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Response of phytoplanktonic nitrogen utilization to the load of eutrophic Barguzin River water in the Barguzin Bay of Lake Baikal

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バイカル湖バルグジン湾におけるバルグジン富栄養河川水の流入負荷が植物プランクトンの窒素利用に与える影響

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Abstract

The response of the utilization ability of ammonia, nitrate and urea nitrogen by phytoplankton to the load of eutrophic river water in its watershed was examined in the Barguzin Bay of Lake Baikal. Concentrations of

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nitrogenous compounds were obviously higher at the inshore stations influenced strongly by the Barguzin River water than at the offshore stations, except for the urea concentration. Chlorophyll a amounts were fairy high at the inshore stations. Photosynthetic rates showed a distribution similar to that of chlorophyll a concentration. Photosynthetic activity was considerably higher at the inshore stations than that at the offshore stations.

The uptake rate of ammonia nitrogen was 1.8 to 9.4 mg N m^{-3} d^{-1}. An appreciable uptake rate of urea nitrogen (0.3 to 3.0 mg N m^{-3} d^{-1}) was also observed. The uptake rates of both nitrogenous compounds were found to be higher in the inshore area. This tendency was a remarkable for the nitrate uptake (7.6 and 6.9 mg N m^{-3} d^{-1} at the inshore stations but 0.00 to 0.06 mg N m^{-3} d^{-1} at the offshore ones). High nitrogen uptake activity was observed in the inshore area. The nitrogen uptake rates were ranked as ammonia > urea > nitrate in the offshore area, in contrast to ammonia > nitrate > urea in the inshore area. Ratios of dark to light values for the uptake rates of ammonia and urea nitrogen were high, but much lower for nitrate uptake. High ratios for these nitrogenous compounds were observed in the inshore waters. These seemed to suggest that the phytoplankton generally utilized these nitrogenous compounds as nitrogen sources more effectively in the day than at night, and that the phytoplankton in the inshore area had a pronounced ability to take up these nitrogenous compounds even at night. The present investigations indicated that the phytoplankton generally preferred the regenerated forms of nitrogenous compounds (ammonia and urea) than the new form (nitrate). The cycling time of ammonia and urea were shorter in both inshore and offshore areas, whereas the cycling of nitrate in the offshore area required much longer periods. The present results indicate that in the offshore area of Barguzin Bay ammonia and urea might serve as significant nitrogenous compounds for the preservation of phytoplankton growth, while conversely, nitrate seemed to be more important in the inshore area.

Key words: Nitrogen uptake, Phytoplankton, Nutrient response, Lake Baikal

摘　要

バイカル湖バルグジン湾において，集水域富栄養河川水の負荷が植物プランクトンによるアンモニア態窒素，硝酸態窒素および尿素態窒素の利用への応答を調べた．窒素化合物濃度は，バルグジン川の影響を受ける沿岸水域では沖域水域に比較して，尿素態窒素を除き，明らかに高かった．クロロフィル a 量と光合成速度は沿岸水域でかなり高かった．光合成活性も沖域水域に比べ沿岸水域の定点でかなり高かった．

アンモニア態窒素の取り込み速度は，1.8～9.4 mg N m^{-3} d^{-1} だった．ある程度の尿素態窒素の取り込み（0.3～3.0 mg N m^{-3} d^{-1}）が認められた．これら窒素化合物の取り込み速度は沿岸水域で高く，この傾向は硝酸態窒素の取り込みではさらに顕著で，沿岸水域で高く（7.6～6.9 mg N m^{-3} d^{-1}）沖域水域で低かった（0.00～0.06 mg N m^{-3} d^{-1}）．取り込み活性も沿岸水域で高かった．窒素化合物の取り込みは，沖域水域ではアンモニア態窒素，尿素態窒素そして硝酸態窒素，沿岸水域ではアンモニア態窒素，硝酸態窒素そして尿素態窒素の順であった．アンモニア態窒素と尿素態窒素の取り込み速度を暗条件の下で測定した値は明条件のそれと比較して大きく低下しなかったが，硝酸態窒素の取り込みの場合は，とくに沖域水域の暗条件下でかなり低かった．これらのことは，一般には，植物プランクトンは夜間より昼間に窒素化合物を利用するが，沿岸水域の植物プランクトンは夜間でさえも窒素化合物を取り込む能力を持っていることを示している．本調査結果は，普通，植物プランクトンは硝酸態窒素のような新生窒素化合物よりもアンモニア態窒素や尿素態窒素のような再生窒素化合物の利用を好むことを示している．水中のアンモニア態窒素と尿素態窒素の回転時間は沿岸・沖域水域ともに短いが，硝酸態窒素の回転時間は沖域水域でかなりの時間を要する．本研究結果は，植物プランクトン増殖を維持するための窒素源として，バルグジン湾の沖域水域ではアンモニア態窒素と尿素態窒素が重要な窒素化合物の役割を果たし，沿岸水域では硝酸態窒素も重要な窒素化合物に加わることを示している．

キーワード：窒素の取り込み，植物プランクトン，栄養塩応答，バイカル湖

Introduction

Lake Baikal has often been classified as an oligotrophic in character (Weiss et al., 1991). Such freshwater bodies also provide high biodiversity, which is supported by various biogeochemical cycling. In recent years, however, due to on-going anthropogenic development in their watershed and coastal areas, severe eutrophication has become a problem (Watanabe and Drucker, 1999).

Much information has been accumulated on the distribution of biogeochemical parameters in Lake Baikal (Votinsev et al., 1972; Nagata et al., 1994; Goldman et al., 1996; Watanabe and Drucker, 1999; Sorokovikova et al., 2000; Genkai-Kato et al., 2002; Yoshioka et al., 2002; Nakano et al., 2003; Yoshida et al., 2003; Katano et al., 2005, 2008a, 2008b; Ueno et al., 2005; Satoh et al., 2006; Kihira et al., 2008). Knowledge of the nitrogen utilization in such a large body of water as Lake Baikal, however, is quite limited, although the limitation of nitrogen for phytoplankton assemblages has already been determined (Watanabe and Drucker, 1999; Genkai-Kato et al., 2002; Satoh et al., 2006; Kihira et al., 2008).

One of the most significant biogeochemical processes in the euphotic layer of freshwater environments is their nitrogen productivity by phytoplankton assemblages. Information regarding the uptake of nitrogenous compounds in freshwater lakes has recently been investigated to elucidate its importance as a nitrogen source for phytoplankton growth and to appreciate its significance in the biogeochemical nitrogen cycle (Miyazaki et al., 1985; Whalen and Alexander, 1986; Mitamura and Saijo, 1986a, 1986b; Takamura et al., 1987; Binhe and Alexander, 1993; Mitamura et al., 1995, 2006; Presing et al., 1998, 2001, 2008; Mitamura, 2000). Such studies have demonstrated that the principal nitrogenous compounds that sustained the standing crop of phytoplankton were ammonia, urea and nitrate, and that phytoplankton preferentially utilized ammonia. Several investigators have reported that the regenerated forms of nitrogenous nutrients such as ammonia and urea play significant roles as nitrogen sources for phytoplankton assemblages (Mitamura, 1986a; Mitamura and Saijo, 1986a, 1986b; Gu et al., 1997; Presing et al., 2001; Mitamura et al., 2006), with the relative nitrogen uptake rates being ranked as ammonia > urea > nitrate in natural lakes (Mitamura and Saijo 1986a; Takahashi et al., 1995; Mitamura et al., 1995, 2006; Presing et al., 2001).

Knowledge of the nitrogen uptake by phytoplankton in freshwater bodies, however, is limited. To obtain further information on the biogeochemical nitrogen cycle in freshwater bodies as it relates to the response of the utilization ability by phytoplankton to the load of eutrophic river water in its watershed, the present study examined the uptake rates of ammonia, nitrate and urea nitrogen by phytoplankton populations in Barguzin Bay, located in the central basin of Lake Baikal.

Materials and methods

Investigation locales

Lake Baikal, located in central Siberia, Russia, is the deepest large lake in the world, covering an area of 31,500 km^2 with a maximum depth of 1,637 m (Kozhov, 1963). The present investigation was carried out in Barguzin Bay, which receives an inflow from the Barguzin River containing significant amounts of nutrients. The study was conducted during the cruise of the RV Vereshchagin of the Limnological Institute, a Siberian Branch of the Russian Academy of Sciences, Irkutsk. The Barguzin River has the third largest flux, i.e., 9% of the total inflow to Lake Baikal arises from its watershed (Tarasova et al., 2003). The waters in Barguzin Bay create a gradient of nutrient concentrations from the river-mouth area to the offshore one. The biogeochemical metabolisms can be expected to respond in relation to this gradient. As described below, the values of the physico-chemical parameters (water temperature and major ionic constituents) in the surface water of Barguzin Bay were influenced by the Barguzin River water. The investigation locales were chosen with a CTD monitoring system. Field investigations were made at six stations, Sta. 1 (53°25.6'N, 108°59.3'E), Sta. 2 (53°26.4'N, 108°58.9'E), Sta. 3 (53°28.5'N, 108°57.7'E), Sta. 4 (53°29.5'N, 108°50.4'E), Sta. 5 (53°28.4'N, 108°47.5'E) and Sta. 6 (53°24.6'N, 108°40.7'E), each of which showed different trophic characters from July 31 to August 1, 1999 (Fig. 1). Water samples at the respective stations were collected with a plastic pail from the surface layer.

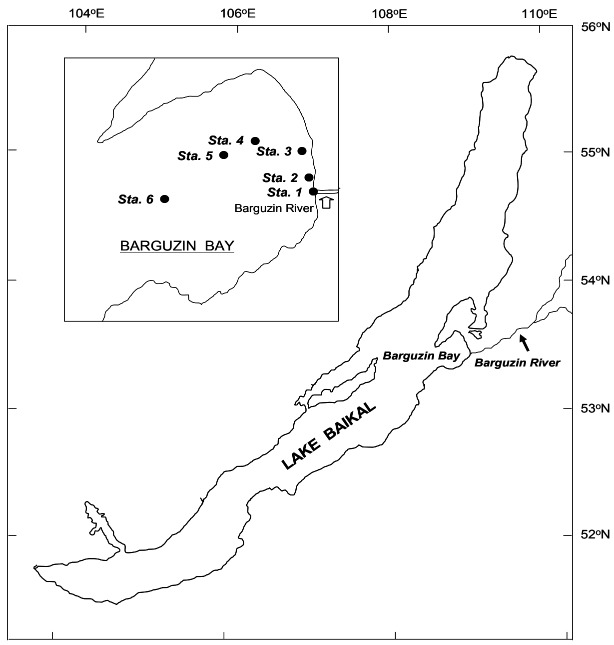

Fig. 1. Investigation stations in Barguzin Bay, Lake Baikal. Map is illustrated based on conic projection.

図1. バイカル湖バルグジン湾における観測定点. 地図は円錐図法で示す.

Measurements of nitrogen uptake

To measure the nitrogen uptake rates, water samples were poured into three series of clear plastic bottles. After adding either ^{15}N-labelled ammonia, nitrate or urea to each bottle, formaldehyde solution was immediately added to a series of control bottles. The second series of bottles was wrapped in a black sheet. The series of transparent and dark bottles were incubated in a water tank placed on the research vessel. The incubation temperature was approximately 20°C, similar to that in the surface water of the sampling stations. After incubating the bottles from 12:35 (approximately noon) to 20:35 (approximately sunset), the biological activity of phytoplankton was terminated by adding formaldehyde solution to each bottle. The weather condition on the incubation day was fine. Sample water in each bottle was filtered through a Whatman GF/F glass fiber filter which was purged of organic matter by ignition at 420°C. The ratio of ^{15}N to ^{14}N in the filter samples was determined using the technique of optical emission spectrometry with a ^{15}N analyzer (Jasco, model NIA-1) after combusting with calcium oxide and copper oxide in a capillary according to the micro-Dumas' method. In the present study, the in situ uptake rates of nitrogenous compounds were calculated using the Michelis-Menten equation, assuming that the rate was related to the concentration of each nitrogenous compound: $V=V_{max}\ S\ (Ks + S)^{-1}$, where V is the nitrogen uptake rate, S is the concentration of nitrogenous compounds, V_{max} is the nitrogen uptake rate at saturating levels of S, and Ks is the half-saturation constant at which $V=V_{max}\ 2^{-1}$. In the present investigations it was assumed that the Ks value was not influenced by irradiance (Anderson and Roels, 1981; Mitamura, 1986a). Nitrogen uptake was correlated with phytoplankton biomass as described below, suggesting that the uptake may have been due to the autotrophs. Heterotrophic bacteria, however, may also have contributed to the nitrogen uptake.

Photosynthetic carbon uptake

The in situ rate of photosynthetic carbon uptake was measured by the ^{14}C technique of Steemann Nielsen (1952), simultaneously with the in situ experiments for the nitrogen uptake measurements. Radioactivity on the filters was measured with a liquid scintillation spectrometer (Aloka LSC-651). The total CO_2 in the sample water was determined with an infrared carbon dioxide analyzer (Horiba VIA-510), as described by Satake et al. (1972).

Chemical analyses

Water temperature was measured on the research vessel with a conductivity meter (Yokogawa, SC-51). Measurements of transparency were taken with a Secchi disk. After filtration with a 0.2 μm membrane filter, the concentrations of major cations (sodium, potassium, magnesium and calcium) and anions (chloride and sulfate) in the sample water were analyzed by an ion chromatographic analyzer (Dionex DX-120). The concentration of bicarbonate was calculated from its alkalinity, assuming that the value was practically equal to the alkalinity, which was determined by acidimetric titration with 0.01 M sulfuric acid to the carbon dioxide end-point, pH 4.8.

For a determination of the concentrations in nutrients and particulate matter, the waters were immediately filtered through glass fiber filters (Whatman GF/F) which were purged of organic matter by ignition at 420°C. The filters and filtrates were then frozen solid until chemical analyses in the laboratory. Samples for the determination of silicate were passed through an Advantec No.5C paper filter and then refrigerated. Ammonia concentrations were determined by the method of Sagi (1966), nitrite after Bendschneider and Robinson (1952), nitrate after Mitamura (1997), Urea after of Newell et al. (1967), phosphate (DIP) after Murphy and Riley (1962) and silicate (DSi) by the method of Mullin and Riley (1955). Chlorophyll a (Chl.a) was determined with a Fluorometer (Turner Designs, 10-AU) according to Holm-Hansen et al. (1965). Particulate carbon (PC) and nitrogen (PN) were determined with a CHN Corder (Yanaco, MT-5).

Results and discussion

Chemical characteristics in Barguzin Bay

Temperatures in the surface waters ranged from 17 to 21°C. The values showed a decreasing tendency with the distance from the river-mouth Sta. 1 toward the offshore Sta. 6. Transparency measured with a Secchi Disk ranged from 0.7 m at Sta. 1 to 8.7 m at Sta. 6. Salinity was 97 to 106 mg L^{-1}, estimated from sum of the major cations (Na^+, Ka^+, Mg^{2+} and Ca^{2+}) and anions (Cl^-, SO_4^{2-} and HCO_3^-), showing a low value at the river-mouth Sta. 1. The concentrations of sodium, magnesium and chloride in the waters at the inshore Stas. 1 and 2, influenced by the inflow from the Barguzin River, were lower than those at the offshore Stas. 5 and 6. However, the concentrations of calcium and sulfate at the inshore stations were higher than at the offshore stations. Barguzin River water showed a high water temperature and low salinity, compared with those in the offshore water of Barguzin Bay. The distributions of these parameters indicated that the river water was swept away on the surface layer in Barguzin Bay, and was gradually mixed with lake water from the river-mouth to the offshore area. Based on the values of major cation and anion concentrations at six respective stations, the mixing rate was estimated from the dilution ratio of the river-mouth water (river-mouth Sta. 1) by the offshore water (offshore Sta. 6), although some portion of the river-mouth water was already mixed by the lake water in Barguzin Bay (Table 1).

Distribution of nitrogenous compounds

Distributions of nitrogenous compounds at the respective stations were listed in times higher than at the offshore stations. The concentrations of urea nitrogen showed no change among stations, and were comparable to or lower than that of ammonia nitrogen. The present investigation detected an appreciable level of urea nitrogen in the TNN was observed, i.e., 5 to 13% in TNN concentration.

Extremely low concentrations of DIP were observed at Stas. 3 to 6, ranging from 0.25 to 0.33 μg P L^{-1}, while on the contrary, an appreciable concentration was obtained at Stas. 1 and 2. The ratio of TNN to DIP displayed low values (9 and 12 of mass ratio) at the inshore Stas. 1 and 2, but high values (56 to 102) at the offshore stations compared with the Redfield ratio (Redfield, 1958). The DSi concentrations ranged from 0.27 to 3.3 mg Si L^{-1}. Based on the DSi concentrations and the TNN:DIP:DSi ratios, the present DSi levels seemed sufficient to serve as a silicon source for phytoplankton growth, even when the phytoplankton was mainly composed of diatoms. The concentrations of these nitrogenous, phosphorus and silicious nutrients at the inshore Stas. 1 and 2 were considerably higher than those at the other stations. The concentrations of TNN and DIP were abruptly reduced at Sta. 3 and exhibited low values at the offshore Stas.

Table 1. Concentrations of dissolved nitrogenous (TNN; sum of ammonia, nitrate and urea nitrogen), phosphorus (DIP) and silicious (DSi) nutrients and particulate carbon (PC) and chlorophyll a (Chl.a), and their ratios at the respective stations in Barguzin Bay. The mixing rate was estimated from the dilution ratio of river-mouth water (Sta. 1) by offshore water (Sta. 6), based on equivalent concentrations of major cations (Na, K, Mg and Ca) and anions (Cl, SO_4 and HCO_3) at the respective stations.

表 1．バルグジン湾の各定点における溶存全窒素（アンモニア態窒素＋硝酸態窒素＋尿素態窒素），リン酸態リン，ケイ酸態珪素，懸濁有機炭素およびクロロフィル a の分布と，各パラメーター間の比．混合率は定点 6 の沖域水による定点 1 の河口水の希釈率を，主要カチオン（Na，Na，Mg，Ca）とアニオン（Cl，SO_4，HCO_3）の当量現存量を基にして求めた．

	Sta. 1	Sta. 2	Sta. 3	Sta. 4	Sta. 5	Sta. 6
Mixing rate (%)	100	63	21	9	2	0
TNN (μg N L^{-1})	120	89	22	19	26	23
Ammonia (μg N L^{-1})	19	14	9	10	11	10
Nitrate (μg N L^{-1})	92	69	10	6	11	9
Urea (μg N L^{-1})	6.6	4.1	1.7	2.0	3.3	2.8
Nitrite (μg N L^{-1})	2.1	1.6	0.6	0.7	0.5	0.6
DIP (μg P L^{-1})	10.4	9.9	0.33	0.33	0.25	0.33
DSi (mg Si L^{-1})	3.3	2.3	0.94	0.33	0.31	0.27
TNN/DIP (mass ratio)	12	9	65	56	102	68
TNN/DSi (mass ratio)	36	40	23	56	84	85
DSi/DIP (mass ratio)	320	230	2800	990	1200	800
PC (mg C L^{-1})	1.27	0.97	0.29	0.24	0.30	0.29
Chl.a (μg chl.a L^{-1})	8.4	7.3	1.9	1.4	1.5	1.4
PC/Chl.a (mass ratio)	150	130	160	170	220	200

4, 5 and 6, suggesting that in Barguzin Bay the load of nutrients from the river water enhanced the primary productivity of phytoplankton, and that the nutrients were rapidly-consumed by the utilization of their nitrogen and phosphorus sources. Watanabe and Drucker (1999) indicated that nitrogenous nutrients were the limiting parameter to phytoplankton growth. Satoh et al. (2006) and Kihira et al. (2008) suggested the limiting nutrient to be phosphorus. Genkai-Kato et al. (2002), on the other hand, suggested that the phytoplankton populations were deficient in neither nitrogenous nor phosphorus nutrients. In the present investigation, the nitrogen and phosphorus levels and their ratio seemed to indicate that both nitrogen and phosphorus were the limiting nutrients for phytoplankton growth in the euphotic zone of the offshore area in Barguzin Bay in summer.

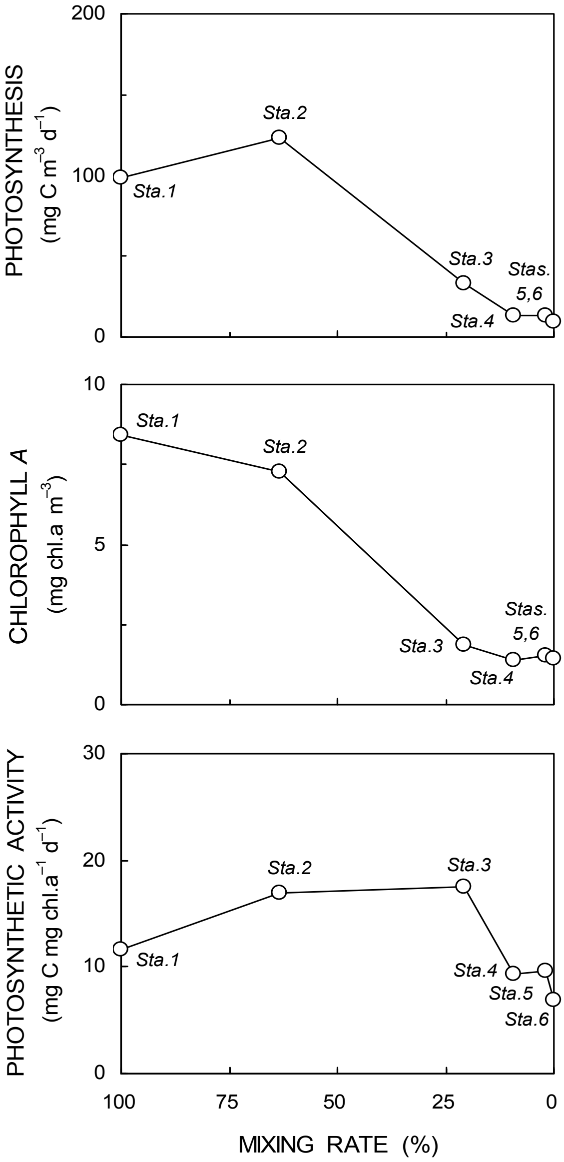

Fig. 2. Distributions in the photosynthetic rate, chlorophyll a concentration and photosynthetic activity at the respective stations. The figure illustrates the relationship between the values in the respective parameters and the mixing rate (dilution ratio of river-mouth water by offshore water).

図 2．各定点における光合成速度，クロロフィル a 現存量，光合成活性．図は各パラメーター値と混合率（河口水と沖域水との混合率）の関係で示す．

Phytoplankton biomass and photosynthetic activity

Chlorophyll a amounts ranged from 1.4 to 8.4 mg chl.a m^{-3} (Fig. 2). Fairly high values were observed at the inshore Sta. 1 and 2. There was a similar distribution in the concentrations of PC and PN. The ratio of PC to Chl.a showed relatively low values of 130 to 220 mg C mg chl.a^{-1} (Table 1), indicating that the waters of Barguzin Bay were characterized by phytoplanktonic particulate matter.

Photosynthetic rates, determined simultaneously during the nitrogen uptake experiments, ranged from 10 to 123 mg C m^{-3} d^{-1} (Fig. 2). The distribution pattern was similar to that of the Chl.a concentration. The present values in the offshore area were in the same range as those previously obtained in Lake Baikal (Kozhova, 1987; Back et al., 1991; Nagata et al., 1994; Yoshida et al., 2003). In the inshore area, however, much higher rates were obtained. Photosynthetic activity (i.e., photosynthetic rate per unit amount of Chl.a during one day) was considerably higher at the inshore Stas. 1, 2 and 3 (ranging from 11.7 to 17.5 mg C mg chl.a^{-1} d^{-1}) than those (6.9 to 9.7 mg C mg chl.a^{-1} d^{-1}) at the offshore Sta. 4, 5 and 6, showing a tendency similar to that in the distribution of the photosynthetic rate. The present activities were in the same range as those observed in natural lakes with a similar water temperature.

The extracellular product, determined simultaneously with the photosynthetic rates, ranged from 2 to 27 mg C m^{-3} d^{-1}. Their values were higher at the inshore and lower at the offshore stations. Their contribution to the total photosynthetic rate (sum of photosynthesis and extracellular product) was calculated to range from 12 to 22%. The percentages showed no appreciable differences among the stations. The distributions in these parameters (Chl.a, photosynthetic rate and photosynthetic activity) and the nutrient concentrations were related to each other, which seemed to indicate that the phytoplankton growth was enhanced by high levels of nutrient loads from the Barguzin River water, as described above.

Uptake rate of ammonia, nitrate and urea nitrogen

Figure 3 shows the distribution of daily rates of ammonia, nitrate and urea nitrogen uptake by microorganisms at the respective stations in Barguzin Bay as estimated from both light and dark values from noon to sunset. The nitrogen uptake rate in the present investigation might have included the bacterial uptake, though that portion could not be estimated from the experiments. The uptake rate in the present investigations was then presumed the nitrogen uptake by phytoplankton. The uptake rate of ammonia nitrogen was 1.8 to 9.4 mg N m^{-3} d^{-1}. An appreciable uptake rate of urea nitrogen was observed, i.e., 0.3 to 3.0 mg N m^{-3} d^{-1}. The uptake rates of both nitrogenous compounds were found to be higher in the inshore area influenced by the eutrophic Barguzin River water than in the offshore area. This revealed a marked tendency in the uptake rate of nitrate nitrogen, i.e., high rates of 7.6 and 6.9 mg N m^{-3} d^{-1} at Stas. 1 and 2, but negligible values of 0.00 to 0.06 mg N m^{-3} d^{-1} at the offshore stations.

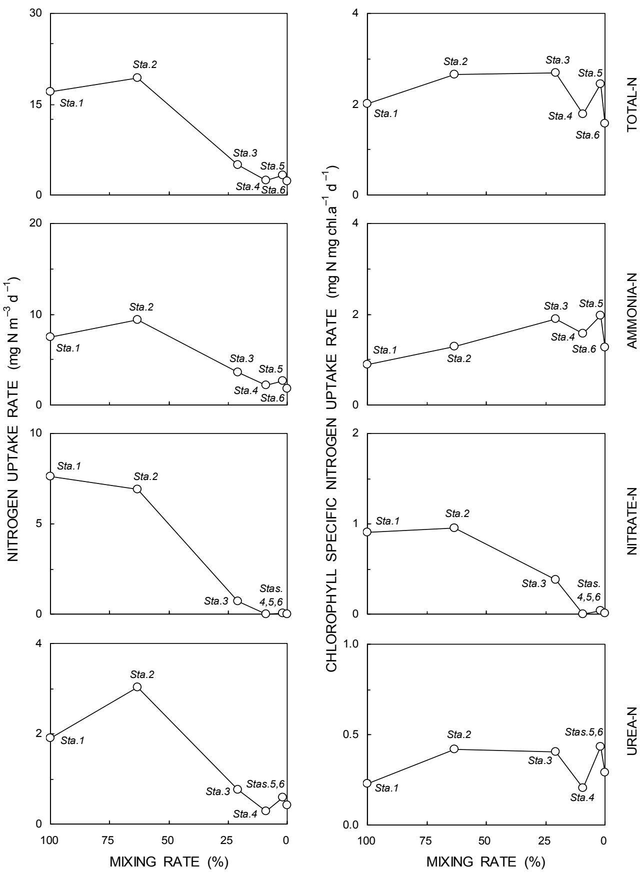

Fig. 3. Distributions in uptake rate of total (sum of ammonia, nitrate and urea), ammonia, nitrate and urea nitrogen, and their chlorophyll specific uptake rate.

図 3．アンモニア態窒素，硝酸態窒素，尿素態窒素およびその合計窒素の取り込み速度の分布と，それらの単位クロロフィル当りの取り込み速度．

The ratios of dark to light value, calculated as the rates in unit time during the incubation under both irradiance regimes, were 0.56 to 0.78 for ammonia uptake, 0.00 to 0.38 for nitrate uptake, and 0.48 to 0.74 for urea uptake (Table 2). Ratios were high in waters from the inshore stations, especially for the ratio of nitrate uptake. The ratios for ammonia and urea uptake were generally higher than those for nitrate uptake, a finding that agreed with the results of the response for the uptake rate of nitrogenous compounds to the irradiance reported by Mitamura (1986b). Mitamura and Saijo (1986a), Cochlan et al. (1991) and Mitamura (2000) reported that the light values were considerably higher than the dark values. On the other hand, Takahashi et al. (1995) observed no clear difference between daytime and nighttime values for ammonia, nitrate and urea nitrogen uptakes, suggesting that light intensity was an insignificant parameter for nitrogen uptake by phytoplankton. The present dark to light values were comparable to or higher than the former results but lower than those in the latter report. This suggested that the phytoplankton in the euphotic zone of Barguzin Bay more effectively utilized these nitrogenous compounds as nitrogen sources during the daytime rather than at night, and that the phytoplankton in the inshore area had a pronounced ability to

Table 2. Contributions of the daily uptake rates of ammonia, nitrate and urea nitrogen to total nitrogen (sum of ammonia, nitrate and urea nitrogen), and the dark to light ratios of respective nitrogen uptake rates from noon to sunset.

表 2．1 日当たりの全窒素取り込み速度に対するアンモニア態窒素，硝酸態窒素，尿素態窒素の寄与と，正中時から日没時間に暗条件下と明条件下で測定した各窒素化合物の窒素取り込み比．

Station	Contribution (%)			Dark/Light ratio		
	Ammonia	Nitrate	Urea	Ammonia	Nitrate	Urea
Sta. 1	44	45	11	0.78	0.31	0.67
Sta. 2	49	36	16	0.61	0.38	0.74
Sta. 3	77	11	12	0.59	0.28	0.52
Sta. 4	89	0	11	0.56	—	0.64
Sta. 5	81	2	18	0.73	0.16	0.55
Sta. 6	81	1	19	0.56	0.06	0.48

take up these nitrogenous compounds even at night.

The contribution of each nitrogenous compound as a nitrogen source for phytoplankton was estimated from the percentages in the uptake rates of ammonia, nitrate or urea nitrogen in the total nitrogen uptake rate (sum of ammonia, nitrate and urea nitrogen uptake rate) (Table 2). The contribution of ammonia was calculated to be 44 to 89% of the total nitrogen uptake rate. Nitrate, on the other hand, made only a minor contribution at the offshore stations (0.0 to 1.7%), whereas a high one at the inshore Stas. 1 to 3 (11 to 45%). In particular, the nitrate uptake in the dark made only a negligible contribution. In the present investigation, an appreciable contribution of 11 to 19% of urea nitrogen was observed. High contributions of urea were observed at the offshore Stas. 5 and 6. At the offshore stations, the range of the present percentages corresponded to those in the previous findings of the freshwater environments (Mitamura and Saijo, 1986a, Priscu et al., 1989, Takahashi et al., 1995, Mitamura et al., 1995). The contributions of each nitrogenous compound differed with each area. In the offshore area, a high contribution for ammonia was observed, in contrast to a negligible one for nitrate. In the inshore area under high concentrations of nitrate, the nitrate contribution rose. A similar tendency of the nitrate contribution to the nitrogen uptake was reported by Chang et al. (1995) in the upwelling region of New Zealand and by Twomey et al. (2005) in the California estuary of USA. Gu et al. (1997) found high contributions of ammonia and nitrate nitrogen in a eutrophic lake. Both present and previous results seem to indicate that phytoplankton assemblages are readily adaptable to circumstances such as rich concentrations of nitrate, and can actively utilize this nitrogen form. This suggests that the role of nitrogenous compounds as a nitrogen source for phytoplankton varied based on the phytoplankton's species composition and/or physiological requirements, as reported in previous studies (Eppley et al., 1969, Mitamura, 1986a, Presing et al., 1999, Fan et al., 2003).

Efficient utilization of regenerated nitrogen

As described above, the concentrations of ammonia and urea nitrogen were fairly low in the offshore area of Barguzin Bay where they efficiently utilized low concentrations of both ammonia and urea nitrogen as their nitrogen source. To evaluate the recycling of regenerated forms of nitrogenous compounds (ammonia and urea) and a new form (nitrate), as defined by Dugdale and Goering (1967), the turnover time of these compounds was estimated. The turnover times of each nitrogenous compound in a steady state can be expressed as the time necessary to utilize an amount of nitrogenous compound equivalent to the ambient concentration. The turnover times of ammonia were calculated to range from 1.5 to 5.6 days in the surface waters of Barguzin Bay (Table 3), while those for urea were from 1.3 to 7.4 days. At the inshore Stas. 1, 2 and 3, the turnover times of nitrate were from 10 to 14 days. At the offshore stations, much longer times were required, ranging from 200 to 550 days. The turnover times of the three nitrogenous compounds were shorter in the inshore than in the offshore area. The present turnover times in Barguzin Bay were comparable to or shorter than those obtained by previous studies (Mitamura and Saijo, 1986b, Mitamura et al., 1995, 2006). The present results indicate that, in the offshore area, the regenerated form of nitrogenous compounds (ammonia and urea) might be a significant factor in the preservation of phytoplankton growth. In the inshore area, on the other hand, the new form of nitrogenous compound (nitrate) as well as the regenerated form of nitrogenous compounds seemed to be equally important.

Residence times of particulate carbon and nitrogen were also estimated from the daily rate in the uptakes of the photosynthetic carbon or total nitrogen together with the

Table 3. Turnover times of ammonia, nitrate and urea calculated from the daily uptake rates of each nitrogenous compound and its ambient concentration, together with the residence times of particulate nitrogen (PN) and carbon (PC) estimated from daily primary nitrogen or carbon productivity and PN or PC amounts in the waters of Barguzin Bay.

表 3. バルグジン湾における各態窒素の 1 日当りの取り込み速度と現存量から推定したアンモニア態窒素，硝酸態窒素，尿素態窒素化合物の回転時間，ならびに 1 日当りの窒素と炭素の基礎生産と懸濁態窒素，と炭素現存量から推定した懸濁態窒素と炭素の滞留時間．

Station	Turnover time			Residence time	
	Ammonia	Nitrate (days)	Urea	PN	PC (days)
Sta. 1	2.6	12	3.5	8.6	12.9
Sta. 2	1.5	10	1.3	6.1	7.9
Sta. 3	1.9	14	2.2	5.5	9.0
Sta. 4	4.4	—	7.4	13.4	18.4
Sta. 5	4.1	200	5.6	13.5	23.2
Sta. 6	5.6	550	6.8	18.8	29.6

concentrations of PC or PN in the water, using the same manner of calculation in the turnover times of the nitrogenous compounds described above. As shown in Table 3, the residence times of PC and PN in Barguzin Bay were shorter in the inshore area but longer offshore.

Chlorophyll a specific carbon and nitrogen uptake rates

Specific nitrogen uptake rates using Chl.a as a cell parameter ranged from 1.6 to 2.7 mg N mg chl.a^{-1} d^{-1} (Fig. 3). A certain degree of nitrogen uptake activity by phytoplankton was observed in Barguzin Bay. Such activity was progressively higher in the inshore area than that in the offshore area. The present Chl.a specific nitrogen uptake rates at both inshore and offshore areas, keeping in mind the ammonia, nitrate and urea nitrogen uptakes, were in the same range as those obtained in freshwater bodies (Mitamura and Saijo, 1986a, McCarthy et al., 1982, Mitamura et al., 2006, Mitamura, 2000). The distribution pattern in the Chl.a specific nitrogen uptake rates was somewhat similar to the specific carbon uptake rates (i.e., photosynthetic activity). This seems to suggest that the carbon and nitrogen uptake rates were reflected in the specific growth rate of phytoplankton in the euphotic layer of Barguzin Bay.

Nitrogen uptakes related to phytoplankton biomass and their photosynthetic rates

The distribution of nitrogen uptake rates bore a striking resemblance to those of the Chl.a or photosynthetic rates. To clarify the relationship between the nitrogen uptake rate and the level of Chl.a amounts, the regression equations of the nitrogen uptake rate (N; mg N m^{-3} d^{-1}) against the Chl.a (C; mg chl.a m^{-3}) were calculated as $N = 2.3C + 0.3$, $R^2=0.72$, $p<0.005$, $n=6$, i.e., for ammonia: $N = 0.9C + 1.7$, $R^2=0.59$, $p<0.05$, for nitrate: $N = 1.1C - 1.5$, $R^2=0.79$, $p<0.005$ and for urea: $N = 0.3C + 0.1$, $R^2=0.55$, $p<0.05$. On the other hand, the regression equations of the nitrogen uptake rate (N; mg N m^{-3} d^{-1}) against the photosynthetic rate (P; mg C m^{-3} d^{-1}) were: $N = 0.16P + 1.0$, $R^2=0.81$, $p<0.005$, $n=6$ (for ammonia: $N = 0.06P + 1.8$, $R^2=0.76$, $p<0.005$, for nitrate: $N = 0.07P - 0.9$, $R^2=0.63$, $p<0.01$ and for urea: $N = 0.02P + 0.1$, $R^2=0.74$, $p<0.005$). The coefficients of determination between nitrogen uptake rates and the photosynthetic carbon uptake rate or Chl.a concentration evidenced a significant level, suggesting that the nitrogen uptake by phytoplankton in Barguzin Bay was associated with the standing crop of phytoplankton and their photosynthetic rate, as noted by Eppley et al. (1979).

It is well known that phytoplankton preferentially utilize ammonia as a nitrogen source. Urea nitrogen is their second choice, being preferred over nitrate (McCarthy et al., 1977, Mitamura and Saijo, 1986a, Takahashi et al., 1995, Mitamura et al., 1995, 2006, Presing et al., 2001). McCarthy and Eppley (1972) and Mitamura (1986b) indicated that the uptake rate of urea and nitrate nitrogen was suppressed at approximately 10 μg N L^{-1} of the ammonia concentration. For the large and deep oligotrophic Lake Baikal no information exists regarding the critical level of ammonia concentration needed for such a suppression in the uptake of either urea or nitrate nitrogen by phytoplankton. The variation in the ratio of the nitrogen uptake to the Chl.a or photosynthetic rate in the present study might be due to the difference in the utilizable activity of phytoplankton.

Productivity of nitrogen and carbon

The principal nitrogenous compounds which sustain the standing crop of natural phytoplankton are considered to be ammonia, nitrate and urea, as reported in several studies (McCarthy et al., 1977, Mitamura and Saijo, 1986a, Presing et al., 2001, Twomey et al., 2005, Mitamura et al., 2006). Natural phytoplankton assemblages can utilize the nitrite nitrogen and amino acids nitrogen, when the appreciable concentrations were existent in water (Wada and Hattori, 1971, Schell, 1971, Wheeler et al., 1977, Berg et al., 1997, Glibert et al., 2004, Zubkov and Tarran, 2005). In Barguzin Bay, the uptake of these nitrogenous compounds in the total nitrogen uptake might amount to only a negligible contribution. In the present investigation, therefore, the uptake of three nitrogenous compounds (ammonia, nitrate and urea) was considered to constitute the total nitrogen uptake. At the inshore Stas. 1 and 2, the total nitrogen uptake rates (sum of ammonia, nitrate and urea nitrogen uptake rates) by phytoplankton were 17.0 and 19.3 mg N m^{-3} d^{-1}., whereas at the offshore Stas. 4, 5 and 6, a low rate of 2.2 to 3.3 mg N m^{-3} d^{-1} was obtained (Table 4). The variations in the distribution of nitrogen uptake rates showed a similar tendency to those of the photosynthetic rate. The present values in the offshore area of Barguzin Bay were lower than those obtained by Mitamura and Saijo (1986a) and Mitamura et al. (2006) in Lake Biwa, Japan, Priscu et al. (1989) in Lakes Fryxell and

Table 4. Carbon and nitrogen productivity and its ratio in Barguzin Bay.

表 4．バルグジン湾における炭素生産と窒素生産，およびその比．

	Sta. 1	Sta. 2	Sta. 3	Sta. 4	Sta. 5	Sta. 6
Carbon production (mg C m^{-3} d^{-1})	98.4	123	32.7	12.8	13.2	9.7
Nitrogen production (mg N m^{-3} d^{-1})	17.0	19.3	6.5	2.4	3.3	2.2
C/N production ratio (mass ratio)	5.8	6.4	5.0	5.3	3.9	4.4
PC concentration (g C m^{-3})	1.27	0.97	0.29	0.24	0.30	0.29
PN concentration (mg N m^{-3})	146	117	36	33	45	42
PC/PN (mass ratio)	8.7	8.3	8.1	7.3	6.7	6.9

Vanda, Antarctica, Mitamura et al. (1995) in tropical lakes in Brazil.

In the pelagic area of lakes, the carbon to nitrogen ratio in particulate matter should be related to the carbon and nitrogen production by phytoplankton (Eppley et al., 1979). The ratio of the daily photosynthetic carbon uptake rate to the daily nitrogen uptake rate was calculated to evaluate the distribution of the particulate carbon to nitrogen ratio in the upper euphotic layer of Barguzin Bay. The ratio of primary carbon production (photosynthetic carbon uptake rate) to nitrogen production (total nitrogen uptake rate) in surface waters was calculated to range from 3.9 to 6.4 by weight (Table 4), thus showing lower values than the Redfield ratio (1958). The mass ratio of particulate carbon to nitrogen, on the other hand, ranged from 6.7 to 8.7. The carbon to nitrogen production ratios exhibited lower values than those of PC to PN, agreeing with those from previous investigations (Mitamura et al., 1995, 2006, Dauchez et al., 1995). The particulate organic carbon and nitrogen at the inshore Stas. 1, 2 and 3 might be influenced by allochthonous organic matter from the Barguzin River. The low ratio of PC to Chl.a and the high Chl.a specific carbon or nitrogen uptake rates, as described above, seemed to indicate that a low PC to PN ratio in particulate organic matters was maintained by active carbon and nitrogen production, even in the inshore areas.

Preferential utilization of regenerated forms of nitrogenous compounds

To evaluate the respective preference of phytoplankton for ammonia, nitrate and urea nitrogen, a Relative Preference Index (RPI) defined by McCarthy et al. (1977) was used. The RPI can be expressed as (e.g., RPI for ammonia): $RPINH_4$ = (NH_4 uptake / ΣN uptake) (NH_4 conc / ΣN conc)$^{-1}$. In the present study, $RPINH_4$ is the RPI for ammonia, NH_4 uptake is the uptake rate of ammonia nitrogen, ΣN uptake is the sum of ammonia, nitrate and urea nitrogen uptakes, NH_4 conc is the ambient concentration of ammonia, and ΣN conc is the sum of ammonia, nitrate and urea concentrations. RPI=1 indicates that the uptake is proportionate to its availability. RPI>1 indicates the preferential uptake, while RPI<1 denotes rejection. The RPI values for ammonia were calculated to range from 1.7 to 3.0, and those for urea ranged from 1.0 to 3.4, whereas the range for nitrate the low values of 0.0 to 0.6 was obtained. In the present investigation, the RPI for ammonia and urea showed high values that were always higher than unity, whereas, the RPI values for nitrate were less than unity. This suggests that ammonia and urea were usually preferred by phytoplankton, although an appreciable uptake rate of nitrate nitrogen was observed at the inshore stations. The RPI for the three nitrogenous compounds showed higher values at the inshore Stas. 1 and 2 than at the offshore Stas. 4, 5 and 6. That seems to indicate that the requirements of nitrogenous compounds as a nitrogen source for phytoplankton may vary among phytoplankton species (Eppley et al., 1969; Fan et al., 2003) and the species clone in a different growth environment (Carpenter and Guillard, 1971).

Summary

Phytoplankton in the offshore area of Barguzin Bay preferentially utilized ammonia and urea nitrogen. The regenerated form of nitrogenous compounds generally plays a significant role in phytoplankton growth. On the other hand, in the inshore area influenced by the nitrogen load from the Barguzin River, nitrate nitrogen as well as ammonia nitrogen was utilized, indicating that new form of nitrogenous compound makes an appreciable contribution as a nitrogen source for phytoplankton in the inshore area.

The present results indicate that biogeochemical nitrogen cycling and its pathways were strongly influenced by the nutrient loads from the eutrophic river water. To fully understand the response of nitrogen metabolism in the Lake Baikal ecosystem to the nutrient load from its watershed, further study is needed of such factors as the physiological state and specific growth rate of phytoplankton populations, the nutrients limitation for phytoplankton growth as well as

the influence of allochthonous input from the watersheds.

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