^{13}\text{C}$  of phytoplankton has been observed in several lakes (Yoshioka et al. 1989, Takahashi et al. 1990a,b, Zohary et al. 1994). However, most previous studies of freshwater phytoplankton stable isotopes involved only 1 or a few field samples, and consequently failed to reveal any seasonal variation in the  $\delta^{13}\text{C}$  of phytoplankton. Few studies have examined the  $\delta^{13}\text{C}$  of benthic diatoms in freshwater lakes because benthic diatoms are difficult to separate from sediments. Recently, however, a

method has been developed for separating benthic diatoms from sediments by using the phototactic movements of diatoms (Coach 1989). Employing this technique, we separated benthic diatoms from sediments obtained from Lake Katanuma in different seasons.

Comparing the carbon isotope data for microalgae in many ecosystems, France (1995) reported that benthic microalgae tend to be more enriched in  $\delta^{13}\text{C}$  than phytoplankton because of boundary layer effects. However, few studies have examined the differences in the  $\delta^{13}\text{C}$  of benthic diatoms and phytoplankton taken from the same natural environment.

Lake Katanuma is a strongly acidic volcanic lake (average pH 2.2). The biotic community of Lake Katanuma is very simple because of the acidity. The

\*Email: h-doi@cneas.tohoku.ac.jp

phytoplankton species *Chlamydomonas acidophila* and the benthic diatom species *Pinnularia acidojaponica* are found in the lake.

Usually, 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  $\delta^{13}\text{C}$  of planktonic and benthic microalgal communities is inevitably influenced by changes in species composition (Fry 1996). Only few studies have reported seasonal variation in the carbon isotope ratio at the species level (Zohary et al. 1994).

This study examines the seasonal changes in the  $\delta^{13}\text{C}$  of phytoplankton and benthic diatoms in a strongly acidic lake and compares the difference in the  $\delta^{13}\text{C}$  between the 2 algal species that dominate it. In Lake Katanuma, because of its limited phytoplankton and benthic algal species, we could analyze the relationships between the carbon isotope ratios of the phytoplankton and benthic diatoms and physiological and environmental factors more accurately than in an environment with a greater variety of species. Moreover, we could determine the difference in the  $\delta^{13}\text{C}$  of benthic diatoms and phytoplankton in the same natural environment.

## MATERIALS AND METHODS

**Study area.** Lake Katanuma is a volcanic lake located in NE Japan ( $38^{\circ}44' \text{N}$ ,  $140^{\circ}43' \text{E}$ ) and is situated 306 m above sea level. Lake Katanuma is strongly acidic, with an average pH of 2.2. The lake surface area is  $0.14 \text{ km}^2$  and the maximum depth is 20 m (Fig. 1). Additionally, there are no inflowing or outflowing streams. The lake is surrounded by an oak forest, but the litter supply is limited because of the small size of the forest and the absence of inflowing streams.

In Lake Katanuma, hydrogen sulfide and heat are supplied from the lake bottom. It is a dimictic lake, with stratification occurring from April to August, and overturn and deep circulation observed between September and December. Between January and mid-March, the lake is almost completely covered with ice.

Zooplankton and nektonic organisms have not been observed in Lake Katanuma. High densities of a chironomid larva (*Chironomus acerbiphilus*: Diptera, Chironomidae), a benthic diatom (*Pinnularia acidojaponica*) and planktonic alga (*Chlamydomonas acidophila*) are often observed (Doi et al. 2001). These species are distributed only in the shallow epilimnion, which has sufficient dissolved oxygen and no hydrogen sulfide during the summer stagnation period. *C. acidophila* tends to accumulate at the water surface near the shore due to phototaxis (Doi et al. 2001).

After overturn (in early September), the dissolved oxygen concentrations immediately fell below detectable levels throughout the water column and hydrogen sulfide was detected briefly. The hydrogen sulfide that accumulated in the hypolimnion during the stratification period was mixed throughout the water column just after the start of overturn. At this time, *Chironomus acerbiphilus* larvae, *Pinnularia acidojaponica* and *Chlamydomonas acidophila* virtually disappeared from the lake because of the deficit of dissolved oxygen and the toxicity of hydrogen sulfide. The subsequent lake overturn restored dissolved oxygen and the hydrogen sulfide disappeared, thereby allowing the distribution of *C. acidophila* throughout the water column and *P. acidojaponica* in the surface sediments in all shallow areas of the lake (Doi et al. 2001).

**Sampling and sample preparation.** To measure particulate organic matter (POM), bottom waters from just above the sediment surface were collected from 4 different stations at 1, 2, 4 and 10 m depths with a Van Dorn water sampler

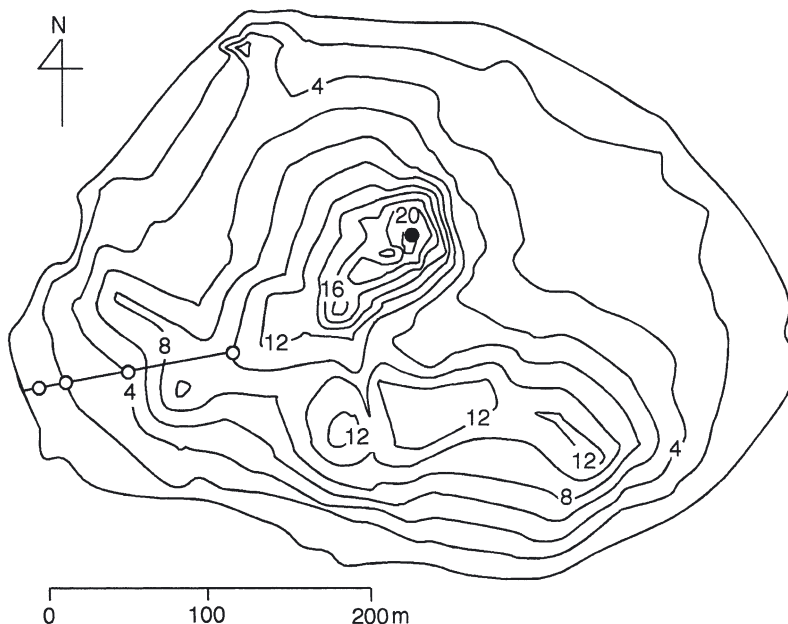


Fig. 1. Lake Katanuma. Bathymetric map showing sampling stations (o) at 1, 2, 4 and 10 m depths for POM and benthic diatoms; (●) sampling point for DIC at 20 m depth

(3 l) from April to December in 2000. We collected POM from just above the lake bottom to compare POM and benthic diatoms with the concentrations of dissolved  $\text{CO}_2$ , oxygen and hydrogen sulfide gases. The bottom waters were filtered through Whatman GF/F glass fiber filters (precombusted at  $500^\circ\text{C}$  for 2 h) in order to collect POM samples.

Sediment samples for *Pinnularia acidojaponica* separation were collected from 3 stations at 1, 2 and 4 m depths with an Ekman-Birge grab from April to December in 2001. *P. acidojaponica* was rarely found in sediments 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. Our method is a combination of Coach (1989) and Currin et al. (1995) with a minor modification. For the separation, the sediment sample was spread to a depth of 1 cm in a Petri dish and a nylon net (75  $\mu\text{m}$  mesh) was placed on the sediment surface. This was covered with a 2 mm layer of silica powder (25 to 65  $\mu\text{m}$  in diameter, precombusted at  $500^\circ\text{C}$  for 2 h). The Petri dishes were illuminated for 24 h, while moisture was maintained with continuous spraying of filtered lake water on the silica. After illumination, the powder containing *P. acidojaponica* was scraped off and mixed with filtered deionized water. Suspended *P. acidojaponica* cells were poured into glass vials and freeze-dried. Samples were stored at  $-20^\circ\text{C}$  until the stable isotope ratios were analyzed.

To measure the  $\delta^{13}\text{C}$  of  $\text{CO}_2$  gas,  $\text{CO}_2$  gas was collected from a fumarole in the lake bottom using a funnel joined to a 100 ml injector containing 5 ml of barium hydroxide solution according to M. Kusakabe (pers. comm.). The  $\text{CO}_2$  gas trapped in the funnel was pulled into the injector and absorbed in the barium hydroxide solution (Fig. 2), then collected as barium carbonate, filtered, washed with deionized water and frozen at  $-20^\circ\text{C}$  until the stable isotope ratios were analyzed.

To determine the abundance of *Chlamydomonas acidophila* and *Pinnularia acidojaponica*, the chlorophyll *a* (chl *a*) content of a water sample and the surface sediments was measured. The water sample used to measure chl *a* was filtered through Whatman GF/F glass filters. Chl *a* was extracted using *N,N*-dimethylformamide from duplicate water samples and measured by fluorometry (10-AU, Turner designs) monthly between April and December in 2000. The chl *a* content of the surface sediments (top 1 cm) was measured using the Whitney & Darley method (Whitney & Darley 1979). Sediment samples for chl *a* determination were collected monthly from 1, 2, 4 and 10 m depths with an Ekman-Birge grab between April and December in 2001. On each sampling day, dissolved inorganic carbon (DIC) profiles were obtained for the deepest

site in the lake. Water samples were collected along a vertical line (1, 2, 5, 10 and 15 m depths) with a Van Dorn water sampler (2 l) and measured using a total organic content (TOC) analyzer (TOC-5000, Shimadzu).

**Carbon content and stable isotope analysis.** The carbon content of the POM at 1, 2, 4 and 10 m depths in July 2000 was measured using a CN analyzer (NA-2500, CE Instruments) with 3 replicates. The samples for the carbon isotope ratios were measured with a DELTA-plus mass spectrometer (Finnigan Mat) connected to an elemental analyzer (NA2500, Fisons Instruments). The results are reported using the standard delta notation:  $\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$  (‰), where  $R = {}^{13}\text{C}/{}^{12}\text{C}$ ; PDB was used as the standard. The analytical error was within  $\pm 0.2$ ‰ for  $\delta^{13}\text{C}$ .

## RESULTS

### POM (mainly phytoplankton)

The seasonal variation in the chl *a* concentration of lake water at 1, 2, 4 and 10 m depths ranged from 0.1 to  $6.3 \mu\text{g l}^{-1}$  (Fig. 3). The chl *a* content in the lake water indicated the presence of *Chlamydomonas acidophila*, the only phytoplankton identified in Lake Katanuma. After the ice cover disappeared in April, the chl *a* content of the water was very low ( $<0.2 \mu\text{g l}^{-1}$ ) at all depths, probably because of the low water temperature. The chl *a* concentrations increased at 1 and 2 m

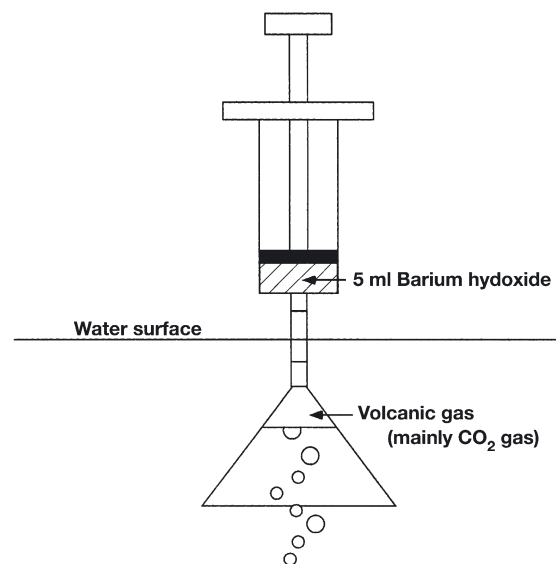


Fig. 2.  $\text{CO}_2$  gas collection from a fumarole in the lake bottom.  $\text{CO}_2$  gas was collected using a funnel joined to a 100 ml plastic syringe containing 5 ml barium hydroxide solution. The  $\text{CO}_2$  gas trapped in the funnel was pulled into the syringe and absorbed by the barium hydroxide

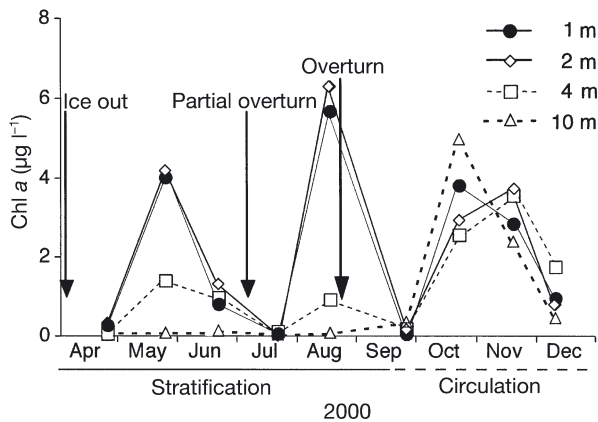


Fig. 3. Seasonal variation in the chl *a* concentration of lake water at 1, 2, 4 and 10 m depths in 2000.  $n = 1$

during the stratification period (from May to August) and approached  $4 \mu\text{g l}^{-1}$ , although the chl *a* concentration decreased to  $<0.2 \mu\text{g l}^{-1}$  once in June 2000. The maximum concentration was  $6.3 \mu\text{g l}^{-1}$  at 2 m in August. On 10 June 2000, a partial overturn of the water column occurred and the concentration of dissolved oxygen in the epilimnion decreased due to the oxidation of hydrogen sulfide supplied from the hypolimnion (S. Shikano unpubl. data); this might have caused the decrease in the chl *a* concentration in June. In September (at the beginning of lake overturn), the chl *a* concentration decreased drastically to 0.2 to  $0.3 \mu\text{g l}^{-1}$  throughout the water column due to the influence of hydrogen sulfide that had accumulated during the stratification period. The chl *a* concentration increased again throughout the water column in October and November ( $>2 \mu\text{g l}^{-1}$ ) and then decreased in December.

The chl *a* concentrations of the water differed significantly with month and depth (2-way ANOVA:  $p < 0.05$ ,  $n = 36$ ). Comparing the chl *a* concentration with depth, the concentrations at 1 and 2 m were significantly higher than those at 10 m from April to August (Scheffé *F*-test:  $p < 0.05$ ,  $n = 5$ ). The concentrations at 1, 2 and 10 m were not significantly different from those at 4 m (Scheffé *F*-test:  $p > 0.05$ ,  $n = 5$ ). The concentrations at 1 and 2 m tended to be higher than those at 4 m from May to August. The chl *a* concentration at 10 m was almost negligible during stratification months, indicating that there was no production of *Chlamydomonas acidophila* at

10 m during these months. During the deep circulation months, the chl *a* concentrations in the water were similar at all depths.

The seasonal variation in the  $\delta^{13}\text{C}$  of POM in 2001 is shown in Fig. 4. The mean  $\delta^{13}\text{C}$  of POM varied within a fairly narrow range from  $-26.4$  to  $-23.7\text{‰}$ . When compared with the  $\delta^{13}\text{C}$  of POM at each depth, the values at 2 and 4 m (from  $-25.8$  to  $-24.1\text{‰}$ ) were significantly higher than those at 10 m (from  $-26.3$  to  $-25.0\text{‰}$ ) from April to August (Scheffé *F*-test:  $p < 0.05$ ,  $n = 15$ ). Seasonally, the  $\delta^{13}\text{C}$  at 1 m in April ( $-25.9 \pm 0.1\text{‰}$ ; mean  $\pm 1$  SD,  $n = 3$ ) was significantly lower than that in October ( $-23.8 \pm 0.3\text{‰}$ ) and November ( $-23.8 \pm 0.2\text{‰}$ ) (Scheffé *F*-test:  $p < 0.05$ ,  $n = 3$ ). The  $\delta^{13}\text{C}$  at 10 m in April ( $-26.3 \pm 0.2\text{‰}$ ) was significantly lower than that in November ( $-24.0 \pm 0.9\text{‰}$ ) (Scheffé *F*-test:  $p < 0.05$ ,  $n = 3$ ).

In July, the measured carbon content of POM was  $27.5 \pm 0.3\%$  (mean  $\pm 1$  SD,  $n = 3$ ),  $25.1 \pm 0.5\%$ ,  $20.3 \pm 0.6\%$  and  $13.6 \pm 0.5\%$  at 1, 2, 4 and 10 m, respectively. The carbon content of POM at 10 m was significantly lower than that at 1, 2 and 4 m (Scheffé *F*-test,  $p < 0.05$ ,  $n = 3$ ).

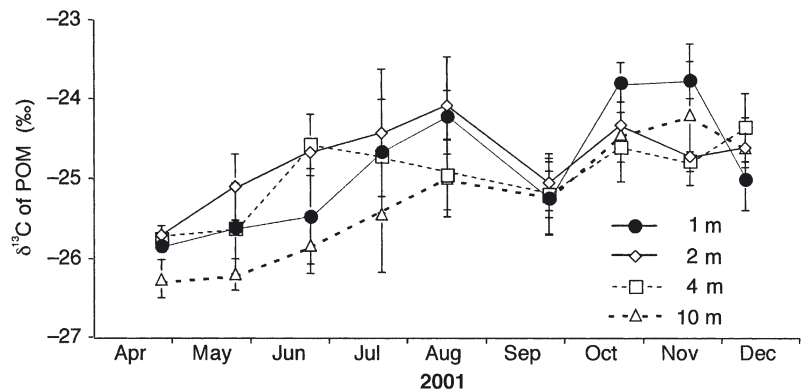


Fig. 4. Seasonal variation in the  $\delta^{13}\text{C}$  of POM at 1, 2, 4 and 10 m depths in 2001. Mean  $\pm 1$  SD ( $n = 3$ )

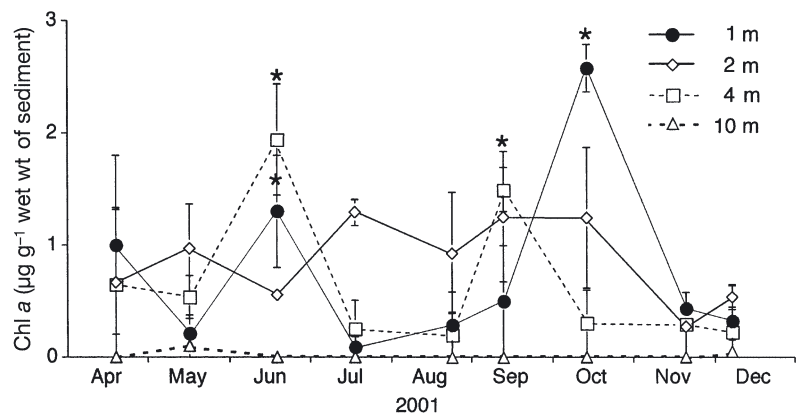


Fig. 5. Seasonal variation in the chl *a* concentration of surface sediments at 1, 2, 4 and 10 m depths in 2001.  $p$ -values are for the treatment effect (Scheffé's *F*-test:  $n = 3$ ): \* $p < 0.05$ , mean  $\pm 1$  SD

### Benthic diatom *Pinnularia acidojaponica*

The seasonal variation in the chl *a* content in the surface sediments at 1, 2, 4 and 10 m depths is shown in Fig. 5. The chl *a* content of the sediments reflected the abundance of *Pinnularia acidojaponica*, the only species of benthic algae in Lake Katanuma. The chl *a* content of the surface sediments at 1 and 4 m varied seasonally, ranging from 0.2 to 2.6  $\mu\text{g g}^{-1}$  wet wt and from 0.3 to 1.9  $\mu\text{g g}^{-1}$  wet wt, respectively. The content at 10 m was almost negligible all year round ( $<0.1 \mu\text{g g}^{-1}$  wet wt), indicating that there was no production of *P. acidojaponica* because of light limitations. Since the light intensity decreases quickly with water depth in the lake, the productivity of *P. acidojaponica* is expected to decrease with depth. The light intensity at 10 m was approximately 1% of that at the surface. The growth of the benthic diatom at 10 m might also be inhibited by hydrogen sulfide that accumulated in the hypolimnion during the stratification period.

The seasonal change in the  $\delta^{13}\text{C}$  of *Pinnularia acidojaponica* in 2001 is shown in Fig. 6. The  $\delta^{13}\text{C}$  of *P. acidojaponica* varied seasonally and vertically over a range from  $-24.6$  to  $-14.0\%$ . The  $\delta^{13}\text{C}$  at 1 m in September and October was significantly higher than in other months (Scheffé *F*-test:  $p < 0.01$ ,  $n = 3$ ), and the  $\delta^{13}\text{C}$  at 4 m in June was also significantly higher than in other months (Scheffé *F*-test:  $p < 0.05$ ,  $n = 3$ ). At 2 m, there was no significant seasonal difference in the  $\delta^{13}\text{C}$  of the diatom, which ranged from  $-18.2$  to  $-22.1\%$  (ANOVA:  $p > 0.05$ ,  $n = 27$ ). Of note, the SD of the  $\delta^{13}\text{C}$  of *P. acidojaponica* was large in May, June, October and December. This may be related to the patchy nature of the distribution of *P. acidojaponica*.

Both the  $\delta^{13}\text{C}$  of the benthic diatom *Pinnularia acidojaponica* and the sedimentary chl *a* content were

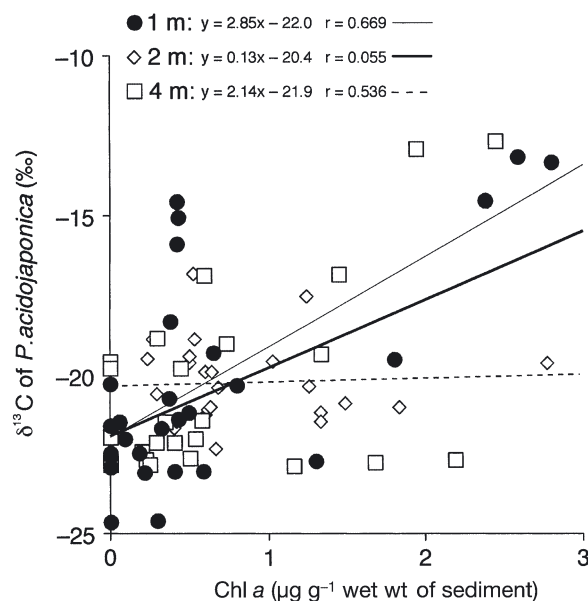


Fig. 7. *Pinnularia acidojaponica*. Correlation between  $\delta^{13}\text{C}$  and chl *a* concentration in surface sediments in Lake Katanuma. *p*-values are for the treatment effect (Pearson's correlation coefficient)

significantly higher at 4 m in June and at 1 m in October (Figs. 5 & 6). This suggests that there is a positive relationship between the  $\delta^{13}\text{C}$  of *P. acidojaponica* and the chl *a* content of the sediments, so we calculated the correlation between the  $\delta^{13}\text{C}$  of *P. acidojaponica* and the chl *a* content of the surface sediments at each sampling depth (Fig. 7). There were significantly positive correlations at 1 and 4 m (Pearson's correlation coefficient:  $p < 0.01$ ; 1 m:  $r = 0.669$ ,  $n = 27$ ; 4 m:  $r = 0.536$ ,  $n = 27$ ), but not at 2 m ( $p > 0.05$ ;  $r = 0.055$ ,  $n = 27$ ).

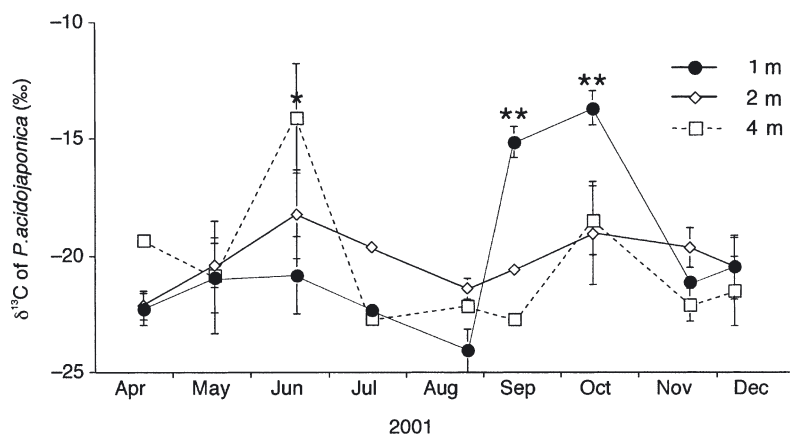


Fig. 6. *Pinnularia acidojaponica*. Seasonal variation in the  $\delta^{13}\text{C}$  of benthic diatoms in 2001. *p*-values are for the treatment effect (Scheffé's *F*-test:  $n = 3$ ): \* $p < 0.05$ , \*\* $p < 0.01$ , mean  $\pm 1$  SD

### DIC

The  $\delta^{13}\text{C}$  of  $\text{CO}_2$  gas from the fumarole on the lake bottom was  $-7.8 \pm 0.3\%$  (mean  $\pm 1$  SD,  $n = 3$ ). This value was the same as the  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  ( $-7.9$  to  $-7.5\%$ ) (Mook et al. 1983). Therefore, the  $\delta^{13}\text{C}$  of  $\text{CO}_2$  in Lake Katanuma did not differ from that in ordinary lakes, although the  $\text{CO}_2$  is derived from 2 sources: the atmosphere and gas from the lake bottom. Zhang et al. (1995) estimated that equilibrium carbon fractionation during  $\text{CO}_2$  gas dissolution in freshwater is  $-1.31 \pm 0.06\%$  at 5 to 25°C. Therefore, the  $\delta^{13}\text{C}$  of dissolved  $\text{CO}_2$  gas in the lake was calculated to be  $-9.1\%$ .

Table 1. Concentration of dissolved inorganic carbon (CO<sub>2</sub> gas; mmol l<sup>-1</sup>) at 0, 1, 2, 5, 10 and 15 m depths in 2001. nd: no data

Water depth (m)	CO <sub>2</sub> gas (mmol l <sup>-1</sup> )				
	20 Apr	18 May	13 Jul	7 Sep	27 Oct
0	0.040	0.132	0.136	0.802	0.463
1	nd	0.148	0.132	0.754	0.353
2	nd	0.145	0.063	0.757	0.369
5	nd	3.13	3.12	0.641	0.437
10	nd	7.13	9.00	0.436	0.468
15	nd	7.69	10.50	0.798	nd

The DIC concentrations are shown in Table 1 and ranged from 0.040 to 0.148 mmol l<sup>-1</sup> at 0 to 2 m, from 3.12 to 10.5 mmol l<sup>-1</sup> at 6 to 15 m during the stratification period, and from 0.353 to 0.802 mmol l<sup>-1</sup> throughout the water column during the circulation period. At pH 2.2, all the DIC is present as dissolved CO<sub>2</sub> gas.

We compared the DIC concentration and the δ<sup>13</sup>C of dissolved CO<sub>2</sub> gas in Lake Katanuma with other lakes. In Lakes Mohonk, Suwa and Kizaki, the DIC concentration and δ<sup>13</sup>C of dissolved CO<sub>2</sub> gas ranged from 0.5 to 35.0 μmol l<sup>-1</sup> and -14 to -9‰, respectively (Herczeg & Fairbanks 1987, Takahashi et al. 1990b, Yoshioka 1997). The δ<sup>13</sup>C of dissolved CO<sub>2</sub> gas in these lakes was lower than that in Lake Katanuma (-9.1‰). Moreover, in 13 volcanic lakes (including Lakes Nyos and Monoun) in the Cameroon Volcanic Line, the DIC concentration ranged from 9.24 to 998 mmol l<sup>-1</sup>, which was markedly higher than the value for Lake Katanuma, and the δ<sup>13</sup>C of dissolved CO<sub>2</sub> gas ranged from -8 to -2‰, which was slightly higher than the value for Lake Katanuma (Tanyileke et al. 1996).

## DISCUSSION

Large seasonal variation in the δ<sup>13</sup>C of phytoplankton has been reported in freshwater ecosystems (Yoshioka et al. 1989, Takahashi et al. 1990a,b, Hollander & McKenzie 1991, Gu et al. 1994, Zohary et al. 1994). The δ<sup>13</sup>C of POM (58 to 100 μm mesh size fraction) fluctuates from -35 to -15‰ in Lake Kizaki (Yoshioka et al. 1989). The seasonal variation in the δ<sup>13</sup>C of phytoplankton in Lakes Kinneret, Greifen, Fukami-ike (predominantly *Chlamydomonas reinhardtii*) and Suwa (only *Microcystis aeruginosa*) was -33 to -18, -39 to -28, -29.2 to -18.0 and -29.0 to -16.7‰, respectively (Takahashi et al. 1990a,b, Hollander & McKenzie 1991, Zohary et al. 1994). By contrast, the seasonal fluctuation in the mean δ<sup>13</sup>C of POM in Lake Katanuma varied within a narrow range from -26.4 to -23.7‰. Compared to Lakes Kinneret, Greifen, Kizaki, Suwa and Fukami-ike,

the δ<sup>13</sup>C of POM in Lake Katanuma is characterized by being essentially constant all year round.

The carbon isotope fractionation of phytoplankton has been explained using carbon isotope fractionation models (O'Leary 1981), which show that the observed overall fractionation between DIC and C fixed by algae is determined by the interaction between CO<sub>2</sub> transport across the cellular membrane by diffusion and carboxylation steps involving RUBISCO. When CO<sub>2</sub> is limited, isotopic fractionation only occurs at the CO<sub>2</sub> transport step and there is virtually no isotopic fractionation at the carboxylation step, since almost all DIC entering the cells is used. Carbon isotope fractionation models also show that a small amount of isotopic fractionation (high algal cell δ<sup>13</sup>C) indicates carbon transport-limited uptake, while a large isotopic fractionation (low algal cell δ<sup>13</sup>C) indicates carboxylation-limited uptake. This is because the fractionation during CO<sub>2</sub> transport is small, while the fractionation in the carboxylation step is potentially large (Fry 1996).

Following the conventions established by O'Leary (1981), the carbon isotope fractionation factor can be expressed as a positive number, ε, calculated as:

$$\varepsilon = (\delta^{13}\text{C}_{\text{DIC}} - \delta^{13}\text{C}_{\text{FIXED C}})/(1 + \delta^{13}\text{C}_{\text{DIC}}/1000) \quad (1)$$

where δ<sup>13</sup>C<sub>DIC</sub> is the δ<sup>13</sup>C of dissolved CO<sub>2</sub> gas (-9.1‰ in Lake Katanuma) and δ<sup>13</sup>C<sub>FIXED C</sub> is the δ<sup>13</sup>C of microalgae. When the δ<sup>13</sup>C<sub>DIC</sub> ranges from -10 to +10‰, Eq. (1) can be simplified with very little loss of accuracy to:

$$\varepsilon = \delta^{13}\text{C}_{\text{DIC}} - \delta^{13}\text{C}_{\text{FIXED C}} \quad (2)$$

Using this equation, the calculated carbon isotope fractionation factor (ε) for POM in Lake Katanuma ranged from 14.6 to 17.3 and the seasonal amplitude was 2.7. In contrast, in Lake Fukami-ike, the ε of *Chlamydomonas reinhardtii* ranged from -7.5 to 9.7 when calculated using CO<sub>2</sub> assimilation (Takahashi et al. 1991). Generally, the CO<sub>2</sub> concentration is very low in strongly acidic lakes (Wetzel 2001). Carbon isotope fractionation models (O'Leary 1988, Fry 1996) should show that the ε of phytoplankton is low in a low CO<sub>2</sub> environment, such as a strongly acidic lake. However, the POM (*C. acidophila*) in Lake Katanuma (strongly acidic lake) had higher ε values than *C. reinhardtii* in Lake Fukami-ike. In Lake Katanuma, the dissolved CO<sub>2</sub> concentration of the lake water exceeded 0.04 mmol l<sup>-1</sup> despite its extremely low pH (Table 1), since CO<sub>2</sub> gas is supplied from fumaroles in the lake bottom. Therefore, the continuous supply of CO<sub>2</sub> gas from these fumaroles might maintain higher CO<sub>2</sub> concentrations, with a consequent higher fractionation in phytoplankton in Lake Katanuma irrespective of its strong acidity.

The seasonal amplitude of  $\epsilon$  for *Chlamydomonas reinhardtii* in Lake Fukami-ike was also greater than for *C. acidophila* in Lake Katanuma. The seasonal amplitude of the carbon isotope fractionation of phytoplankton ranges from 5.3 to 20.0 in lakes (see Zohary et al. 1994 for a review). Therefore, the  $\epsilon$  of POM in Lake Katanuma had smaller seasonal fluctuations than in other lakes.

The primary reason for the stable  $\delta^{13}\text{C}$  and  $\epsilon$  values of POM in Lake Katanuma seems to be the maintenance of the high  $\text{CO}_2$  concentration, despite the extremely low pH (Table 1). A difference in DIC species generally produces considerable variation in the fractionation factor of phytoplankton in a lake (Yoshioka 1997). With a low  $\text{CO}_2$  concentration, phytoplankton transport  $\text{HCO}_3^-$  into the cell, where it is converted into  $\text{CO}_2$  by intracellular carbonic anhydrase (CA) (Lucas & Berry 1985). At the CA step, the carbon isotope fractionation was 10, which is higher than that with passive  $\text{CO}_2$  diffusion (Deines et al. 1974, Paneth & O'Leary 1985). In Lake Katanuma, however, DIC was composed only of  $\text{CO}_2$  gas; consequently, DIC assimilation by *Chlamydomonas acidophila* only occurred via passive  $\text{CO}_2$  diffusion. This suggests that the relatively constant high  $\text{CO}_2$  concentration caused the small seasonal fluctuation in isotope fractionation by *C. acidophila*.

A second reason might be the lower photosynthetic rate and biomass of *Chlamydomonas acidophila* in Lake Katanuma. In Lakes Kizaki and Fukami-ike, the chl *a* concentrations fluctuated from 25 to 100  $\mu\text{g l}^{-1}$ , especially during the spring bloom (Yoshioka et al. 1989, Takahashi et al. 1990a,b). As the phytoplankton biomass increases, the supply of DIC would be limited; consequently, the carbon would be more enriched in  $^{13}\text{C}$  due to the reduced carbon isotope fractionation during photosynthesis. The  $^{13}\text{C}$ -enrichment with a higher phytoplankton biomass caused the large seasonal fluctuation in the  $\delta^{13}\text{C}$  of POM (phytoplankton) in Lakes Kizaki and Fukami-ike (Yoshioka et al. 1989, Takahashi et al. 1990a). However, the chl *a* concentrations in Lake Katanuma remained lower (less than 7  $\mu\text{g l}^{-1}$ ) than in Lakes Kizaki and Fukami-ike (Fig. 3). The photosynthetic activity might be reduced by the acidity of the lake water, since the capacity for photosynthesis is limited 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 chl *a* concentrations in the lake compared with other lakes.

The  $\delta^{13}\text{C}$  of POM at 10 m tended to be lower than in the littoral zone (1, 2 and 4 m depths) in the stratification period (Fig. 4). This suggests that the organic matter composition of POM differed between the epilimnion and hypolimnion, since the thermocline was generally situated at about 5 m. Consequently, higher

chl *a* concentrations were observed only in the epilimnion (Fig. 3), while the chl *a* at 10 m (hypolimnion) was negligible during the stratification period (Fig. 3). The carbon content of POM at 10 m also tended to be lower than at 1, 2 and 4 m in July. In July, the carbon content of POM at 10 m ( $13.6 \pm 0.5\%$ ) was significantly lower than at 1 m ( $27.5 \pm 0.3\%$ ) (Scheffé *F*-test:  $p < 0.05$ ,  $n = 3$ ). In June, the estimated carbon content of phytoplankton *Chlamydomonas acidophila* was 27.6%, when an almost pure sample of *C. acidophila* was collected from the lakeshore of Lake Katanuma and measured (H. Doi unpubl. data). Since green surface water due to the presence of *C. acidophila* was observed along the lakeshore from May to July, the POM in the epilimnion was mostly phytoplankton (*C. acidophila*) and the contribution of phytoplankton to the POM was low at 10 m. The lower  $\delta^{13}\text{C}$  of POM at 10 m suggests that there is another source of POM in the hypolimnion from April to July. Satake & Saijo (1973) reported high levels of microbial  $\text{CO}_2$  fixation in the anoxic zone (hypolimnion) in Lake Katanuma. This bacterial anoxic  $\text{CO}_2$  fixation may produce the lower  $\delta^{13}\text{C}$  of POM at 10 m from April to July. In addition, the higher  $\delta^{13}\text{C}$  of POM at 1 to 4 m suggests that *C. acidophila* has a higher  $\delta^{13}\text{C}$ . The  $\delta^{13}\text{C}$  of POM tended to increase during the stagnation period (from April to August). This suggests the accumulation of phytoplankton-derived carbon with a higher  $\delta^{13}\text{C}$  in POM.

We found significant positive correlations between the  $\delta^{13}\text{C}$  of *Pinnularia acidojaponica* and chl *a* in surface sediments at 1 and 4 m (Fig. 5). Benthic algae have diffusive boundary layers over 1 mm thick (Jørgensen & Revsbech 1985, Riber & Wetzel 1987). In Lake Katanuma, *P. acidojaponica* often formed high density patches (algal mats) on the sediment surface in the photic zone. These *P. acidojaponica* mats were sometimes 0.5 to 1.0 mm thick (Satake & Saijo 1978). Therefore, *P. acidojaponica* might promote  $^{13}\text{C}$ -enrichment because its supply of DIC is limited by its patch thickness (Wheeler 1980, France 1995). Consequently, the  $\delta^{13}\text{C}$  of *P. acidojaponica* was higher when algal biomass was high (Fig. 5).

In contrast, the  $\delta^{13}\text{C}$  of *Pinnularia acidojaponica* at 2 m did not differ significantly with month. The seasonal fluctuation in the  $\delta^{13}\text{C}$  was narrower than at 1 and 4 m depths. This was caused by the lower seasonal variation of chl *a* content in sediments compared to other depths. The narrower variation of both the  $\delta^{13}\text{C}$  of benthic diatoms and the chl *a* content of the sediments might cause the lack of correlation at 2 m depth.

In Lake Katanuma, the  $\delta^{13}\text{C}$  of benthic diatoms was significantly higher than that of POM (Student's *t*-test:  $p < 0.001$ ,  $n = 81$ ); the mean  $\delta^{13}\text{C}$  and isotope fractionation of POM in Lake Katanuma was  $-25.0 \pm 0.7\%$  ( $\epsilon =$

15.9 ± 0.7) (mean ± 1 SD) and that of benthic diatoms was -20.3 ± 2.7‰ ( $\epsilon = 11.2 \pm 2.7$ ). Therefore, the  $\delta^{13}\text{C}$  of benthic diatoms was higher than the POM (phytoplankton), although both appear to assimilate the same carbon source,  $\text{CO}_2$  gas in the lake water. France (1995) also reported that benthic microalgae tend to be more enriched in  $\delta^{13}\text{C}$  than phytoplankton, and suggested that the difference in  $\delta^{13}\text{C}$  was caused by diffusive boundary layer effects, because benthic algae have thicker diffusive boundary layers that are over 1 mm thick (Jørgensen & Revsbech 1985, Riber & Wetzel 1987). However, France (1995) did not compare sympatric benthic algae and phytoplankton, which use the same carbon source, but only compared the percentage frequency distributions and average stable carbon isotope ratios of algae reported in the literature from different environments. Ours, therefore, may be the first study to detect a difference in the  $\delta^{13}\text{C}$  of benthic diatoms and phytoplankton in the same natural environment.

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