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Urea uptake kinetics of a midsummer planktonic community on the SW coast of Finland

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ABSTRACT: Urea uptake of the planktonic community was studied in a series of experiments on the SW coast of Finland, the Baltic Sea, in midsummer. Uptake was measured with ^{14}C-urea incubations, where respired $^{14}CO_2$ was found to represent 98 to 99% of total uptake. Uptake kinetics were characterized by high affinity for urea (0.97 to 1.84 µg N l^{-1}), fast turnover rates (29 to 99% h^{-1}) and moderate maximum uptake velocities (0.52 to 1.31 µg N l^{-1} h^{-1}). Light stimulated urea uptake; parallel dark incubations usually yielded uptakes 60 to 80% of those in the light. No surge uptake of urea was observed, as all kinetic parameters remained constant in a short-term time series experiment. Size-fractionation studies and light/dark incubations indicated that phytoplankton dominated urea uptake. The time course of urea depletion in an enrichment experiment followed the diel primary production pattern, and the depletion rate was of the same order as the ammonium depletion rate. Inorganic nitrogen additions (ammonium, nitrate) did not produce immediate inhibitions of urea uptake, but with a time lag of 1 night, ammonium inhibition was observed. Phosphate addition stimulated urea uptake. Evidence on stimulated bacterioplankton uptake of urea was found the next night after organic carbon source addition. Alternative nitrogen sources (NO_3, NH_4, urea) were utilized mainly according to their availability. Urea proved to be an important nitrogen source for phytoplankton during the regenerated phase of plankton succession.

KEY WORDS: Urea · Uptake kinetics · Phytoplankton · Bacterioplankton Nitrogen sources Preference · Inhibition · Baltic Sea

INTRODUCTION

Studies of nitrogenous nutrition of the planktonic community have shown the importance of organic nitrogen sources, especially urea and amino acids. In culture studies, several algal species have been shown capable of utilizing urea as their sole nitrogen source (e.g. Antia et al. 1975).

During the regenerated phase of plankton succession, urea has usually been reported as the most favoured nitrogen source after ammonium for the plankton community in offshore (Eppley et al. 1971, 1973, 1977, Kanda et al. 1985), coastal (McCarthy 1972, Harvey & Caperon 1976, McCarthy et al. 1977, Paasche & Kristiansen 1982, Kristiansen 1983, Furnas et al. 1986, Sahlsten et al. 1988) and freshwater (McCarthy et al. 1982, Mitamura & Saijo 1986) environments. On several occasions, urea equalled or exceeded NH_4 in preference during the regenerated production phase. In size-fractionation experiments accompanied by specific inhibitors, Wheeler & Kirchman (1986) found that picoplankton (<1 µm), representing both bacterioplankton and procaryotic and eucaryotic phototrophs, did not take up urea.

In the Baltic Sea, virtually nothing is known of the role of urea in planktonic nutrition. In this paper we summarize the results from a series of experiments on urea uptake, including assimilation kinetics, time course experiments, size-fractionation studies, and stimulation or inhibition by inorganic and organic nutrient additions. The data are based on measurements of ^{14}C-urea uptake, chemical urea analyses and comparisons with parallel chlorophyll analyses and primary and bacterial productivity measurements.

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MATERIAL AND METHODS

Sampling and experimental conditions. The water for all experiments originated from the surface layer of a pelagic sampling station outside the outer archipelago line, some 6 km off the Tvärminne Zoological Station, at the entrance to the Gulf of Finland, the Baltic Sea. The experimental period covered 2 wk (21 June to 1 July 1988) of the midsummer stage of the planktonic succession, characterized by exceptionally calm and warm weather, which caused the surface temperature to rise from 16 to 22.5°C during the period.

Experiments were performed at *in situ* temperature in a thermostated water bath which followed the natural irradiation cycle as it was situated outdoors, sheltered from direct sunlight. Irradiation level was ca 13% of surface irradiance, corresponding to light levels of 3 to 4 m at the sampling location.

Prefiltrations in the experiments were performed by filtering water through polycarbonate filters (Nuclepore 3 and 0.8 µm) or filters cut from nylon plankton net (10 and 20 µm mesh size). Filter diameter was 50 mm and no additional vacuum was applied in prefiltrations.

Chemical analyses. Chemical analyses of dissolved nutrients (urea, ammonium, phosphate) were performed manually in duplicate according to the methods presented in Grasshoff (1976). Particulate organic nitrogen was determined after filtering (<100 mm Hg) the samples onto acid-washed and precombusted (4 h at 500°C) glass-fibre filters (Whatman GF/F). In organic nitrogen analyses, the measurement of nitrate after sample oxidation according to Grasshoff (1976) was performed by Flow Injection Analysis (FIA). The FIA procedure was slightly modified after Johnson & Petty (1983), and a Tecator 5020 FIAstar apparatus was used for the analyses. Glassware for organic N filtrations was acid-washed and precombusted. Chlorophyll *a* (chl *a*) was measured fluorometrically (Sequoia-Turner 450) after sonication and ethanol extraction (24 h at room temperature) of samples filtered onto glass-fibre filters (Whatman GF/F).

Urea uptake. Urea uptake was measured after incubations with ^{14}C-urea (CFA.41, Amersham International, Buckinghamshire, UK; specific activity 56 mCi mmol^{-1}), in closed incubation bottles with a glass cup installed in a rubber stopper for the collection of respired $^{14}CO_2$ (Kuparinen & Uusi-Rauva 1980). Incubations were terminated by injecting 200 µl of 4.5 M H_2SO_4 into the 10 ml sample through the rubber stopper, whereafter 300 µl of ethanolamine was similarly injected into the glass cup. After 24 h, incubation bottles were opened and the glass cup was carefully removed into a scintillation vial for the measurement of respired $^{14}CO_2$.

A scintillation cocktail of 5 ml ethanol and 10 ml PCS (Amersham International) was added to the vial, and the radioactivities were measured with a LKB-Wallac 1219 RackBeta liquid scintillation counter with the external channel ratio method. When particulate ^{14}C was also measured, the 10 ml samples were filtered (<100 mm Hg) onto 0.2 µm membrane filters (Sartorius) after the removal of the respiration cup. A 10 ml volume of PCS was added on the filters placed in scintillation vials, and radioactivities were measured as above.

A blank sample was prepared by adding 50 µl of 38% formaldehyde in the 10 ml sample before the addition of radioactivity, after which the blank was treated similarly to the samples. Duplicate incubations with a blank were used routinely for all incubations, including the different added concentrations in kinetic studies.

Other rate measurements. Primary productivity was measured with the ^{14}C method (Steemann Nielsen 1952, the modification presented by Niemi et al. 1983) as apparent net productivity (particulate + dissolved organic ^{14}C), after incubations of 20 ml samples with 2 µCi addition of $NaH^{14}CO_3$ (CFA.3, Amersham International). Incubation time was ca 2 h, duplicates with a dark blank were incubated for each sample, and incubations were terminated by the addition of 38% formaldehyde (100 µl per 20 ml sample). A 4 ml subsample was acidified with 100 µl of 1 N HCl, and after 24 h, 7 ml of PCS was added and radioactivities were counted as above.

Bacterial productivity was measured with the ^{3}H-thymidine method (Fuhrman & Azam 1980, 1982). ^{3}H-thymidine (TRK.418, Amersham International) was added to 20 ml samples in final concentration of 10 nM. A blank was prepared by adding 100 µl of 38% formaldehyde into a 20 ml subsample, and duplicates with a blank were incubated for each sample. Incubation time was approximately 2 h, and incubations were terminated by the addition of formaldehyde. After ice-cold TCA extraction, samples were filtered onto 0.2 µm membrane filters (Sartorius). Radioactivities of ^{3}H samples were counted similarly to particulate ^{14}C samples, presented above.

Experiments. A time series of urea uptake (both particulate and respired ^{14}C) was measured to define appropriate incubation times for further experiments. The urea addition was 2.3 µg N l^{-1}, and sampling times were from 0.5 to 12 h. After 4 h, uptake started to deviate significantly from linearity over time, as over 50% of the added radioactivity had been taken up. Therefore, incubation times of 2 h were selected for next experiments, but after urea turnover rates started to increase during the experimental period, incubation times were shortened, down to 30 min at the end of the period.

Measurements of uptake kinetics of urea were performed with 4 ^{14}C-urea addition concentrations between 0.05 and 22.6 µg N l^{-1}. On each addition level, duplicate incubations with a blank were used. Uptake followed closely Michaelis-Menten kinetics, and the kinetic parameters describing urea uptake were calculated with a linear transformation (Wright & Hobbie 1966).

In the first experiment on uptake kinetics, the $K+S_n$ value, which describes the affinity of the community for the substrate, was 1.75 µg N l^{-1}. It has been shown (Kuparinen et al. 1984) that this parameter can be used to estimate appropriate concentrations for the determination of natural turnover rate $1/T$ (or turnover time T) and maximum uptake rate (V_{max}) with single concentration assays, these concentrations being approximately 0.1 and 10 times the $K+S_n$ value, respectively. Consequently, a concentration of 0.23 µg N l^{-1} was chosen for the experiments to determine the turnover rate of urea. The turnover rate ($1/T$) was calculated with the equation $[c/(Ct)] \times 100$, where c is measured radioactivity, C is added radioactivity, and t is incubation time, the formula yielding the turnover rate as % h^{-1}.

The experimental designs for enrichment experiments are presented in Tables 1 to 3.

In Expt 1 (Table 1), incubations started immediately after prefractionations and nutrient additions to 1 l experimental units (22 June, around noon). Urea turnover rate in the light and in the dark was measured from all experimental units. Primary productivity was measured from the units with no or inorganic nutrient additions (1 to 9), bacterial productivity from the units with no or organic C (glucose) additions (1 to 3, 10 to 12) After 12 h, at midnight, urea turnover rate and bacterial productivity were measured from the control and glucose addition units.

Table 1. Design for the experiment on the immediate effects of ammonium, nitrate and glucose addition on urea turnover rate, primary productivity and bacterial productivity in different size fractions. Nutrient additions were 20 µg N l^{-1} for ammonium and nitrate, and 50 µg N l^{-1} for glucose

Unit	Addition	Prefractionation (µm)
1	–	–
2	–	20
3	–	0.8
4	NH_4	–
5	NH_4	20
6	NH_4	0.8
7	NO_3	–
8	NO_3	20
9	NO_3	0.8
10	Glucose	–
11	Glucose	20
12	Glucose	0.8

Table 2. Design for the experiment on the delayed effects of ammonium, phosphate and glucose addition on urea turnover rate, primary productivity, bacterial productivity and chl *a*. Size fractionations were preformed from Units 1, 4, 5 and 8 (see text). Nutrient additions were 20 µg N l^{-1}, 5 µg P l^{-1} and 50 µg C l^{-1} + and – indicate 'added' and 'not added' respectively

Unit	NH_4	PO_4	Glucose
1	–	–	–
2	+	–	–
3	–	+	–
4	+	+	–
5	–	–	+
6	+	–	+
7	–	+	+
8	+	+	+

In Expt 2 (Table 2), nutrients were added into 1 l experimental units in the late afternoon (27 June), and incubations for urea turnover rate, primary productivity and bacterial productivity measurements were started the next day, at noon, when chl *a* was also measured. Prefractionations were performed for samples from Units 1, 4, 5 and 8, immediately before the start of the incubations. Prefractionations were 20, 10, 3 and 0.8 µm for urea turnover rate and primary productivity, and 3 and 0.8 µm for bacterial productivity.

In Expt 3 (Table 3), urea turnover rate was measured approximately 24 h after nutrient additions into 2 l experimental units, which took place 30 June at noon.

In Expt 4, depletion rate of nonlabelled urea was calculated after addition of urea (20 µg N l^{-1}) into a 6 l sample in a 8 l polycarbonate bottle. Both this sample and a control without urea addition were prefiltered through 20 µm to remove larger zooplankton. Urea-N, ammonium-N and particulate organic N were assayed from the samples over 39 h after the addition of urea late at night, 28 June. Urea depletion rate was calcu-

Table 3. Design for the experiment on the delayed effects of nitrate, ammonium and phosphate on urea turnover rate. Nutrient additions in µg N l^{-1} or µg P l^{-1}

Unit	NO_3	NH_4	PO_4
1	–	–	–
2	50	–	–
3	–	5	–
4	–	20	–
5	–	50	–
6	–	100	–
7	–	50	20

lated as the difference between successive urea analyses, expressed per hour.

The depletion rates for NH_4 and NO_3 in Expts 1 and 3 were calculated similarly to the urea depletion rate.

RESULTS

Respiration percentage of ^{14}C-urea

In several experiments, both particulate and respired ^{14}C was measured after urea uptake incubations. The percentage of $^{14}CO_2$ was consistently 98 to 99% of total uptake ($\bar{x}$ = 99.1%, SD = 0.7, n = 76), and the ratio remained the same in the light and in the dark, during a time series experiment, as well as with different added concentrations of urea. Therefore, in most enrichment experiments only respired $^{14}CO_2$ was measured after incubations, as a close approximation of total uptake. In order to retain comparability, all results in this paper refer to $^{14}CO_2$ measurements unless otherwise stated.

Urea uptake kinetics

Kinetic experiments showed that uptake closely followed Michaelis-Menten kinetics, and the saturation level was reached with added urea concentrations in the order of 15 to 20 µg N l^{-1} (e.g. Fig 2C). When the absolute urea uptake rate was calculated by multiplying turnover rates ($1/T$) with the sum of chemically analyzed and added urea concentrations, a decreasing curve over increasing urea additions was obtained (Fig. 1).

This kind of curve is usually interpreted as an indication of erroneously high results from nutrient analysis (e.g Eppley et al. 1977), and the phenomenon was observed with urea uptake measurements by Eppley et al. (1977) and Sahlsten et al. (1988). If the kinetic $K+S_n$ value, or a fraction of it as an approximation of S_n, was used instead of urea concentration from the chemical analysis, a more realistic curve was obtained (Fig. 1).

A short-term time series of urea uptake kinetics was measured in order to reveal possible enhanced or

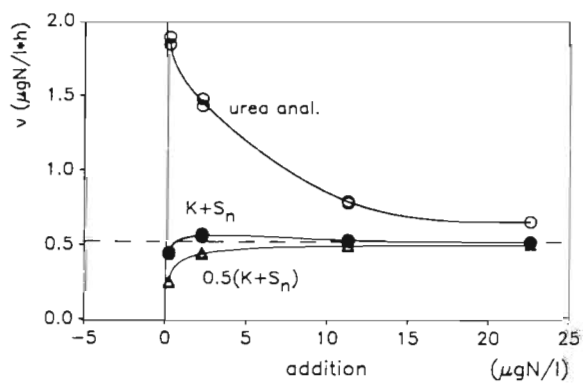

Fig. 1. Urea uptake rate [$v = (S_n + A)/T$], calculated with chemically analyzed urea concentration, kinetic sum ($K+S_n$), and $0.5(K+S_n)$ as estimates for ambient urea concentration (S_n). Dashed line denotes kinetic maximum uptake velocity (V_{max})

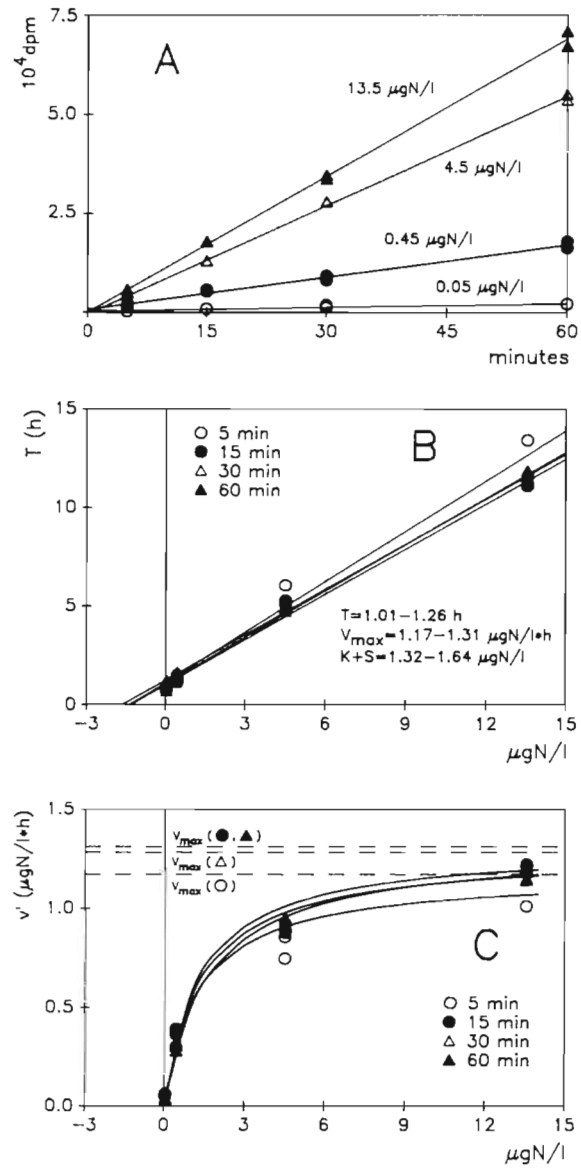

Fig. 2. Urea uptake kinetics during a time series experiment, 1 July 1988. (A) Uptake (dpm) during the experiment at different added concentrations. (B) Turnover time (T) vs urea addition (A) with different incubation times. (C) Uptake rate ($v' = A/T$) vs urea addition (A) with different incubation times. Dashed lines denote respective kinetic maximum uptake velocities (V_{max})

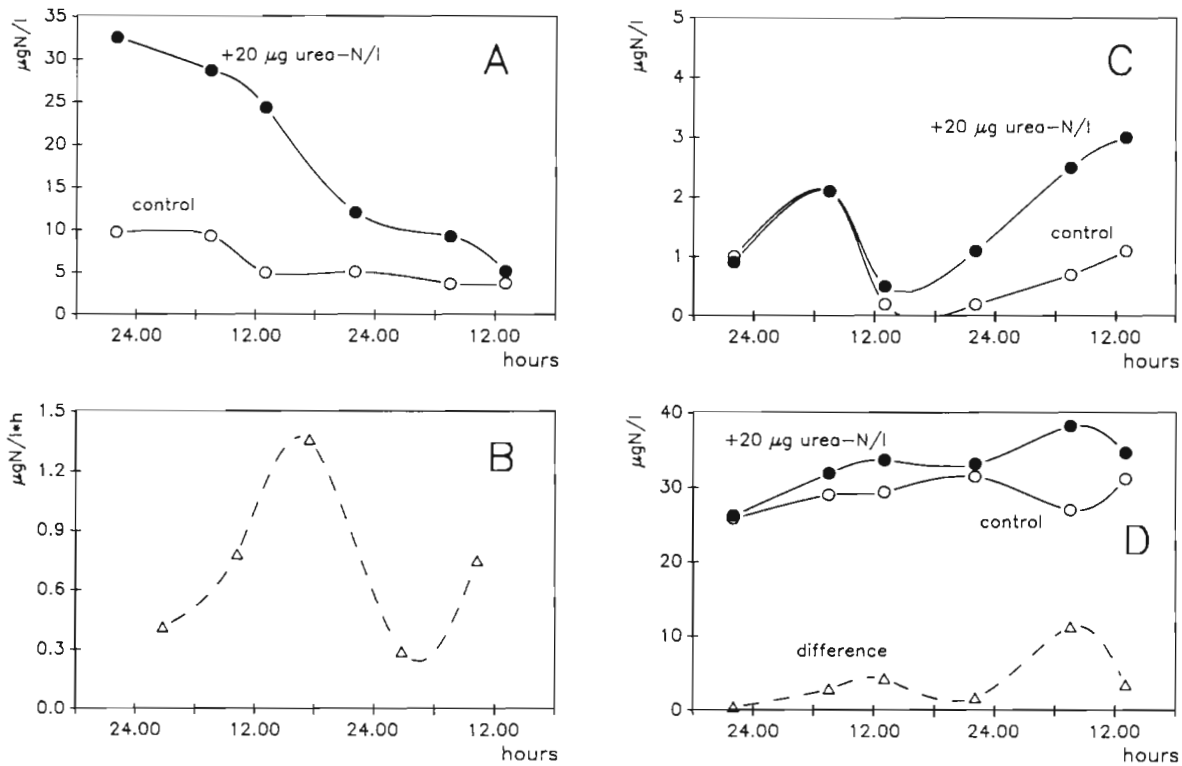

Fig. 3. Experiment on nonlabelled urea depletion, 28 to 30 June 1988. (A) Urea concentrations in the control and urea addition units. (B) Urea depletion (v = dS/dt) in the urea addition unit, plotted in the midpoints of successive sampling times. (C) Ammonium concentration in the control and urea addition units. (D) Particulate organic nitrogen concentration in the control and urea addition units. Dashed curve denotes the difference between the units

surge uptake of urea immediately after substrate addition. Conway & Harrison (1977), McCarthy & Goldman (1979) and Glibert & Goldman (1981) have presented evidence for this phenomenon in ammonium uptake, and Horrigan & McCarthy (1981) found that the length of the incubation period affected the observed V_{max} for urea uptake in cultures.

However, our results did not support this in urea uptake of natural phytoplankton, as it remained quantitatively and qualitatively constant during the experiment (Fig. 2). Uptake was completely linear over time with all added concentrations (Fig. 2A), and the kinetic properties remained essentially unaltered (Fig. 2B, C).

The kinetic parameters describing urea uptake in the light varied from 0.52 to 1.31 µg N l^{-1} h^{-1} (V_{max}), 1.01 to 3.41 h (T), and 0.97 to 1.84 µg N l^{-1} (K+S_n), and they showed a clear succession during the experimental period. With the warming of the surface layer (from 16 to 22.5°C), turnover times decreased and maximum uptake rates increased. The turnover times correspond to turnover rate ($1/T$) values of 29 to 99% h^{-1}. Uptake in the dark was 60 to 85% of that in the light, in close agreement with the results of Kristiansen & Lund (1989).

Urea depletion rate

Urea addition (20 µg N l^{-1}) in the experiment on nonlabelled urea depletion rate (Expt 4) was chosen on the basis of the kinetic data, in order to start the experiment from the saturation level with maximum urea uptake rate.

Fig. 3A & B shows that the depletion rate had a clear diurnal pattern: during nighttime, it showed the lowest values, and a distinct peak was found in the afternoon. NH_4 accumulation in the unit with urea addition started only after the first light period, and it did not exceed 10% of the original urea addition (Fig. 3C).

The accumulation of particulate organic nitrogen in the urea-enriched unit appeared more slowly than urea was depleted, as was the case with NH_4 accumulation (Fig. 3D). In the beginning of the second light period, the depleted urea nitrogen (14.4 µg N l^{-1}) could be traced from particulate (11.3 µg N l^{-1}) and NH_4 (1.8 µg N l^{-1}) fractions almost completely (91%). However, these fractions covered less than 30% of the depleted urea at the end of the experiment.

The highest depletion rate for urea in this experiment (1.36 µg N l^{-1} h^{-1}) corresponds closely to the

maximum uptake rates observed in kinetic experiments.

Enrichment studies: immediate effects

The incubations in the first enrichment experiment were performed immediately after prefractionations and nutrient additions according to Table 1. The results are shown in Fig. 4 for urea turnover rate, primary and bacterial productivity. The 20 µm prefiltration did not diminish primary productivity systematically, but only a negligible amount of primary productivity was measured from the 0.8 µm filtrate (Fig. 4B). Roughly the same size-distribution pattern was found with urea turnover rate (Fig. 4A), in contrast to bacterial productivity, of which a significant fraction was found to pass 0.8 µm (Fig. 4B). These results clearly indicate that phytoplankton was dominating urea uptake.

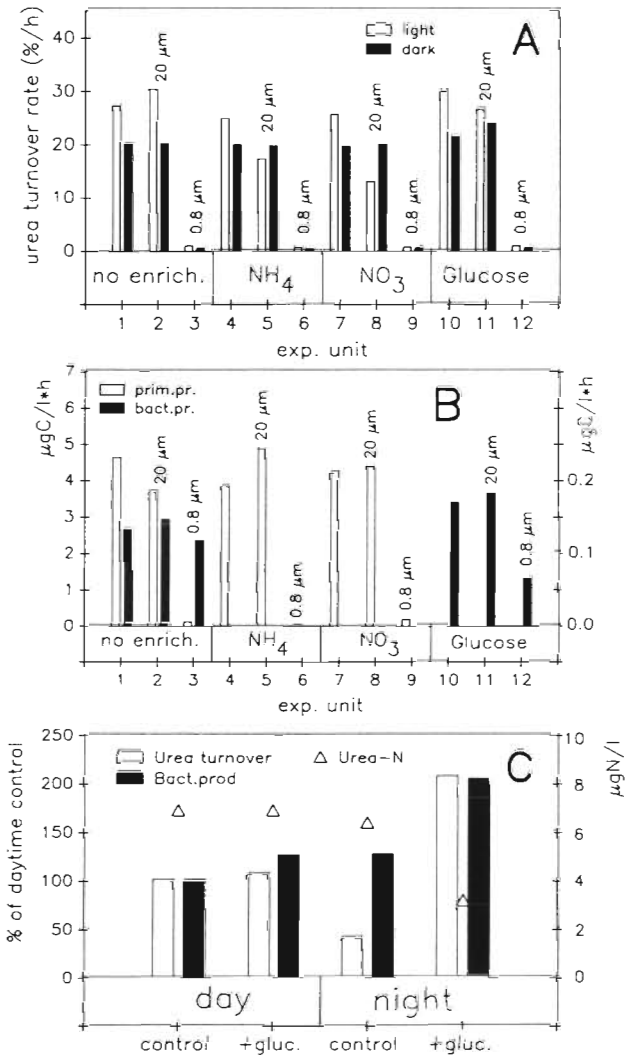

Fig. 4. Experiment on effects of nutrient additions in different size fractions, 22 June 1988 (see Table 1). (A) Urea turnover rate ($1/T$) in the light and in the dark. (B) Primary productivity (left y-axis) and bacterial productivity (right y-axis). Primary productivity was measured from Units 1 to 9, bacterial productivity from Units 1 to 3 and 10 to 12. (C) Urea turnover rate ($1/T$) and bacterial productivity in the control and glucose addition units, immediately (day) and 12 h after (night) nutrient addition, expressed as % of daytime control. Respective urea concentrations in mg $N\,l^{-1}$

None of these rate measurements showed a straightforward immediate stimulation or inhibition due to nutrient additions. Urea turnover rate measurements in the dark (Fig. 4A) were uniform in all experimental units except for a slight increase in the glucose addition units. In these units, bacterial productivity was also somewhat elevated (Fig. 4B).

The parallel incubations in the light showed generally faster urea turnover rates than in the dark (Fig. 4A), as in the kinetic experiments, with the exception of prefractionated (<20 µm) units with inorganic nitrogen additions. Here the urea turnover rates were lower than either in parallel nonfractionated or dark incubations.

After 12 h, at midnight, urea turnover rate and bacterial productivity were measured from the control and glucose addition units (Fig. 4C). In accordance with the diurnal cycle observed in the urea depletion experiment (Fig. 3B), urea turnover rate at night was 40% of the daytime measurement in the control unit. In the glucose addition unit, urea turnover rate was over 200% of the daytime control, as was the case with bacterial productivity (Fig. 4C). In this unit urea concentration had also clearly decreased compared to other units (Fig. 4C). These results suggest that the addition of organic carbon source had stimulated bacterial uptake of urea.

Enrichment studies: effects after overnight delay

In Expt 2, the effects of nutrient additions were studied after an overnight delay (Table 2). Inorganic nutrients (NH_4, PO_4) had been depleted in the units with combined additions, but in the units with single N or P additions, the concentrations were still higher than ambient levels (Fig. 5A). Urea concentrations had again decreased in the units with organic carbon additions (Fig. 5A).

Inorganic nutrient additions, especially when combined, had clearly stimulated phytoplankton. This could be seen both in chl *a* and primary productivity (Fig. 5B). The combined addition of PO_4 and organic carbon stimulated bacterial productivity (Fig. 5B).

Urea turnover rate showed a pattern which clearly differed from both primary and bacterial productivity

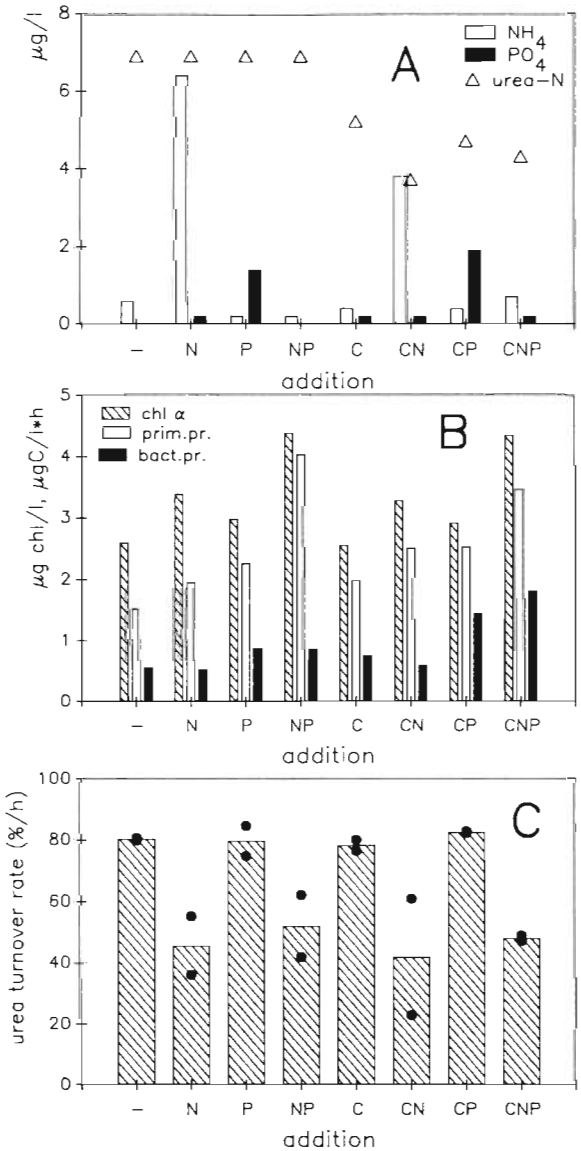

Fig. 5. Experiment on delayed effects of nutrient additions, 27 to 28 June 1988 (see Table 2). N = 20 mg NH_4-N l^{-1}, P = 5 mg PO_4-P l^{-1}, C = 50 mg glucose-C l^{-1}. (A) Nutrient concentrations at the start of incubations. (B) Chlorophyll *a*, primary and bacterial productivity. (C) Urea turnover rate ($1/T$). Circles: replicates, bars: averages

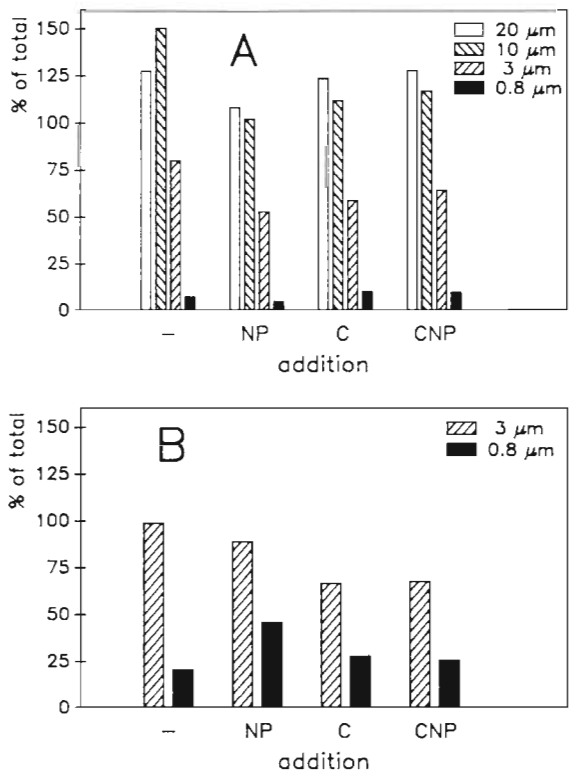

Fig. 6. Primary and bacterial productivity in prefractionated incubations in Expt 2, 28 June 1988 (see Table 2)

measurements, as it had systematically decreased in the units with NH_4 additions (Fig. 5C).

The prefractionation results from this experiment showed that phytoplankton production was dominated by smallest nanoplankton or picoplankton, as 50 to 75% of primary productivity passed 3 µm prefiltration. Less than 10% passed 0.8 µm (Fig. 6A). With bacterial productivity, over 60% passed 3 µm, and 20 to 50% was found below 0.8 µm (Fig. 6B).

The urea turnover rate derived from the prefractionated samples produced controversial results in this experiment (data not shown). In 3 out of 4 units, all prefractionations drastically reduced urea turnover rate. In the unit with all nutrient additions, the pattern was different and comparable to prefractionation results from Expt 1 (Fig. 4A). These results indicate again that phytoplankton was responsible for urea uptake.

The reduction of urea turnover rate after other prefractionations is difficult to explain satisfactorily, as both primary and bacterial productivity incubations were performed from the same filtrates and no corresponding reductions were found (Fig. 6A, B). This seems to rule out the possibility of some maltreatment of the organisms during the prefiltrations. It is possible that prefractionation had damaged fragile zooplankton thus affecting urea concentrations in the filtrate.

Enrichment studies: effects after 24 h delay

In Expt 3, the effects of inorganic nutrient additions were measured after a 24 h delay. Emphasis was laid on NH_4 inhibition of urea uptake on several NH_4 addition levels (Table 3). After 24 h, only the units with large single NH_4 additions (50 and 100 µg N l^{-1}) had clearly elevated NH_4 concentrations left (10.9 and 60.2 µg N l^{-1}), and the depletion rates of NH_4 in these

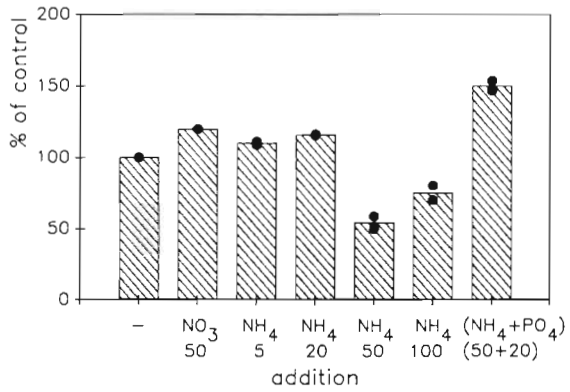

Fig. 7. Urea turnover rate ($1/T$) in the experiment on delayed effects of nutrient additions, 30 June to 1 July 1988 (see Table 3), expressed as % of control unit. Circles: replicates, bars: averages

units were 1.50 and 1.53 µg N l^{-1} h^{-1}, respectively. In the unit with the combined addition of PO_4 and 50 µg N l^{-1} of NH_4, the depletion rate for NH_4 was 1.83 µg N l^{-1} h^{-1}.

Urea turnover rate results showed clearly the inhibition by NH_4 additions in the units where NH_4 concentrations were still elevated (Fig. 7). The corresponding NO_3 addition (50 µg N l^{-1}) did not cause inhibition of urea turnover rate, although a NO_3 concentration of 19.5 µg N l^{-1} was left at the time of the incubation. The depletion rate for NO_3 was 1.20 µg N l^{-1} h^{-1}. The unit with the combined PO_4 and NH_4 addition showed the highest urea turnover rate, over 150% of control (Fig. 7).

DISCUSSION

Respiration and particulate uptake of ^{14}C-urea

We found a uniformly high respiration percentage (98 to 99%) after ^{14}C-urea incubations in a variety of experiments. This result is identical to the observations from cultured phytoplankton by Carpenter et al. (1972) and Horrigan & McCarthy (1981), but contradicts several other results both from culture and natural phytoplankton studies.

Mitamura & Saijo (1986) and Mitamura (1986a, b) reported in their studies on lacustrine phytoplankton that the percentage showed diurnal variation, the particulate percentage being 11 to 47% during daytime. From a coastal area, Mitamura & Saijo (1975) reported particulate uptake percentages of 38 to 84% in light incubations. Price & Harrison (1988) noted that in their short-term time series incubations with *Thalassiosira pseudonana* the particulate percentage was 63% during the first minutes, whereafter it decreased down to approximately 20% as estimated from their Fig. 3. Both Mitamura & Saijo (1986) and Price & Harrison (1988) suggested a 2-phase model for ^{14}C-urea uptake on the basis of these results.

We suspect that a methodological artifact is at least partly responsible for the higher particulate ^{14}C percentages referred to above. Both in the experiments by Horrigan & McCarthy (1981) and in this study, the respired fraction was trapped immediately after incubations from closed incubation bottles, leading to respiration percentages of 98 to 99%.

The studies which report higher particulate percentages (Mitamura & Saijo 1975, 1986, Mitamura 1986a, b, Price & Harrison 1988) have utilized a procedure where filtration is performed first, and the respired fraction is trapped thereafter from the filtrate. If respired $^{14}CO_2$ is not collected directly from closed incubation bottles, a significant part of it will obviously be lost, especially during the efficient aeration which is inevitable when filtrating the sample. In fact, Price & Harrison (1988) noted a discrepancy between their ^{14}C-urea uptake rates and the disappearance of urea in their experiments, and proposed '... that some of the ^{14}C is lost to a compartment not included in our analysis.' They hypothesized a dissolved organic ^{14}C loss during the incubations, but an alternative explanation could be incomplete trapping of the respired $^{14}CO_2$.

A possible cause for higher particulate ^{14}C percentages in the light than in the dark is naturally reassimilation of respired $^{14}CO_2$ in photosynthesis. This artifact has been found insignificant because of rapid dilution of $^{14}CO_2$ in the medium of marine phytoplankton cultures (Horrigan & McCarthy 1981), but in lakes it could be a more serious source of error than with marine phytoplankton because of far lower CO_2 background levels.

Chemical and kinetic urea analyses

The kinetic maximum uptake rates, obtained with ^{14}C-urea, and the depletion rate of urea, obtained with chemical analyses (diacetyl monoxime method), fit together quite well (range for kinetic values 0.5 to 1.3 µg N l^{-1} h^{-1}, for chemical daytime values 0.8 to 1.3 µg N l^{-1} h^{-1}; see Figs. 1 to 3). However, a considerable discrepancy between the kinetic $K+S_n$ value and chemical urea analyses was consistent, the latter being several times higher than the concentration sum $K+S_n$. This sum should be composed of the ambient substrate concentration (S_n) and the half-saturation constant K, describing the affinity of the community for the substrate.

This kind of result is rather common in uptake studies with radioactive organic substrates (glucose, amino

acids, urea, etc.), PO_4 and inorganic nitrogen compounds (e.g. Gocke et al. 1981, Kristiansen & Lund 1989), and it is often explained by introducing the concept of 'biologically available' substrate in question. Chemical analyses often involve drastic manipulations of the sample (e.g. pH), which can bring some absorbed, chelated, biologically loosely bound etc. compartments into solution. Therefore, chemical analyses easily overestimate the 'biologically available' (that is, truly dissolved) compartment of the substrate, while the kinetic analysis is based on the biologically available substrate during the incubation.

This explanation seems valid also for the abovementioned results: if the chemical analyses systematically overestimate the prevailing dissolved urea concentration because of sample manipulation, the kinetic concentration sum ($K+S_n$) should be consistently smaller than measured urea concentration, as it was. However, this artificially increased background should not affect the depletion rate calculations, as they are based on the differences between successive chemical analyses — the background is counted out. Therefore the kinetic and chemical results agree in this case.

Another indication of overestimated urea concentrations in chemical analysis was the nonsense curve which was produced when chemically analyzed urea concentration was included in urea uptake rate calculations (Fig. 1).

The fate of urea N

Urease or a corresponding enzymatic system for urea degradation is considered to be intracellular in phytoplankton (Berns et al. 1966, Leftley & Syrett 1973). If urea is degraded within the cell, the rapid (practically immediate) and almost complete liberation of ^{14}C as $^{14}CO_2$ after urea uptake indicates that enzymatic uptake through the cell membrane is closely coupled to enzymatic degradation of urea within the cell. Therefore, intracellular storage pools of urea seem to be nonexistent or at least quantitatively nonsignificant. Wheeler et al. (1982) have presented evidence for a similar pattern in NH_4 assimilation, where uptake through the cell membrane would be directly related to NH_4-N utilization in macromolecule synthesis.

Price & Harrison (1988) proposed an urea uptake model for *Thalassiosira pseudonana* which included efflux of both urea, and NH_3 after urea degradation, followed by reabsorption to a varying degree. They also refer to 'evidence that in the ocean much of the urea N taken up is not retained by phytoplankton and over the short term is not reabsorbed'. This evidence is based on observed discrepancies between ^{15}N and ^{14}C urea assimilation patterns in short-term incubations. We have already suspected that this discrepancy may at least partly arise from methodological sources.

In Expt 4, NH_4 accumulation was followed during urea depletion. A small but significant difference in NH_4 concentrations developed between the control and urea addition units (Fig. 3C), with a time lag from the peak of urea depletion (Fig. 3B). At the end of the experiment when all added urea had been taken up, a maximum of 10% of the urea N enrichment was found as NH_4-N in the medium, in accordance with the urea depletion experiments by Eppley et al. (1971).

It seems therefore that after urea molecules are transported through the cell membrane, they are immediately degraded into NH_3 and CO_2, a major part of the produced NH_3 is retained by the cells and CO_2 is liberated in the medium. Our results thus indicate that urea was a genuine nitrogen source for phytoplankton, in contrast to the urea uptake model by Price & Harrison (1988).

N source preferences

Since Dugdale & Goering (1967) introduced the concepts of new and regenerated planktonic production, the relative uptake preferences of the major nitrogen sources for the planktonic community have been extensively studied by comparing *in situ* uptake rates and concentrations of NH_4, NO_3 and urea (e.g. the Relative Preference Index, RPI, of McCarthy et al. 1977).

Widely different mechanisms control the availability of new and regenerated nitrogen sources. For nitrate, spatially extensive physical transport or accumulation over long periods of time are most important, while immediate regeneration within the planktonic food web governs ammonium and urea availability. It is therefore doubtful whether ambient concentrations could represent actual relative availability of the N sources. Experimental manipulation of availability appears thus essential for preference evaluations (Tamminen 1995).

In our experiments, NO_3 did not suppress urea turnover (Figs. 4A & 7). The experiments also showed the transient nature of NH_4 suppression on urea turnover rate. Immediately (0 to 2 h) after the NH_4 addition, no suppression of urea uptake was observed in unfractionated experimental units (Fig. 4A).

With a time lag of 20 h from enrichments, urea turnover rate had decreased in the experimental units which had received NH_4 (Fig. 5C), no matter whether traces of the addition were present or not at the start of the incubation (Fig 5A). After 24 h, urea turnover rate had recovered in the units where NH_4 had been depleted, but clear suppression was observed if traces

of the additions were still present (Fig. 7), in accordance with the NH_4 inhibition results presented by Kokkinakis & Wheeler (1988).

The depletion rates for each nitrogen source, when they were added alone, were rather similar (1.20, 1.36, and 1.50 µg N l^{-1} h^{-1} for NO_3, urea, and NH_4, respectively). This suggests that nitrogen sources were interchangeable to a high degree, and uptake of each source was regulated mainly by availability, and only secondarily by preference. Although this might not necessarily be the case for any single phytoplankton species, for the community as a whole it seems obvious. However, Horrigan & McCarthy (1981) presented evidence for similar lack of preference between nitrogen sources in diatom cultures as well.

When PO_4 was also added in the experiments, nitrogen sources were utilized clearly more efficiently: NH_4 depletion rate increased in Expts 2 and 3 (Fig. 5A), and urea turnover rate increased in Expt 3 (Fig. 7). Moreover, organic carbon addition stimulated bacterioplankton production, and obviously also bacterial utilization of urea (Fig. 4C), which was otherwise almost negligible. Kirchman et el. (1990) have presented analogous evidence for organic C limitation of bacterial NH_4 uptake.

These nutrient uptake results indicate that the planktonic community was in a state of multiple substrate limitation, phytoplankton by nitrogen and phosphorus, and bacterioplankton by carbon and phosphorus. This is supported by the primary productivity, chl *a* and bacterial productivity responses in multiple enrichment assays (Fig. 5B).

Hierarchical regulation of nitrogen uptake thus seemed to operate during the research period: (1) the availability of other growth factors (for phytoplankton, mainly phosphate) defined the nitrogen need in cellular metabolism; (2) the availability of different forms of nitrogen governed the pick of choice in uptake; (3) if several alternative nitrogen sources were available, the preference of reduced forms of nitrogen according to the order NH_4 > urea > NO_3 seemed to prevail.

Under natural marine conditions, when nitrogen often is the primary limiting nutrient, and when nutrients are scarce compared to uptake saturation levels and thus not freely selected upon (that is, there is no room for the third-level regulation to occur), the commonly used preference measures (e.g. RPI; McCarthy et al. 1977) probably mainly reflect the relative availability of nitrogenous nutrients, not preference by phytoplankton in any physiological sense. During regenerated production periods, the relative proportions of NH_4 and urea in remineralization might thus be the underlying cause behind observed apparent preferences in uptake of alternative nitrogen sources.

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