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A study of the nitrogen stable isotope dynamics of phytoplankton in a simple natural ecosystem

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ABSTRACT: We investigated fluctuations in the nitrogen stable isotope ratio ($\delta^{15}N$) of *Chlamydomonas acidophila* (Chlorophyceae) by short-interval sampling during a bloom that occurred from 11 May to 29 July 2001 in Lake Katanuma, Japan. In this lake, *C. acidophila* uses NH_4^+ as the primary inorganic nitrogenous nutrient, since only trace amounts of other inorganic nitrogenous compounds are present. The $\delta^{15}N$ values obtained for *C. acidophila* ranged from –7.8 to –3.4‰ during the study period. The lowest values occurred during periods characterized by high concentrations of NH_4^+ (>15 µmol l^{-1}), and our results showed that $\delta^{15}N$ values for *C. acidophila* were negatively correlated with concentrations of NH_4^+. The results suggest that this nutrient plays a major role in the variation of the $\delta^{15}N$ value for this species. The nitrogen isotope fraction factor (ε) of *C. acidophila* was much lower than that of many NH_4^+-based primary producers from other eutrophic environments.

KEY WORDS: Nitrogen · Stable isotope · Phytoplankton · Isotope fractionation · Inorganic nutrient · Acidic lake · Spring bloom · Lake Katanuma

INTRODUCTION

Nitrogen stable isotope ratios ($\delta^{15}N$) have become an important tool for the study of both the sources and the biogeochemical processing of organic matter in aquatic ecosystems (Minagawa & Wada 1986, Cifuentes et al. 1989, Meyers 1994). Recently, the factors that determine the $\delta^{15}N$ value of many phytoplankton species have been studied using pure algal cultures in the laboratory (e.g. Pennock et al. 1996, Waser et al. 1998, 1999). Laboratory culture experiments have shown wide fluctuations in the $\delta^{15}N$ values of different algal species and their isotope fractionation factors (ε), which can be used as indicators of ^{15}N-enrichment and the assimilation of dissolved inorganic nitrogen (DIN) by phytoplankton. Variation in $\delta^{15}N$ values has been reported to depend on a range of factors, such as the species of algae and culture conditions (nutrient supply, light intensity, temperature, etc.) (Wada & Hattori 1978, Wada 1980, Hoch et al. 1992, Waser et al. 1998, 1999). Moreover, the experiments investigating changes in the $\delta^{15}N$ values of phytoplankton using laboratory cultures have been conducted only on short time scales (usually hours), since under culture conditions nutrient concentrations decrease and the biomass of phytoplankton increases quickly (Pennock et al. 1996, Waser et al. 1998, 1999).

To date, there have been no detailed studies of the $\delta^{15}N$ value dynamics of phytoplankton in natural lakes, and little is known about the factors that determine the $\delta^{15}N$ values of phytoplankton in natural systems. There is a clear need to investigate fluctuations in $\delta^{15}N$ values of phytoplankton in lakes, with reference to changes in biomass and environmental factors. Such information would be useful for determining the factors that affect variation of $\delta^{15}N$ values in nature.

Lake Katanuma is a volcanic, strongly acidic lake (annual average pH of 2.2). The phytoplankton community in the lake is very simple, with only 1 domi-

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nant species, *Chlamydomonas acidophila* (Chlorophyceae), which often accumulates at the water surface, producing dark-green scum close to the lakeshore. During these periods, we were able to obtain almost pure samples of *C. acidophila* for use in our experiments. In general, lake phytoplankton communities are composed of many microalgal species, which usually complicates determination of the relationships between the $\delta^{15}N$ value of individual phytoplankton species and the prevailing physiological and environmental conditions. As a result, $\delta^{15}N$ values have frequently been measured in whole phytoplankton communities (e.g. Pennock et al. 1996). In this study, however, we were able to analyze the relationships between the $\delta^{15}N$ value of a single phytoplankton species and the controlling environmental conditions.

In the present study, we investigated changes in the $\delta^{15}N$ value and biomass of *Chlamydomonas acidophila* and DIN concentrations in Lake Katanuma from May to July (during the spring bloom). We also conducted a short-interval sampling experiment from the start to the peak of the bloom, to reveal the short-term fluctuations in the $\delta^{15}N$ value of this phytoplankton species under natural conditions.

MATERIALS AND METHODS

Study area. Lake Katanuma is a volcanic lake in northeastern Japan (38° 44′ N, 140° 43′ E), at an altitude of 306 m above sea level. It is highly acidic, with annual variation in pH ranging from 2.0 to 2.4. The lake has a surface area of 0.14 km^2 and a maximum depth of 20 m. It is essentially a closed system, with no inflowing or outflowing streams, and is dimictic, with stratification and circulation periods from April to August and from September to December, respectively. Ice usually covers the lake from January to March. Zooplanktonic and nektonic organisms have not been observed in Lake Katanuma (Doi et al. 2001); therefore, herbivores for phytoplankton do not exist in the lake. Moreover, the dissolved CO_2 concentration of the lake water exceeds 0.04 mmol l^{-1} despite its extremely low pH, since CO_2 gas is supplied from fumaroles in the lake bottom. The continuous supply of CO_2 gas from these fumaroles might maintain higher CO_2 concentrations for phytoplankton photosynthesis in Lake Katanuma (Doi et al. 2003a,b).

Collecting and preparation of samples. Green surface water attributed to the presence of *Chlamydomonas acidophila* was observed along the lakeshore from 15 May to 29 July 2001. Lake surface water samples were collected for analysis from 11 May to 17 July 2001. The green coloration of the surface water was not observed on 11 May. Water samples were collected where *C. acidophila* had accumulated near the shore of the lake. Triplicate water samples were filtered through Whatman GF/F glass-fiber filters (precombusted at 500°C for 2 h) to collect phytoplankton. To determine the abundance of *C. acidophila*, chlorophyll *a* (chl *a*) concentrations of the water samples were measured using a fluorometer (10-AU, Turner Designs), following extraction using triplicate GF/F filters with N,N-dimethylformamide. The concentrations of inorganic nutrients (NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-}) were determined for triplicate samples of filtered lake water collected from the surface near the lakeshore between 11 May and 17 July and from 10 m depth at the center of the lake on 15 May. The concentration of NH_4^+ was determined according to the method of Scheiner (1976). The concentrations of NO_2^-, NO_3^-, and PO_4^{3-} were measured using the colorimetric analyses described by Strickland & Parsons (1972), following neutralization of the filtered water samples with sodium hydroxide. The water temperature of the lake was measured using a multiple water quality sensor (U-22, Horiba). Sampling was carried out daily from 15 to 22 May, at 2 to 5 d intervals from 25 May to 5 June, and weekly or biweekly from 5 June to 17 July.

To measure $\delta^{15}N$ values for NH_4^+, surface water was collected on 15 May, 19 June, and 17 July from the *Chlamydomonas acidophila* sampling area. We were able to measure $\delta^{15}N$ values for NH_4^+ only for the lake water collected from the surface (near the lakeshore) and at a depth of 10 m (at the center of the lake) on 15 May, since the NH_4^+ concentrations of the water samples collected on 19 June and 17 July were too low to measure the $\delta^{15}N$ values. The $\delta^{15}N$ values for NH_4^+ were measured in triplicate samples, using the following method, described by Holmes et al. (1998). The filtered lake water (2 l) was placed in a diffusion bottle, and 100 g NaCl was added. Then, a precombusted Whatman GF/D glass filter that had been soaked with 2 mol l^{-1} H_2SO_4 was placed in the headspace of the diffusion bottle. After adding 6 g MgO to the diffusion bottle, the bottle was incubated for 2 wk on a shaker. Moreover, we measured the standard diffusion samples using the lake surface water to correct for the fractionation of samples with refer to Holmes et al. (1998). The $\delta^{15}N$ value of the phytoplankton and NH_4^+ on the glass-fiber filter was measured using a mass spectrometer (Delta plus, Finnigan Mat) connected to an elemental analyzer (NA2500, CE Instruments). Results were reported in delta notation as: $\delta^{15}N = ({}^{15}N/{}^{14}N_{sample}/{}^{15}N/{}^{14}N_{standard} - 1) \times 1000$ (‰), where air N_2 was used as the standard. The analytical error was estimated to be approximately ± 0.2‰.

RESULTS

Concentrations of inorganic nutrients in Lake Katanuma

The concentrations of NH_4^+ and PO_4^{3-} in Lake Katanuma are shown in Fig. 1A. The concentrations of NH_4^+ between 11 and 20 May ranged from 6.6 to 9.4 µmol l^{-1}. The concentrations of NH_4^+ increased to 15 to 20 µmol l^{-1} between 21 and 29 May. This increase was probably because of vertical mixing of water at the lakeshore, since the water collected at a depth of 10 m in the hypolimnion had high concentrations of NH_4^+ (25.1 ± 7.5 µmol l^{-1} on 15 May, mean ± 1 SD, n = 3). The concentration of NH_4^+ in the lake water then decreased from 29 May to 19 June, and thereafter remained at low concentrations (0.5–4.4 µmol l^{-1}), due to consumption by *Chlamydomonas acidophila*. The concentrations of NO_3^- and NO_2^- in Lake Katanuma were below detection limits throughout the study period; thus, NH_4^+ was the only detectable form of inorganic nitrogen in the lake. The concentrations of PO_4^{3-} were <3 µmol l^{-1} between 11 May and 17 July (except for 19 June, 7.4 ± 1.0 µmol l^{-1}). However, there was no evidence that PO_4^{3-} limited the growth of *C. acidophila*, since the mean N/P was 6.9 ± 4.8 (mean ± 1 SD) over the study period. These ratios are lower than the Redfield ratio value of 16, which is used as an indicator of PO_4^{3-} limitation (Redfield 1963, Hecky et al. 1993).

Biomass of *Chlamydomonas acidophila*

The concentrations of chl *a* reflected the biomass of *Chlamydomonas acidophila* in the lake. Concentrations of chl *a* were low (0.3 ± 0.2 µg l^{-1}, mean ± 1 SD, n = 3) on 11 May, before the spring bloom (Fig. 1B). At the beginning of the bloom, the concentration increased to 154.5 ± 86.3 µg l^{-1} on 16 May, then decreased to 16.5 ± 1.0 µg l^{-1} on 19 May. Values then increased rapidly to 122.5 ± 39 µg l^{-1} on 20 May, coinciding with an increase in NH_4^+ concentration, then decreased to around 200 µg l^{-1} from 22 to 25 May, before increasing and peaking on 29 May at a concentration of 989.9 µg l^{-1}. Thereafter, the chl *a* concentrations gradually decreased to around 250 µg l^{-1}. This chl *a* fluctuation resulted from both growth at the shore site and the vertical and horizontal migration of phytoplankton with water movement.

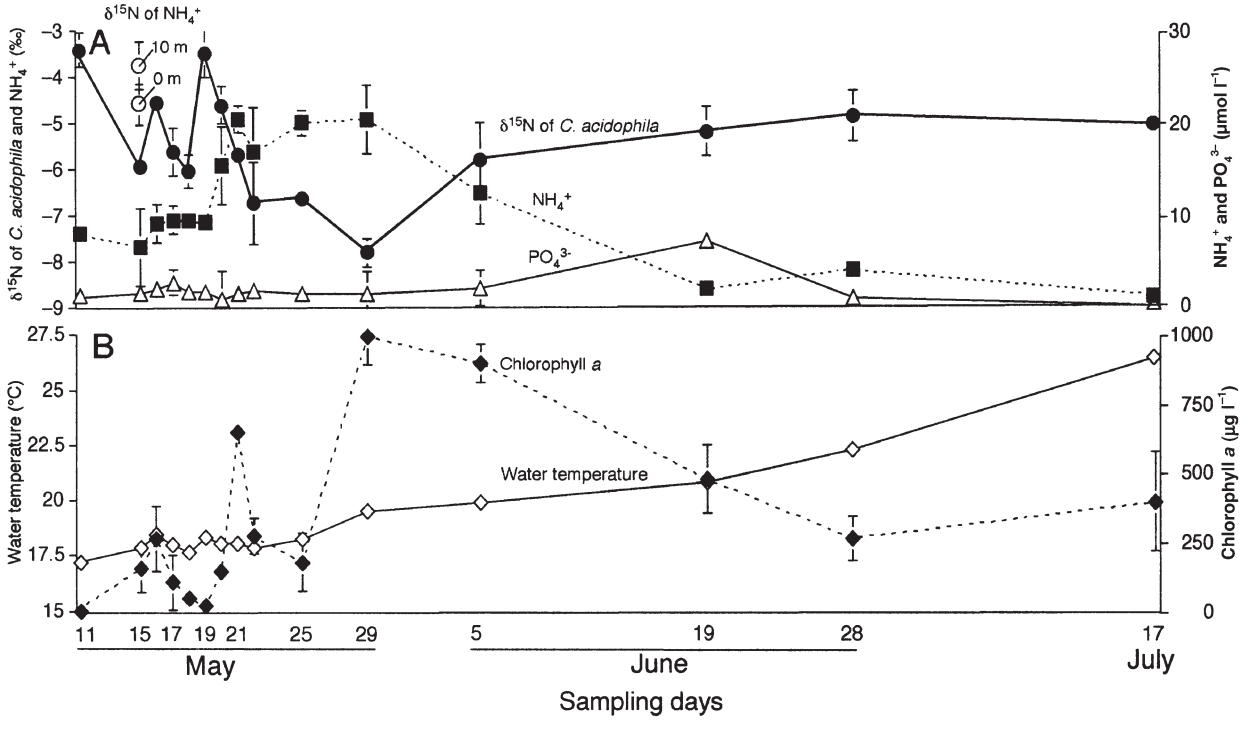

Fig. 1. *Chlamydomonas acidophila*. (A) $\delta^{15}N$ values for *C. acidophila*, NH_4^+, and the concentrations of NH_4^+ and PO_4^{3-} in the surface water. (●,○) $\delta^{15}N$ values obtained for *C. acidophila* and NH_4^+, (■,△) concentrations of NH_4^+ and PO_4^{3-}, respectively. Left *y*-axis: $\delta^{15}N$ values obtained for *C. acidophila* and NH_4^+; right *y*-axis: concentrations of NH_4^+ and PO_4^{3-}. Error bars indicate ±1 SD (n = 3). (B) Concentrations of chl *a* and water temperature in Lake Katanuma. (◆,◇) Concentrations of chl *a* and water temperature, respectively. Left *y*-axis: water temperature; right y-axis: concentrations of chl *a*. Error bars indicate ±1 SD (n = 3)

Water temperature in Lake Katanuma

Variations in the surface water temperature of Lake Katanuma are shown in Fig. 1B. A seasonal increase in water temperature was observed over the sampling period from 11 May to 17 July (from 17.2 to 26.6°C), although there was a slight decrease in the water temperature in the middle of May. Water temperatures between 15 and 29 May were 17.8 to 19.5°C, similar to those used in a laboratory study investigating the $\delta^{15}N$ values of some phytoplankton species (Pennock et al. 1996, Waser et al. 1998).

Variation in the $\delta^{15}N$ value of *Chlamydomonas acidophila* and NH_4^+ in Lake Katanuma

The $\delta^{15}N$ values obtained for *Chlamydomonas acidophila* are shown in Fig. 1A. Values varied during the sampling period, ranging from −7.8 to −3.4‰. On 11 May, before the spring bloom, the value was high at −3.4 ± 0.4‰ (mean ± 1 SD, n = 3), but the low concentration of chl *a* detected in the sample (0.3 ± 0.2 µg l^{-1}) suggests that the sample might have included some bacterial organic matters. After 11 May, $\delta^{15}N$ values were presumed to reflect the presence of *C. acidophila*, since the concentrations of chl *a* remained high (from 16.5 to 989.9 µg l^{-1}). The $\delta^{15}N$ value of *C. acidophila* fluctuated within the range of −7.8 to −3.5‰ from the start (15 May) to the peak (5 June) of the bloom, and showed high values of −4.6, −3.5, and −4.6‰, on 16, 19, and 20 May, respectively. Low values (−7.8 to −6.4‰) were recorded between 22 and 29 May. Thereafter, from 19 June to 17 July, values tended to be higher, ranging from −5.2 to −4.8‰. The $\delta^{15}N$ values of NH_4^+ in lake water collected on 15 May from the surface and at a depth of 10 m were −4.9 ± 0.3 and −3.6 ± 0.5‰ (mean ± 1 SD, n = 3), respectively.

DISCUSSION

Natural phytoplankton populations usually assimilate NO_3^-, the most common inorganic nitrogenous compound in most lakes (Wetzel 2001). In Lake Katanuma, however, NH_4^+ is the most common form of nitrogen, and concentrations of NO_3^- are below the limits of detection. The most likely explanation for this is that nitrification proceeds slowly under acidic conditions (< pH 5), leading to a build-up of NH_4^+ (Wetzel 2001). Mitamura & Saijo (1986) suggested that the relative nitrogen assimilation rates of inorganic nitrogenous compounds by phytoplankton are in the following order: ammonium > urea > nitrate. Therefore, the available evidence suggests that the *Chlamydomonas acidophila* populations in Lake Katanuma use NH_4^+ as the substrate for conversion into proteins.

Very few $\delta^{15}N$ values have been obtained for NH_4^+ of lake water (e.g. Holmes et al. 1998). The $\delta^{15}N$ values obtained for NH_4^+ for Lake Katanuma water on 15 May were −4.9 ± 0.3 and −3.6 ± 0.5‰, which were lower than those in Miles Pond (2.8 ± 0.6‰) (Holmes et al. 1998). Lake Katanuma does not have any inflowing streams and is located on a mountain top, suggesting only a limited supply of rainwater (Shikano et al. 2004). It therefore seems likely that the high NH_4^+ concentrations of the lake water are derived from the remineralization of organic matter in the lake. The $\delta^{15}N$ value of NH_4^+ for the lake water on 15 May might indicate that re-mineralized NH_4^+ is supplied from the hypolimnion. However, the NH_4^+ in Lake Katanuma water might originally be derived from rainwater, since there is no other water inflow. The $\delta^{15}N$ value obtained for NH_4^+ in rainwater range from −10 to 0‰ (Kendall 1998). The $\delta^{15}N$ values obtained for NH_4^+ for Lake Katanuma water from the surface and at 10 m depth (−4.9 ± 0.3 and −3.6 ± 0.5‰) equal the medium values for rainwater. Moreover, at a pH of 2.2, the fractions of NH_4^+ and NH_3 gas were 5.4×10^8 and 1, respectively (Emerson et al. 1975). Therefore, the influence of low pH on the isotope fractionation between NH_4^+ and NH_3 gas was negligible in the low pH water of Lake Katanuma, since NH_4^+ dominated exclusively.

The $\delta^{15}N$ value of phytoplankton is determined by physiological characteristics such as growth rates and biomass, as well as the $\delta^{15}N$ value and concentration of nitrogenous compounds (Wada 1980, Waser et al. 1998). In the present study, we examined the correlations between the $\delta^{15}N$ values obtained for *Chlamydomonas acidophila* and the concentrations of chl *a*, and environmental factors such as temperature and NH_4^+ and PO_4^{3-} concentrations (Table 1).

The results showed that the $\delta^{15}N$ values obtained for *Chlamydomonas acidophila* were negatively correlated with NH_4^+ concentrations (Pearson's correlation coefficient, r = −0.637, $p < 0.05$), suggesting that high

Table 1. Correlation (r) between $\delta^{15}N$ values obtained for *Chlamydomonas acidophila*, water temperature (WT), concentrations of NH_4^+, PO_4^{3-}, and chl *a* in the surface water between 15 May and 17 July. *Significant r-values ($p < 0.05$, n = 14, Pearson's correlation coefficient)

	$\delta^{15}N$	WT	NH_4^+	PO_4^{3-}	Chl *a*
$\delta^{15}N$		0.057	−0.637*	−0.030	−0.426
WT			−0.605*	−0.057	0.377
NH_4^+				−0.249	0.018
PO_4^{3-}					0.181
Chl *a*					

NH_4^+ values result in low particulate $\delta^{15}N$ values. The low $\delta^{15}N$ values occurred only during periods characterized by high NH_4^+concentrations (>15 µmol l^{-1}) (Fig. 2). Thus, our results suggest that the concentration of NH_4^+ is a major determinant of the $\delta^{15}N$ value of *C. acidophila* in Lake Katanuma. The high NH_4^+ values correlate to low particulate $\delta^{15}N$ values, owing to isotopic fractionation during ammonium assimilation. In studies using laboratory cultures, similar relationships have been reported. Hoch et al. (1992) reported low $\delta^{15}N$ values for phytoplankton at high concentrations of NH_4^+ (50 to 200 µmol l^{-1}). Pennock et al. (1996) found that the isotopic fractionation of NH_4^+ by phytoplankton varied with ambient NH_4^+ concentration over the range from 5 to 100 µmol l^{-1}, and lower fractionation was observed at lower concentrations of NH_4^+ (5 to 20 µmol l^{-1}).

In Lake Katanuma, water temperature was negatively correlated with concentrations of NH_4^+ ($r = -0.605$, $p < 0.05$) (Table 2). This study was carried out at the beginning of the spring bloom period, and the abundance of *Chlamydomonas acidophila* increased with increasing water temperature from May to July. Therefore, the negative correlation obtained in this study may show only that the concentration of NH_4^+ tended to decrease with the increasing biomass of *C. acidophila*, because of assimilation by the phytoplankton.

Higher $\delta^{15}N$ values obtained for phytoplankton have been reported to be accompanied by higher growth rates and greater biomass (Waser et al. 1998). This did not appear to be the case in Lake Katanuma. Lower

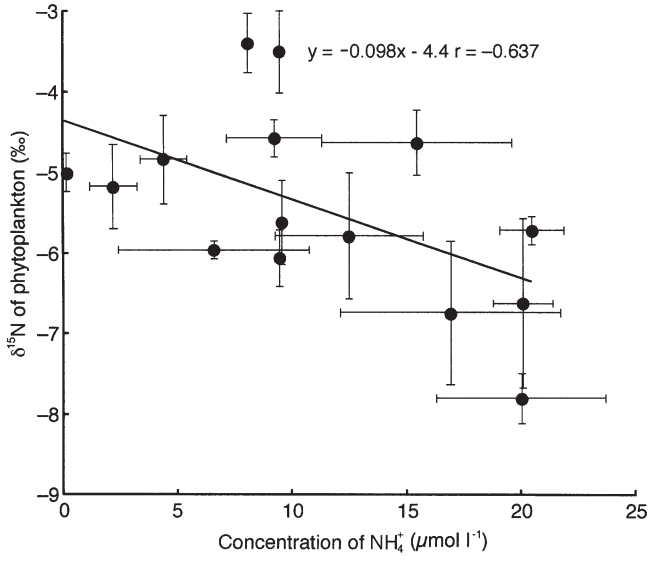

Fig. 2. *Chlamydomonas acidophila*. Relationship between $\delta^{15}N$ values of *C. acidophila* and concentration of NH_4^+ between 15 May and 17 July. Correlation line gave an r-value of −0.637 ($p < 0.05$, n = 14). Error bars indicate ±1 SD (n = 3)

Table 2. *Chlamydomonas acidophila*. Isotope fractionation (ε: mean ± 1 SD, n = 3) obtained between 15 May and 28 June. Isotope fractionation factor (ε) was determined using the equation described by Mariotti et al. (1981) and Waser et al. (1998)

Sampling date	ε (‰)
15 May	1.2 ± 0.1
16 May	0.3 ± 0.2
17 May	1.1 ± 0.4
18 May	0.2 ± 0.3
19 May	−1.0 ± 0.5
20 May	0.5 ± 0.4
21 May	1.9 ± 0.2
22 May	2.1 ± 0.4
25 May	2.8 ± 0.8
29 May	3.5 ± 0.9
5 Jun	1.2 ± 0.5
19 Jun	2.5 ± 1.0
28 Jun	1.1 ± 0.4

$\delta^{15}N$ values of the phytoplankton were observed following a rapid increase in chl *a* concentration from 21 to 29 May (Fig. 1A), and there was no significant correlation between the $\delta^{15}N$ value of the phytoplankton and concentrations of chl *a*, which is indicative of the biomass of *Chlamydomonas acidophila* (Table 1).

The isotope fractionation factor (ε) was determined, as described by Mariotti et al. (1981), as an indicator of ^{15}N enrichment. To calculate ε, the $\delta^{15}N$ values of NH_4^+ were necessary, and were calculated using the following isotope mixing model equation, since the NH_4^+ of surface water was derived from the hypolimnion:

on 15 May:

$$\delta^{15}N_{DIN} = \delta^{15}N_{DIN} \text{ (initial at 15 May)}$$

from 16 May to 5 June:

$$\delta^{15}N_{DIN} \text{ (sampling day)} = (1 - f_N)\delta^{15}N_{DIN} \text{ (1 d before sampling day)} + f_N\delta^{15}N_{DIN} \text{ (hypolimnion), and}$$

$$f_N = [NH_4^+ \text{ (sampling day)} - NH_4^+ \text{ (1 d before sampling)}] \times [NH_4^+ \text{ (sampling day)}]^{-1}$$

where $\delta^{15}N_{DIN}$ (initial at 15 May) and $\delta^{15}N_{DIN}$ (hypolimnion) were assumed to be −4.9 and −3.6‰, respectively, since the DIN in Lake Katanuma is composed almost entirely of NH_4^+. Since $f_N \geq 0$, DIN was calculated for 16, 17, 20, 21, 25 May, and 5 June, while on the other sampling days ($f_N < 0$) $\delta^{15}N_{DIN}$ was assumed to have the same values as those of the previous samples, since the NH_4^+ supply from the hypolimnion may be negligible.

Assuming that the initial $\delta^{15}N$ value of NH_4^+ was −4.9‰, the isotope fractionation factor (ε) was calculated for the sampling period from 16 May to 18 May and from 22 May to 28 June using the model for closed-system conditions described by Mariotti et al. (1981) and Waser et al. (1998) as follows:

on 15 May:

$$\varepsilon = \delta^{15}N_{DIN}\ (\text{initial at 15 May}) - \delta^{15}N_{phytoplankton}$$

for the periods from 16 May to 18 May and from 22 May to 28 June:

$$\varepsilon = (\delta^{15}N_{DIN} - \delta^{15}N_{phytoplankton}) / [-f\ (1 - f)^{-1}]\ \ln f$$

$$f = NH_4^+\ (\text{sampling day}) \times [NH_4^+\ (\text{initial at 15 May})]^{-1}$$

where $\delta^{15}N_{phytoplankton}$ and $\delta^{15}N_{DIN}$ indicate $\delta^{15}N$ values of phytoplankton and NH_4^+, respectively. f means the fraction of unreacted NH_4^+ on sampling days during our study. On 17 July, the ε values of *Chlamydomonas acidophila* may not be accurate, since NH_4^+ concentrations were extremely low and below detection limits; thus unreacted NH_4^+ could not be calculated accurately.

For the period from 19 to 21 May, ε was calculated using the model for open-system conditions, with the following equation (Mariotti et al. 1981), since partial mixing of lake water was observed during 19 to 21 May, the surface water system for phytoplankton was not closed, and the NH_4^+ concentration increased significantly:

$$\varepsilon = \delta^{15}N_{phytoplankton} - \delta^{15}N_{DIN}\ (\text{sampling day})$$

The isotope fractionations (ε) obtained for *Chlamydomonas acidophila* ranged from –1.0 to 3.9‰ between 15 May and 28 June (Table 2). These values are lower than those reported for many other NH_4^+-based primary producers in other water bodies, which usually range from 6.5 to 9.1‰ (Cifuentes et al. 1989, Montoya et al. 1991). The low values obtained for *C. acidophila* in this study indicate low ^{15}N-depletion in phytoplankton in Lake Katanuma. Research using laboratory cultures has shown isotope fractionation by phytoplankton to decrease during nutrient starvation (Hoch et al. 1992, Waser et al. 1998, 1999). Waser et al. (1998) suggested that isotope fractionation by phytoplankton decreases with decreasing concentrations of NH_4^+. The concentrations of NH_4^+ in Lake Katanuma were <20 µmol l^{-1}, much lower than those used in laboratory culture experiments (23 to 190 µmol l^{-1}; Hoch et al. 1992, Waser et al. 1998, 1999). Therefore, the low concentration of NH_4^+ in Lake Katanuma may be the reason for the low isotope fractionation values.

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