

Uptake of Carbon and Nitrogen by *Microcystis* Algal Assemblages in the Seonakdong River

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Carbon (^{14}C) and nitrogen ($^{15}\text{NH}_4$ and $^{15}\text{NO}_3$) uptake were measured at two stations in the hypertrophic zone of the Seonakdong River, where *Microcystis aeruginosa* explosively bloomed in September 1998. Significant nitrogen limitation occurred in the period of *Microcystis* bloom, while phosphorus limitation was common in the river. The specific nitrogen ($\text{NH}_4 + \text{NO}_3$) uptake was $12\text{--}50 \mu\text{mol mg chl-}a^{-1} \text{ hr}^{-1}$ at two stations, showing substantially higher than for any other freshwaters. The specific nitrogen uptake was higher at the GAR Station of the nitrogen-limited area and this high nitrogen uptake resulted in low $^{14}\text{C}:^{15}\text{N}$ atomic ratios of algal uptake. Carbon uptake was dependent upon irradiance, decreasing gradually toward the bottom in the euphotic zone, whereas the nitrogen uptake increased slightly toward the bottom. NH_4 preferable uptake against NO_3 was hardly discernible due to the fact that it exceeded the NH_4 ambient concentration. The $^{14}\text{C}:^{15}\text{N}$ atomic ratios of algal uptake in the surface waters approached the Redfield C:N ratio.

Key Words: algal uptake, ^{14}C and ^{15}N , hypertrophic water, *Microcystis*, NH_4 and NO_3

INTRODUCTION

Microcystis blooms in the Seonakdong River have been annual summer events, and have brought aesthetic nuisance and resulted in the social problems. Blue-green algae abundantly occurred in summer, while diatoms persistently bloomed in the cold season. The river has been eutrophic and has shown typical seasonal variations (Cho and Shin 1998; Cho *et al.* 2002). Generally, phosphorus is a strong limiting factor in the Seonakdong River except during the period of *Microcystis* bloom (Cho and Shin 1998). The success of *Microcystis* over the other algae is reported to be associated with low N:P ratios and low NO_3 nitrogen with sufficient NH_4 nitrogen concentration (Jacoby *et al.*, 2000). The isotopic method was introduced by Dugdale *et al.* (1961) to determine the nitrogen uptake of marine phytoplankton and, with improved instrumentation, has frequently been applied to understand the regenerated production in the ocean (Dugdale and Wilkerson 1986). Though the nitrogen isotope is a rapid tool in determining nitrogen mobility in water columns, there

have been many difficulties and limitation in conducting the ^{15}N experiment in Korea.

The aim of this study is to determine, by the isotopic method, the carbon and nitrogen uptake efficiency of algal assemblages dominated by *Microcystis aeruginosa* in the productive freshwaters of the Seonakdong River.

MATERIALS AND METHODS

The experiments were carried out at two stations (GAR and GIM Stations) on the Seonakdong River, which is a tributary of the Nakdong River. The river is one of the most eutrophic (or productive) freshwaters in Korea (Kim *et al.* 1996). The GIM Station is located in the upper zone of the river and the GAR Station 1.5 km downstream from the GIM Station. The water depth was 1.5 m at the GIM Station and 8.0 m at the GAR Station. Topography and descriptions of the Seonakdong River were presented in detail in Jung and Cho (2003).

Field experiments for the determination of carbon and nitrogen uptake were conducted in the Seonakdong Rivers in September 1998. In this period, *Microcystis aeruginosa* and related algal groups bloomed after the heavy rains of the summer. Incubations for the carbon and nitrogen uptake measurements were carried out to

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Table 1. Chlorophyll-*a* (chl-*a*) and nutrient concentrations at the GAR Station on the Seonakdong River in September 1998

Depth (m)	Temp (°C)	Chl- <i>a</i> (mg · l ⁻¹)	POC (mg C · l ⁻¹)	PON (mg N · l ⁻¹)	C:N	NH ₄ -N (μM)	NO ₃ -N (μM)	NO ₂ -N (μM)	PO ₄ -P (μM)
0.0	26.8	94	7.64	1.08	8.3	14.3	5.9	0.07	3.39
0.2	26.8	97	8.28	1.01	9.6	14.3	5.6	0.07	3.26
0.4	26.7	107	8.02	0.95	10.0	13.7	5.9	0.07	3.48
0.8	26.4	93	7.37	0.88	9.7	13.9	5.9	0.07	3.61
1.5	26.1	87	6.95	0.91	8.9	15.9	5.9	0.07	2.97

Table 2. Chl-*a* and nutrient concentrations at the GIM Station on the Seonakdong River in September 1998

Depth (m)	Temp (°C)	Chl- <i>a</i> (mg · l ⁻¹)	POC (mg C · l ⁻¹)	PON (mg N · l ⁻¹)	C:N	NH ₄ -N (μM)	NO ₃ -N (μM)	NO ₂ -N (μM)	PO ₄ -P (μM)
0.0	28.3	402	20.92	3.09	7.9	14.3	6.1	0.14	1.94
0.2	27.8	425	21.27	2.99	8.3	13.9	5.9	0.14	1.97
0.4	27.8	338	18.05	2.47	8.5	14.3	9.6	0.43	1.94
0.8	27.3	273	14.59	1.97	8.7	18.8	37.5	1.43	1.94
1.5	26.6	91	5.51	0.63	10.2	20.4	6.1	0.07	1.94

the 1% irradiance penetration depth (about 1.0 m). Underwater irradiance was measured with a quantum sensor (Li-Cor 190SA). Fifty ml of water at the selected depth were transferred to incubation tubes, 1 μCi NaH¹⁴CO₃ and 0.15 μmol ¹⁵NH₄Cl (99%) were injected into a tube and 0.30 μmol K¹⁵NO₃ (99%) into another incubation tube. Three light tubes and one dark tube for NH₄ and NO₃ uptake were incubated at the corresponding depths for 3.5 hours (from 12:00 to 15:30). The algal materials were fixed in 0.3% formaldehyde solution after incubation and filtered through GF/C and membrane filters. The membrane filters were exposed to HCl fume and dissolved in a cocktail solution in order to measure the ¹⁴C activity with a liquid scintillation counter (Tri-Carb 2000CA, Packard Co.). Twenty five ml of water for ¹⁴NH₄ analysis and 50 ml for the ¹⁴NO₃ analysis were filtered with GF/C filters, which were precombusted in a muffle furnace for 2 hours, and stored in incubator (60°C) until the samples were analyzed. The atomic ratios and amounts of carbon and nitrogen were determined with a gas chromatography – mass spectrometer system (Roboprep-Tracermass, Europa Co.). The nitrogen uptake (ρ , unit: μmol · l⁻¹ · hr⁻¹) and transport rate (V , unit: hr⁻¹) ($V = \rho \text{ PON}^{-1}$) of algae were calculated by the equation of Dugdale and Wilkerson (1986) and Legendre and Gosselin (1996). The nitrogen uptake of algae collected in the Seonakdong River was measured in the laboratory for 3 hours under the controlled irradiance (0-900 μmol · m⁻² · s⁻¹) and temperature (25°C). The water was filtered with GF/C (ϕ 25 mm) in order to determine the particulate organic

carbon (POC) and particulate organic nitrogen (PON) by a Perkin Elmer model 2400 CHN analyzer.

RESULTS AND DISCUSSION

Water quality

Tables 1 and 2 present the chemical properties of the water: chlorophyll-*a* (chl-*a*), inorganic nutrients, POC and PON concentrations at the two stations on the Seonakdong River in September. NH₄, NO₃ and PO₄ concentrations were recorded as 192-286 μg N · l⁻¹, 79-525 μg N · l⁻¹ and 60-112 μg P · l⁻¹, respectively. Phytoplankton biomass determined as chl-*a* concentration were 90-110 μg · l⁻¹ at the GAR Station and 270-430 μg · l⁻¹ at the GIM Station. In this period, *Microcystis aeruginosa* and related algae occurred predominantly, forming scum over the water surface. The POC concentration ranged from 7.0 to 8.3 mg C · l⁻¹ at the GAR Station and from 5.5 to 21.3 mg C · l⁻¹ at the GIM Station, respectively, and the PON concentration was 0.9-1.1 mg N · l⁻¹ at the GAR Station and 2.0-3.1 mg N · l⁻¹ at the GIM Station. Organic carbon and nitrogen concentrations are directly correlated with algal biomass, and chl-*a*, POC and PON concentrations at the GIM Station were 3.8 fold, 2.4 fold and 2.3 fold compared with those at the GAR Station.

The C:N atom ratios ranged from 7.9 to 10.2 (an average of 9.0 at the two stations), showing little variation between stations or for depth. Considering that the optimum C:N ratio of algal biomass is in the 7.7-12.5 range (Healey and Hendzel 1980), and considering also the Redfield C:N ratio, the algae of the study area may

Table 3. ^{14}C , $^{15}\text{NH}_4$ and $^{15}\text{NO}_3$ uptakes or assimilation during *Microcystis* bloom at the GAR Station on the Seonakdong River in September 1998. The irradiance extinction coefficient (k) = 3.6

Depth (m)	Irradiance ($\text{mmol} \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$)	POC ($\text{mg C} \cdot \text{l}^{-1}$)	PON ($\text{mg N} \cdot \text{l}^{-1}$)	Uptake or assimilation		
				$^{14}\text{CO}_2$ ($\text{mg C mg chl-}a^{-1} \cdot \text{hr}^{-1}$)	$^{15}\text{NH}_4$ ($\mu\text{mol} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$)	$^{15}\text{NO}_3$ ($\mu\text{mol} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$)
0.0	4,902	7.64	1.08	3.51	2.16	1.77
0.2	2,386	8.28	1.01	3.08	2.29	1.52
0.4	1,161	8.02	0.95	1.96	2.19	2.11
0.8	275	7.37	0.88	0.60	3.15	1.42
1.5	22	6.95	0.91	0.16	3.60	0.77

Table 4. ^{14}C , $^{15}\text{NH}_4$ and $^{15}\text{NO}_3$ uptake or assimilation during *Microcystis* bloom at the GIM Station on the Seonakdong River in September 1998. The irradiance extinction coefficient (k) = 6.6

Depth (m)	Irradiance ($\text{mmol} \cdot \text{m}^{-2} \cdot \text{hr}^{-1}$)	POC ($\text{mg C} \cdot \text{l}^{-1}$)	PON ($\text{mg N} \cdot \text{l}^{-1}$)	Uptake or assimilation		
				$^{14}\text{CO}_2$ ($\text{mg C mg chl-}a^{-1} \cdot \text{hr}^{-1}$)	$^{15}\text{NH}_4$ ($\mu\text{mol} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$)	$^{15}\text{NO}_3$ ($\mu\text{mol} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$)
0.0	4,011	20.92	3.09	3.06	2.81	2.29
0.2	1,071	21.27	2.99	1.37	2.79	2.32
0.4	286	18.05	2.47	0.35	3.31	2.90
0.8	20	14.59	1.97	0.19	4.63	2.66
1.5	0.2	5.51	0.63	0.11	1.36	0.01

not suffer from nitrogen deficiency. Particulate C:N atom ratios were close to the typical or optimum stoichiometry of planktonic algae. However, the inorganic N:P atom ratios ranged from 5.9 to 7.4 at the GAR Station and from 10.1 to 13.7 at the GIM Station, showing significant nitrogen limitation at the GAR Station. Though phosphorus limitation commonly persisted in the Seonakdong River (Cho *et al.* 2002), nitrogen limitation might occur in the period of *Microcystis* bloom.

Carbon and nitrogen uptake

The ^{14}C and ^{15}N uptakes by *Microcystis* algae are shown in Tables 3 and 4. Uptake experiments were conducted from the surface to a 1.5 m depth in the euphotic zone. Specific carbon assimilation (assimilation number) decreased from 3.1 to 3.5 $\text{mg C mg chl-}a^{-1} \cdot \text{hr}^{-1}$ at the surface to below 0.1 $\text{mg C mg chl-}a^{-1} \cdot \text{hr}^{-1}$ at the 1.5 m depth. The depth profiles for chl-*a* concentration and the uptake were similar at the two stations.

The total nitrogen (NH_4 and NO_3) uptakes were 3.9-4.4 $\mu\text{mol}^{-1} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$ at the GAR Station and 5.1-7.3 $\mu\text{mol}^{-1} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$ at GIM Station. The NH_4 and NO_3 uptakes by algae at the GAR and GIM Stations were 2.2-2.3 $\mu\text{mol}^{-1} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$ and 2.8-3.3 $\mu\text{mol}^{-1} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$, and the NO_3 uptakes were 1.5-2.1 $\mu\text{mol}^{-1} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$ and 2.3-2.9 $\mu\text{mol}^{-1} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$, respectively. The NH_4 and NO_3 uptakes

showed a gradually increasing (an average of 1.5 fold) pattern towards the bottom, while uptake rates significantly dropped at the euphotic-limit depth. However, the surface irradiance was low at the 1.5 m depth: 0.45% at the GAR Station, and 0.01% at the GIM Station. The nitrogen and carbon uptakes were dependent upon irradiance and NO_3 was more irradiance-dependent than NH_4 . The maximum NH_4 uptake occurred at the 0.8-1.5 m depths, the maximum NO_3 uptake at the 0.4 m depth.

The NH_4 uptakes in the dark bottles at the GAR and GIM stations were 2.8 $\mu\text{mol}^{-1} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$ and 3.8 $\mu\text{mol}^{-1} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$, and the NO_3 uptakes were 1.0 $\mu\text{mol}^{-1} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$ and 1.2 $\mu\text{mol}^{-1} \cdot \text{l}^{-1} \cdot \text{hr}^{-1}$, respectively. The NH_4 uptakes for the dark bottles were 111% that for the light bottles, compared with NO_3 at 47-61%, showing a greater dependence on irradiance. Many authors have reported the relationship between nitrogen uptake and irradiance conditions in the sea waters and freshwaters (MacIssac and Dugdale 1972; Kanda *et al.* 1985; Takamura *et al.* 1987). As NH_4 uptakes by algae in the dark bottles increased more than in the light, it is supposed that NH_4 would be actively taken up with the accumulation of carbohydrate in algal cells. Miyazaki *et al.* (1987) showed that NH_4 uptake and cellular carbohydrate content have a negative relation through

Table 5. Uptake rate (V) of NH₄ and NO₃ by planktonic algae (e.g. Takamura *et al.* 1987)

Algae or locality		Uptake rate (hr ⁻¹)		Reference
		NH ₄	NO ₃	
Fresh water	Lake 885	0.028-0.037	-	Murphy and Brownlee (1981)
	Lake Castle	0.004-0.013	-	Axler <i>et al.</i> (1981)
	Lake Castle	0.001-0.003	0.0002-0.0008	Axler <i>et al.</i> (1982)
	Lake Kinneret	0.035	0.009	Berman <i>et al.</i> (1984)
	Lake Kinneret	0.02-0.03	-	Sherr <i>et al.</i> (1982)
	Lake Taupo	0.009-0.012	0.005	Priscu and Priscu (1984)
	<i>Microcystis</i> spp.	0.15-0.17	0.025-0.046	Takamura <i>et al.</i> (1987)
	<i>Microcystis</i> spp.	0.015-0.059	0.012-0.036	The present study
Marine water	<i>Skeletonema costatum</i>	0.05-0.10	0.02-0.26	Collos and Slawyk (1979)
	<i>Phaeodactylum tricornutum</i>	0.15	-	Goldman and Glibert (1982)
	<i>Thalassiosira weissflogii</i>	0.10	-	Goldman and Glibert (1982)
	<i>Chaetoceros simplex</i>	0.10	-	Goldman and Glibert (1982)
	<i>Dunaliella tertilecta</i>	0.05	-	Goldman and Glibert (1982)
	Oligotrophic Sea	0.008-0.009	0.01-0.04	MacIsaac and Dugdale (1972)
	New York Bight	0.052	0.027	Garside (1981)
	Sargasso Sea	0.01-0.05	0.002-0.02	Glibert and McCarthy (1984)
	Chesapeake Bay	0.02-0.03	0.01	Gilbert and McCarthy (1984)
	Chesapeake Bay	0.02-0.03	-	Wheeler <i>et al.</i> (1982)
	Caribbean Sea	0.01-0.055	-	Glibert and McCarthy (1984)
	Pacific Ocean	< 0.014	< 0.006	Kanda <i>et al.</i> (1985)
	Arctic Ocean	0.003-0.006	0.0015-0.003	Whalen and Alexander (1984)

the diurnal cycles. Nitrogen availability in the algal cells increases with carbohydrate decomposition and the transformation of carbohydrates to protein or fat through cellular metabolism. The NO₃ uptake may be less dependent on cellular carbohydrates than NH₄ uptake and more sensitive to irradiance conditions because NO₃ has to be reduced by NADPH before assimilation within cells (Miyazaki *et al.* 1987; Takamura *et al.* 1987).

Microcystis aeruginosa and nitrogen availability

The specific nitrogen (NH₄ + NO₃) uptake was 39-50 $\mu\text{mol} \cdot \text{mg} \cdot \text{chl-}a^{-1} \cdot \text{hr}^{-1}$ at the GAR Station and 12-27 $\mu\text{mol} \cdot \text{mg} \cdot \text{chl-}a^{-1} \cdot \text{hr}^{-1}$ at the GIM Station. It was high at the GAR Station in the nitrogen-limited area. These specific rates were substantially higher than 4.0-23.0 $\mu\text{mol} \cdot \text{mg} \cdot \text{chl-}a^{-1} \cdot \text{hr}^{-1}$ of other domestic lakes (Lake Soyang etc., Mitamura *et al.* 1993), 1.1-14.2 $\mu\text{mol} \cdot \text{mg} \cdot \text{chl-}a^{-1} \cdot \text{hr}^{-1}$ of Lake Kinneret (McCarrthy *et al.* 1982) and 1.3-24.7 $\mu\text{mol} \cdot \text{mg} \cdot \text{chl-}a^{-1} \cdot \text{hr}^{-1}$ of Lake Biwa (Mitamura and Saijo 1986). Nitrogen uptake rates increase as nitrogen deficiency occurs and nitrogen limitation is enhanced (Goldman and Glibert 1982). Like the Seonakdong River, Lake Kasumigaura persisted in sustaining a strong nitrogen limitation during *Microcystis* outbreak (Takamura *et al.* 1987). Furthermore, nitrogen uptake at

the GIM Station may be depressed by the extremely high biomass of phytoplankton.

The NH₄ uptake rates (V) measured in the Seonakdong River were 0.051-0.064 hr⁻¹ at the GAR station and 0.013-0.026 hr⁻¹ at the GIM Station. The V values of the Seonakdong River were higher than those of the oligotrophic lakes (Lake Castle, Lake Kinneret and Lake Taupo) and less than those of the eutrophic lake (Lake Kasumigaura, 0.15-0.17 hr⁻¹) (Table 5). Nitrogen uptake rates may be significantly different according to the season, the extent of the nitrogen deficiency, the cellular cycles of algae, among other factors. Algae growing in oligotrophic waters may have lower V and K_s values (half saturation coefficients) than those in polluted or eutrophic areas (MacIsaac and Dugdale 1972).

Most algae can generally utilize NH₄, NO₃ and urea as nitrogen sources; however, they preferably uptake NH₄ and urea, and have a higher affinity for NH₄ than NO₃ (Mitamura *et al.* 1993; Miyazaki *et al.* 1995). In our experiments, the NO₃ uptakes at the GAR and GIM Stations were constituted of about 56% and 75% NH₄ uptake, respectably (Tables 3 and 4). The total nitrogen uptakes of NH₄ and NO₃ during the incubation times (3.5 hrs) exceeded the available NH₄ content of the water

by over 4% at the GAR Station and by 30% at the GIM Station. It is assumed that after the primary NH_4 uptake, additional NO_3 may be uptaken into the algal cells. Pennock (1987) reported the inhibition of NO_3 uptake in the presence of NH_4 in concentrations above $2 \mu\text{M}$ and this suppression was not as universal as has been reported by other authors. As a consequence of the affinity of algae for NH_4 , no relationship was observed between NO_3 and chl-*a* concentration, whereas a clear reciprocal relationship existed between NH_4 and chl-*a* (Kappers 1980). The NH_4 uptake of *Microcystis aeruginosa* may be enhanced with increased NH_4 concentrations as this algal group has a high affinity for NH_4 . However, in this study, NH_4 preferable uptake against NO_3 was hardly discernible due to the fact that nitrogen uptake exceeded the NH_4 ambient concentration.

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