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In situ Assimilation Rate of Nitrogenous Compounds by Phytoplankton in the Euphotic Layer of Reservoirs

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人工湖 生産層에서 植物플랑크톤의 질소화합물 동화속도

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ABSTRACT

The nitrogen assimilation rate of nitrogenous nutrients by reservoir phytoplankton was measured in the *in situ* condition in the euphotic layer of Lakes Soyang, Chuncheon and Uiam located on the upper reaches of the North Han River System in August, 1983, Korea.

The assimilation rate of ammonia, nitrate and urea nitrogen in surface water was 13, 2 and 13μg at. $N \cdot m^{-3} \cdot (12:10 \sim 18:15)^{-1}$ in Lake Soyang, 325, 27 and 59μg at. $N \cdot m^{-3} \cdot (12:30 \sim 18:30)^{-1}$ in Lake Chuncheon, and 174, 12 and 45μg at. $N \cdot m^{-3} \cdot (12:30 \sim 19:30)^{-1}$ in Lake Uiam. Ammonia and urea were preferentially utilized by reservoir phytoplankton. The dark /light ratios of nitrate assimilation were much lower than those of ammonia and urea assimilation. The contribution of ammonia in total nitrogen assimilation was 47 to 79%. The assimilation of nitrate showed little contribution. The primary production was estimated as 59 mg $C \cdot m^{-2} \cdot day^{-1}$ and 6.9mg $N \cdot m^{-2} \cdot day^{-1}$ in Lake Soyang, 217mg $C \cdot m^{-2} \cdot day^{-1}$ and 26mg $N \cdot m^{-2} \cdot day^{-1}$ in Lake Chuncheon, and 110mg $C \cdot m^{-2} \cdot day^{-1}$ and 13mg $N \cdot m^{-2} \cdot day^{-1}$ in Lake Uiam, with production ratios of 8.6, 8.4 and 8.4, respectively. The turnover time of ammonia and urea in the upper euphotic layer was 2 to 47 days and 4 to 38 days, respectively. Nitrate required much longer periods.

In the euphotic layer of reservoirs, ammonia and urea played significant roles in the biogeochemical nitrogen metabolism.

Key words : Nitrogen assimilation, C /N assimilation ratio, Turnover time, Reservoirs

INTRODUCTION

Information regarding nitrogen assimilation has been accumulated to elucidate the importance of nitrogen sources for phytoplankton growth, and contributed to an appreciation of their significance in the biogeochemical cycle (Axler and Gersberg 1981, Axler *et al.* 1981, 1982, Takahashi and Saijo 1981, McCarthy *et al.* 1982, Miyazaki *et al.* 1985, Mitamura 1986a, 1986b, Mitamura and Saijo 1986a, 1986b, Whalen and Alexander 1986, Takamura *et al.* 1987, Priscu *et al.* 1989, Binhe and Alexander 1993). Mitamura (1986a) and Mitamura and Saijo (1986a) demonstrated that the principal nitrogenous compounds which sustain the standing crop of phytoplankton in the euphotic layer of Lake Biwa were ammonia, nitrate and urea, and that relative nitrogen assimilation rates by phytoplankton were ammonia > urea > nitrate. It is possible that these three nitrogenous compounds play an important role in the nitrogen metabolism not only in freshwater lake but also in reservoir ecosystems.

Knowledge of the nitrogen assimilation by phytoplankton populations in the reservoirs, however, is quite limited. In this paper, the significance of the assimilation of nitrogenous compounds in three reservoirs located on the upper reaches of the North Han River System is described for the first time. The *in situ* assimilation rate of ammonia, nitrate and urea nitrogen by phytoplankton was measured in the euphotic layer of reservoirs to clarify the nitrogen source for reservoir phytoplankton, the preference of phytoplankton for nitrogenous nutrients, and the turnover time of each nitrogenous compound.

MATERIALS AND METHODS

Lake Soyang, Lake Chuncheon and Lake Uiam are the reservoirs located on upper reaches of the North Han River System, in Korea, covering areas of 70, 14 and 15 km^2 at full water level. The *in situ* experiments were carried out at station near the dam of three resevoirs in August, 1983(Fig. 1).

To measure the *in situ* rate of nitrogen assimilation in the euphotic layer, water samples were taken with a Van Dorn type plastic sampler of 6 l capacity from depths of 0, 3, 7 and 15 m in Lake Soyang and 0, 2, 5 and 10 m in Lakes Chuncheon and Uiam. Water samples were poured into three series of 200 ml glass-stoppered bottles to measure the nitrogen assimilation rates of ammonia, nitrate and urea. After adding five concentrations of ^{15}N-labelled ammonia, nitrate and urea solution to each glass bottle, considering the ambient concentrations of these nitrogenous compounds in the surface water, 1 ml of concentrated formaldehyde solution was immediately added to a series of control bottles. The second series of bottles were wrapped in a black sheet to measure the dark nitrogen assimilation rate. Light and dark series of bottles were suspended from a buoy at the respective depths from which sample waters were taken. After leaving the bottles from noon to sunset, biological activity of phytoplankton was stopped by adding formaldehyde solution to each bottle. The experiment was incubated *in situ* during 12:10 to 18:50 in Lake Soyang on Aug. 31, 12:30 to 18:30 in Lake Chuncheon on Aug. 27 and 12:10 to 18:50 in Lake Uiam on Aug. 12, respectively. Sample water in each glass bottle was filtered through a Whatman GF/C glass fiber filter which was free of organic matter by ignition

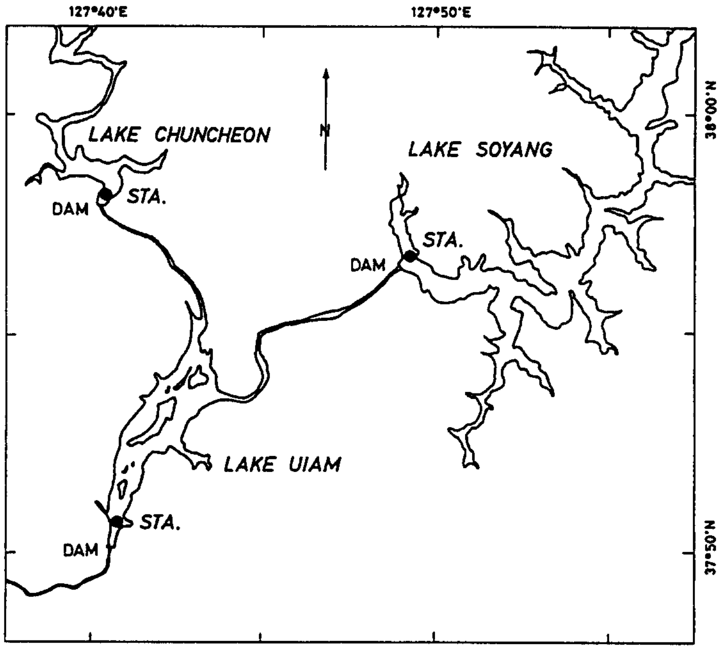

Fig. 1. Map of station location in Lake Soyang, Lake Chuncheon and Lake Uiam, located on the upper reaches of the North Han River System, Korea.

at 450℃. The ratio of ^{15}N to ^{14}N in filter sample was determined by the technique of optical emission spectrometry with a Nihonbunko ^{15}N analyzer (type NIA-1) after dry combusting with calcium oxide and copper oxide in a capillary by micro-Dumas' method. The *in situ* nitrogen assimilation rates of nitrogenous compounds were extrapolated using a linear transformation of the following Michaelis-Menten equation, assuming that the rate was related to the ambient concentration of each nitrogenous compound:

$$\rho = \rho_{max} \frac{S}{K_s + S}$$

where ρ is the nitrogen assimilation rate, S is the concentration of nitrogenous compounds, ρ_{max} is the nitrogen assimilation rate at saturating levels of S, and K_s is the half-saturation constant at which $\rho=\rho_{max}/2$.

The photosynthetic carbon assimilation rate was measured by the technique of Steemann Nielsen (1952), simultaneously with *in situ* experiments for the nitrogen assimilation measurements. The total CO_2 in the sample water was determined with an infra-red CO_2 analyzer, as described by Satake *et al.* (1972).

For the chemical analysis of lake water, the collected sample waters were immediately filtered through glass fiber filters (Whatman GF/C) treated by ignition at 450℃. The filters and filtrates were then stored at −20℃ in a deep freezer until chemical analysis in the laboratory. Ammonia concentration was determined by the method of Sagi (1966), nitrite after Bendschneider and Robinson (1952), nitrate by the method of Wood *et al.* (1967), and urea after Newell *et al.* (1967) with a modification by Mitamura (1987). Particulate carbon and nitrogen were determined with a Yanagimoto MT-2 type CHN Corder. Chlorophyll-a concentration was determined by the method of SCOR/Unesco (1966).

RESULTS AND DISCUSSION

Concentration of nitrogenous nutrients and particulate matter

The concentrations of nitrogenous nutrient compounds and particulate matters in the euphotic layer of three reservoirs are shown in Table 1. The concentration of dissolved total nitrogenous nutrient (sum of ammonia, nitrite, nitrate and urea nitrogen) ranged from 20 to 45 μg at. $N \cdot l^{-1}$. The greater part of total nitrogenous nutrient was nitrate. On the other hand, the concentration of ammonia and urea only took 4 to 11% and 1 to 5% of total nitrogenous nutrient, respectively. The concentrations of nitrate in Lake Uiam were much higher than those in Lake Soyang and Lake Chuncheon.

The concentrations of particulate carbon(PC), nitrogen (PN) and chlorophyll-a were considerably low. The amount of particulate matter in surface layer of Lake Soyang showed a lower level than in the other two reservoirs. The weight ratio of PC to PN concentration was calculated as 6 to 12 in the surface layer of three reservoirs. The PC/Chl.a ratio was 110 to 240. High ratios for these parameters were obtained in Lake Soyang.

Table 1. Concentrations of ammonia, nitrate and urea nitrogen, and the particulate carbon(PC), nitrogen (PN) and chlorophyll-a amount in the euphotic layer of three reservoirs.

Reservoir	Depth (m)	Ammonia	Nitrate	Urea	PC	PN	Chl.a
		(μg at. $N \cdot l^{-1}$)			(mg·m^{-3})		
L. Soyang	0	1.2	19	1.0	245	22	1.1
(Aug. 31)	3	1.5	18	1.1	271	23	1.1
	7	2.3	18	0.9	280	26	1.1
	15	2.1	26	1.1	156	15	0.7
L. Chuncheon	0	1.4	26	1.0	443	70	3.0
(Aug. 27)	2	1.5	27	1.0	401	63	3.5
	5	1.3	32	0.9	265	33	0.8
	10	1.0	34	0.7	238	22	0.2
L. Uiam	0	1.8	42	0.4	365	67	2.9
(Aug. 12)	2	1.9	42	0.8	264	40	1.9
	5	2.0	43	0.5	162	21	0.8
	10	1.8	43	0.7	127	12	0.3

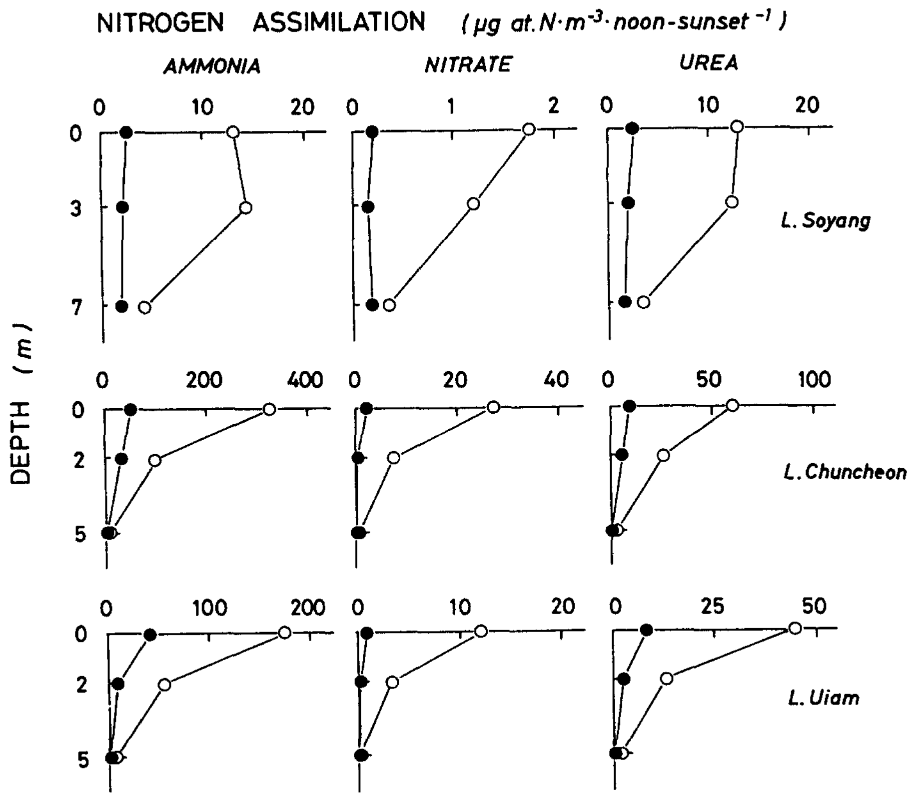

Fig. 2. Vertical distributions of ammonia, nitrate and urea nitrogen assimilation rate in the euphotic layer of Lakes Soyang, Chuncheon and Uiam. (○) and(●) indicate the nitrogen assimilation rate in the light and dark, respectively.

Assimilation rate of ammonia, nitrate and urea nitrogen

Fig. 2 shows the distribution of ammonia, nitrate and urea nitrogen assimilation rates measured *in situ* in the three reservoirs. The photosynthetic carbon assimilation rate in surface water of Lakes Soyang, Chuncheon and Uiam was 6, 55 and 35 mg $C \cdot m^{-3} \cdot (\text{incubation time})^{-1}$, respectively, and decreased with depth. In the light condition, the assimilation rate of ammonia, nitrate and urea nitrogen was 13, 2 and 13 μg at. $N \cdot m^{-3} \cdot (12{:}10 \sim 18{:}15)^{-1}$ in surface water of Lake Soyang, 325, 27 and 59 μg at. $N \cdot m^{-3} \cdot (12{:}30 \sim 18{:}30)^{-1}$ in Lake Chuncheon, and 174, 12 and 45 μg at. $N \cdot m^{-3} \cdot (12{:}30 \sim 19{:}30)^{-1}$ in Lake Uiam, respectively. The rates of nitrate assimilation in the present study show the rate at saturating levels of nitrate. The assimilation rates of these nitrogenous compounds decreased with depth and showed negligible values in the deeper euphotic layer below 5 m depth.

The ratio of dark to light value in the nitrogen assimilation rate of ammonia, nitrate and urea was calculated as 0.14 to 0.29, 0.03 to 0.11 and 0.15 to 0.21, respectively. The dark /light ratios of nitrate assimilation were much lower than those of ammonia and urea assimilation. The present results agree with those reported earlier by Mitamura and Saijo (1986a).

The contribution of ammonia to total nitrogen assimilation (sum of ammonia, nitrate and urea assimilation) was 47 to 79% in the light, and 47 to 84% in the dark condition in three reservoirs. The assimilation of nitrate in the dark, on the other hand, showed a negligible contribution. The contribution of urea in the nitrogen assimilation was considerable, namely 15 to 50%. These percentages are comparable to those obtained in Lake Biwa by Mitamura and Saijo (1986a) and in the central gyre of the North Pacific Ocean by Eppley *et al.* (1973). The phytoplankton populations in Lake Soyang utilized half of their nitrogen source from urea, although there was no appreciable difference between the ambient concentration of ammonia and urea. The nitrogen assimilation rates varied with the concentrations of these nitrogenous compounds. This suggests that the role of nitrogen compound as a nitrogen source for phytoplankton varies by species composition or with the physiological requirements of phytoplankton, as reported by Eppley *et al.* (1969).

The assimilation activity of reservoir phytoplankton for these nitrogenous compounds was higher in Lakes Chuncheon than in Lakes Soyang and Uiam, calculated from the nitrogen assimilation rate by the unit amount of chlorophyll-a or nitrogen assimilation velocity. The photosynthetic rate by unit amount of chlorophyll-a in the surface layer of Lake Chuncheon also appeared to be several times higher than in Lake Soyang. This seems to be reflected in the specific growth rate of phytoplankton. The specific transfer rate (considering the ammonia, nitrate and urea assimilation using chlorophyll-a as a cell parameter) in the light and dark at surface water was calculated as 4 and 1 μg at. N·mg chl.a^{-1}·hr.$^{-1}$ in Lake Soyang, 23 and 3 μg at. N·mg chl.a^{-1}·hr.$^{-1}$ in Lake Chuncheon, and 12 and 2 μg at. N·mg chl. a^{-1}· hr.$^{-1}$ in Lake Uiam. The present specific transfer rates are in the same range as those obtained in Lake Kinneret (1.1 to 14.2 μg at. N·mg chl.a^{-1}·hr.$^{-1}$) by McCarthy *et al.* (1982), and in Lake Biwa (1.3 to 24.7 in the light and 1.1 to 17.6 μg at. N·mg chl.a^{-1}·hr.$^{-1}$ in the dark) by Mitamura and Saijo (1986a).

Ratio of carbon to nitrogen assimilation

The daily rate of total nitrogen assimilation was estimated from the *in situ* assimilation rate of ammonia, nitrate and urea nitrogen in the light and dark during the incubation period. The daily rates of total nitrogen assimilation in surface water of Lakes Soyang, Chuncheon and Uiam were 0.9, 13.2 and 7.5 mg N·m^{-3}·day^{-1}, respectively (Fig. 3). These values decreased with depth. The vertical profile of the total nitrogen assimilation rate was similar to the distribution of the daily photosynthetic carbon assimilation rate. Primary production was estimated as 59 mg C·m^{-2}·day^{-1} and 6.9 mg N·m^{-2}·day^{-1} in Lake soyang, 217 mg C·m^{-2}·day^{-1} and 26 mg N·m^{-2}·day^{-1} in Lake Chuncheon, and 110 mg C·m^{-2}·day^{-1} and 13 mg N·m^{-2}·day $^{-1}$ in Lake Uiam, calculated from both carbon and nitrogen assimilation rates.

To determine the distribution of PC / PN ratio in particulate matter in water, the ratio

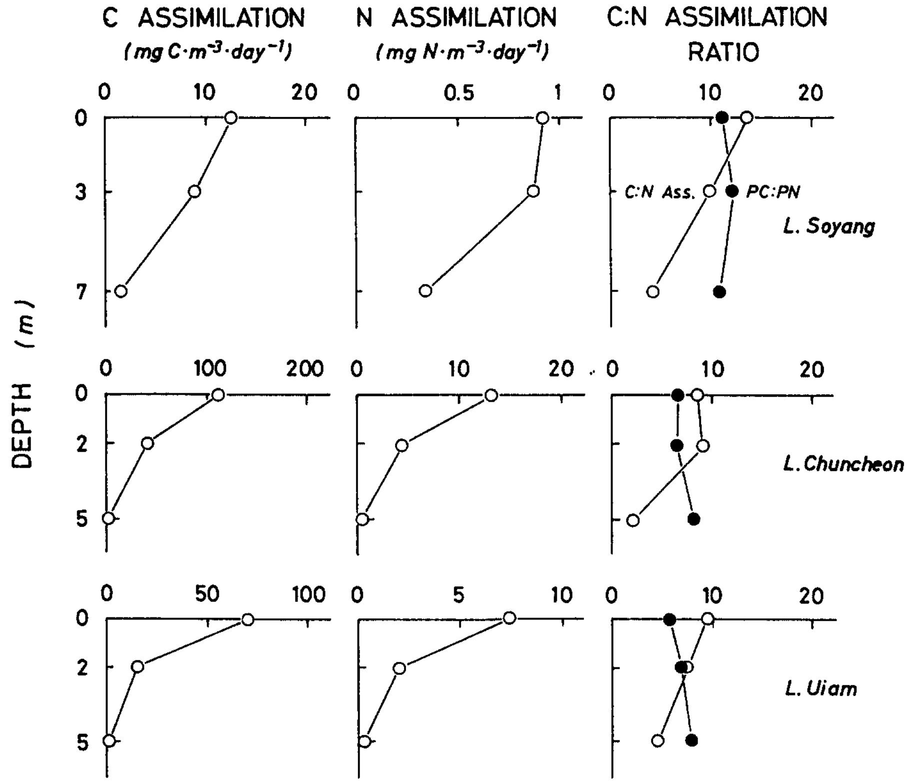

Fig. 3. Daily rate of photosynthetic carbon assimilation rate and total nitrogen assimilation rate (as sum of ammonia, nitrate and urea nitrogen assimilation rates), and carbon/nitrogen assimilation ratio (○) and PC/PN ratio (●) in particulate matter.

of the daily photosynthetic carbon assimilation to the total nitrogen assimilation rate was calculated. The C/N assimilation ratio was 9.9 to 13.6 (by weight) in Lake Soyang, 8.4 to 9.2 in Lake Chuncheon, and 7.5 to 9.4 in Lake Uiam. These C/N assimilation ratios showed a decreasing tendency with depth. The C/N assimilation ratios in the surface layer were higher than those of the PC/PN ratio in the particulate matter, whereas lower C/N assimilation ratios compared to the PC/PN ratios were observed in the deeper euphotic layer. The ratio of primary carbon production to nitrogen production was calculated as 8.6 in Lake Soyang, 8.4 in Lake Chuncheon, and 8.4 in Lake Uiam. There were no differences among the three reservoirs. These production ratios were similar to the PC/PN ratio in the euphotic layer of reservoirs. These trends agree with those obtained in Lake Biwa as reported by Mitamura and Saijo (1986a).

Preference of phytoplankton for ammonia, nitrate and urea

To evaluate the preference of phytoplankton for nitrogenous nutrients in three reservoirs, the Relative Preference Index (RPI), defined by McCarthy *et al.* (1977), was calculated. RPI can be calculated as (e.g., RPI for ammonia):

$$RPI_{ammonia} = \frac{\dfrac{\text{ammonia N assimilation}}{\sum \text{N assimilation}}}{\dfrac{\text{ambient ammonia N concentration}}{\text{ambient} \sum \text{N concentration}}}$$

In the present study, total nitrogen is the sum of ammonia, nitrate and urea nitrogen. RPI=1 indicates that assimilation may be equated with availability. RPI>1 indicates preferential assimilation, while RPI<1 indicates rejection. As can be seen in Fig. 4, the RPI values were calculated as 6 to 23 for ammonia, 0.0 to 0.1 for nitrate and 4 to 24 for urea in the three reservoirs. The RPI values for ammonia and urea were never less than unity and usually both nitrogenous compounds were strongly preferred. The RPI values for nitrate, however, were always less than unity. Nitrate was never preferred to other nitrogenous compounds, although high concentrations of nitrate in the euphotic layer were observed. Thus, in the three reservoirs, ammonia and urea are preferentially utilized by phytoplankton in the euphotic layer. Takahashi and Saijo(1981), McCarthy *et al.* (1982), Berman *et al.* (1984) and Binhe and Alexander (1993) found that ammonia is a more

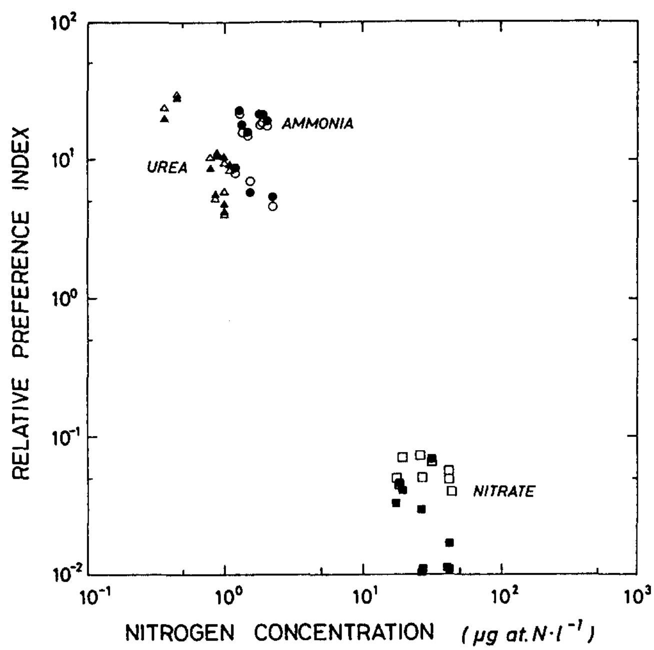

Fig. 4. Relative Preference Index (RPI) for assimilation of ammonia, nitrate and urea nitrogen. (○) and (●), (△) and (▲) and (□) and (■) indicate RPI values for ammonia, nitrate and urea in light and dark conditions, respectively.

significant compound as a nitrogen source of phytoplankton growth than nitrate. It is generally known that phytoplankton prefer ammonia to urea and nitrate as a nitrogen source. Urea nitrogen is utilized second to ammonia, and is the choice over nitrate for the requirement of phytoplankton (McCarthy *et al.* 1977, Mitamura 1986b, Mitamura and Saijo 1986a). Whalen and Alexander (1986), Harrison *et al.* (1987) and Dortch (1990) reported that phytoplanktonutilize the nitrogenous compounds at rates proportional to their availability. On the other hand, the suppression of nitrate assimilation in the presence of ammonia has often been observed with natural phytoplankton populations in freshwater lakes (Procházková *et al.* 1970, Toetz 1981, Takhashi and Saijo 1981, Mitamura 1986b). The assimilation of nitrate seemed to be suppressed by the preferential utilization of reservoir phytoplankton for ammonia and urea nitrogen in the presence of both nitrogenous compounds in water. It seems to indicate that the preference of phytoplankton for each nitrogenous compound may vary by species composition of phytoplankton and trophic levels in lakes, as suggested by Eppley *et al.* (1969) and Binhe and Alexander (1993).

Table 2. Turnover time of ammonia and urea, and doubling time of particulate carbon and nitrogen.

Reservoir	Depth	Turnover time		Doubling time	
	(m)	Ammonia	Urea	PC	PN
		(days)		(days)	
L. Soyang	0	39	33	14	16
	3	47	38	22	18
	7	450	200	130	130
L. Chuncheon	0	2	7	3	4
	2	6	16	7	10
	5	230	930	310	240
L. Uiam	0	5	4	4	7
	2	16	30	12	13
	5	510	500	300	200

Turnover time of ammonia, nitrate and urea

The turnover time of nitrogenous compounds, in steady state, can by expressed as the time necessary to utilize an amount of nitrogenous compound equivalent to the ambient concentration. As seen in Table 2, the turnover times of ammonia and urea in the surface water were 2 to 39 days and 4 to 33 days. The turnover times tended to increase with depth. Much shorter turnover times for ammonia and urea were obtained in Lakes Chuncheon and Uiam. On the other hand, nitrate required much longer periods. Several investigators have noted a brief turnover time for ammonia and urea in the euphotic layer of freshwater lakes (Axler *et al.* 1981, Axler and Gersberg 1981, McCarthy *et al.* 1982, Mitamura and Saijo 1986a, Suttle and Harrison 1988). The present turnover times resemble or are longer than the values obtained by previous investigators. The brief turnover time of ammonia and urea in the euphotic layer of reservoirs indicates that both nitrogenous compounds, as reflected in the term "regenerated production", are associated with rapidly recycled forms, as reported by Dugdale and Goering (1967).

Doubling time of particulate nitrogen was calculated from the total nitrogen assimilation velocity:

$$T_D = \frac{\ln 2}{V}$$

where T_D is the doubling time of particulate nitrogen and V is the total nitrogen assimilation velocity. The present values of the doubling time of phytoplankton populations in reservoirs were conservative estimates because of the difficulty in determining the living cell in particulate nitrogen which contains detrital matter.

The doubling times of particulate nitrogen were calculated as 4 to 18 days in the upper euphotic layer, and they increased with depth. These doubling times were similar to those of particulate carbon calculated from the photosynthetic carbon assimilation rate. The values in the present study were similar to those obtained by Mitamura and Saijo (1986a) in a freshwater lake.

In the euphotic layer of reservoirs located on the upper reaches of the North Han River System, ammonia and urea in the regenerated form of nitrogen compounds make a major contribution as nitrogen sources for the growth of phytoplankton populations, and play a significant role in the biogeochemical nitrogen cycle.

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적 요

北漢江 上流에 位置한 昭陽湖, 春川湖, 衣岩湖에 있어서 生産層 植物플랑크톤에 의한 窒素化合物 同化速度를 現場法에 의해 測定했다.

암모니아, 질산 및 尿素態窒素의 표면 수중 同化速度는 昭陽湖에서 각각 13, 2 및 13μg at. N·m^{-3}·(12:10~18:15)$^{-1}$, 春川湖는 325, 27 및 59μg at. N·m^{-3}·(12:30~18:30)$^{-1}$ 그리고 衣岩湖에서는 174, 12 및 45μg at. N·m^{-3}·(12:30~19:30)$^{-1}$로 나타났다. 植物플랑크톤은 암모니아와 尿素態窒素를 우선적으로 이용하고 있다고 본다. 三態의 窒素化合物 중 窒酸態窒素의 同化速度는 暗條件下에서 매우 낮았다. 植物플랑크톤의 窒素源으로서 암모니아態窒素는 47에서 79%를 점유하고 있으나 질산태질소의 역할은 매우 낮았다. 각각 人工湖의 炭素와 窒素의 基礎生産은 昭陽湖에서 59와 6.9, 春川湖에서 217과 26, 衣岩湖는 110mg C·m^{-2}·day^{-1}와 13mg N·m^{-2}·

day^{-1}로 推定되어 基礎生産의 炭素와 窒素比는 각각 8.6, 8.4 및 8.4였다. 암모이나와 尿素의 回轉時間은 生産層 上層에서 각각 2~47日 및 4~38日로 계산되고 있으나 窒酸이온은 매우 긴 回轉時間을 必要로 하고 있다.

人工湖의 生産層에서 암모니아이온과 尿素는 生物地球化學的 窒素代謝 중 매우 중요한 역할을 하고 있다.

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