Estimation of Nitrogen Uptake Rate of Small Zooplankton Using ^{15}N tracer

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Abstract

The uptake rate of nitrogen by freshwater zooplankton was measured by use of a ^{15}N tracer technique under experimental conditions. To detect the low quantities of excess ^{15}N in less than several hundreds of small zooplankton individuals (as small as $5\text{ }\mu\text{gN}$), an isotope dilution technique was adopted using a ratio mass spectrometer. The analytical differences between natural ^{15}N abundance method and result obtained by this technique were -0.00210 to $+0.00113$ ^{15}N atom % (mean $\pm$ S.D. = 0.00048 ± 0.0010 ^{15}N atom %). We obtained uptake rates of $8.5 \times 10^{-8} \text{ mg atom-N} \cdot \text{indiv.}^{-1} \cdot \text{day}^{-1}$ by *Kellicottia longispina* in Lake Nakatsuna and 4.2×10^{-7} by *Keratella quadrata* in Lake Suwa. Net production rates calculated from the data of the uptake rates, carbon contents and nitrogen contents were $5.1 \times 10^{-6} \text{ mgC} \cdot \text{indiv.}^{-1} \cdot \text{day}^{-1}$ for *K. longispina* and $2.5 \times 10^{-5} \text{ mgC} \cdot \text{indiv.}^{-1} \cdot \text{day}^{-1}$ for *K. quadrata*. Using a combination of the ratio mass spectrometer and the ^{15}N tracer experiment, the nitrogen uptake rates from nutrient salt to phytoplankton and further to zooplankton can be detected directly at the same time and on the same space scale.

Key words: ^{15}N tracer technique; zooplankton; nitrogen uptake rate; lacustrine ecosystems

1. Introduction

Small zooplankton, such as rotifers, have key roles as secondary producer in lacustrine ecosystems. Feeding habits of zooplankton have been investigated with various methods, such as *in situ* experiments using radio isotopes, as tracers (HANEY, 1973; LAMPERT, 1975; LAMPERT and GABRIEL, 1984; GILBERT and BOGDAN, 1985; WALLACE and STARKWEATHER, 1985; BJØRNSSEN, 1986; BOSSELMANN and RIEMANN, 1986). However, our knowledge of and information on the nitrogen flow through small zooplankton are still limited. Up to now nitrogen uptake kinetics of small zooplankton have been determined indirectly by the conversion from dry weight or carbon to nitrogen (WATERS, 1977; FASHAM *et al.*, 1990). Although phytoplankton have been an object of study using a stable

nitrogen isotope tracer (WADA *et al.*, 1977; TAKAHASHI *et al.*, 1981; KANDA *et al.*, 1985), only a few experiments have been carried out to determine the nitrogen flow through small zooplankton (OTSUKI *et al.*, 1985). This is partly due to technical difficulties in the detection of very small quantities of tracers, such as ^{15}N , transferred to small zooplankton species.

The ratio-mass spectro-analysis allows the detection of very small amounts of natural stable isotopes such as ^{15}N , and may be applicable to a micro scale tracer experiment (WADA *et al.*, 1977). Nitrogen flowing through zooplankton can be measured directly, using a combination of the mass spectro-analysis and the ^{15}N tracer technique. This technique allows us to show the nitrogen transferred from phytoplankton to zooplankton simultaneously and within the same system.

This paper describes the experimental procedure of the ^{15}N analysis, including the isotope dilution with nitrogen carrier, and the results of experiments carried out with samples originating from two Japanese lakes.

2. Materials and methods

2-1. Tracer experiment

Lake water was collected with a column sampler ($\phi 40\text{mm} \times 200\text{cm}$, about 2 liters in volume) from 0 to 4 m depth in Lake Nakatsuna (max. depth 12.0 m, surface area 0.14 km^2) on 12 May 1987, and in Lake Suwa (6.3 m, 13.3 km^2) on 30 April 1987. Both lakes are located in the central part of Honshu, Japan. Zooplankton were collected by vertical tows with plankton nets of 40 or 98 μm mesh size.

Water of Lake Nakatsuna, collected on 12 May 1987 was put in glass bottles (10 liters each in volume) immediately after sampling without any filtration. One bottle was used as a control (time 0 hr), while the other bottles were enriched with 9.97 μg atom-N of $(^{15}\text{NH}_4)_2\text{SO}_4$ (atomic percentage of $^{15}\text{N}=50.47\%$), and incubated in a water bath at the same temperature as that of the sampling depth (7°C) under a 13 hr light (3,500 lux) and 11 hr dark photocycle. After incubation for 24, 48 and 96 hr, one of these bottles was set aside for ^{15}N analyses of particulate organic matter (POM) and zooplankton, as shown in Figure 1. Appropriate amounts of the water samples (200 to 1000 ml) were filtered through precombusted (450°C , 1 hr) Whatman GF/C glass-fiber filters. These filters were used to measure the organic carbon and nitrogen contents of POM, and the filtered water samples were used for ammonium-N analysis. From the remaining water samples, POM and zooplankton were fractionated with 20, 40, 64 and 98 μm mesh sized nets to differentiate the nitrogen uptake rates of each size fraction. Zooplankton from the 64 to 98 μm and $>98\ \mu\text{m}$ fractions were further sorted to species level by using glass capillary under a stereo-microscope. These plankton samples were collected on precombusted (450°C , 1 hr) Whatman GF/F glass-fiber filters, and washed with a small amount of 0.005 N HCl and deion-

Fig. 1. Schematic presentation of tracer experiment. Time course of ^{15}N tracer from $^{15}\text{NH}_4^+-\text{N}$ into organic matter.

ized water to remove free $^{15}\text{NH}_4^+-\text{N}$, before being dried at 60°C . They were weighed and kept in a desiccator. ^{15}N contents were analyzed with mass-spectrometry as described below. Organic carbon and nitrogen contents were determined with a Yanaco MT-2 CHN analyzer. Ammonium-N was analyzed using a Technicon Auto-Analyzer II (TECHNICON INDUSTRIAL METHODOLOGY, 1974).

Similar experiments were carried out on 30 April 1987 with samples from Lake Suwa. The lake water samples were incubated outside the Suwa Hydrobiological Station, Shinshu University at about 15°C which corresponds to *in situ* temperature.

2-2. Determination of nitrogen isotope ratio

To each of the samples for nitrogen analysis L-alanine (2.00 mg, Kokusan Chemical Co., Inc.) was added as a nitrogen carrier. Organic nitrogen of the sample enriched with alanine was converted to N_2 gas by the combustion

method (MINAGAWA *et al.*, 1984). After cryogenic purification in a vacuum line, the N_2 gas was introduced into a Hitachi RMU-6R mass spectrometer fitted with double collector and double inlet system for ratiometry.

The nitrogen isotope ratio was presented as permill deviation from standard, as defined by the following equation:

$$\delta^{15}N(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right) \times 1000, \quad (1)$$

where R denotes $^{15}N/^{14}N$. The atmospheric nitrogen was used as a standard. R_{std} value is 0.3663 ^{15}N atom %. The analytical error of the mass spectrometric measurement of $\delta^{15}N$ value itself was found to be less than $\pm 0.2 \text{‰}$ (S. D.), or ± 0.00007 ^{15}N atom % (S. D.). In our method, in which alanine was added, the equation (1) was reformulated as follows,

$$\delta^{15}N_{\text{obs}}(\text{‰}) = \left(\frac{R_{(\text{sample}+\text{alanine})}}{R_{\text{std}}} - 1 \right) \times 1000, \quad (2)$$

where $\delta^{15}N_{\text{obs}}$ is the nitrogen isotope ratio of the sample with alanine. Therefore the δ value for the sample nitrogen ($\delta^{15}N_s$) was calculated by the following equation.

$$\delta^{15}N_s(\text{‰}) = \frac{(N_s + N_a) \delta^{15}N_{\text{obs}} - N_a \cdot \delta^{15}N_a}{N_s}, \quad (3)$$

where N_s , N_a and $\delta^{15}N_a$ are the amounts of nitrogen in the sample, the alanine, and the

isotope ratio of alanine, respectively. Since N_s was not measured directly during the ^{15}N analysis, it was calculated from the dry weight of samples supplied by isotope measurements and from the nitrogen content in each of the fractions of the samples without incubation (0 hr samples). The $\delta^{15}N_a$ and N_a are $-5.3 \pm 0.2 \text{‰}$ (mean $\pm$ S. D., $n=17$) and 0.315 mgN (calculated from the chemical formula of alanine), respectively. The ^{15}N atom % in samples was calculated from the δ value by the following equation.

$$^{15}N \text{ atom \%} = \frac{R_{\text{std}}(\delta^{15}N_s/1000+1)}{R_{\text{std}}(\delta^{15}N_s/1000+1)+1} \times 100, \quad (4)$$

The variation caused by the analytical procedure in the determination of ^{15}N contents of several plankton samples collected from Lake Suwa was examined by comparison between the $\delta^{15}N_s$ without incubation (0 hr samples, alanine was added) and the natural abundance of nitrogen isotope ($\delta^{15}N$).

3. Results and discussion

The results of the comparison between the $\delta^{15}N_s$ without incubation and the $\delta^{15}N$ for Lake Suwa samples are presented in Table 1. The differences in ^{15}N contents between $\delta^{15}N_s$ and $\delta^{15}N$ values were -5.8 to $+3.1 \text{‰}$ (mean $\pm$ S. D. = $-1.46 \pm 2.86 \text{‰}$, $n=8$, Table 1) beyond the wide range of the taxonomic groups of plank-

Table 1. Comparison of δ values (‰) and ^{15}N atom % with data for natural nitrogen isotope abundance in Lake Suwa. Δ values are difference between natural abundance and sample with alanine added.

Sample Name	Date	Natural abundance		After alanine addition		Differences	
		$\delta^{15}N$	atom %	$\delta^{15}N$	atom %	$\Delta \delta^{15}N$	Δ atom %
<i>Keratella quadrata</i>	1987, 5.2	7.8	0.36783	2.0	0.36573	-5.8	-0.00210
<i>Bosmina fatalis</i>	1987, 6.4	9.3	0.36838	9.3	0.36838	0	0.00000
<i>Leptodora kindtii</i>	1987, 6.4	13.5	0.36991	7.9	0.36788	-5.6	-0.00203
<i>Eodiaptomus japonicus</i>	1987, 6.4	13.2	0.36980	10.6	0.36886	-2.6	-0.00094
<i>Cyclops vicinus</i>	1987, 5.2	10.4	0.36878	13.5	0.36991	3.1	0.00113
<i>Microcystis aeruginosa</i>	1987, 6.4	6.7	0.36744	7.2	0.36762	0.5	0.00018
POM (1-98 μm fraction)	1987, 6.4	7.9	0.36787	7.0	0.36755	-0.9	-0.00032
POM (3-10 μm fraction)	1987, 6.4	7.5	0.36773	7.1	0.36758	-0.4	0.00025
Mean $\pm$ S. D	-	-	-	-	-	-1.46 ± 2.86	-0.00048 ± 0.00106

ton. These differences corresponded to -0.00210 to $+0.00113$ ^{15}N atom % (-0.00048 ± 0.00106 ^{15}N atom %). This variance was in the same range as the data of ^{15}N analysis which OTSUKI (1985) obtained by mass spectrometer, though his samples contained more nitrogen (>35 μgN) than ours (5 μgN).

The experimental results of Lake Nakatsuna samples are given in Figure 2. In POM samples, the "1-20 μm " fraction mainly consisted of *Peridinium* sp., some *Asterionella* sp. and *Uroglenopsis americana*. The "20-98 μm " fraction consisted principally of *Uroglenopsis americana*, *Asterionella* sp. and some zooplankton (nauplii and rotifer). The ^{15}N atom % in every POM fraction increased meaning for the first 48 hr (Fig. 2a). The ^{15}N atom % in *Kellicottia longispina* and "Rotifer mix" (*K. longispina*: *Filinia* sp.: *Keratella quadrata* = 4: 3: 1, individual numbers) increased up to 96 hr (Fig. 2b). A chronological difference of the ^{15}N increase between POM and zooplankton was also obtained by OTSUKI *et al.* (1985). The level of ^{15}N atom % of zooplankton was lower than that of POM. Since the nitrogen content of zooplankton was 2 to 3 times higher than POM (Table 2), replacing ^{14}N in zooplankton bodies with ^{15}N tracer from phytoplankton may require a longer time than that for phytoplankton.

kton.

The experimental results of Lake Suwa are shown in Figure 3. In POM samples, "1-3 μm " and "3-20 μm " fractions mainly consisted of *Cyclotella* sp.; "24-40 μm " was made up of *Asterionella* sp. and *Cyclotella* sp.; "40-100 μm " consisted of *Fragilaria* sp., *Synedra* sp. and some zooplankton (nauplii and small rotifers). The content of ^{15}N in POM increased during the first 24 hr and decreased thereafter, except for the largest fraction which continued to increase slightly during this period (Fig. 3a). These results are in contrast to those for Lake Nakatsuna. The ^{15}N atom % of the smaller POM fraction at 24 hr was higher than that of the larger one.

The increment of ^{15}N atom % during the incubation period of the zooplankton fraction of Lake Suwa is shown in Figure 3b. The ^{15}N atom % in *Keratella quadrata* and *Brachionus calyciflorus* were different at 48 hr from each other. The rate of increase of ^{15}N of both rotifers declined during the 24 to 48 hr, compared with those between 0 and 24 hr. These decreases may reflect a reduction of the ^{15}N atom % in the POM fraction.

The amount of nitrogen transfer per unit of time (nitrogen uptake rates) of POM and zooplankton fractions are shown in Table 3.

Fig. 2. Changes in ^{15}N atom % of POM fractions (a) and zooplankton (b) samples after addition of $(^{15}\text{NH}_4)_2\text{SO}_4$ to Lake Nakatsuna water. In POM samples: "1-20 μm " fraction contained *Peridinium* sp., some *Asterionella* sp. and *Uroglenopsis americana*; "20-98 μm " fraction contained *Uroglenopsis americana*, *Asterionella* sp. and some zooplankton. In zooplankton, "Rotifer mix" fraction consisted of *Kellicottia longispina*, *Filinia* sp., and *Keratella quadrata* in ratio of about 4: 3: 1. Symbols: $\blacktriangle$, 1-20 μm ; $\bullet$, 20-98 μm ; $\circ$, *Kellicottia longispina*; $\triangle$, "Rotifer mix".

Table 2. Dry weight, N content and C/N of zooplankton species and POM fractions in Lake Nakatsuna and Lake Suwa.

Sample name	Date	Sampling lake	Dry weight ($\mu\text{g}\cdot\text{indiv.}^{-1}$)	N content (%·D. W.)	C/N (weight ratio)
Zooplankton					
<i>Kellicottia longispina</i>	1987.5.12	Lake Nakatsuna	0.09	8.0	4.3
<i>Keratella quadrata</i>	1987.4.30	Lake Suwa	0.12	10.0	3.0
<i>Brachionus calyciflorus</i>	1987.4.30	Lake Suwa	0.39	15.5	2.7
POM					
1-20 μm fraction	1987.5.12	Lake Nakatsuna	-	4.1	3.9
20-98 μm fraction	1987.5.12	Lake Nakatsuna	-	4.3	6.1
1-3 μm fraction	1987.4.30	Lake Suwa	-	3.7	5.9
3-20 μm fraction	1987.4.30	Lake Suwa	-	1.7	7.0
20-40 μm fraction	1987.4.30	Lake Suwa	-	4.2	6.3
40-100 μm fraction	1987.4.30	Lake Suwa	-	7.0	4.5

Fig. 3. Changes in ^{15}N atom % of POM fractions (a) and zooplankton (b) samples after addition of $(^{15}\text{NH}_4)_2\text{SO}_4$ to lake water from Lake Suwa. In POM samples: "1-3 μm " and "3-20 μm " fractions mainly consisted of *Cyclotella* sp.; "20-40 μm " made up of *Asterionella* sp. and *Cyclotella* sp.; "40-100 μm " of *Fragilaria* sp., *Synedra* sp. and some zooplankton (nauplii and small rotifera). Symbols: $\blacktriangle$, 1-3 μm ; $\bullet$, 3-20 μm ; $\blacksquare$, 20-40 μm ; $\times$, 40-100 μm ; $\circ$, *Keratella quadrata*; $\triangle$, *Brachionus calyciflorus*; $\square$, *Cyclops vicinus*.

Uptake rates for the time period 0 to 24 hr of POM and zooplankton were calculated after OTSUKI (1985) and OTSUKI *et al.* (1987), respectively. POM nitrogen uptake rates in Lake Nakatsuna and Lake Suwa ranged from 2.2 to 6.1×10^{-4} and 1.7 to 5.2×10^{-4} $\text{mg atom-N} \cdot \text{I}^{-1} \cdot \text{day}^{-1}$, respectively (Table 3). Ammonium concentration during incubation in Lake Suwa was higher than in Lake Nakatsuna (Table 4). This might reflect the fact that ammonium regeneration (or isotope dilution) was higher in Lake Suwa than in Lake Nakatsuna. It

seemed that high ammonium regeneration might be responsible for an underestimation of the nitrogen uptake rates in Lake Suwa.

Nitrogen uptake rates for *K. longispina* in Lake Nakatsuna and for *K. quadrata* in Lake Suwa were 8.5×10^{-8} and 4.2×10^{-7} $\text{mg atom-N} \cdot \text{indiv.}^{-1} \cdot \text{day}^{-1}$, respectively (Table 3). Relative nitrogen uptake rates per nitrogen content was $16.5 \text{ \%} \cdot \text{day}^{-1}$ for *K. longispina*, and $48.4 \text{ \%} \cdot \text{day}^{-1}$ for *K. quadrata*. The value implies that *K. longispina* and *K. quadrata* need at least 6 and 2 days, respectively, to double their body

Table 3. Nitrogen uptake rates of POM fractions, and nitrogen uptake rates and turnover times of zooplankton species. POM and zooplankton nitrogen uptake rates calculated using equations of OTSUKI (1985) and OTSUKI *et al.* (1987), respectively, for time period 0 to 24 hr in Tracer Experiment. Nitrogen turnover times calculated from nitrogen contents and nitrogen uptake rates.

Sample name	Sampling lake	Nitrogen uptake rates	
		mg atom-N $\cdot$ l $^{-1}\cdot$ day $^{-1}$	mg atom-N $\cdot$ indiv. $^{-1}\cdot$ day $^{-1}$
POM			
1-20 μ m fraction	Lake Nakatsuna	6.1 $\times$ 10 $^{-4}$	-
20-98 μ m fraction	Lake Nakatsuna	2.2 $\times$ 10 $^{-4}$	-
3-20 μ m fraction	Lake Suwa	5.2 $\times$ 10 $^{-4}$	-
20-40 μ m fraction	Lake Suwa	1.7 $\times$ 10 $^{-4}$	-
Zooplankton			
<i>Kellicottia longispina</i>	Lake Nakatsuna	-	8.5 $\times$ 10 $^{-8}$
<i>Keratella quadrata</i>	Lake Suwa	-	4.2 $\times$ 10 $^{-7}$

Table 4. Changes in ammonium concentrations (μ g atom-N $\cdot$ l $^{-1}$) in lake water during incubation.

	Incubation time		
	0 hr	24 hr	48 hr
Lake Suwa	0.97	2.48	36.7
Lake Nakatsuna	1.29	1.51	1.95

nitrogen.

Since the nitrogen uptake rate measured does not include respiration loss and excretion, it approximately corresponded to the net assimilation rate. Net production rates calculated from the nitrogen uptake rates, carbon contents and nitrogen contents (Table 2) in *K. longispina* and *K. quadrata* were 5.1×10^{-6} and 2.5×10^{-5} mgC $\cdot$ indiv. $^{-1}\cdot$ day $^{-1}$, respectively. On the other hand, the rates calculated from body dry weight and environmental temperature after IKEDA and MOTODA (1977) were 4.4×10^{-6} for *K. longispina* and 1.9×10^{-5} mgC $\cdot$ indiv. $^{-1}\cdot$ day $^{-1}$ for *K. quadrata*. The net production rates were similar to those calculated from our nitrogen uptake rates, though these estimated values based on different factors. This approximate coincidence suggests that our nitrogen uptake rate is reasonable.

The ^{15}N atom % for 0 hr and 24 hr of *K. longispina* was 0.36784 and 0.47440 %, respectively. Incubation periods shorter than 24 hr

are sufficient to detect changes in ^{15}N content of small zooplankton, because the analytical variance with alanine addition is less than 0.00106 ^{15}N atom % (see section of Materials and Methods). A detailed knowledge of the feeding habit of small zooplankton may be obtained using this method.

It is important to investigate nitrogen flows not only among the compartments of aquatic ecosystems (*e.g.* phytoplankton, zooplankton and bacteria) but also within each compartment among species or individuals. However, nitrogen uptake rates of each species have yet to be examined. We could detect the nitrogen uptake rate of zooplankton at the species level by using the combination of the ratio mass spectrometer and the ^{15}N tracer experiment. This method can be used to clarify secondary production and strictly measure nitrogen flows in the aquatic food web.

Acknowledgements

We are indebted to Miss Y. KABAYA for her assistance with ^{15}N analysis, as well as Dr. T. OKINO and his colleagues at Suwa Hydrobiological Station, Shinshu University for help during the experiment. This study could not have been performed without the generous support of the staff and students of the authors' laboratories. This work was partly supported by a Grant-in-Aid for Special Pro-

ject Research No. 62124040 from the Ministry of Education, Science and Culture, Japan.

摘 要

¹⁵N トレーサー実験による微小動物プランクトンの窒素取り込み速度の見積

窒素安定同位体 (¹⁵N) を用いたトレーサー実験を行い、淡水のプランクトンの窒素の取り込み速度を測定した。数 100 個体以下 (5 μgN 程度) での微小動物プランクトンの微量な ¹⁵N 変化の検出のため、同位体比既知の物質 (アラニン) を N キャリアーとして用い、比率直読式質量分析計で測定を行った。キャリアー添加によって生ずる誤差は -0.00210 ~ +0.00113 ¹⁵N atom % (平均 0.00048 ± 0.0010 S. D.) の範囲にあった。この方法で測定を行った結果、中綱湖の *Kellicottia longispina* について $8.5 \times 10^{-8} \text{mg} \cdot \text{atom-N} \cdot \text{indiv}^{-1} \cdot \text{day}^{-1}$ 、諏訪湖の *Keratella quadrata* について $4.2 \times 10^{-7} \text{mg} \cdot \text{atom-N} \cdot \text{indiv}^{-1} \cdot \text{day}^{-1}$ の値が得られた。取り込み速度、窒素含量及び炭素含量から計算した純生産速度は *K. longispina* で $5.1 \times 10^{-6} \text{mgC} \cdot \text{indiv}^{-1} \cdot \text{day}^{-1}$ 、*K. quadrata* で $2.5 \times 10^{-5} \text{mgC} \cdot \text{indiv}^{-1} \cdot \text{day}^{-1}$ となった。質量分析と ¹⁵N トレーサー実験の組合わせにより、動物プランクトンの種のレベルで、栄養塩から植物プランクトンを経た窒素の取り込みが、同時に、かつ同じ空間で測定が可能であることが示された。

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Received: 10 June 1991

Accepted: 6 May 1992