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In Situ Measurement of Diel Periodicity in Urea Decomposition in a Reed Zone of Lake Biwa, Japan

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Diel change in urea decomposition activity of epiphytic algae on *Phragmites* stems and phytoplankton in a shallow littoral reed zone in the south basin of Lake Biwa was investigated with an *in situ* technique using ^{14}C-labelled urea. The daily rates of urea decomposition (sum of urea carbon incorporation rate and CO_2 liberation rate) by epiphytic and planktonic algae were calculated as 180 μ mole urea surface shoot area m^{-2} day^{-1} and 210 μ mole urea m^{-3} day^{-1}. The chlorophyll *a* specific urea decomposition rates of epiphytic and planktonic algae were 4.7 to 6.4 and 4.4 to 6.2 μ mole urea mg chl. a^{-1} incubation $time^{-1}$ in daytime and 4.2 to 5.7 and 2.4 to 3.5 μ mole urea mg chl. a^{-1} $time^{-1}$ in nighttime, respectively. High values were obtained during 12:00~18:00 and low values during 00:00~06:00 for both epiphytic and planktonic algal communities. A clear diel periodicity in the urea decomposing activity of the planktonic algae was observed. The activity of the epiphytic algae, on the other hand, showed no distinctive variation during a day. The present results indicate that epiphytic algae are one of the significant urea decomposers in a reed zone, and that the diel patterns are quite difference between both algal communities.

Key words : urea decomposition, diel periodicity, epiphytic algae, reed zone, Lake Biwa

INTRODUCTION

A dense biomass of emergent and submerged macrophytes, and epiphytic algae on submerged parts of their stems, are often observed in shallow near-shore areas. Epiphytic algae contribute to the biogeochemical cycle in the macrophytic zone. In Lake Biwa, *Phragmites australis* covers an area of 3 km^2 (including lagoons), which comprises 60% of submerged macrophytes. Throughout the year, *Phragmites* has a large substratum for epiphytic algal colonization in the reed zone. In the pelagic-littoral zone, the standing crop of these epiphytic algae is generally higher than that of phytoplankton. The contribution of the epiphytic algae to the biogeochemical cycling is related to the available surface area of reed stems as the epiphytic substratum. The epiphytic primary production on reed stems has been measured by several investigators. Some available biological data on the reed zones of Lake Biwa have been reported (Tanimizu *et al*., 1981; Ohtsuka *et al*., 1996). There is, however, little information

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on the biogeochemical cycling in the reed zone.

On the other hand, diel variations in urea decomposition have been observed in lacustrine environments (Mitamura and Saijo, 1986a; Park *et al*., 1997). Mitamura and Saijo (1986a) recognized higher rates of urea decomposition during the daytime than during the night. Park *et al*. (1997) found that the rates at night were higher than those in the daytime in two thirds of the rates examined, but higher during the daytime in one case.

Knowledge of urea decomposition by epiphytic algae, however, is quite limited. The purpose of this study has been to provide information on the contribution of epiphytic algae to the urea decomposition and on the diel periodicity in urea decomposing activity in a shallow littoral reed zone of Lake Biwa.

MATERIALS AND METHODS

The present investigations were carried out at a typically large−scale reed zone at the end of September (Fig. 1). The field observations were conducted on calm days at the boundary between the littoral and pelagic zones, where the *Phragmites* zone extended from land to water. In the present investigation, a eutrophic reed zone (35°02′40″N, 135°52′30″E) in the south basin cover 300 m^2 with 80 stem m^{-2}. At the center station in the reed zone, the water depth of the submerged portion of the stems was 0.6 m, and the bottom sediments were composed of sand.

In advance of the present investigations, no vertical changes in the chemical parameters were observed using a cylindrical water sampler (Mitamura, 1991). Water samples, therefore, were drawn gently with a plastic tube from a 0.2 m depth at stations inside the reed zone (Sta. I) and one off−shore station outside the reed zone (Sta. O). To determine the concentration of nutrients and chlorophyll *a*, the collected water samples were immediately filtered through glass fiber filters (Whatman GF/C) treated by ignition at 420°C. The filters and filtrates were then stored at −20°C in a deep freezer until chemical analyses in the laboratory. Urea was determined by the method of Newell *et al*. (1967) with a modification. Ammonia was determined by the method described by Sagi (1966), nitrite after Bendschneider and Robinson (1952), and nitrate after Wood *et al*. (1967).

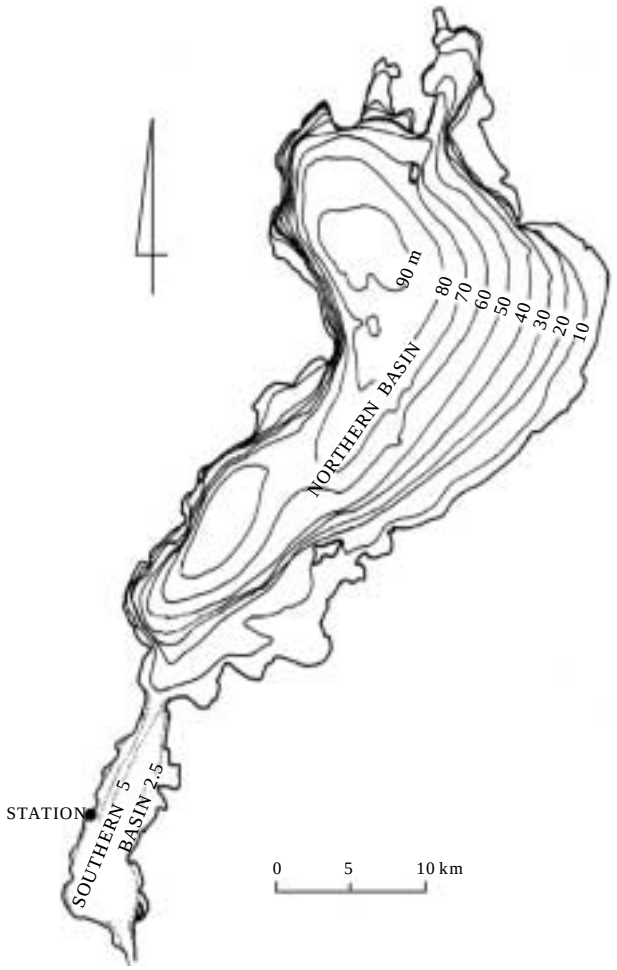

Fig. 1. Map showing the reed zone investigated in Lake Biwa.

In the reed zone of Lake Biwa, dead *Phragmites* generally remains for three years. The dead submerged stems accounted for approximately 60% of the total reed stems in the present investigation. The standing crop of epiphytic algae on dead reed stems was generally greater than that on living reed stems. Accordingly, the reed stems employed in this experiment were chosen in proportion to the ratio of living to dead numbers. At the observation site, both living and dead stems of *Phragmites* were cut above the rhizomes and returned to the laboratory. The random sections of the stem were used for the experiments and the substrate of epiphytic algae was employed for a length of approximately 5 cm at a depth of 0.2 m from the water surface. Periphyton was gently removed from the surface

of reed stems using a paintbrush with filtered water through a glass fiber filter (Whatman GF /C). The stripped periphyton was subdivided for *in situ* measurements of urea decomposition and photosynthesis, and chlorophyll *a* analysis. The chlorophyll *a* concentration was measured, using 90% acetone as the extraction solvent according to the method of SCOR/Unesco (1966).

A preliminary examination before the present experiments was carried out for determinations of the rates of urea decomposition and photosynthesis of epiphytic algae. The submerged parts of reed stems were cut into several sections and placed in incubation bottles. The results provided high rates caused by the enriching effects of nutrients exuded from the cut sections. In the present investigation, therefore, the epiphytic algae were stripped from the reed stems. Mitamura and Tachibana (1999) reported that the photosynthetic activity decreased by self-shading from the dense architecture of the periphytic layer. It was a complex procedure to manage the water movement for the present examinations. No appreciable difference was found between the photosynthetic rates in the samples of low epiphytic algal concentrations (less than 50 mg chl. *a* m^{-3}) and the rates under water movement using no stripped epiphytic algae. In the present study, therefore, the dilute samples were used to determine the urea decomposition and photosynthesis of epiphytic algae. Damage to epiphytic algae may result when stripping them off the substrate.

In situ measurements of urea decomposition and photosynthesis were carried out at the center station in the reed zone. During the experiments, there was fair weather and the water temperature was 23°C. To measure the *in situ* rate of urea decomposition, water samples containing epiphytic algae were poured into three series of clear glass bottles. After adding 0.5 mL of the diluted ^{14}C labeled urea solution (containing 2 nmol ^{12}C urea and 3.7 kBq ^{14}C urea) to each bottle, 0.2 mL of concentrated formaldehyde solution was immediately added to a series of control bottles. The second series of bottles was wrapped in a black sheet for determination of the urea decomposing activity in the dark. The series of transparent (light) and dark bottles was suspended from a buoy at 0.2 m depth where samples were taken. In the present field experiments, sunrise and sunset were at 05:40 and 18:00, respectively. After leaving the bottles in the incubation depth during time (12:00～18:00, 18:00～00:00, 00:00～06:00 and 06:00～12:00), biological activity was terminated by adding formaldehyde solution. Sample water in each glass bottle was then filtered through a Millipore HA type filter. The filter was put in a scintillation vial, with 10 mL of Bray scintillation fluid (Bray, 1960). The radioactivity was then measured with a liquid scintillation spectrometer (Aloka LSC-651) to determine the rate of urea carbon incorporation into the particulate organic matter. The filtrate of each sample was poured into a separate 50 mL glass bottle with a screw cap, and a CO_2 absorption tube containing 0.5 mL of n-ethanolamine was inserted into each bottle to absorb the $^{14}CO_2$ liberated from the sample solution by acidification. After adding 0.5 mL of 2 M sulfuric acid solution to each filtrate, the bottles were sealed tightly and left for four days at room temperature. After adding the scintillation fluid to n-ethanolamine (containing $^{14}CO_2$ liberated from sample water in the glass bottle), the radioactivity was determined as described above. The urea decomposition rate by phytoplankton in water of the reed zone was measured in the same way as in the urea decomposition measurement of epiphytic algae.

Photosynthetic rate was measured by the ^{14}C technique of Steemann Nielsen (1952) simultaneously with the experiments for the measurements of urea decomposition rate. The concentration of total carbon dioxide in the sample water was determined with an infrared carbon dioxide analyzer, as described by Satake *et al.* (1972).

RESULTS AND DISCUSSION

Diel changes of urea and biogeochemical parameters

Figure 2 shows the diel variations of nitrogenous nutrients in the reed zones. Total dissolved nitrogenous nutrients (TNN; sum of urea, ammonia, nitrite and nitrate nitrogen) ranged from 16 to 31 μM inside Station I and 5 to 17 μM outside Station O. The predominant component of nitrogenous nutrients was nitrate. High ammonia concentrations were observed in the reed zone. The urea was generally at low concentrations of 0.38 to 0.73 μM at Sta. I and 0.21 to 0.43 μM at Sta. O, respectively, much lower

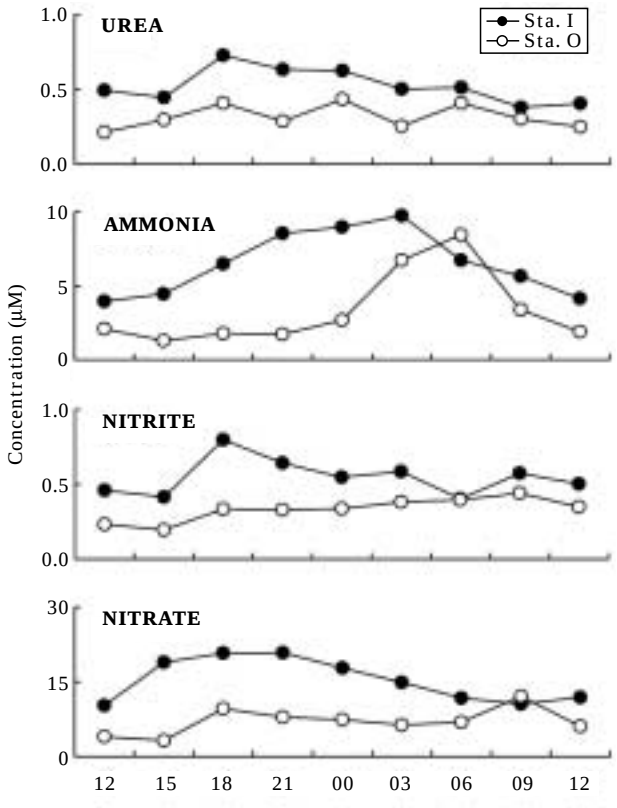

Fig. 2. Diel variations of urea and other nitrogenous nutrients (ammonia, nitrite and nitrate) concentration at a depth of 0.2 m depth at stations in- and outside the reed zone. Closed and open symbols represent the inside (Sta. I) and outside (Sta. O) stations, respectively.

than those of ammonia and nitrate. An appreciable contribution of urea nitrogen was observed, ranging from 3.6 to 11.2% of TNN. The importance of urea as a nitrogen source for phytoplankton has been demonstrated by several authors (*e.g.*, Mitamura and Saijo, 1986a). Present results indicate that the urea in water of reed zones is also one of the essential nitrogenous compounds for epiphytic and planktonic algal growth. Concentrations of these parameters inside a station showed values higher than those at outside station.

As can be seen in Fig. 2, a clear diel periodicity was recognized. The urea concentration tended to be high in latter half of the afternoon and to decrease during daytime at Sta. I. The concentrations of nitrite and nitrate showed a similar tendency with the diel pattern of urea concentration. Ammonia levels, on the other hand, were high at midnight.

Table 1. Photosynthetic rate and assimilation number of epiphytic and planktonic algae in the reed zone of Lake Biwa.

Incubation time	Photosynthesis		Assimilation number	
	Epiphyton	Plankton	Epiphyton	Plankton
	(mg C m^{-2} $time^{-1}$)	(mg C m^{-3} $time^{-1}$)	(mg C mg chl. a^{-1} hr^{-1})	
12:00～18:00	16	221	0.31	2.6
06:00～12:00	15	189	0.31	2.3

Epiphytic and planktonic algal biomass and their photosynthesis

Chlorophyll *a* amounts of epiphytic and planktonic algae in samples used for the *in situ* diel examination ranged from 7.4 to 8.7 mg chl. *a* surface shoot area m^{-2} and 10.5 to 14.0 mg chl. *a* m^{-3}. During the diel observation, the chlorophyll *a* concentrations showed an almost uniform level.

The photosynthetic rate of epiphytic and planktonic algae, measured simultaneously with the experiment of urea decomposition rates, was 15 to 16 mg C m^{-2} incubation $time^{-1}$ and 189 to 211 mg C m^{-3} incubation $time^{-1}$, respectively (Table 1). Daily primary productions of epiphytic and planktonic algae were calculated as 31 mg C m^{-2} day^{-1} and 400 mg C m^{-3} day^{-1}. The diel variations in the photosynthetic rate of planktonic algae, showing higher values during 12:00～18:00 than during 06:00～12:00, showed somewhat similar diel patterns to that of the chlorophyll *a* concentration. On the other hand, the rate of epiphytic algae was fairly constant during the observation. The photosynthetic carbon assimilation number of both algal communities was calculated as 0.3 mg C mg chl. a^{-1} hr^{-1} for epiphytic algae and 2.3 to 2.6 mg C mg chl. a^{-1} hr^{-1} for planktonic algae. The assimilation number of epiphytic algae showing same values during diel observation was considerably lower than those of planktonic algae.

Diel periodicity in urea decomposing activity of epiphytic and planktonic algae

The daily rate of urea decomposition was estimated from each value obtained during the

incubation. The urea decomposition rate by epiphytic algae was 178 μ mole urea surface shoot area m^{-2} day^{-1} and the rate by planktonic algae was 209 μ mole urea m^{-3} day^{-1}, respectively. There was no appreciable difference between the values decomposed by both algal communities.

The decomposition rate of urea by unit amount of chlorophyll *a*, using chlorophyll *a* as a cell parameter, was calculated to eliminate the influence of varying the standing crop of both algal communities, especially in the epiphyton samples taken from *Phragmites* stems, at each sampling during the investigation. Diel variations in the chlorophyll *a* specific decomposition rate of urea (sum of incorporation rate of urea carbon and CO_2 liberation rate from urea) measured with the *in situ* technique were illustrated in Fig. 3. The daily variations in the present specific decomposing activity of epiphytic algae ranged from 4.4 to 6.4 μ mole urea mg chl. a^{-1} incubation $time^{-1}$ in the transparent (light) bottles, and 3.8 to 6.3 μ mole urea mg chl. a^{-1} incubation $time^{-1}$ in the dark bottles, respectively. The decomposition activity was higher during the afternoon than the morning, although the variation showed a weak diel change. The incorporation rate of urea carbon into epiphytic algal cells ranged from 0.02 to 0.26 μ mole urea mg chl. a^{-1} $time^{-1}$ in the light and 0.01 to 0.03 μ mole urea mg chl. a^{-1} $time^{-1}$ in the dark. On the other hand, the rate of CO_2 liberation into the water from urea was 4.4 to 6.2 μ mole urea mg chl.a^{-1} $time^{-1}$ and 3.7 to 6.3 μ mole urea mg chl. a^{-1} $time^{-1}$ in the light and dark, respectively. The present chlorophyll *a* specific urea decomposition rate was in the same range as those obtained using ^{14}C–labeled urea with *in situ* experiment in Lake Biwa by Mitamura (1986a).

The chlorophyll *a* specific urea decomposition rate by planktonic algae, on the other hand, ranged from 2.8 to 5.7 μ mole urea mg chl. a^{-1} $time^{-1}$ in the light, and 0.9 to 3.3 μ mole urea mg chl. a^{-1} $time^{-1}$ in the dark. The incorporation rate of urea carbon into phytoplankton cell ranged from 0.07 to 1.20 μ mole urea mg chl. a^{-1} $time^{-1}$ in the light and 0.01 to 0.02 μ mole urea mg chl. a^{-1} $time^{-1}$ in the dark. On the other hand, the CO_2 liberation rate was 2.7 to 4.5 μ mole urea mg chl. a^{-1} $time^{-1}$ and 0.8 to 3.2 μ mole urea mg chl. a^{-1} $time^{-1}$ in the light and dark conditions, respectively. A distinguishable diel variation in the urea decomposition rate was recognized, as

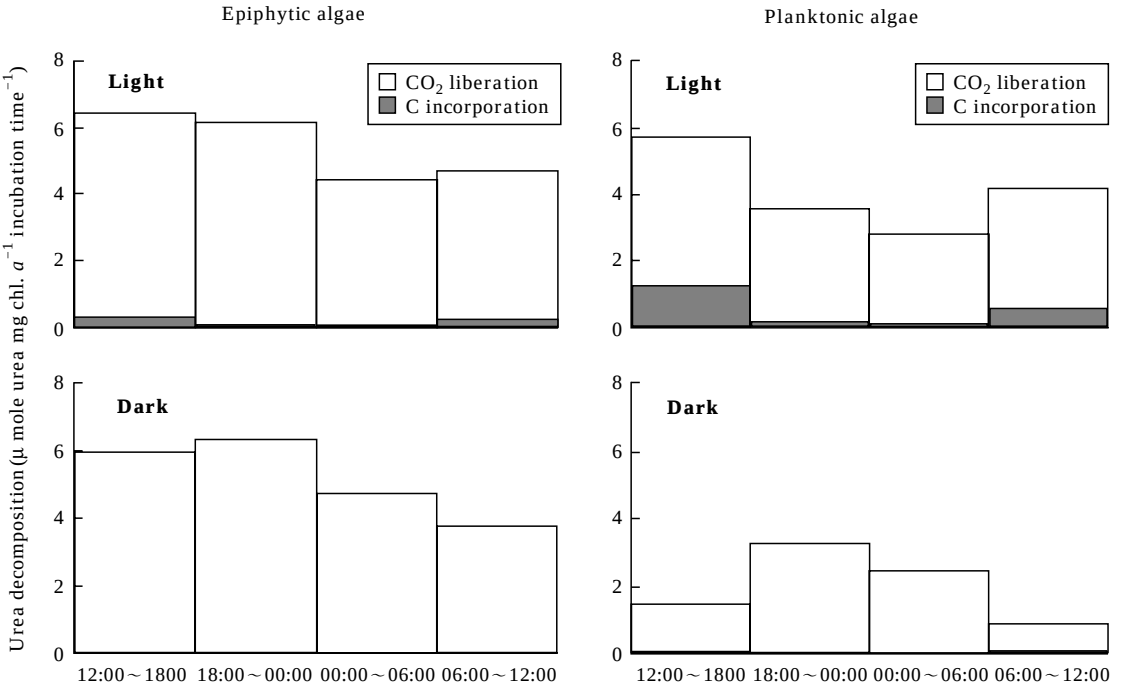

Fig. 3. Diel periodicity in the chlorophyll *a* specific urea decomposition rate of epiphytic algae on the reed stems and planktonic algae in a eutrophic reed zone of Lake Biwa. Shaded and white sections represent the values of urea carbon incorporation into epiphytic and planktonic algal cell and CO_2 liberation from urea, respectively.

Table 2. Diel variations in the contribution of urea carbon incorporation into epiphytic and planktonic algal cell in urea decomposition, the ratio of dark to light value in the rates of urea decomposition, urea carbon incorporation and CO_2 liberation from urea, and the ratio of urea decomposition (as carbon unit) to photosynthetic carbon assimilation for epiphytic and planktonic algal communities in the reed zone.

		Incubation time			
		12:00～18:00	18:00～00:00	00:00～06:00	06:00～12:00
Carbon incorporation / Urea decomposition (%)					
Epiphytic algae	Light	4.0	0.7	0.5	3.8
	Dark	0.5	0.1	0.1	0.5
Planktonic algae	Light	21.1	3.0	2.6	13.0
	Dark	1.5	0.3	0.3	2.2
Dark / Light ratio					
Epiphytic algae	Decomposition	0.92	1.02	1.07	0.80
	C incorporation	0.10	0.22	0.30	0.11
	CO_2 liberation	0.96	1.03	1.08	0.83
Planktonic algae	Decomposition	0.26	0.92	0.86	0.21
	C incorporation	0.00	0.10	0.10	0.04
	CO_2 liberation	0.32	0.94	0.88	0.23
Urea decomposition / Photosynthesis (%)					
Epiphytic algae	Decomposition	4.16	ND	ND	3.06
	C incorporation	0.17	ND	ND	0.12
	CO_2 liberation	3.99	ND	ND	2.94
Planktonic algae	Decomposition	0.44	ND	ND	0.37
	C incorporation	0.09	ND	ND	0.05
	CO_2 liberation	0.34	ND	ND	0.32

shown in Fig. 3. The rates of chlorophyll *a* specific urea decomposition observed were high during daytime and low during nighttime. A similar diel pattern in the absolute rates of urea decomposition was also observed.

As can be seen in Table 2, the greater part of urea decomposition by epiphytic algae occurred during the phase of CO_2 liberation. Some 3.8 to 4.0% urea decomposition took place in the carbon incorporation phase in the daytime, but it was negligible in the dark. An appreciable contribution of carbon incorporation in urea decomposition of 13 to 21% was obtained in daytime planktonic algal samples. The present values were similar to those obtained by previous investigators in lakes (*e.g.*, Mitamura, 1986a). The contribution of the carbon incorporation phase in urea decomposition showed a higher tendency during the daytime than at night, although the contributions of epiphytic algae were much lower than those of planktonic algae. This seemed to indicate that the phase of carbon incorporation was closely related to the physiological light/dark cycle of both algal communities.

The urea decomposition rate was obviously higher in the transparent bottles than in the dark ones, especially for plankton samples (Fig. 3). The ratio of dark to light values was calculated as 0.80 to 0.92 in urea decomposition for the epiphyton samples, 0.21 to 0.26 for plankton samples, 0.10 to 0.11 and 0.00 to 0.04 in carbon incorporation, and 0.83 to 0.96 and 0.23 to 0.32 in the CO_2 liberation phase, respectively (Table 2). During daytime the dark to light ratio in the CO_2 liberation phase revealed markedly high values, but only negligible levels in the phase of carbon incorporation, although the nighttime ratios exhibited rather higher values than otherwise. This indicates that in the light urea decomposes in two phases whereas in the dark it decomposes only in the CO_2 liberation phase, especially in the decomposing activity of planktonic algae. The present results agreed with the earlier two-phase model for urea decomposition by Mitamura and Saijo (1986a), and Price and Harrison (1988). The present results seemed to suggest that in the reed zone of Lake Biwa, planktonic algae decomposed urea more effectively during the daytime than the nighttime, whereas epiphytic algae decomposed urea throughout the day, and its activity showed no relation to the natural light/dark cycles.

The ratios of the urea decomposition rate (as carbon units) to the photosynthetic carbon assimilation rate were calculated as 3.1% to 4.2% for epiphyton samples, and as extremely low values of 0.37% to 0.44% for plankton samples. Almost no change in these ratios was observed during the investigation period. The carbon incorporation into epiphytic algal cells from urea was calculated as 0.12% to 0.17% photosynthetic carbon incorporation. The carbon incorporation into phytoplankton cells, on the other hand, was 0.05% to 0.09% photosynthesis. The present percentages were similar to the earlier findings of Mitamura and Saijo (1986a) and Mitamura *et al.* (1994). The ratios of the carbon incorporation rate to the photosynthetic rate gave almost constant values in each algal community. The contribution of urea carbon to the carbon source of phytoplankton was negligible for both epiphytic and planktonic algae in the reed zone of Lake Biwa. Urea is more effectively decomposed by phytoplankton than by bacteria in most marine and freshwater environments (Remsen *et al.*, 1972; Mitamura and Saijo, 1986a; Tamminen and Irmisch, 1996), except for polluted eutrophic waters (Mitamura *et al.*, 1994). The previous results and ours suggest that specific urea decomposing activity can be modified primarily by the photosynthetic activity of algae, even in the epiphytic algal community.

Mitamura (1986b) found that the urea decomposition rate by freshwater phytoplankton increased with increasing light intensity up to some asymptotic value. A similar tendency was obtained for the urea decomposition by epiphytic algae taken from *Phragmites* stems (Mitamura and Tachibana, unpublished). The present results suggest that the natural light/dark cycle is one of the most important environmental parameters affecting the diel periodicity in the rate of urea decomposition in the reed zone, implying that the present diel variation in urea decomposition was influenced by both the endogenous and exogenous rhythm.

In the present study, the daily variations in the urea concentration showed a narrow range, although high decomposing activity was measured as described above. This suggests the existence of a quasi-steady state in the balance between the urea consumption due to the decomposition by epiphytic and planktonic algae and the urea supply which comes from the excretion of zooplankton and zoobenthos and microbial mineralization in the water and on the reed stems, as indicated by Mitamura and Saijo (1986b) in Lake Biwa. This dynamic balance seems to play an important role in urea cycling in the reed zone of Lake Biwa.

In summary, the present investigation indicates that the diel variation in urea decomposing activity took place in close association with the algal photosynthetic carbon assimilation activity in the euphotic reed zone of Lake Biwa. To accurately elucidate the biogeochemical dynamics of urea in the reed zones, further investigation of several variables is needed, including the influences of light intensity, nutrient concentration and bacterial contribution on urea decomposing activity.

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