RELATIONSHIP BETWEEN UREA DECOMPOSITION AND CELL CLASSES OF RESERVOIR PHYTOPLANKTON IN THE NORTH HAN RIVER SYSTEM

Mitamura, O.[1], Cho, K.S.[2], Hong, S.U.[3] and Lee, H.K.[4]

1) Division of Natural Sciences, Osaka Kyoiku University, Tnnnoji-ku Osaka 543, Japan
2) College of Natural Science, Kangweon National University, Chuncheon 200-701, Korea
3) College of Pharmacy, Seonkyoonkwan University, Myeonryum-dong, Seoul 110-745, Korea
4) Kangweon Province Institute of Health and Environment, Chuncheon 200-093, Korea

ABSTRACT

The influence of natural phytoplankton cell classes upon the response of urea decomposition was investigated in four reservoirs in the North Han River System. The decomposition rate of urea was 0.3 to 29.4 μ mol urea$\cdot m^{-3}\cdot hr.^{-1}$ in the light and 0.2 to 14.9 μ mol urea$\cdot m^{-3}\cdot hr.^{-1}$ in the dark. Much higher decomposition rates were observed at the eutrophic stations in Lake Euiam. The urea decomposition in the smaller fraction (<5 μm) exceeded that in the middle (5-25 μm) and the larger fraction (>25 μm). No differences in the ratios of urea decomposition to chlorophyll-a or photosynthesis among three fractions were observed. This might be mainly due to the difference in the standing crop of phytoplankton. These trends were no different between sampling areas and reservoirs. The greater part of urea decomposition was the phase of CO_2 liberation rate into the water. Eight to 50% of the urea decomposition was incorporated into the particulate phase in the light, but this was much lower in the dark. The results of the present study indicate that urea in reservoirs decomposes by phytoplankton rather than bacteria and the phytoplankton would be competitive to bacteria.

KEY WORDS : Urea decomposition, Size fraction, Phytoplankton, Reservoir,

INTRODUCTION

Several workers have recently been studied the phytoplankton cell size to clarify problems regarding the distribution of phytoplankton cell classes, the influence of cell size on physiological processes such as photosynthesis, nutrient requirement, growth, and the influence of particulate size on zooplankton grazing (2,11,12,16).

Information has been accumulated to elucidate the distribution and metabolism of urea in natural waters because of its importance as one of the major nitrogen sources for phytoplankton and the appreciation of its role in the biogeochemical cycle of nitrogenous compounds. Urea decomposition by freshwater phytoplankton has been demonstrated using ^{14}C-labelled urea (3,6,8,9). Urea was presumably

decomposed more effectively by phytoplankton than bacteria, although urea-decomposing bacteria was extensively distributed in natural waters.

Using ^{14}C-labelled urea, several workers (3,9,14,20) measured a urea decomposition rate in fractionated water sample from coastal sea areas and a freshwater lake. They reported high rates of urea decomposition in the smaller phytoplankton cell classes (less than 15-25 μm). Mitamura (3) suggested that the smaller phytoplankton would have a competitive advantage over larger phytoplankton in urea decomposition in Lake Biwa.

However, there has been very little research on influence of cell size on the response of urea decomposition in reservoirs. This study is to clarify the influence of cell size on the response of urea decomposition by reservoir phytoplankton located in the North Han River System, Korea, as a means to obtain further information on the urea metabolism in natural waters.

MATERIALS AND METHODS

The present study was carried out at station near the dam of Lake Paro, Lake Soyang, Lake Chuncheon and Lake Euiam. To obtain further informations on the influence of the eutrophication of water region upon the response of urea decomposition, sample waters were taken from six stations showing a different trophic character in Lake Euiam (Fig. 1).

To measure the activity of urea decomposition by a different cell classes of phytoplankton, sample waters taken from surface layer at stations in four reservoirs in May and June 1981 were separated into three fractions by sieving the water with a 5 μm, 25 μm Nitex mesh screen. An appropriate amount of phytoplankton on each mesh screen was resuspended in the filtered water through glass fiber filters.

The water samples of smaller fraction (Fraction A; <5 μm), middle fraction (Fraction B; 5-25 μm) and larger fraction (Fraction C; >25 μm) were poured into three series of 55 ml clear glass-stoppered bottles for the measurement of urea

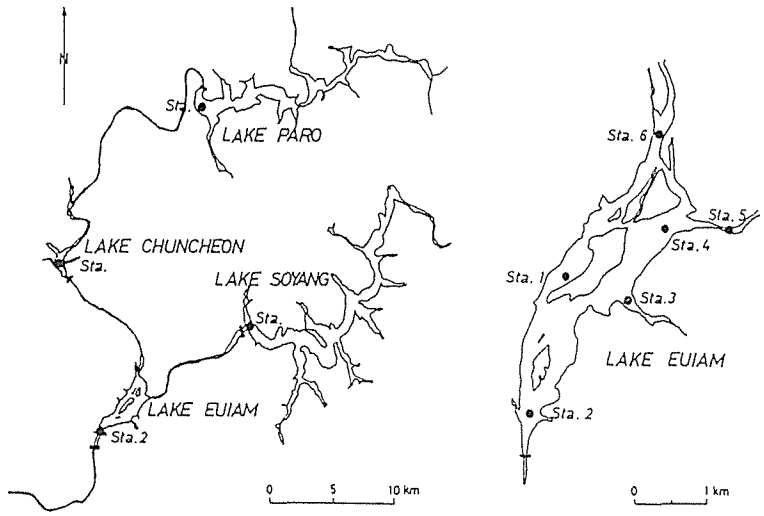

Fig.1 Map of sampling station in Lakes Paro, Soyang, Chuncheon and Euiam located in the North Han River System.

decomposition rate. Crystalline ^{14}C-labelled urea (Daiichi Pure Chemicals; sp act, 50 mCi·m mol^{-1}; 1.85 GBq·m mol^{-1}) was dissolved in sterile, deionized distilled water and then the stock ^{14}C-labelled urea solution was stored at -20 °C. After adding 0.5 ml of ^{14}C-labelled urea solution (containing 0.002 μ mol ^{12}C-urea and 0.1 μCi ^{14}C-urea) to each 50 ml sample water, 0.2 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 rate of urea decomposition. Light and dark series of bottles were incubated in a water tank under 7,000 lux using daylight type fluorescent lamps at 16.0 °C. (water temperature similar to that of sampling stations). After six hours incubation, biological activity was stopped by adding formaldehyde solution to each bottle.

Sample water in each glass bottle was filtered through a Millipore HA type filter of 24 mm diameter. To measure the rate of urea carbon incorporation into phytoplankton cell, the filter was put in a scintillation vial, and 10 ml of Bray scintillation fluid was added. The radioactivity was then measured with Aloka Model LSC-651 liquid scintillation spectrometer. The filtrate of each sample was poured into a separate 100 ml Erlenmeyer flask and a CO_2 absorption tube containing 0.5 ml of n-ethanolamine was placed in each flask to absorb the $^{14}CO_2$ liberated from the sample solution by acidification. After adding 0.5 ml of 3N H_2SO_4 solution to each filtrate, the flasks were sealed tightly and left for four days at room temperature, Then the radioactivity of $^{14}CO_2$ absorbed into n-ethanolamine solution was determine as described above.

Photosynthetic activity was measured by the technique (19), simultaneously with the experiment of urea decomposition determination. The total CO_2 in the sample water was determined with an infra-red CO_2 analyzer (17).

For the chemical analyses of lake water, the collected sample waters were immediately filtered through glass fiber filters (Whatman GF/C) treated by ignition at 450 °C. Then the filters and filtrates were stored at -20 °C in a deep freezer until chemical analyses in the laboratory. Urea concentration was determined by the method of Newell *et al.* (13) with a modification (5), ammonia after Sagi (15), nitrite after Bendschneider and Robinson (1), nitrate by the method of Wood *et al.* (21), and phosphate after Murphy and Riley (10). Particulate carbon (PC) and nitrogen (PN) were determined with a Yanagimoto MT-2 type CHN Corder. Chlorophyll-a concentration was determined by the method of SCOR/Unesco (18).

RESULTS AND DISCUSSION

Concentration of urea and nutrients

Distribution of urea, ammonia, nitrite, nitrate and phosphate concentration in the surface layer at the stations in reservoirs was listed in Table 1. Concentration of dissolved inorganic nitrogen (DIN; sum of ammonia, nitrite and nitrate nitrogen) at station near the dam was 42 to 56 μg at.N·l^{-1}. The percentage of ammonia in DIN was calculated as 6 to 10%. The nitrite was only 0.6 to 0.8%. In four reservoirs, the greater part of DIN was nitrate. The phosphate concentration ranged from 0.14 to 0.16 μg at.P·l^{-1} and the values were generally low. In Lake Euiam, the concentrations of ammonia showed much higher values at Stations 3, 4 and 5 than at other stations. This tendency also observed in the

Table 1 Concentrations of urea and nutrients in surface layer of reservoirs.

Reservoir	Station	Urea	Ammonia	Nitrite	Nitrate	Phosphate
		(μ mol·l^{-1})		(μg at.N·l^{-1})		(μg at.P·l^{-1})
L. Paro		0.45	3.7	0.40	52	0.14
L. Soyang		0.23	2.7	0.24	39	0.14
L. Chuncheon		0.61	3.8	0.39	45	0.16
L. Euiam	Sta.1	0.85	5.4	0.35	50	0.13
	Sta.2	0.58	5.1	0.41	48	0.16
	Sta.3	4.30	59.4	2.62	52	0.83
	Sta.4	5.35	21.4	0.67	50	0.55
	Sta.5	8.55	30.5	0.79	57	0.74
	Sta.6	1.13	4.5	0.41	51	0.13

distribution of phosphate concentrations. This indicates that Stations 3, 4 and 5, where affected by the sewage from Chuncheon City, have an eutrophic character. The concentration of urea was 0.23 to 0.61 μ mol·l^{-1} at station near the dam of four reservoirs. High concentrations of urea were observed at the stations showing eutrophic character in Lake Euiam. The concentration of urea nitrogen was comparable to that of ammonia, but much lower than the nitrate concentration.

Size distribution of particulate matter

Table 2 shows the amounts of particulate carbon (PC), nitrogen (PN) and chlorophyll-a in fractionated sample at the stations of four reservoirs. The particulate matter at the stations in Lake Euiam showed higher values than in other reservoirs. A greater part of particulate matter was occupied by the smaller Fraction A, ranging from 70 to 92% in PC of total fraction, 66 to 91% in total PN and 71 to 88% in total chlorophyll-a, while the amounts in the middle Fraction B and the larger Fraction C were usually low. There were no appreciable differences among the ratios of PC/PN and PC/Chl.a in three fractions.

Table 2 Amounts of particulate carbon (PC), nitrogen (PN) and chlorophyll-a in Fractions A (<5 μm), B (5-25 μm) and C (>25 μm) in four reservoirs.

Reservoir	Station	PC			PN			Chl.a		
		A	B	C	A	B	C	A	B	C
		(mg C·m^{-3})			(mg N·m^{-3})			(mg chl.a·m^{-3})		
L. Paro		134	10	16	14	1.2	1.9	0.34	0.03	0.05
L. Soyang		171	6	15	23	0.9	2.1	0.96	0.05	0.12
L. Chuncheon		141	8	16	20	1.3	2.5	0.82	0.05	0.09
L. Euiam	Sta.1	296	20	21	39	2.4	2.6	2.26	0.15	0.16
	Sta.2	368	57	56	46	6.8	6.9	2.57	0.35	0.30
	Sta.3	1705	171	416	254	27.7	69.3	15.6	1.46	3.84
	Sta.4	861	38	33	99	5.7	3.9	1.69	0.17	0.14
	Sta.5	841	103	259	79	11.3	28.6	2.19	0.24	0.60
	Sta.6	296	18	59	36	2.6	6.8	0.86	0.06	0.29

Photosynthetic activity in phytoplankton cell classes

As can be seen in Fig. 2, the photosynthetic carbon assimilation rate, determined simultaneously with the experiments for the measurement of the urea decomposition rate, was 0.7 to 8.4 mg C·m^{-3}·hr.$^{-1}$ at the station near the dam in reservoirs. At eutrophic Station 3 in Lake Euiam, high photosynthetic rate was obtained. 66 to 84% of photosynthetic rate was the Fraction A. The photosynthetic carbon assimilation rate by unit amount of chlorophyll-a concentration was calculated as 1.3 to 3.8 mg C·mg chl.a^{-1}·hr.$^{-1}$ and showed no differences among the phytoplankton cell classes.

Urea decomposition in fractionated phytoplankton

The decomposition rate of urea, incorporation rate of urea carbon into particulate matter, and the CO_2 liberation rate into the water from urea in the fractionated experiments measured under continuous light and dark condition are illustrated in Fig. 2. The decomposition rate of urea (as sum of incorporation rate of urea and CO_2 liberation rate) was 0.3 to 29.4 μ mol urea·m^{-3}·hr.$^{-1}$ in the light and 0.2 to 14.9 μ mol urea·m^{-3}·hr.$^{-1}$ in the dark, respectively. Much higher decomposition rates were observed at eutrophic areas in Lake Euiam.

The percentage of urea decomposition in the smaller Fraction A to that in the total fraction (as sum of Fractions A, B and C) ranged from 66 to 90% (average; 80%) for decomposition rate of urea, 41 to 92% (average; 74%) for carbon incorporation rate, and 66 to 90% (average; 80%) for CO_2 liberation rate, re-

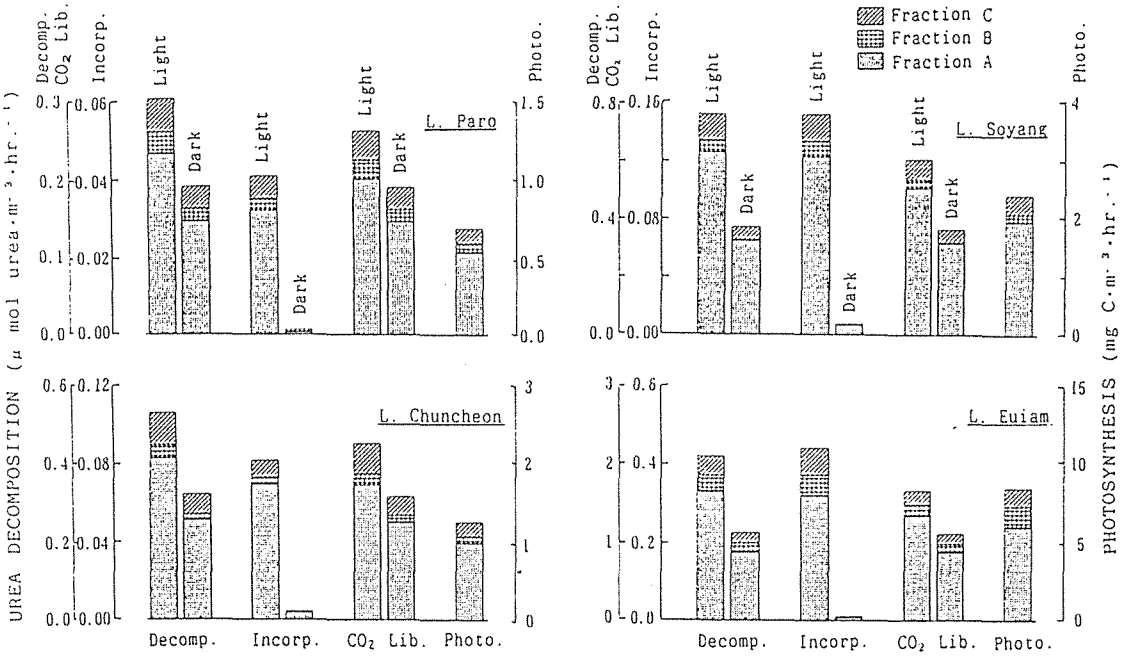

Fig.2a Rate of urea decomposition (sum of urea carbon incorporation rate into particulate matter and CO_2 liberation rate into water from urea) in light and dark condition, and rate of photosynthetic carbon assimilation, in Fractions A (<5 μm), B (5-25 μm) and C (>25 μm) at station near the dam of four reservoirs.

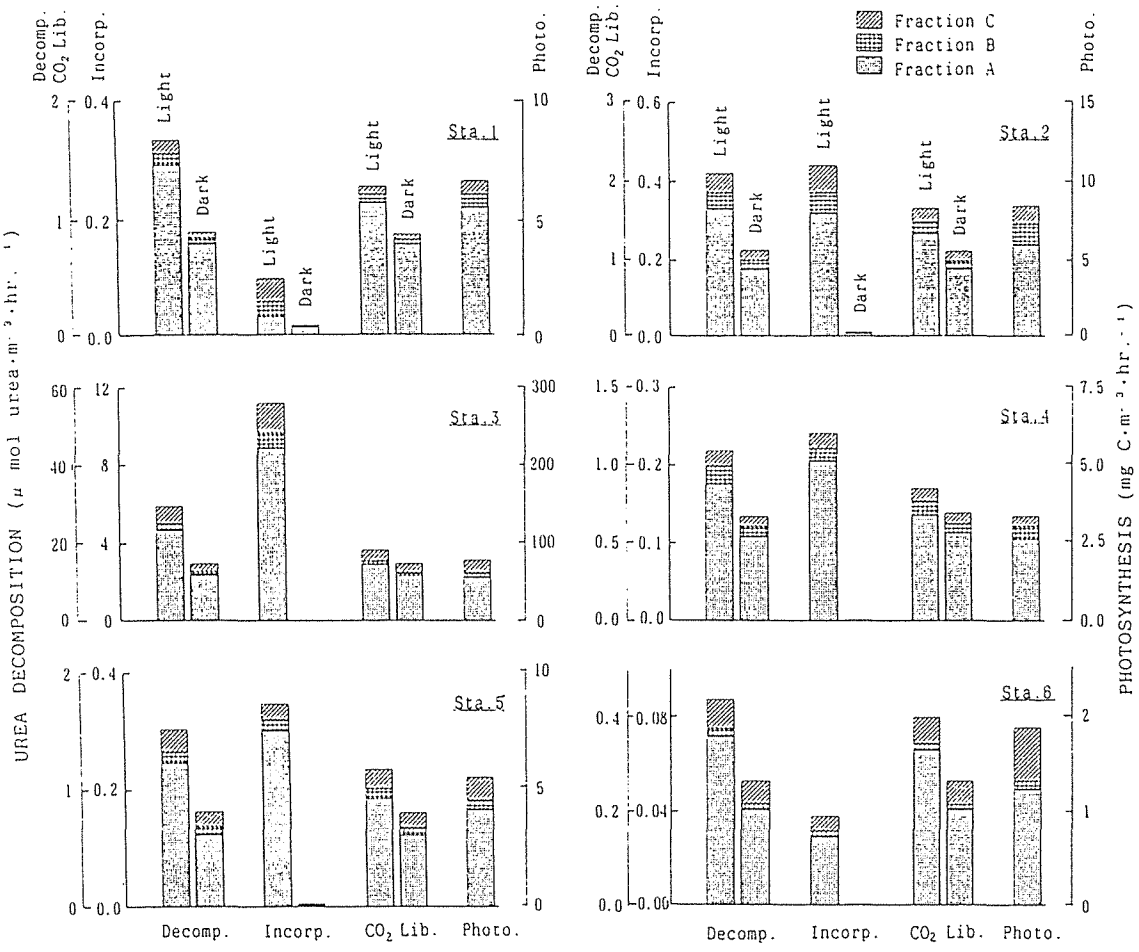

Fig.2b Rate of urea decomposition and photosynthesis in Fractions A, B and C at six stations in Lake Euiam.

spectively. While, the contributions of decomposition rate of urea in Fractions B and C to that in the total fraction were considerably low, ranging from 4 to 10% for Fraction B and 6 to 19% for Fraction C. A similar tendency was observed in the contribution for the carbon incorporation rate and CO_2 liberation rate. These results were no different between sampling areas and reservoirs. No appreciable differences between the contribution of each fraction in the light and in the dark were observed.

The major part of urea decomposition was the phase of CO_2 liberation rate into the water (Table 3). Eight to 50% of the urea decomposition was incorporated into the particulate phase in the light, but this was much lower in the dark. There were no appreciable differences among the size fractions. These percentage in the light are similar to those obtained by previous workers (3,6,8,9) in freshwater lake and reservoirs.

The urea decomposition rates were obviously higher in the light condition than in the dark one. Mitamura and Saijo (7) and Mitamura (4) examined the influence

Table 3 Percentage of carbon incorporation rate into particulate matter and CO_2 liberation rate into water from urea in decomposition rate of urea, and the dark/light ratio of urea decomposition in three fractions.

Reservoir		Fraction	C Incorporation Decomposition (%)		CO_2 Liberation Decomposition (%)		Dark/Light		
			Light	Dark	Light	Dark	Decomp.	Incorp.	CO_2 Lib.
L. Paro		A	14	0.3	86	100	0.63	0.02	0.73
		B	11	1.1	89	99	0.60	0.10	0.67
		C	13	1.6	87	98	0.67	0.12	0.76
L. Soyang		A	20	1.2	80	99	0.52	0.06	0.63
		B	25		75			0.02	
		C	22	0.6	78	99	0.51	0.03	0.64
L. Chunch		A	17	1.0	83	99	0.62	0.06	0.73
		B	12	0.6	88	99	0.50	0.05	0.55
		C	10	1.1	90	99	0.58	0.11	0.64
L. Euiam	Sta.1	A	22	1.0	78	99	0.55	0.05	0.69
		B	29	2.6	71	97	0.46	0.09	0.60
		C	36	0.8	64	99	0.45	0.02	0.69
	Sta.2	A	19	0.6	81	99	0.54	0.03	0.66
		B	26	1.0	74	99	0.53	0.04	0.70
		C	27	0.5	73	99	0.54	0.02	0.73
	Sta.3	A	38	0.1	62	100	0.51	0.00	0.82
		B	50	0.5	50	100	0.44	0.01	0.86
		C	35	0.2	65	100	0.51	0.00	0.78
	Sta.4	A	23	0.1	77	100	0.64	0.01	0.83
		B	15	0.8	85	99	0.56	0.06	0.65
		C	18	0.6	82	99	0.65	0.03	0.79
	Sta.5	A	25	0.4	75	100	0.51	0.01	0.67
		B	19	0.4	81	100	0.65	0.02	0.79
		C	15	1.0	85	99	0.66	0.07	0.76
	Sta.6	A	8	0.2	92	100	0.58	0.02	0.62
		B	13	0.5	87	99	0.58	0.04	0.66
		C	11	0.2	89	100	0.80	0.02	0.90

of irradiance of the response of urea decomposition under different light intensity. They reported that the urea decomposition rate increased with increasing light intensity up to some asymptotic value. As seen in Table 3, the ratio of dark to light values was calculated as 0.44 to 0.80 in the decomposition rate of urea, 0.00 to 0.12 in the carbon incorporation rate, and 0.60 to 0.90 in the CO_2 liberation rate. The dark/light ratio in the CO_2 liberation rate revealed considerably high values, but only negligible levels in the phase of carbon incorporation. This suggests that in the light urea decomposes into two phases of carbon incorporation and CO_2 liberation, while in the dark it decomposes only into CO_2 liberation.

The decomposition rate of urea carbon by unit amount of chlorophyll-a was calculated as 2.6 to 18.0 μg urea C·mg chl.a^{-1}·hr.$^{-1}$ in the light and 2.0 to 9.2 μg urea C·mg chl.a^{-1} hr.$^{-1}$ in the dark (Table 4). High ratios were obtained at the eutrophic stations in Lake Euiam. No appreciable differences in the ratios among the fractions were observed. The values in the present study proved to be in the same range as those obtained in Lake Biwa (8,9), and Lakes Soyang, Chun-

Table 4 Ratio of urea decomposition rate, carbon incorporation rate and CO_2 liberation rate to chlorophyll-a amount in three fractions.

Reservoir		Urea Decomposition Chlorophyll-a			C Incorporation Chlorophyll-a			CO_2 Liberation Chlorophyll-a		
		A	B	C	A	B	C	A	B	C
		(μg urea C·mg chl.a^{-1}·hr.$^{-1}$)								
L. Paro	Light	8.2	10.3	10.2	1.1	1.2	1.4	7.1	9.1	8.9
	Dark	5.2	6.2	6.9	0.0	0.1	0.2	5.2	6.1	6.7
L. Soyang	Light	7.9	9.3	8.6	1.5	2.3	1.9	6.3	7.0	6.8
	Dark	4.1		4.4	0.1	0.0	0.1	4.0		4.3
L. Chuncheon	Light	6.1	7.6	11.9	1.0	0.9	1.2	5.1	6.7	10.7
	Dark	3.8	3.8	6.9	0.1	0.1	0.1	3.7	3.7	6.8
L. Euiam Sta.1	Light	7.8	7.8	8.1	1.7	2.2	2.9	6.1	5.6	5.2
	Dark	4.3	3.6	3.7	0.1	0.2	0.1	4.2	3.4	3.6
Sta.2	Light	7.7	7.0	9.7	1.5	1.8	2.6	6.3	5.2	7.1
	Dark	4.2	3.7	5.2	0.1	0.1	0.1	4.1	3.6	5.2
Sta.3	Light	18.0	14.3	13.4	6.8	7.1	4.7	11.2	7.2	8.7
	Dark	9.2	6.2	6.8	0.0	0.1	0.0	11.2	7.2	8.7
Sta.4	Light	6.3	7.5	9.2	1.5	1.1	1.7	4.8	6.4	7.5
	Dark	4.0	4.2	6.0	0.0	0.1	0.1	4.0	4.2	6.0
Sta.5	Light	6.7	5.1	3.8	1.7	1.0	0.6	5.1	4.2	3.2
	Dark	3.4	3.3	2.5	0.0	0.0	0.0	3.4	3.3	2.4
Sta.6	Light	5.0	3.5	2.6	0.4	0.5	0.3	4.6	3.1	2.3
	Dark	2.9	2.0	2.1	0.0	0.0	0.0	2.9	2.0	2.0

cheon and Euiam (6). As can be seen in Table 4, the decomposition rate of urea, the carbon incorporation rate, and the CO_2 liberation rate, except for the values of the carbon incorporation rate obtained in the dark condition, appear to be related to the chlorophyll-a amount. This suggests that the urea decomposition rate was proportional to the standing crop of phytoplankton.

The ratio of urea carbon decomposition rate to photosynthetic carbon assimilation rate was 0.13 to 0.56% (Table 5). The carbon incorporation rate from urea was calculated as 0.01 to 0.19% of photosynthetic carbon incorporation rate. The ratio of CO_2 liberation rate to photosynthesis was 0.12 to 0.50% There were no differences among three fractions. The contribution of urea carbon in the carbon source of reservoir phytoplankton was negligible. These percentages were similar to the findings in Lake Biwa (3,8,9).

Remsen *et al.* (14) measured a urea decomposition rate in the smaller fraction (<20 μm) and the total fraction in coastal waters. Webb and Haas (20) reported a considerable contribution of the smaller fraction (15 μm) in the total fraction for urea decomposition in estuary waters. Mitamura and Saijo (9) reported that the urea decomposition by smaller fraction (<25 μm) accounted for the great part of that by the total fraction in the south basin, whereas the contribution of smaller phytoplankton was low in the north basin of Lake Biwa, as measured by *in situ* experiments. Mitamura (3) reported that the urea decomposition by phytoplankton populations in the smaller fraction (<25 μm) exceeded that in the middle (25-70 μm) and larger fraction (>70 μm) in different trophic areas of Lake Biwa. He reported that the ratios of urea decomposition to chlorophyll-a or photosynthetic carbon assimilation were higher in the smaller fraction than in the middle or larger one in Lake Biwa. The results of the present fractionated experiment show that the urea decomposition rate by the smaller phytoplankton accounted for a considerable part of that by natural reservoir phytoplankton

Table 5 Ratio of urea decomposition, carbon incorporation and CO_2 liberation to photosynthesis in three fractions.

Reservoir		Urea Decomposition / Photosynthesis			C Incorporation / Photosynthesis			CO_2 Liberation / Photosynthesis		
		A	B	C	A	B	C	A	B	C
			(%)			(%)			(%)	
L. Paro		0.52	0.56	0.54	0.07	0.06	0.07	0.45	0.50	0.47
L. Soyang		0.38	0.38	0.33	0.08	0.09	0.07	0.31	0.29	0.26
L. Chuncheon		0.51	0.45	0.55	0.09	0.05	0.05	0.42	0.40	0.49
L. Euiam	Sta.1	0.32	0.23	0.23	0.07	0.06	0.08	0.25	0.16	0.15
	Sta.2	0.33	0.19	0.25	0.06	0.05	0.07	0.27	0.14	0.18
	Sta.3	0.49	0.37	0.33	0.19	0.18	0.12	0.31	0.19	0.21
	Sta.4	0.41	0.31	0.40	0.10	0.05	0.07	0.31	0.26	0.33
	Sta.5	0.35	0.31	0.24	0.09	0.06	0.03	0.27	0.25	0.20
	Sta.6	0.35	0.26	0.13	0.03	0.03	0.01	0.32	0.23	0.12

populations, while no differences in the ratios of urea decomposition to chlorophyll-a or photosynthesis among three fractions were observed. This might be mainly due to the difference in the standing crop of phytoplankton. The decomposition of urea in natural waters occurred in connection with the photosynthesis of phytoplankton as suggested by several workers. The results of the present study indicate that the urea in reservoirs decomposes by phytoplankton rather than bacteria and the phytoplankton would be competitive to bacteria in terms of the urea decomposition.

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