Article

The Significance of Regenerated Nitrogenous Compounds as a Nitrogen Source for Phytoplankton in the Whitewater of the Pre-Amazonian Floodplain in Brazil

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To obtain information on the biogeochemical nitrogen cycle in tropical turbid water bodies of the Turiacu and Pindare aquatic systems, a pre-Amazonian floodplain ecosystem in Baixada Maranhense, Brazil, uptake rates of ammonia, nitrate and urea nitrogen by phytoplankton were measured using a ^{15}N-labelled nitrogenous compound. Turbidity in the waters was very high, indicating that both aquatic systems were of the Whitewater type. Salinity in the Pindare waters was high, whereas those in the Turiacu were fairly low. Concentrations of ammonia, nitrate and urea nitrogen were relatively low in the two aquatic systems, indicating that in both systems the supply of nitrogenous compounds from their watersheds was at a low level, and/or microorganisms were rapidly consuming the compounds as a nitrogen source.

The uptake rates of ammonia nitrogen were 19 to 30 mg N m^{-3} d^{-1} in the Pindare and 36 to 56 N m^{-3} d^{-1} in the Turiacu waters, which exhibited close to the maximum rates. In the case of nitrate uptake, low rates of 0.0 to 2.5 mg N m^{-3} d^{-1} and 1.6 to 2.2 mg N m^{-3} d^{-1} were obtained. An appreciable uptake rate of urea nitrogen was observed, ranging from 3 to 11 mg N m^{-3} d^{-1} in the Pindare and 23 to 37 in the Turiacu aquatic systems. The contribution of ammonia to the total nitrogen uptake was high. Nitrates generally played a minor role as a nitrogen source. Urea was a mediating factor in the utilization between ammonia and nitrates. Ammonia and urea were usually preferred by phytoplankton. The ratio of dark to light values was high for ammonia and urea uptakes but low for nitrate uptake. Specific nitrogen uptake rates using chlorophyll a as a cell parameter ranged from 1.6 to 2.2 mg N mg chl. a^{-1} d^{-1} in the Pindare and 3.1 to 4.2 mg N mg chl. a^{-1} d^{-1} in the Turiacu. The chlorophyll-a-specific nitrogen uptake rates were somewhat similar to the photosynthetic carbon assimilation rates. Correlation coefficients were obtained at a statistically significant level between the nitrogen uptake rate and the chlorophyll a amount or photosynthetic rate. The nitrogen uptake rate may be primarily modified by the standing crop of phytoplankton and their photosynthetic activity. Much shorter turnover times of ammonia and urea were calculated. The present results suggest that the regenerated form of nitrogenous compounds was a significant factor in sustaining phytoplankton growth.

Keywords: nitrogen uptake, phytoplankton, turbid water, pre-Amazonian floodplain

1. Introduction

The limnology of the Amazon River and its floodplain ecosystems has been thoroughly researched (Fisher & Parsley 1979; Sioli 1984; Forsberg *et al.* 1988; Melack & Fisher 1990; Richey *et al.* 1990; Devol *et al.* 1995; Junk & Weber, 1996; Ibanez 1997; Junk 1997; Bozelli & Garrido 2000; Carneiro *et al.* 2002; Farjalla *et al.* 2002; Roland *et al.* 2002; Guenther & Bozull 2004). Freshwater bodies in the floodplain provide high levels of productivity and biodiversity, which are sustained by various biogeochemical cycling. We also have some data from rivers and lakes in the pre-Amazonian floodplain, Baixada Maranhense, Brazil (Reid & Turner 1988; Barbieri *et al.* 1989; Ibanez *et al.* 2000; Mitamura *et al.* 2009).

Much information regarding the uptake of nitrogenous compounds has been accumulated to elucidate their importance as a nitrogen source for phytoplankton growth and to foster appreciation for their significance in the nitrogen cycle (Miyazaki *et al.* 1985; Whalen & Alexander 1986; Mitamura & Saijo 1986a, 1986b; Takamura *et al.* 1987; Fisher *et al.* 1988; Binhe & Alexander 1993; Dickson & Wheeler 1995; Gu *et al.* 1997; Presing *et al.* 1998, 2001, 2008). The significant nitrogenous compounds that sustain the standing crop of phytoplankton were found to be ammonia, urea and nitrate, while the relative nitrogen uptake rates were identified as ammonia > urea > nitrate in temperate and tropical natural lakes, as demonstrated by Mitamura & Saijo (1986a), Takahashi *et al.* (1995), Mitamura *et al.* (1995) and Presing *et al.* (2001). Several studies have reported that the regenerated form of nitrogenous compounds plays an important role as a nitrogen source for phytoplankton assemblages (Mitamura 1986a; Mitamura & Saijo 1986b; Presing *et al.* 2001; Mitamura *et al.* 2006).

Knowledge of the nitrogen uptake by phytoplankton populations in tropical freshwater bodies, however, is limited (Fisher *et al.* 1988; Mitamura *et al.* 1995; Collos *et al.* 2001). To obtain further information on the biogeochemical nitrogen cycle in freshwater bodies, the present study set up to examine the uptake rates of ammonia, nitrate and urea nitrogen by phytoplankton in the Turiacu and Pindare systems, that differ in salinity and turbidity in a typical pre-Amazonian floodplain ecosystem located in the southern part of the Amazon River estuary.

2. Methods

2.1. Study areas

Baixada Maranhense, with an area of 18,000 km^2 (2°00'–4°00'S; 44°20'–45°30'W), has distinct dry and rainy seasons. This wetland area is subject to a seasonal flooding cycle with small-scale river water level fluctuations. During our investigation period, the aquatic systems were entering the rainy phase just after the dry season; water levels, therefore, were considerably low. Field investigations were carried out of extensive water bodies located in the middle-to-lower reaches of the Pindare and Turiacu Rivers in March 2002 (Figure 1).

Measurements of the nitrogen uptake were carried out on the surface waters at seven stations (water depth of 1.4–9.0 m) of the Pindare, and at five (2.5–4.7 m) of the Turiacu (Table 1). No appreciable differences were observed in the vertical distributions of the physico-chemical parameters (water temperature, electric conductivity, pH and turbidity) determined by a water quality meter at the respective investigation stations, indicating that water at those stations was well mixed vertically. Present investigation areas were lentic in character, exhibiting the geomorphometric property of a shallow lake located at the middle-lower reaches of the aquatic systems.

2.2. Chemical analyses

Water temperature, pH and electric conductivity were measured in the field on a research vessel equipped with a water quality monitoring system (YSI, model 33). Measurements of transparency were taken with a Secchi disk. Electric conductivity was equated to the value at 25°C. Water samples were collected from the surface layer

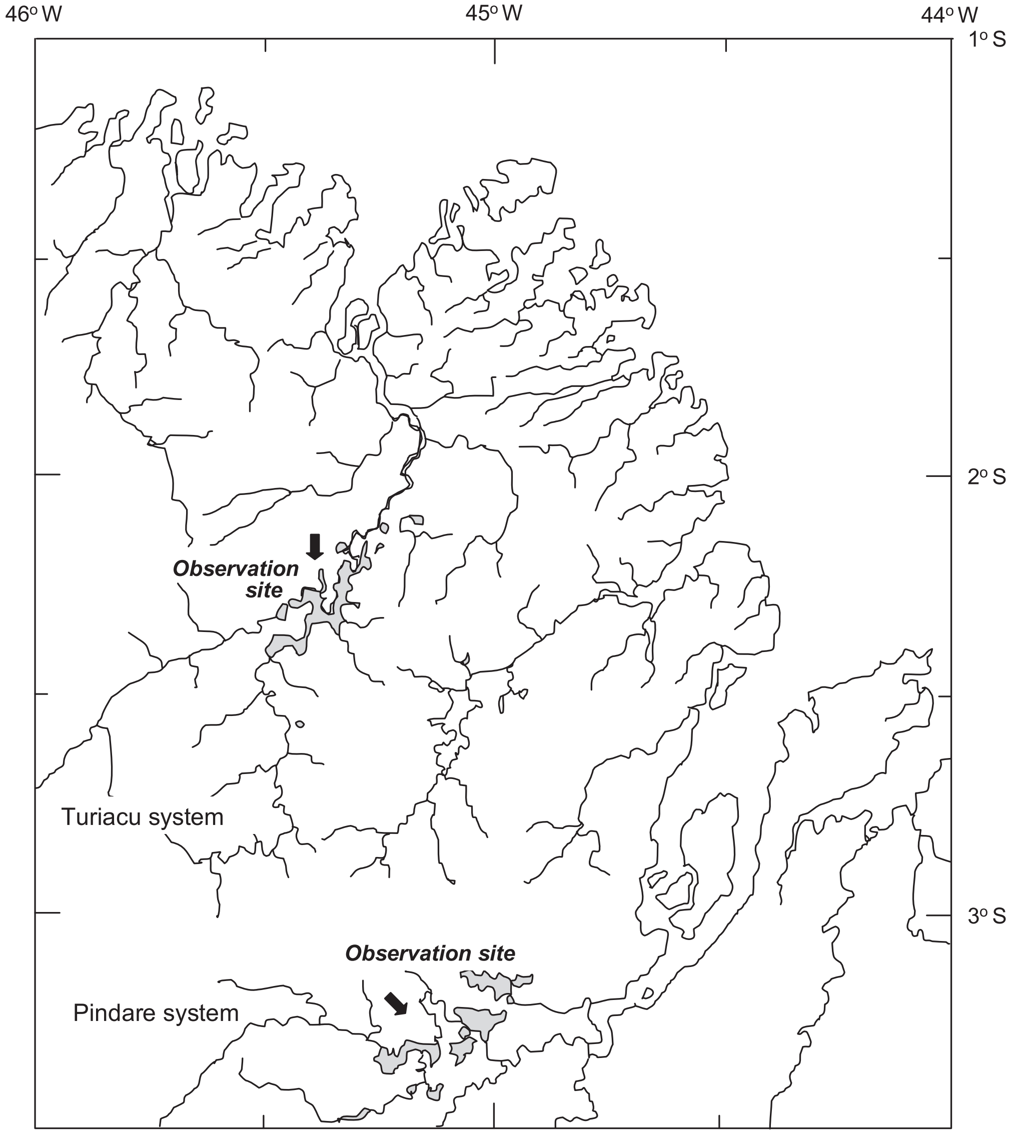

Figure 1 Map showing the investigation areas in the Pindare and Turiacu aquatic systems, Baixada Maranhense, Maranhao, Brazil.

(approximately 0.2 m depth) at respective stations with a plastic pail. The samples were then used for measurements of turbidity, which was measured in the laboratory using a Turbidmeter (Hach, 2100N). After filtration with a 0.2 µm membrane filter, the concentrations of six major elements (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}) were determined with an Ion Chromatograph (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 the determination of biogeochemical constituents, the waters were immediately filtered through glass fiber filters (Whatman GF/F) purged of organic matter by ignition at 420°C. Both the filters and filtrates were then frozen solid at –20°C until chemical analyses in the laboratory. Ammonia concentrations were determined by the method of Sagi (1966), nitrite after Bendschneider & Robinson (1952), nitrate after Mitamura (1997), urea after of Newell *et al.* (1967), and phosphate (DIP) after Murphy & Riley (1962). Chlorophyll *a* amounts were 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) after the samples were free of carbonate carbon using 1M HCl solution.

2.3. Measurement of nitrogen uptake

To measure 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 both transparent and dark bottles were incubated in a water tank located in the field. The incubation temperature was similar to that at the sampling stations. After incubating the bottles from sunrise to sunset, the biological activity of phytoplankton was terminated by adding formaldehyde solution to each bottle. The prevailing weather on respective incubation days was a pallid sky. Sample water in each bottle was filtered through a Whatman GF/F glass fiber filter purged of organic matter by ignition at 420°C. The ratio of ^{15}N to ^{14}N in the filter samples was determined by optical emission spectrometry with a ^{15}N analyzer (Jasco, model NIA-1) after combusting with calcium oxide and copper oxide in a capillary according the micro-Dumas' method. In the current experiments, as a final ^{15}N concentration, 2 μM of ^{15}N-labelled ammonium sulfate, 5 μM of sodium nitrate and 2 μM of urea were added to the water samples. The ^{15}N concentration in some samples was comparable to or greater than the amounts of ambient concentration of nitrogenous compounds. The present nitrogen uptake rate seemed to be only a rough estimation, since the detrital nitrogen was contained in particulate matter. In the case of nitrate uptakes, in addition, the rates might be rough due to the detection limit of the analytical method. Nitrogen uptake was correlated with phytoplankton biomass (see Figure 4), suggesting that the uptake might have been due to autotrophs. We found that the flinders of macrophytes supplied from the dense macrophyte zones in both aquatic systems may have contained a partial fraction of autotrophs, with heterotrophic bacteria possibly contributing to the nitrogen uptake.

Photosynthetic activity was measured by the ^{14}C technique of Steemann Nielsen (1952), simultaneously with experiments for the measurements of nitrogen uptake rates. 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).

3. Results and Discussion

3.1. Physico-chemical features

Temperature in the surface waters ranged from 27.4 to 31.6°C. The water exhibited acidic properties, showing pH values of 5.4 to 6.3 in the Pindare and 6.7 to 6.9 in the Turiacu aquatic systems. Electric conductivity revealed a considerable difference between the two aquatic systems, ranging from 22 to 28 mS m^{-1} in the Pindare and 4.5 to 4.8 mS m^{-1} in the Turiacu waters. Transparency measured with a Secchi Disk ranged from 0.1 to 0.2 m in the Pindare and 0.4 to 0.5 m in the Turiacu aquatic systems. Turbidity ranged from 82 to 107 NTU in the Pindare and 19 to 23 NTU in the Turiacu waters (Table 1). High turbidities in the Pindare and Turiacu waters, i.e., the Whitewater propounded by Sioli (1984), were in the same range as those in the Solimoes River during the low water season, as reported by Schmidt (1972). Salinity in the Pindare waters was 134 to 152 mg L^{-1}, as estimated from the sum of the six major ionic elements, while in the Turiacu it was fairly low (26 to 28 mg L^{-1}). The chemical characteristics of the waters differed between the two aquatic systems. The Turiacu waters were characterized by Na, HCO_3 and Cl ions, and the Pindare by Na and Cl ions. Thus, the major ionic constituents in the waters of both aquatic systems varied depending on the type of chemicals present.

Table 1 Turbidity and concentration of nitrogenous and phosphorus nutrients in the waters at respective stations in the Pindare and Turiacu systems.

Aquatic system	Station	(latitude, longitude)	Turbidity (NTU)	Ammonia	Nitrite	Nitrate	Urea	Phosphate (μg P L^{-1})
				(μg N L^{-1})				
Pindare	Sta. 1	(3° 19'31"S, 45° 00'21"W)	96	43	2.8	20	19	5.7
	Sta. 2	(3° 19'18"S, 45° 01'00"W)	94	29	2.3	16	17	4.0
	Sta. 3	(3° 13'42"S, 45° 00'57"W)	97	26	2.1	31	14	2.6
	Sta. 4	(3° 13'30"S, 44° 59'59"W)	107	24	2.7	30	13	3.0
	Sta. 5	(3° 15'50"S, 44° 59'44"W)	93	19	2.2	26	10	1.9
	Sta. 6	(3° 14'37"S, 45° 01'42"W)	82	13	2.9	23	12	2.4
	Sta. 7	(3° 14'09"S, 45° 03'29"W)	89	25	3.2	34	11	3.5
Turiacu	Sta. 1	(2° 17'44"S, 45° 22'59"W)	22	17	3.7	15	18	3.7
	Sta. 2	(2° 18'40"S, 45° 22'44"W)	19	14	3.3	12	11	2.7
	Sta. 3	(2° 18'57"S, 45° 20'35"W)	22	14	2.8	10	16	4.0
	Sta. 4	(2° 17'08"S, 45° 20'05"W)	21	8	3.0	13	9	3.2
	Sta. 5	(2° 15'50"S, 45° 19'24"W)	23	11	2.5	20	10	4.3

3.2. Distribution of nitrogenous compounds

Distributions of nitrogenous compounds in the respective water systems are listed in Table 1. Concentrations of total nitrogenous nutrients (TNN; as the sum of ammonia, nitrite, nitrate and urea nitrogen) were 51 to 84 μg N L^{-1} in the Pindare and 32 to 54 μg N L^{-1} in the Turiacu waters. In both aquatic systems, ammonia nitrogen ranged from 33 to 65 μg N L^{-1} (26 to 51% in TNN) and 8 to 17 μg N L^{-1} (24 to 34%), nitrate nitrogen from 16 to 34 μg N L^{-1} (24 to 46%) and 10 to 19 μg N L^{-1} (23 to 46%), and urea nitrogen from 10 to 19 μg N L^{-1} (15 to 27%) and 9 to 18 μg N L^{-1} (24 to 38%). Contributions of nitrite nitrogen to TNN were fairly low at 3 to 9%. These findings seem to indicate that in both aquatic systems the supply of nitrogenous compounds from their watersheds was low levels and/or the microorganisms consumed rapidly the compound as a nitrogen source. Urea nitrogen concentrations displayed no change among the respective stations in the two aquatic systems. The urea nitrogen showed amounts comparable to those of ammonia and nitrate nitrogen. In our investigations, an appreciable amount of urea nitrogen was observed in the TNN.

The DIP concentration in both aquatic systems was 1.9 to 5.7 μg P L^{-1} (Table 1). No appreciable change was observed at stations in either aquatic system. The ratio of TNN to DIP by weighs was calculated to be 10 to 31, showing a slightly higher value than that in the Redfield stoichiometric ratio (Redfield, 1958). Low concentrations of TNN and DIP and their ratios seemed to indicate that both nitrogen and phosphorus nutrient compounds were the limiting parameters for phytoplankton growth in the Pindare and Turiacu aquatic systems.

3.3. Standing crop of phytoplankton and its photosynthetic activity

As shown in Table 2, the respective concentrations of PC and PN were 8.5 to 15.0 mg C L^{-1} and 0.64 to 1.21 mg N L^{-1} in the Pindare and 3.7 to 7.2 mg C L^{-1} and 0.54 to 0.81 mg N L^{-1} in the Turiacu water systems. Chlorophyll *a* amounts were 13 to 20 mg chl.a m^{-3} and 18 to 24 mg chl.a m^{-3} in the Pindare and Turiacu waters, showing a distribution similar to those of PC and PN. This indicates that the standing crop of phytoplankton in both aquatic systems was high up to a certain level, though their photosynthetic activity might be low in the water column due to limited irradiance in the deeper layers resulting in turbidity. The ratio of PC concentration to turbidity or chlorophyll *a* amounts was calculated to elucidate the characteristics of particulate matter in both aquatic systems. The Pindare waters, with their low ratio of PC to turbidity and high ratio of PC to chlorophyll *a*, seemed to be characterized by low levels of organic carbon content and phytoplankton assemblages in the particulate matter. However, the Turiacu waters, with their high ratio of PC to turbidity and low ratio of PC to chlorophyll *a*, were the turbid water, including phytoplanktonic particulate

Table 2 Photosynthetic rate of phytoplankton and its activity, together with particulate chlorophyll *a*, organic carbon (PC) and nitrogen (PN) at respective stations in the Pindare and Turiacu waters.

Aquatic system	Station	Photosynthetic rate ($mg\ C\ m^{-3}\ d^{-1}$)	Photosynthetic activity ($mg\ C\ chl.a^{-1}\ d^{-1}$)	Chlorophyll *a* ($mg\ chl.a\ m^{-3}$)	PC ($g\ C\ m^{-3}$)	PN ($g\ N\ m^{-3}$)	PC/Chl.*a* (wt/wt)
Pindare	Sta. 1	233	11.4	20	15.0	1.21	739
	Sta. 2	163	11.0	15	11.7	0.81	794
	Sta. 3	158	10.0	16	12.2	0.87	770
	Sta. 4	215	12.4	17	15.7	1.09	904
	Sta. 5	163	9.9	16	12.3	0.95	746
	Sta. 6	105	8.1	13	8.5	0.71	660
	Sta. 7	165	11.7	14	10.3	0.64	730
Turiacu	Sta. 1	371	19.2	19	5.3	0.57	273
	Sta. 2	505	21.9	23	5.4	0.67	233
	Sta. 3	344	19.4	18	3.7	0.54	208
	Sta. 4	325	16.7	19	5.2	0.61	267
	Sta. 5	473	19.8	24	7.2	0.81	303

matter that might contain the flinders of macrophytes. Marlier (1967) and Sioli (1984) classified the Amazonian tropical waters into three types: Clearwater, Whitewater and Blackwater. They noted that the Whitewater is characteristic of a water system with a high concentration of suspended solid (SS) and low primary productivity, and often exhibits high concentrations of nutrients. The Pindare and Turiacu waters, as mentioned in the present study, are considered to be Whitewater systems.

Photosynthetic rates, determined simultaneously during our nitrogen uptake experiments, ranged from 105 to 233 mg C m^{-3} d^{-1} in the Pindare and 325 to 505 mg C m^{-3} d^{-1} in the Turiacu waters (Table 2). Photosynthetic activity (photosynthetic rate per unit amounts of chlorophyll *a* during one day) was fairly low, ranging from 8.1 to 12.4 mg C mg $chl.a^{-1}$ d^{-1} in the Pindare and 16.7 to 21.9 mg C mg $chl.a^{-1}$ d^{-1} in the Turiacu samples, with activity levels generally lower than those observed in natural clear lakes.

3.4. Uptake rates of ammonia, nitrate and urea nitrogen

Figure 2 shows the distribution of the uptake rates of ammonia, nitrate and urea nitrogen by microorganisms at respective stations in the two aquatic systems. The nitrogen uptake rate in the present investigations might have been affected by the uptake the macrophyte flinders and bacteria, though to what degree could not be estimated from our experiments. As described below, the nitrogen uptake rate was related to the standing crop of phytoplankton and their photosynthetic rate. Thus, the uptake rate in our investigations was presumed to be the nitrogen uptake by phytoplankton. The uptake rates of ammonia nitrogen were 19 to 30 mg N m^{-3} d^{-1} in the Pindare and 36 to 56 mg N m^{-3} d^{-1} in the Turiacu waters, which were approximately the maximum rates. In the case of nitrate uptake, low rates of 0.0 to 2.5 mg N m^{-3} d^{-1} and 1.6 to 2.2 mg N m^{-3} d^{-1} were obtained in the Pindare and Turiacu aquatic systems, respectively. An appreciable uptake rate of urea nitrogen was observed in both turbid waters, i.e., 3 to 11 mg N m^{-3} d^{-1} in the Pindare and 23 to 37 mg N m^{-3} d^{-1} in the Turiacu systems.

The ratios of dark to light value, calculated as the rates in time units during incubation under both irradiance regimes, were 0.82 to 0.94 and 0.68 to 0.79 for ammonia uptake, 0.00 to 0.65 and 0.13 to 0.36 for nitrate uptake, and 0.71 to 0.96 and 0.62 to 0.72 for urea uptake in the Pindare and Turiacu aquatic systems, respectively, with higher ratios in waters from the former. This suggests that the phytoplankton in both aquatic systems have the ability to take up nitrogen under twilight light intensity due to high turbidity. The ratios for ammonia and urea uptakes were generally higher than those for nitrate uptake, a finding that agreed with the results of the response of uptake rates of

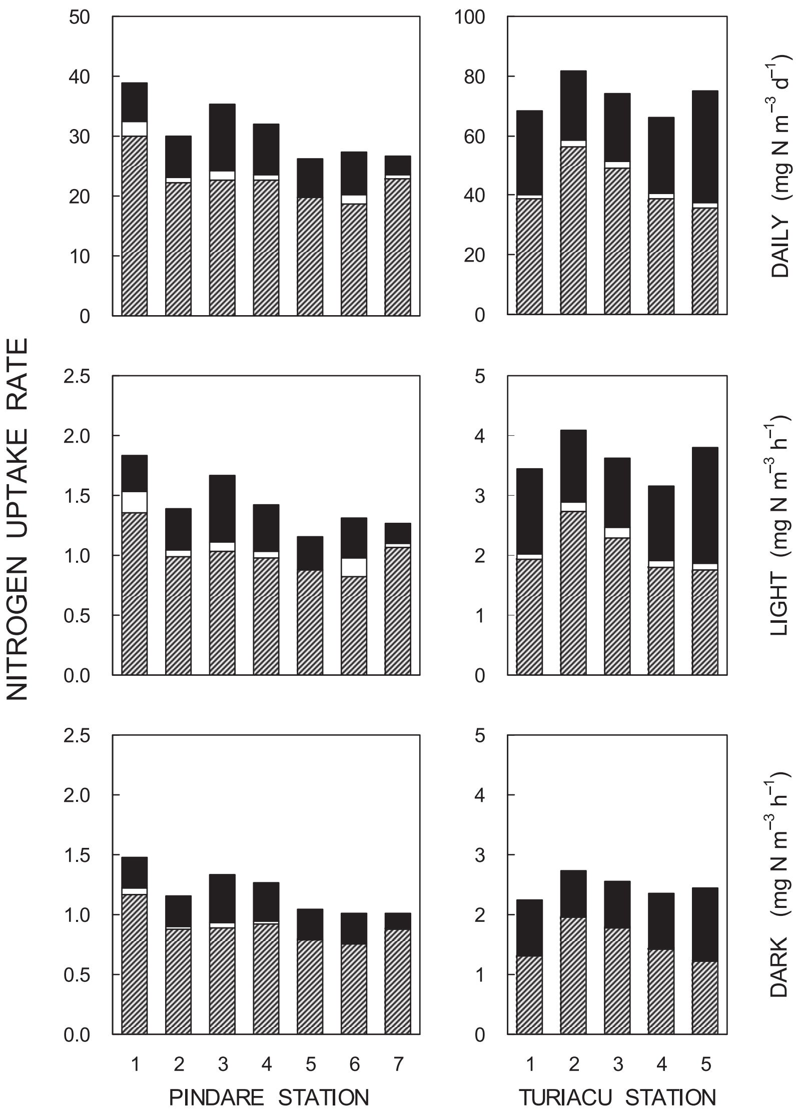

Figure 2 Distributions in uptake rates of ammonia (hatched column), nitrate (white column) and urea (black column) nitrogen at respective stations in the Pindare and Turiacu waters. The uptake rate under light and dark conditions indicates the values in units of time during the incubation under both irradiance regimes.

nitrogenous compounds to the irradiance reported by Mitamura (1986b). Fisher *et al.* (1988) also reported that the utilization of nitrate nitrogen by phytoplankton was strongly influenced by irradiance, but that the uptake of ammonia nitrogen was influenced less by light intensity in the tropical Lake Calado, Brazil. Mitamura & Saijo (1986a), Cochlan *et al.* (1991) and Mitamura (2000) reported that the light value in the nitrogen uptake rate was considerably higher than the dark uptake. On the other hand, Takahashi *et al.* (1995) observed no clear difference between daytime and nighttime values for ammonia, nitrate and urea nitrogen uptakes, and concluded that light intensity was an insignificant parameter for nitrogen uptake by phytoplankton. The present dark to light values were comparable to or higher than those of the above authors, but lower than that by Takahashi *et al.* (1995). This suggests that phytoplankton in the current two water systems effectively utilized these nitrogenous compounds as nitrogen sources more in the daytime than at night, particularly for the nitrate uptake, and that the contribution of nitrate nitrogen to the total nitrogen uptake diminished with depth due to the abrupt attenuation of light intensity in the turbid water.

The principal nitrogenous compounds which sustain the standing crop of phytoplankton populations in natural waters are thought to be ammonia, nitrate and urea, as reported in several studies (McCarthy *et al.* 1977; Mitamura & Saijo

1986a; Presing *et al.* 2001; Twomey *et al.* 2005; Mitamura *et al.* 2006), though nitrite and amino acids nitrogen are utilized by natural phytoplankton when appreciable concentrations are present in water (Wada & Hattori 1971; Schell 1971; Wheeler *et al.* 1977; Berg *et al.* 1997; Glibert *et al.* 2004; Zubkov & Tarran 2005). In the current aquatic systems, the roles of these nitrogenous compounds in the total nitrogen uptake might be somewhat less than those of ammonia, nitrate and urea nitrogen. Therefore, the nitrogen uptakes of the present three nitrogenous compounds were considered to be the total nitrogen uptake. The total nitrogen uptake rate (sum of ammonia, nitrate and urea nitrogen uptake rates) by phytoplankton ranged from 26 to 39 mg N m^{-3} d^{-1} in the Pindare and 66 to 82 mg N m^{-3} d^{-1} in the Turiacu waters. It was of considerable interest that the phytoplankton in both the Whitewater Pindare and Turiacu aquatic systems showed a ready ability to take up the nitrogen as a nitrogen source, though the values were lower than those reported by Mitamura & Saijo (1986a) in Lake Biwa, Priscu *et al.* (1989) in Lakes Fryxell and Vanda, and Mitamura *et al.* (1995) in Brazilian tropical lakes.

The contribution of ammonia to the total nitrogen uptake as a nitrogen source for phytoplankton was calculated to range from 64 to 86% (62 to 85% in the light and from 66 to 87% in the dark regimes) in the Pindare waters and 48 to 69% (46 to 67% and 50 to 72%) in the Turiacu waters. Nitrate, on the other hand, generally played a minor role as a nitrogen source, ranging from 0 to 6% (0 to 11% in the light, 0 to 4% in the dark). The contributions were greater at stations in the Pindare water, showing considerable fluctuations among them. Nitrate uptake in the dark made a particularly negligible contribution. An appreciable contribution of urea was observed, i.e. 12 to 30% (12 to 32% in the light, 11 to 29% in the dark) in the Pindare and 29 to 50% (29 to 51% in the light, 27 to 49% in the dark) in the Turiacu systems; major contributions were observed in the Turiacu waters. This seems to suggest that the role of each nitrogenous compound as a nitrogen source for phytoplankton varied by their species composition and/or by the physiological requirements of phytoplankton, as reported in several studies (Eppley *et al.* 1969; Mitamura 1986a; Presing *et al.* 1999; Fan *et al.* 2003). The present contribution of each nitrogenous compound was comparable to those in previous investigations of freshwater environments (Mitamura & Saijo 1986a; Priscu *et al.* 1989; Mitamura *et al.* 1995).

The specific nitrogen uptake rate, using chlorophyll *a* as a cell parameter, ranged from 1.6 to 2.2 mg N mg $chl.a^{-1}$ d^{-1} in the Pindare and 3.1 to 4.2 mg N mg $chl.a^{-1}$ d^{-1} in the Turiacu systems. The chlorophyll *a* specific nitrogen uptake rates in both the Pindare and Turiacu were in the same range as those obtained in natural lakes (Mitamura & Saijo 1986a; McCarthy *et al.* 1982). In the present Whitewaters systems, a certain level of nitrogen uptake activity of phytoplankton was observed. These specific nitrogen uptake rates were somewhat similar to the specific carbon uptake rates (i.e. photosynthetic activity), which seems to suggest that the carbon and nitrogen uptake rates were reflected in the specific growth rates of phytoplankton in the euphotic zone of both aquatic systems.

3.5. Cycling time of ammonia, nitrate and urea

In our investigations, the amounts of three ^{15}N-labelled nitrogenous compounds added to the water samples were comparable to or higher than those in the ambient concentration. Therefore, the uptake rates measured in the two aquatic systems were likely to show much higher values than those in the in situ rate. The turnover time 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, nitrate and urea were calculated from the daily nitrogen uptake rate of each nitrogenous compound and its concentration. Table 3 shows the distribution of turnover times of ammonia, nitrate and urea in the waters at respective stations in the two aquatic systems. The ranges of ammonia turnover times were 0.7 to 1.4 days and 0.2 to 0.4 days in the surface layers of Pindare and Turiacu waters, while the times for urea were 1.3 to 3.3 days and 0.3 to 0.7 days in both aquatic systems. The turnover of nitrate, on the other hand, required longer times than those of ammonia and urea. Such turnover times in the field

Table 3 Turnover times (day) of ammonia, nitrate and urea in the waters at respective stations in the Pindare and Turiacu systems.

Aquatic system	Station	Ammonia	Nitrate	Urea
Pindare	Sta. 1	1.42	7.9	2.84
	Sta. 2	1.29	21.1	2.40
	Sta. 3	1.13	20.8	1.28
	Sta. 4	1.06	35.0	1.54
	Sta. 5	0.97	–	1.60
	Sta. 6	0.69	15.2	1.66
	Sta. 7	1.09	60.3	3.33
Turiacu	Sta. 1	0.44	9.7	0.64
	Sta. 2	0.24	5.5	0.49
	Sta. 3	0.28	4.4	0.69
	Sta. 4	0.20	7.2	0.33
	Sta. 5	0.29	11.0	0.28

might require longer periods than our present estimation, due to the calculations for the nearly maximum rate of nitrogen uptake. The present turnover times in both Whitewater systems were comparable to or shorter than those obtained in previous studies (Mitamura *et al.* 1995; Mitamura 2000). Our results indicate that the regenerated form of nitrogenous compounds (ammonia and urea) might be a significant factor in sustaining the phytoplankton growth.

3.6. Preference of phytoplankton for 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): RPI_{NH_4} = (NH_4 uptake / ΣN uptake) (NH_4 conc / ΣN conc)$^{-1}$. In the present study, RPI_{NH_4} is the RPI for ammonia, NH_4 uptake is the uptake rate of ammonia nitrogen, ΣN uptake is the sum of the 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. As shown in Figure 3, the RPI values for ammonia in two aquatic systems were calculated to range from 1.5 to 2.5, while those for urea were 0.7 to 1.9. However, the range for nitrate was extremely low RPI value (0.0 to 0.3). The RPI values for ammonia and urea were generally high, and those for ammonia were always higher than unity, whereas those for nitrate were less than unity. In the present study, the RPI for nitrate was never greater than that for ammonia or urea nitrogen, suggesting that ammonia and urea were usually preferred by phytoplankton, though the requirement of nitrogenous compounds as a nitrogen source for phytoplankton may vary by the species of phytoplankton, the growth environment, or physiological requirements.

3.7. Nitrogen uptake related to phytoplankton biomass and its photosynthetic rate

The distribution of nitrogen uptake rates bore a striking resemblance to those of the chlorophyll *a* amount or the photosynthetic rate. Data were plotted to clarify the relationship between the nitrogen uptake rate and the chlorophyll *a* amount or photosynthetic rate (Figure 4). The linear regression equations of the nitrogen uptake rate (N; mg N m^{-3} d^{-1}) against chlorophyll *a* (C; mg chl.a m^{-3}) were: N=5.2C–45, R^2=0.64, $p<0.005$ (N=1.5C+7, R^2=0.57, $p<0.05$ in the Pindare, and N=1.4C+45, R^2=0.35, $p<0.5$ in the Turiacu system). On the other hand, 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+6, R^2=0.91, $p<0.001$ (N=0.076P+18, R^2=0.44, $p<0.1$ in the Pindare, and N=0.063P+48, R^2=0.69, $p<0.05$ in the Turiacu system). In both aquatic

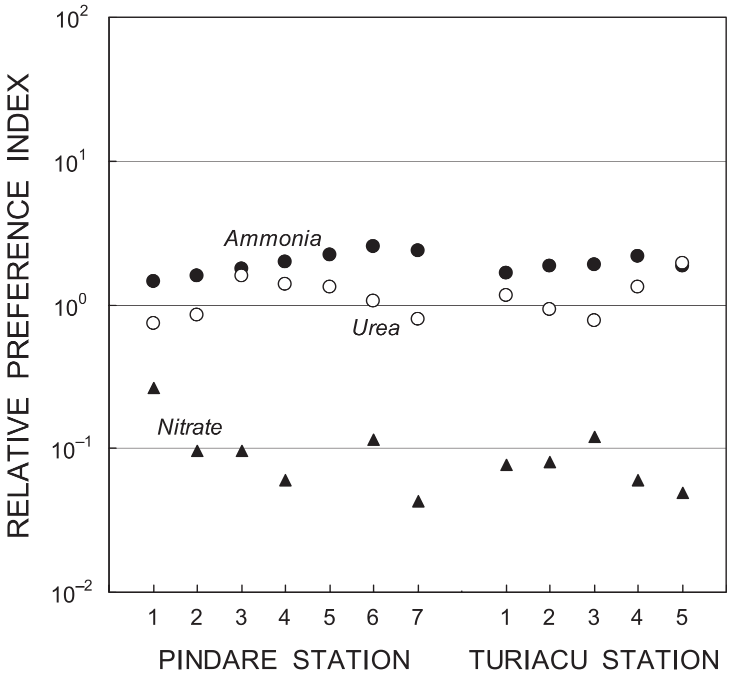

Figure 3 Relative Preference Index (RPI) for uptakes of ammonia, nitrate and urea nitrogen. Filled circles, open circles, and filled triangles indicate the RPI values for ammonia, urea and nitrate uptakes, respectively.

systems, the correlation coefficients at a statistically significant level indicate that the nitrogen uptake rate could be modified primarily by 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, followed by nitrate (McCarthy *et al.* 1977; Mitamura & Saijo 1986a, Takahashi *et al.* 1995; Mitamura *et al.* 1995, 2006; Presing *et al.* 2001). McCarthy & Eppley (1972) and Mitamura (1986b) indicated that the uptake rates of urea and nitrate nitrogen were suppressed at approximately 10 μg N L^{-1} of the ammonia concentration. No information was available regarding the critical level of ammonia concentration needed for the suppression of both nitrogenous nutrients for phytoplankton in the Whitewater of the Amazon River and its neighboring aquatic system. The preference for nitrogenous compounds might differ among phytoplankton species (Eppley *et al.* 1969; Fan *et al.* 2003) and among the species clones in a different growth environment (Carpenter & Guillard 1971). This suggests that the present variation in the ratios of urea or nitrate nitrogen uptake to chlorophyll *a* or photosynthesis accounted for the difference in the utilizable activity of phytoplankton.

The ratio of primary carbon production (daily photosynthetic rate) to nitrogen production (daily total nitrogen uptake rate) was calculated to be 3.8 to 6.7 by weight in the Pindare and 4.6 to 6.3 in the Turiacu waters, which was similar to those reported in previous findings (Mitamura *et al.* 1995, 2006; Dauchez *et al.* 1995). The carbon to nitrogen ratios in particulate matter, on the other hand, ranged from 12.0 to 16.0 and 6.8 to 9.2 in both aquatic systems. The Pindare waters with their high turbidity showed high PC:PN ratios, compared with the Redfield stoichiometric ratio (Redfield 1958). The carbon to nitrogen production ratios showed values lower than those of PC to PN. In natural lakes, the production ratio and particulate ratio should be equal in the euphotic zone. The nitrogen production in the current study showed approximately the maximum rates. Therefore, the production ratios in the field must be close to the particulate ratios. The particulate organic carbon and nitrogen in the current Whitewaters may be affected by allochthonous organic materials from its watershed. The high ratios of PC to chlorophyll *a* and low chlorophyll *a* specific

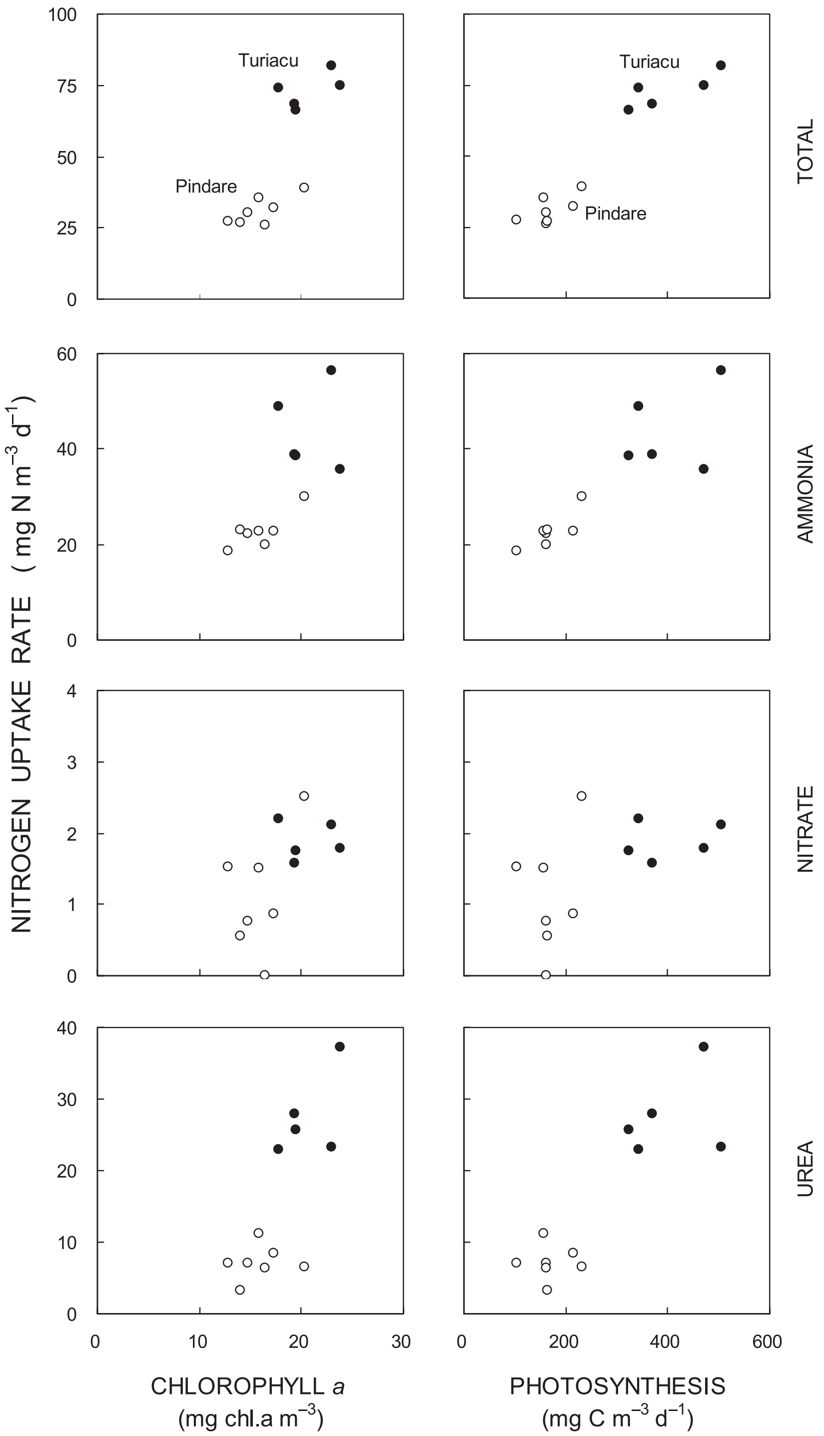

Figure 4 The relationship between the nitrogen uptake rate (total, ammonia, nitrate and urea nitrogen) and the chlorophyll *a* amount or photosynthetic rate. Open and filled circles indicate the Pindare and Turiacu waters, respectively.

carbon or nitrogen uptake rates, as described above, demonstrate that phytoplankton assemblages are among one of the major constituents of particulate organic matter. In our present investigations, the uptake rates of carbon and nitrogen were measured for surface waters. The turbidity in the Pindare and Turiacu water systems was extremely high. The compensation depth in both water systems was roughly estimated as 0.5 m and 1 m, based on the values of transparency, indicating that the areal primary productivity of carbon and nitrogen in the Whitewater aquatic system was considerably low. Those current results seem to suggest that the contribution of regenerated forms of nitrogen (ammonia and urea nitrogen) as a nitrogen source for phytoplankton increased in the lower layer, while in contrast, the role of new form of nitrogen (nitrate nitrogen) decreased with depth. It is interesting to note that any type of productivity served to maintain the high chlorophyll *a* amounts in the Whitewater.

In summary, phytoplankton in the Whitewater of the pre-Amazonian floodplain were found to preferentially utilize ammonia and urea nitrogen. The regenerated nitrogenous compounds generally play significant roles as nitrogen sources for phytoplankton growth. The present results indicate that, in terms of nitrogen cycling, the Whitewater ecosystems were rich in diversity in the pre-Amazonian floodplain. To fully understand the nitrogen metabolism in that floodplain, further study of such factors as the physiological state and specific growth rate of phytoplankton, the contribution of dense macrophytes assemblages to the nitrogen cycling, as well as the influences of allochthonous detritus from the watersheds must be incorporated into the investigations.

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References

Binhe, G. & V. Alexander. 1993. Seasonal variations in dissolved inorganic nitrogen utilization in a subarctic Alaskan lake. Arch. Hydrobiol., 126: 273–288.

Barbieri, R., M. S. R. Ibanez, F. J. Aranha, M. M. F. Correia, J. W. Reid & P. Turner. 1989. Plankton, primary production and some physico-chemical factors of two lakes from Baixada Maranhense. Rev. Brasil. Biol., 49: 399–408.

Bendschneider, K. & R. J. Robinson. 1952. A new spectrophotometric method for the determination of nitrite in sea water. J. Mar. Res., 11: 87–96.

Berg, G. M., P. M. Glibert, M. W. Lomas & M. A. Burford. 1997. Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. Mar. Biol., 129: 377–387.

Bozelli, R. L. & A. V. Garrido. 2000. Gradient of inorganic turbidity and responses of planktonic communities in an Amazonian lake, Brazil. Verh. Internat. Verein. Limnol., 27: 147–151.

Carneiro, L. S., R. L. Bozelli & F. A. Esteves. 2002. Long-term changes in the density of the copepod community in an Amazonian lake impacted by bauxite tailings. Amazoniana, 17: 553–566.

Carpenter, E. J. & R. R. L. Guillard. 1971. Intraspecific differences in nitrate half-saturation constants for three species of marine phytoplankton. Ecology, 52: 183–185.

Cochlan, W. P., P. J. Harrison & K. L. Denman. 1991. Diel periodicity of nitrogen uptake by marine-phytoplankton in nitrate-rich environments. Limnol. Oceanogr., 36: 1689–1700.

Collos, Y., A. Vaquer, A. M. Johnston, V. Pons, B. Bibent & S. Richard. 2001. Carbon fixation, ammonium uptake and regeneration in an equatorial lake: Biological versus physical control. J. Plankton Res., 23: 263–270.

Dauchez, S., L. Legendre & L. Fortier. 1995. Assessment of simultaneous uptake of nitrogenous nutrients (^{15}N) and inorganic carbon (^{13}C) by natural phytoplankton populations. Mar. Biol., 123: 651–666.

Devol, A. H., B. R. Forsberg, J. E. Richey & T. P. Pimentel. 1995. Seasonal variation in chemical distributions in the Amazon (Solimoes) River: A multiyear time series. Global Biogeochemical Cycles, 9: 307–328.

Dickson, M. & P. A. Wheeler. 1995. Nitrate uptake rates in a coastal upwelling regime: A comparison of PN-specific, absolute, and Chl. a-specific rates. Limnol. Oceanogr., 40: 533–543.

Eppley, R. W., E. H. Renger, W. G. Harrison & J. J. Cullen. 1979. Ammonium distribution in southern California coastal waters and its role in the growth of phytoplankton. Limnol. Oceanogr., 24: 495–509.

Eppley, R. W., J. N. Rogers & J. J. McCarthy. 1969. Half-saturation constant for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr., 14: 912–920.

Fan, C., P. M. Glibert, J. Alexander & M. W. Lomas. 2003. Characterization of urease activity I three marine phytoplankton species, *Aureococcus anophagefferens, Prorocentrum minimum,* and *Talassiosira weissflogi*. Mar. Biol., 142: 949–958.

Farjalla, V. F., F. A. Esteves, R. L. Bozelli & F. Roland. 2002. Nutrient limitation of bacterial production in clear water Amazonian

ecosystem. Hydrobiologia, 489: 197–205.
Fisher, T. R., K. M. Morrissey, P. R. Carlson, L. F. Alves & J. M. Melack. 1988. Nitrate and ammonium uptake by plankton in an Amazon River floodplain lake. J. Plankton Res., 10: 7–29.
Fisher, T. R. & P. E. Parsley. 1979. Amazon lakes: water storage and nutrient stripping by algae. Limnol. Oceanogr., 24: 74–92.
Forsberg, B. R., A. H. Devol, J. E. Richey, L. A. Martinelli & H. Dos Santos. 1988. Factors controlling nutrient concentrations in Amazon floodplain lakes. Limnol. Oceanogr., 33: 41–56.
Glibert, P. M., C. A. Heil, D. Hollander, M. Revilla, A. Hoare, J. Alexander & S. Murasko. 2004. Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. Mar. Ecol. Prog. Ser., 280: 73–83.
Gu, B. H., K. E. Havens, C. L. Schelske & B. H. Rosen. 1997. Uptake of dissolved nitrogen by phytoplankton in a eutrophic subtropical lake. J. Plankton Res., 19: 759–770.
Guenther, M. & R. Bozull. 2004. Factors influencing algae-clay aggregation. Hydrobiologia, 523: 217–223.
Holm-Hansen, O., C. J. Lorenzen, R. W. Holmes & J. D. H. Strickland. 1965. Fluorometric determination of chlorophyll. J. Cons. perm. int. Explor. Mer, 30: 3–15.
Ibanez, M. S. R. 1997. Phytoplankton composition and abundance of a central Amazonian floodplain lake. Hydrobiologia, 362: 79–83.
Ibanez, M. S. R., P. R. S. Cavalcante, J. P. Costa Neto, R. Barbieri, J. P. Pontes, S. C. C. Santana, C. L. M. Serra, N. Nakamoto & O. Mitamura. 2000. Limnological characteristics of three aquatic system of the pre-Amazonian floodplain, Baixada Maranhense（Maranhao, Brazil）. Aquatic Ecosystem Health and Management, 3: 521–531.
Junk, W. J. 1997. The central Amazon floodplain, Ecology of a pulsing system. Springer: 523pp.
Junk, W. J. & G. E. Weber. 1996. Amazonian floodplains: a limnological perspective. Verh. Internat. Verein. Limnol., 26: 149–157.
Marlier, G. 1967. Ecological studies of some lakes of the Amazon Valley. Amazoniana, 1: 91–115.
McCarthy, J. J. & R. W. Eppley. 1972. A comparison of chemical, isotopic, and enzymatic methods for measuring in nitrogen assimilation of marine phytoplankton. Limnol. Oceanogr., 17: 371–382.
McCarthy, J. J., W. R. Taylor & J. L. Taft. 1977. Nitrogenous nutrition of the plankton in Chesapeake Bay. I. Nutrient availability and plankton preferences. Limnol. Oceanogr., 22: 996–1001.
McCarthy, J. J., D. Wynne & T. Berman. 1982. The uptake of dissolved nitrogenous nutrients by Lake Kinneret（Israel）microplankton. Limnol. Oceanogr., 27: 673–680.
Melack, J. M. & T. R, Fisher. 1990. Comparative limnology of tropical floodplain lakes with an emphasis on the central Amazon. Acta Limnol. Bras., 3: 1–48.
Mitamura, O. 1986a. Urea metabolism and its significance in the nitrogen cycle in the euphotic layer of Lake Biwa. II. Half-saturation constant for nitrogen assimilation by fractionated phytoplankton in different trophic areas. Arch. Hydrobiol., 107: 167–182.
Mitamura, O. 1986b. Urea metabolism and its significance in the nitrogen cycle in the euphotic layer of Lake Biwa. III. Influence of the environmental parameters on the response of nitrogen assimilation. Arch. Hydrobiol., 107: 167–182.
Mitamura, O. 1997. An improved method for the determination of nitrate in freshwaters based on hydrazinium reduction. Mem. Osaka Kyoiku Univ. Ser. III, 45: 297–303.
Mitamura, O. 2000. Nitrogen uptake by pond phytoplankton in riverbed of the Yodo River System, Japan. Verh. Internat. Verein. Limnol., 27: 2922–2928.
Mitamura, O., H. Maeda, Y. Seike, K. Kondo, N. Goto & T. Kodama. 2006. Seasonal changes of carbon and nitrogen productivity in the north basin of Lake Biwa, Japan. Verh. Internat. Verein. Limnol., 29: 1913–1920.
Mitamura, O., N. Nakamoto, M. R. S. Ibanez, J. P. Costa Neto & R. Barbieri. 2009. Grain size distribution of particulate matter and sediment in extensive freshwater bodies in pre-Amazonian floodplain, Baixada Maranhense, Brazil. Verh. Internat. Verein. Limnol., 30: 964–970.
Mitamura, O. & Y. Saijo. 1986a. Urea metabolism and its significance in the nitrogen cycle in the euphotic layer of Lake Biwa. I. In situ measurement of nitrogen assimilation and urea decomposition. Arch. Hydrobiol., 107: 23–52.
Mitamura, O. & Y. Saijo. 1986b. Urea metabolism and its significance in the nitrogen cycle in the euphotic layer of Lake Biwa. IV. Regeneration of urea and ammonia. Arch. Hydrobiol., 107: 425–440.
Mitamura, O., Y. Saijo, K. Hino & F. A. R. Barbosa. 1995. The significance of regenerated nitrogen for phytoplankton productivity in the Rio Doce Valley Lakes, Brazil. Arch. Hydrobiol., 134: 179–194.
Miyazaki, T., Y. Honjo & S. Ichimura. 1985. Applicability of the stable isotope method using ^{13}C and ^{15}N simultaneously to the estimation of carbon and nitrogen assimilation in a eutrophic, freshwater lake, Lake Nakanuma, Japan. Arch. Hydrobiol., 102: 355–365.
Murphy, J. & J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta, 27: 31–36.
Newell, B. S., B. Morgan & J. Cundy. 1967. The determination of urea in seawater. J. Mar. Res., 25: 201–202.
Presing, M., S. Herodek, T. Preston & L. Voros. 1998. The significance of various nitrogen sources for phytoplankton growth in shallow lake and hypertrophic reservoirs. Internat. Rev. Hydrobiol., 83: 361–368.
Presing, M., S. Herodek, L. Voros, T. Preston & G. Abrusan. 1999. Nitrogen uptake by summer phytoplankton in Lake Balaton. Arch. Hydrobiol., 145: 93–110.
Presing, M., T. Preston, A. Takatsy, P. Sprober, A. W. Kovacs, L. Voros, G. Kenesi & I. Kober. 2008. Phytoplankton nitrogen demand and significance of internal and external nitrogen sources in a large shallow lake（Lake Balaton, Hungary）. Hydrobiologia, 599:

87–95.

Presing, M., L. Voros, S. Herodec & G. Abrusan. 2001. Nitrogen uptake and the importance of internal nitrogen loading in Lake Balaton. Freshwat. Biol., 46: 125–139.

Priscu, J. C., W. F. Vincent & C. H. Williams. 1989. Inorganic nitrogen uptake and regeneration in perennially ice-covered Lakes Fryxell & Vanda, Antarctica. J. Plankton Res., 11: 335–351.

Redfield, A. C. 1958. The biological control of chemical factors in the environment. Am. Sci., 46: 205–221.

Reid, J. W. & P. N. Turner. 1988. Planktonic Rotifera, Copepoda and Cladocera from Lake Acu and Viana, State of Maranhao, Brazil. Rev. Bras. Biol., 48: 485–495.

Richey, J. E., J. I. Hedges, A. H. Devol, P. D. Quay, R. Victoria, L. Martinelli & B. R. Forsberg. 1990. Biogeochemistry of carbon in the Amazon River. Limnol. Oceanogr., 35: 352-371.

Roland, F., F. A. Esteves & F. A. R. Barbosa. 2002. Relationship between antropogenically caused turbidity and phytoplankton production in a clear Amazonian floodplain lake. Amazoniana, 17: 65–77.

Sagi, T. 1966. Determination of ammonia in sea water by the indophenol method and its application to the coastal and off-shore waters. Oceanogr. Mag., 18: 43–51.

Satake, K., Y. Saijo & H. Tominaga. 1972. Determination of small quantities of carbon dioxide in natural waters. Jap. J. Limnol., 33: 16–20.

Schell, D. M. 1971. Uptake and generation of free amino acids in marine waters of southeast Alaska. Limnol. Oceanogr., 19: 260–270.

Schmidt, G. W. 1972. Amounts of suspended solids and dissolved substances in the middle reaches of the Amazon over the course of one year (August 1969-Juky1970) . Amazoniana, 3: 208–223.

Sioli, H. 1984. The Amazon, Limnology and landscape ecology of a mighty tropical river and its basin. Dr. W. Junk Publishers: 763pp.

Steemann Nielsen, E. 1952. Use of radio active carbon (^{14}C) for measuring organic production in the sea. J. Cons. int. Explor. Mer, 18: 117–140.

Takahashi, M., T. Hama, K. Matsunaga & N. Handa. 1995. Nitrogenous nutrient uptake by phytoplankton and ammonium regeneration by microbial assemblage in Lake Biwa. J. Plankton Res., 17: 1027–1037.

Takamura, N., T. Iwakuma & M. Yasuno. 1987. Uptake of ^{13}C and ^{15}N (ammonium, nitrate and urea) by Microcystis in Lake Kasumigaura. J. Plankton Res., 9: 151–165.

Twomey, L. J., M. F. Piehler & H. W. Paerl. 2005. Phytoplankton uptake of ammonia, nitrate and urea in the Neuse River Estuary, NC, UAS. Hydrobiologia, 533: 123–134.

Wada, E. & A. Hattori. 1971. Nitrite metabolism in the euphotic layer of the central North Pacific Ocean. Limnol. Oceanogr., 16: 766–772.

Whalen, S. C. & V. Alexander. 1986. Seasonal inorganic carbon and nitrogen transport by phytoplankton in an arctic lake. Can. J. Fish. Aquat. Sci., 43: 1177–1186.

Wheeler, P., B. North, M. Littler & G. Stephens. 1977. Uptake of glycine by natural phytoplankton communities. Limnol. Oceanogr., 22: 900–910.

Zubkov, M. V. & G. A. Tarran. 2005. Amino acid uptake of *Prochlorococcus* spp. in surface waters across the South Atlantic Subtropical Front. Aquatic Microbial Ecol., 40: 241–249.