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Diel Cycles of Nitrogen Uptake by Marine Phytoplankton in NO_3^--high and -low Environments

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To test the roles of NO_3^- concentration and light as controlling factors of NO_3^- uptake in the NO_3^--low and -high environments and to assess the significance of night-time nitrogen (N) uptake in estimating the daily N uptake rate, 2 diel studies of N uptake were conducted in NO_3^--low (the eastern part of the Yellow Sea) and NO_3^--high (the marginal ice zone of the northwestern Weddell Sea) environments on June 14 to 15, 1996 and January 15 to 16, 1995, respectively. Our observations confirmed that NO_3^- uptake by phytoplankton is mainly determined by ambient NO_3^- concentration in NO_3^--low environment and by light in NO_3^--high environment, respectively. Our results suggest the need for diel studies to accurately estimate the daily N uptake rates and thus new and regenerated production because the daily rates calculated without considering the night-time N uptake would be significantly underestimated (up to 41%), particularly in the NO_3^--low environment.

INTRODUCTION

New production is the portion of primary production supported by newly available nitrogen (N) forms (mainly, NO_3^-), whereas regenerated production is supported by nutrients recycled (mainly, NH_4^+ and urea) within the euphotic zone (Dugdale and Goering, 1967; Eppley and Peterson, 1979). The ratio of NO_3^- uptake to total N uptake, i.e. *f*-ratio (Eppley and Peterson, 1979) is an important measure because it can provide insights both to analyze the structure and function of the marine ecosystems and to model global biogeochemical fluxes (Platt *et al.*, 1992). While the importance of *f*-ratio is well recognized, several biases in estimating the *f*-ratio remain to be resolved (Harrison *et al.*, 1987; Murray *et al.*, 1989). One is the diel variation in NO_3^- uptake, which may underestimate the true *f*-ratio (Murray *et al.*, 1989), especially when night-time NO_3^- uptake is considerable over the natural light and dark cycles. Dark N uptake by phytoplankton is not trivial, especially for NH_4^+ (Olson, 1980; Fisher *et al.*, 1982; Glibert, 1982; Koike *et al.*, 1986; Hanson and Robertson, 1988; Sahlsten *et al.*, 1988; Wheeler and Kokkinakis, 1990). But, dark N uptake rates reported in many studies (e.g., Cochlan *et al.*, 1991b and references therein) were obtained with light and dark incubation method only during daytime, and thus may not necessarily reflect the *in situ* night-time uptake rates. In this context, JGOFS (1994) recently recommended that the uptake measurements should be done at least twice a day, once during the daytime and once during the night-time. To accurately estimate the daily N uptake rates and *f*-ratio, diel study of N uptake needs to be done.

Two time-course experiments of N uptake were conducted in the euphotic zone in 2 distinct environments: the eastern part of the Yellow Sea (YS) with low NO_3^- concentration and low phytoplankton biomass, and the northwestern Weddell Sea (WS) with high NO_3^- concentration and high biomass. The magnitudes of NO_3^-, NH_4^+ and urea uptake by phytoplankton on a diel scale at two mooring stations were measured and factors controlling the magnitude of diel variations of N uptake were addressed. Here, we report that significant night-time uptake of NO_3^- did occur in the eastern part of

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the Yellow Sea with relatively low NO_3^- concentration, and that the estimates of daily N uptake rates (and thus new and regenerated production) calculated without considering the night-time N uptake would be underestimated up to 41% in this area. Diel variations of N (especially those for NO_3^-) uptake were mainly controlled by NO_3^- concentration in the YS and light availability in the WS, respectively.

MATERIALS AND METHODS

Study area and sample collection

Two time-course experiments of N uptake were conducted aboard the R/Vs *Eardo* and *Yuzhmorgeologiya* on June 14 to 15, 1996 in the earstern part of the Yellow Sea (36°15'N, 125°40'E) and on January 15 to 16, 1995 in the marginal ice zone of the northwestern Weddell Sea (63°30'S, 53°00'W) as a part of the 8th Korea Antarctic Research Program (KARP) cruise. Water temperature and salinity were measured with a SBE-911 or a Neil-Brown CTD system mounted on a rosette sampler.

Seawater samples in the YS were collected at 4 h intervals for 24 h with 5 l Niskin bottles mounted on a rosette sampler for depths corresponding to 100, 30, and 1% surface light penetration depths (LPDs) within the euphotic zone, as determined by the Secchi disc. In the WS, samples were collected at 5 h intervals for 24 h for depths corresponding to 100, 49, 30, 14.5, 3.5, and 1% LPDs. The solar irradiance was measured over 10 min intervals using a LI-190SA quantum sensor and recorded with a LI-1000 DataLogger (LI-COR, Inc.).

Measurements of N uptake

All N uptake experiments began within 0.5 h of collection. Seawater samples were transferred into 250 ml polycarbonate bottles wrapped with perforated nickel screens (Stork Veco, Bedford, MA, USA) to simulate *in situ* light intensity at which the samples were collected, and inoculated with either $^{15}NH_4Cl$ or $K^{15}NO_3$ (all 99 atom% ^{15}N; Cambridge Isotope Lab., Woburn, MA, USA) to bring the final tracer addition to 0.2 and 1 μM, respectively. Samples were incubated for 4 (in the YS) or 5 h (in the WS) in on-deck incubators cooled with continuously flowing surface seawater. After the incubation, samples were filtered onto pre-combusted (4 h at 450°C) Whatman GF/F filters (diameter 25 mm) and stored dry at 60°C until the analysis of $^{15}N/^{14}N$ ratio with a mass spectrometer (Europa Scientific GC-MS; Owens, 1988). Urea uptake rates were determined using ^{14}C-urea (Remsen *et al.* 1972) and were described in detail in Shim *et al.* (1996). Particulate nitrogen (PN)-specific (V) and absolute (ρ) uptake rates were calculated according to Dugdale and Wilkerson (1986). Chlorophyll *a* (Chl *a*)-specific N uptake rate was calculated by dividing ρ by the chl *a* concentration and was designated as V_{NO3}^{Chl} (for NO_3^-), V_{NH4}^{Chl} (for NH_4^+), and V_{Urea}^{Chl} (for urea).

Other analytical methods

Samples for nutrient analyses were filtered through Whatman GF/F filters and the filtrates were stored frozen (-20°C) and analyzed later with a SKALAR 5100 autoanalyzer or manually by the methods of Parsons *et al.* (1984) and Solorzano (1969). Urea was determined using the diacetyl monoxime thiosemicarbazide method described by Price and Harrison (1987). Chl *a* concentrations were measured by a spectrophotometric method (Parsons *et al.*, 1984) after filtration (< 100 mm of Hg) onto Whatman GF/F filters. Samples for the quantitative analysis of phytoplankton were fixed with Lugol's solution and were identified after Yamaji (1984) and Tomas (1996). Bacterial abundance was measured by epifluorescence microscopy of DAPI-stained samples (Porter and Feig, 1980). Bacterial production was measured basically by the method of Ducklow *et al.* (1992). To estimate bacterial biomass and production, 20 fg C $cell^{-1}$ (Lee and Fuhrman, 1987) was used. Bacterial turnover time was calculated by dividing bacterial biomass with bacterial production. The kinetic parameters for NO_3^- uptake with respect to irradiance were obtained by a direct fit of the data to a Michaelis-Menten hyperbola using a computerized, iterative, non-linear least-squares technique (TableCurve™ 2D, Jandel Scientific).

RESULTS

The Yellow Sea

Diel variations of temperature, salinity, and density are shown in Fig. 1a-c. In the eastern part of the Yellow Sea, a strong thermocline (as well as pycnocline) was developed between 10 and 20 m depth, and its position almost overlapped with the

base of the euphotic zone (the euphotic depth, 15 m). The density field did mirror the temperature variations rather than salinity variations. The maximal light intensity at the surface was ca. 1400 μE m^{-2} s^{-1} (Fig. 1d), and the photoperiod was 15 h. Chl *a* concentrations showed a subsurface maximum and were usually less than 1 μg l^{-1} in the euphotic zone (Fig. 1e). Chl *a* concentrations did not show marked diel variation. Autotrophic nanoflagellate *Cryptomonas* sp. and tychopelagic diatom *Paralia sulcata* dominated the phytoplankton community at the surface and at the bottom of the euphotic zone during the diel study, respectively (Table 1). After the pycnocline was strongly disturbed in the aphotic zone (0545-1000 hours and 1820-2200 hours; Fig. 1a-c), however, surface phytoplankton community was temporarily dominated by *Paralia sulcata* and thereafter was again replaced by *Cryptomonas* sp. over the time. NO_3^- concentrations in the euphotic zone were usually less than 2 μM (Fig. 1f). NH_4^+

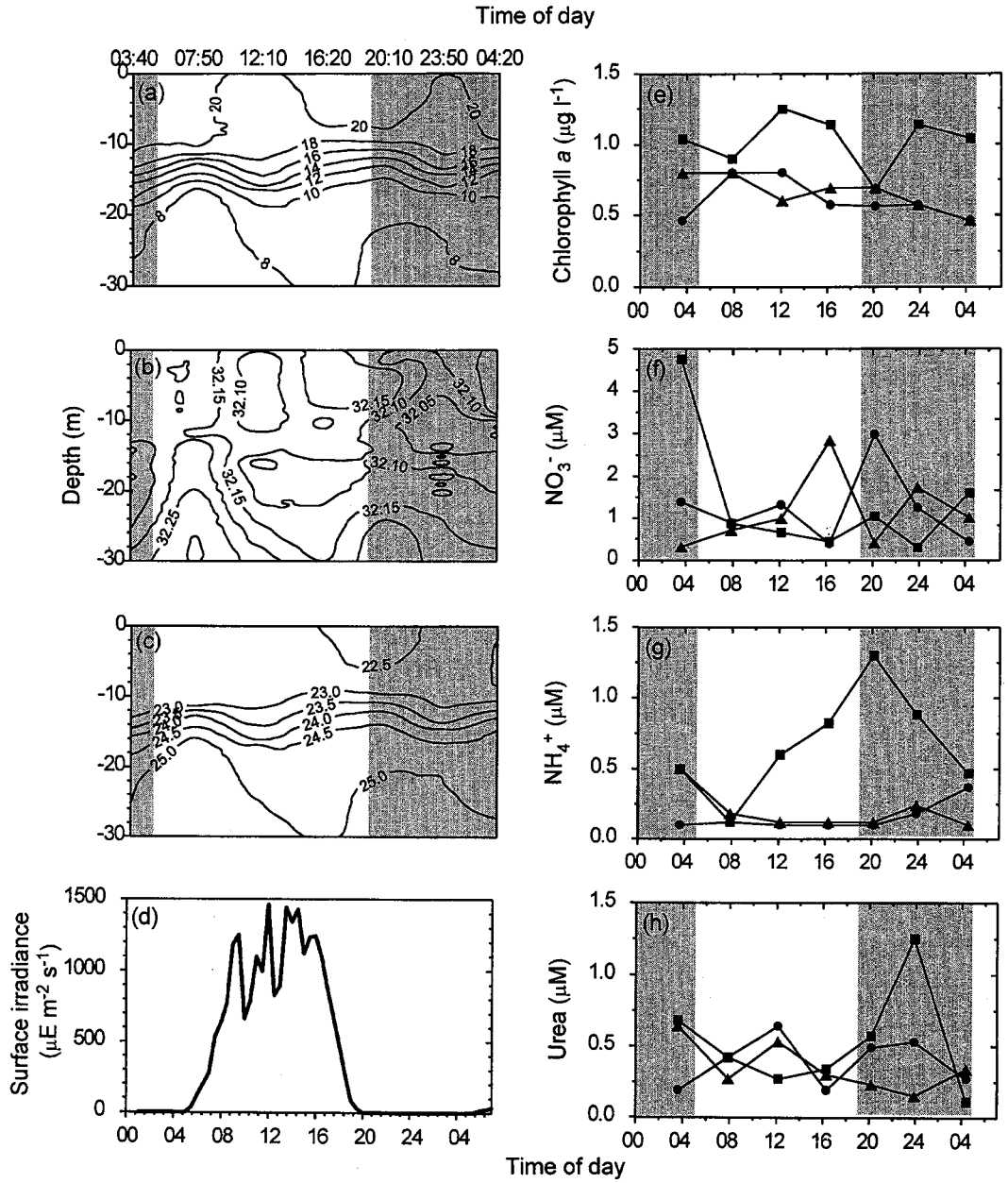

Fig. 1. Diel variations of (a) water temperature (°C), (b) salinity (‰), (c) sigma-t, (d) surface irradiance, (e) chlorophyll *a*, (f) NO_3^-, (g) NH_4^+, and (h) urea concentrations in the Yellow Sea. Shaded areas represent the dark periods. Closed circles, triangles, and squares represent the samples from depths corresponding to 100, 30, and 1% surface light penetration depths, respectively.

concentrations tended to increase with depth and were usually less than 0.8 μM in the euphotic zone, with no marked diel pattern (Fig. 1g). Urea concentrations also showed no marked diel variation and were usually less than 0.5 μM in the euphotic zone (Fig. 1h).

Diel variations of PN-specific, absolute, and chl *a*-specific N uptake rates are shown in Fig. 2. V_{NO3} values did not show a clear diel pattern (Fig. 2a) even though they showed a weakly decreasing pattern with depth, suggesting that diel variation of V_{NO3} values was not closely associated with the light/dark cycles. The surface maximal (2.2×10^{-3} h^{-1}) and minimal (0.37×10^{-3} h^{-1}) V_{NO3} values were observed in the early part of the dark period (1820-2200 hours) and in the afternoon, respectively. Diel variations of both ρ_{NO3} and V^{Chl}_{NO3} were all similar to that of V_{NO3} values (Fig. 2b & c). V_{NH4} values showed a strong diel periodicity at the surface and 30% LPD, with maximal values around the noon (1000-1415 hours), reduced values in the morning and the afternoon, and minimal values during the night-time (Fig. 2d). But, there was no obvious diel pattern of V_{NH4} values at 1% LPD. Both ρ_{NH4} and V^{Chl}_{NH4} values also showed the similar patterns to V_{NH4} values (Fig. 2e & f). Although V_{Urea} values were not calculated in this study due to the use of ^{14}C-urea (see Materials and Methods), both ρ_{Urea} and V^{Chl}_{Urea} values also showed the similar diel variations to the uptake parameters of NH_4^+, with maximal values around the noon and minimal values during the night-time (Fig. 2g & h).

NH_4^+ was a predominant N source utilized by phytoplankton, followed by NO_3^- and urea (Fig. 2). The diel variation of each N uptake was also reflected in the diel variation of the *f*-ratio, i.e. absolute NO_3^- uptake over total absolute N (NO_3^-, NH_4^+ and urea) uptake (Fig. 3). The *f*-ratios showed the large variation, ranging from 0.029 (at 1% LPD) in the afternoon to 0.328 (at the surface) in the early part of dark period. The *f*-ratios for uptake rates integrated over the euphotic zone also showed a large variation from 0.038 around the noon (when NH_4^+ uptake was greatest) to 0.205 in the afternoon. The *f*-ratios for uptake rates integrated over the euphotic zone were on average 0.143 ± 0.091 (coefficient of variation, CV=63.9%) and 0.112 ± 0.002 (CV= 2.1%) during daytime and night-time, respectively.

The mean absolute N uptake rates during the daytime and the night-time are summarized in Table 2. Mean night-time ρ_{NO3} values at the surface and 30% LPD were 130% and 28% of mean daytime values, respectively, whereas mean night-time ρ_{NH4} and ρ_{Urea} values were 35-40% and 37-54% of the mean daytime values, respectively. At 1% LPD, mean night-time uptake values were all greater than those during the daytime. Mean daytime and night-time uptake rates integrated over the euphotic zone were 0.49 ± 0.05 (CV=11.1%) and 0.36 ± 0.15 mg N m^{-2} h^{-1} (CV=40.4%) for NO_3^-, 5.24 ± 5.54 (CV= 105.8%) and 2.85 ± 1.20 mg N m^{-2} h^{-1} (CV=42.2%)

Table 1. Dominant species of phytoplankton as percentage of total cell numbers during this study in the eastern part of the Yellow Sea

Sampling time	% LPD	Dominant species	Cell numbers (cells l^{-1})	%
01:30	100	*Cryptomonas* sp.	9,091	62
		Dictyocha fibula	1,364	9
		Pseudo-nitzschia delicatissima	1,364	9
	1	*Paralia sulcata*	15,086	46
		Cylindrotheca closterium	8,686	26
		Thalassiosira oestrupii	2,286	7
05:45	100	*Dictyocha fibula*	2,344	36
		Prorocentrum minimum	977	15
		Gymnodinium sp.	781	12
		pennate diatom (20 μm)	586	9
	1	*P. sulcata*	3,922	48
		pennate diatom (20 μm)	588	7
		Gymnodinium sp.	392	5
		Pseudo-nitzschia delicatissima	392	5
10:00	100	*Cryptomonas* sp.	12,600	78
		Dictyocha fibula	1,000	6
		Gymnodinium sp.	1,200	7
	1	*P. sulcata*	15,417	33
		C. closterium	8,750	19
		Th. Oestrupii	4,167	9
14:15	100	*P. sulcata*	4,587	40
		Cryptomonas sp.	917	8
		Dictyocha fibula	1,101	10
		Gymnodinium sp.	734	6
	1	*P. sulcata*	10,101	29
		C. closterium	10,101	29
		Cryptomonas sp.	2,020	6
18:20	100	*Cryptomonas* sp.	18,596	62
		Dictyocha fibula	3,036	10
	1	*P. sulcata*	1,923	9
		Cryptomonas sp.	10,385	49
		Prorocentrum sp.	1,923	9
22:00	100	*P. sulcata*	4,696	15
		Cryptomonas sp.	15,130	49
		Dictyocha fibula	2,087	7
	1	*P. sulcata*	18,795	41
		C. closterium	4,819	11
		pennate diatom (20 μm)	2,892	6
01:45	100	*Cryptomonas* sp.	6,303	37
		P. minimum	2,424	14
		Amphidinium crassum	4,606	27
	1	*P. sulcata*	6,563	26
		C. closterium	2,813	11
		Th. oestrupii	2,344	9
		Amphipora sp.	2,344	9

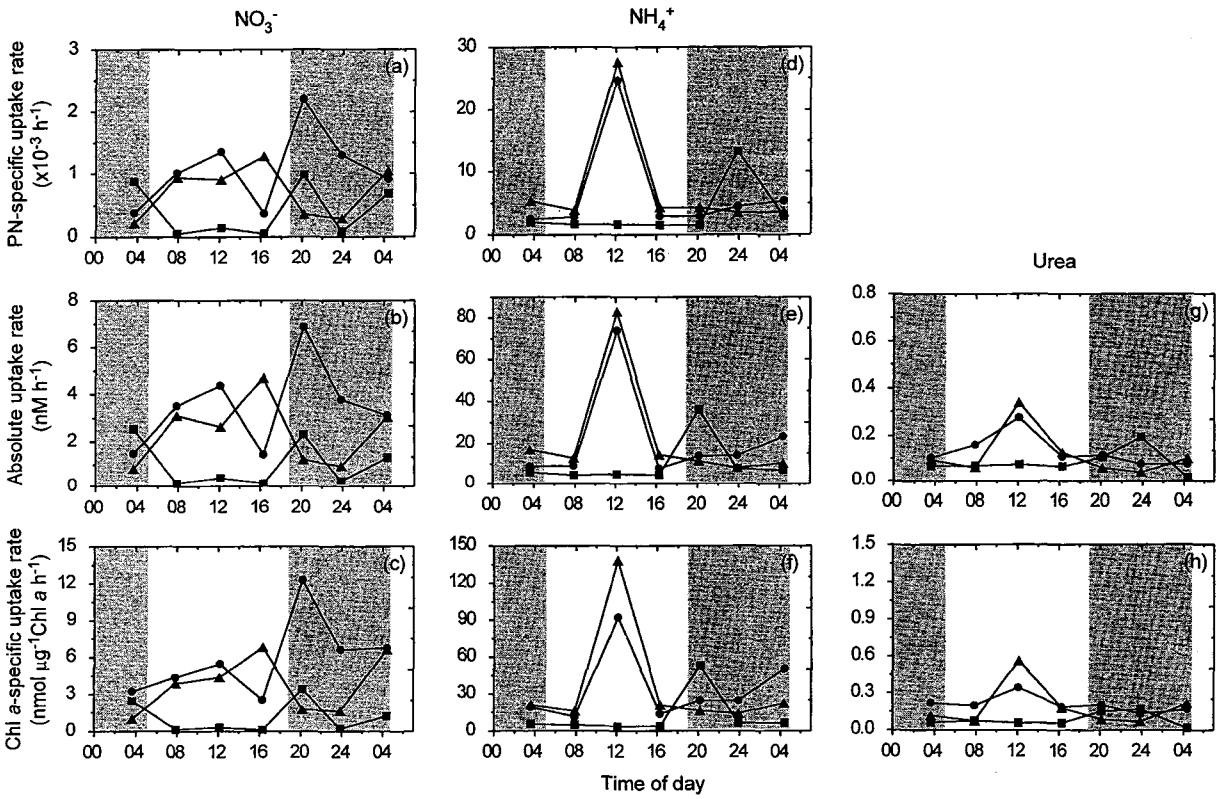

Fig. 2. Diel variations of particulate nitrogen (PN)-specific (top), absolute (middle), and chlorophyll *a* (chl *a*)-specific (bottom) uptake rates of (a-c) NO_3^-, (d-f) NH_4^+, and (g-h) urea in the Yellow Sea. Shaded areas represent the dark periods. Note that samples from the final sampling time were incubated until after the sunrise, and the data are not included in Table 2. The symbols as for Fig. 1.

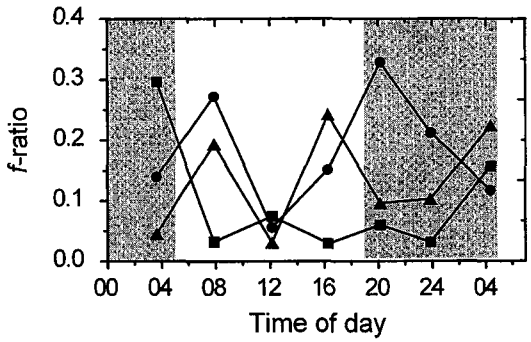

Fig. 3. Diel variations of *f*-ratios (ratios of absolute NO_3^- uptake rates over absolute total N uptake rates) in the Yellow Sea. Shaded areas represent the dark periods. The symbols as for Fig. 1.

for NH_4^+, and 0.06 ± 0.04 (CV=62.1%) and 0.04 ± 0.00 mg N m^{-2} h^{-1} (CV=7.8%) for urea, respectively. Mean night-time uptake rates integrated over the euphotic zone were equivalent to 74 (for NO_3^-), 54 (for NH_4^+), and 63% (for urea) of those during the daytime, respectively.

Bacterial abundance was almost constant during the diel cycle (Table 2). Bacterial abundance at 1% LPD was one half of those at the surface and 30% LPD during the daytime and the night-time. Bacterial production also showed a similar pattern to bacterial abundance. Bacterial turnover times ranged from 11.3 to 13.4 d at the surface and 30% LPD and were slightly longer (17.5 to 18.8 d) at 1% LPD during this study.

The Weddell Sea

In the marginal ice zone of the Weddell Sea (WS), the diel variation of water temperature was very small (less than 0.8°C), ranging from -0.26 to 0.53°C (Fig. 4a). The density field did mirror the salinity variations rather than temperature (Fig. 4b & c), unlike the case in the Yellow Sea. The maximal light intensity at the surface was ca. 1400 µE m^{-2} s^{-1} (Fig. 4d), and the photoperiod was approximately 17 h. In the WS, in which Prymnesiophyte *Phaeocystis antarctica* (colonial stage) bloom occurred, chl *a* concentrations were very high (from 4 to 12 µg l^{-1}) throughout the euphotic zone (Fig. 4e). NO_3^- concentrations in the euphotic zone were very rich (mean of 9 µM, range of 3-13 µM). NO_3^- con-

Table 2. Summary of mean absolute (ρ) N uptake rates, bacterial abundance (BA) and production (BP), bacterial N demand (BND), and bacterial turnover times during the daytime (n=3) and the night-time (n=3) in the Yellow Sea. Data from the final sampling time, when samples were incubated until after the sunrise, were not included in calculating for mean absolute N uptake rates

%LPD	Time	ρ_{NO3}	ρ_{NH4}	ρ_{Urea}	BA	BP	BND	Bacterial turnover time (d)			
		(ng N l^{-1} h^{-1})			($\times 10^9$ l^{-1})	(μg C l^{-1} h^{-1})	(ng N l^{-1} h^{-1})	*In situ*	NO_3^-	NH_4^+	Urea
100	Day	43.5	423.6	5.0	1.77	0.11	27.5 (18.3)[a]	13.4	5.7 (56.5)[b]	0.6 (1.9)[c]	49.2 (98.3)[d]
	Night	56.6	170.9	2.8	1.70	0.12	30.0 (20.0)	11.8	4.2 (41.7)	1.4 (4.6)	84.3 (168.7)
30	Day	48.7	514.8	4.8	1.76	0.12	30.0 (20.0)	12.2	5.0 (50.2)	0.5 (1.6)	50.9 (101.9)
	Night	13.7	168.7	1.7	1.49	0.11	27.5 (18.3)	11.3	15.1 (151.1)	1.2 (4.1)	121.7 (243.5)
1	Day	3.2	64.1	2.0	0.90	0.04	10.0 (6.7)	18.8	39.1 (390.6)	2.0 (6.5)	62.5 (125.0)
	Night	23.9	231.3	3.4	0.84	0.04	10.0 (6.7)	17.5	4.9 (48.8)	0.5 (1.7)	34.3 (68.6)

[a] The N demand in bacteria collected onto GF/F filters when assuming the retention efficiency of ca. 2/3 of natural bacterial numbers
[b, c, d] Bacterial turnover times when assuming bacterial contribution of 10% (Kirchman *et al.*, 1994), 30% (Kirchman, 1994) and 50% (Park *et al.*, 1997) to the NO_3^-, NH_4^+, and urea uptake, respectively

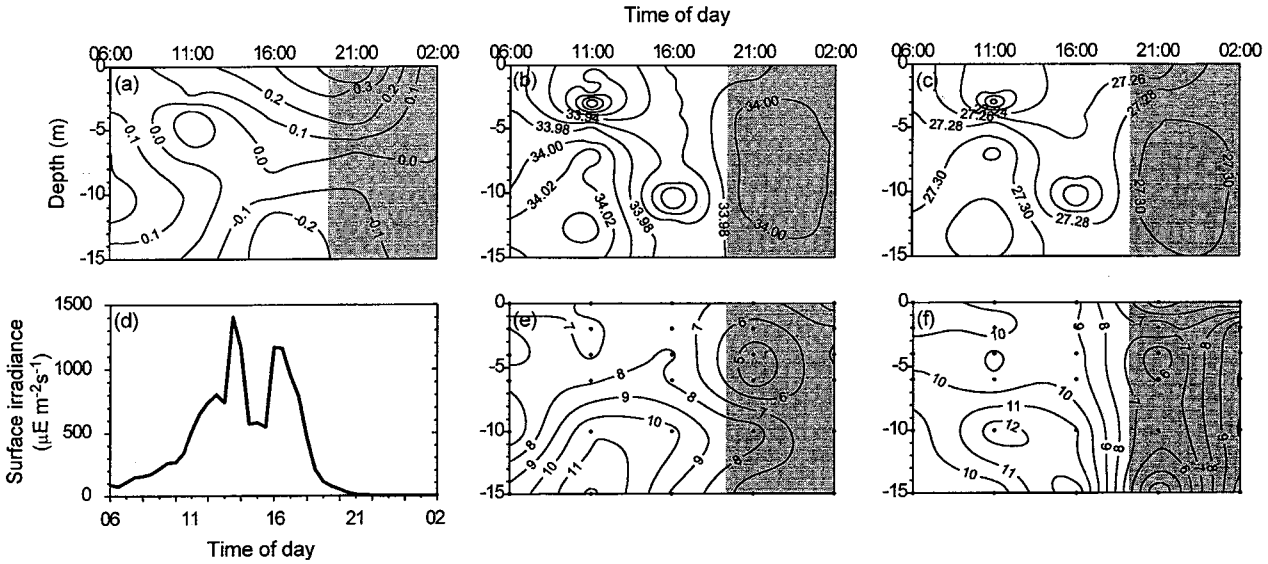

Fig. 4. Diel variations of (a) water temperature (°C), (b) salinity (‰), (c) sigma-t, (d) surface irradiance, (e) chlorophyll *a* (μg l^{-1}), and (f) NO_3^- (μM) concentrations in the Weddell Sea. The dots represent sampling depths. Shaded areas represent the dark periods.

centrations tended to be high during the daytime and low during the night-time, apparently due to the active uptake during the daytime (Fig. 4f).

V_{NO3} values showed a strong diel periodicity at light levels above 14.5% LPD, with the maximal values around the noon and the minimal values during the night-time (Fig. 5a). At low light levels below 3.5% LPD, however, the diel periodicity of V_{NO3} values was not obvious, presumably due to the light limitation. Diel variations of both ρ_{NO3} and V^{Chl}_{NO3} were all similar to that of V_{NO3} values (Fig. 5b & c). Mean night-time V_{NO3} values at light levels above 14.5% LPD were only less than 7% of the mean daytime values, whereas at low light levels below 3.5% LPD they were equivalent to 28-48% of the mean daytime values. Mean daytime and night-time NO_3^- uptake rates integrated over the euphotic zone were 6.76 ± 1.89 (CV=28.0%) and 0.64 mg N m^{-2} h^{-1}, and night-time NO_3^- uptake rates integrated over the euphotic zone was equivalent to 9.5% of mean daytime uptake rate.

Correlation between N uptake and environmental parameters

The plots of V_{NO3} values versus NO_3^- concentrations or light intensity for all data during 2 time-course experiments are shown in Fig. 6. The correlation between V_{NO3} values and NO_3^- concentrations was positively significant (r^2=0.46, $p < 0.01$) for data in the YS (Fig. 6a), but was not significant for data in the WS (Fig. 6b). The V_{NO3} values in the WS could be related to light intensities averaged during the incubation by a rectangular hyperbola, similar to

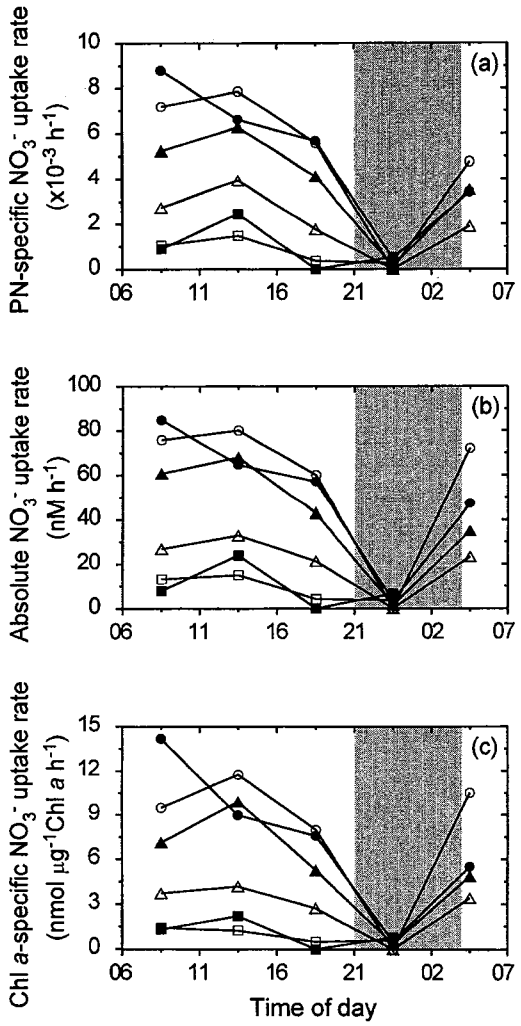

Fig. 5. Diel variations of (a) particulate nitrogen (PN)-specific, (b) absolute, and (c) chlorophyll *a* (chl *a*)-specific uptake rates of NO_3^- in the Weddell Sea. Shaded areas represent the dark periods. Closed and open circles, triangles, and squares represent the samples from depths corresponding to 100, 49, 30, 14.5, 3.5 and 1% surface light penetration depths, respectively. Note that samples from the final sampling time were incubated until after the sunrise, and the data are not included in Table 2.

the Michaelis-Menten equation. In this hyperbola, the kinetic parameters V_{max} (maximum V_{NO3} value at saturating light intensity) and K_{LT} (the half-saturation constant for light, equivalent to light intensity at 0.5 V_{max}) were estimated to be 7.13×10^{-3} h^{-1} and 38.8 μE m^{-2} s^{-1}, respectively (Fig. 6d). We could not find any significant relationship between V_{NO3} values and chl *a* concentrations or water temperature for both study areas and for all pooled data (not shown). Both V_{NH4} and ρ_{Urea} values in the YS were not correlated to their concentrations and the other parameters (not shown).

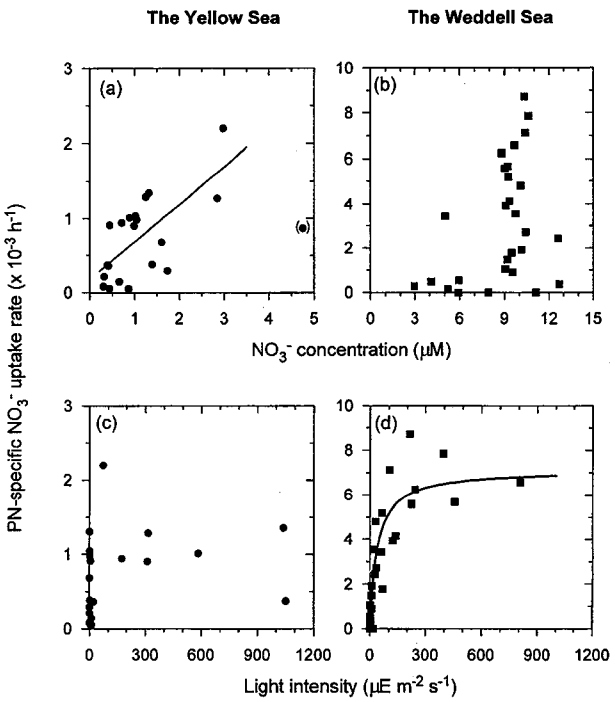

Fig. 6. The plots of particulate nitrogen (PN)-specific NO_3^- uptake rates versus (a, b) NO_3^- concentrations or (c, d) light intensities averaged during the study. The linear regression equation is $y=0.00018+0.0005x$ ($r^2=0.46$, $p < 0.01$). An exceptional datum was not included in the linear regression analysis; The curved plot is fitted directly to the Michaelis-Menten equation for all data in the Weddell Sea ($V_{max}=7.13\times10^{-3}$ h^{-1}, $K_{LT}=38.8$ μE m^{-2} s^{-1}).

DISCUSSION

Diel variation of N uptake and its controlling factor

The NO_3^- uptake by phytoplankton is usually known to be more light-dependent than those of NH_4^+ or urea, presumably due to the greater energy requirements for NO_3^- assimilation (MacIsaac and Dugdale, 1972; Fisher *et al.*, 1982; Lipschultz *et al.*, 1985). Thus, diel periodicity of NO_3^- uptake can be expected to be more prominent than those of NH_4^+ and urea uptake. In NO_3^--rich (mean 9 μM) environment of the Weddell Sea, NO_3^- uptake showed an obvious diel variation with the maximal during the daytime and the minimal during the night-time, and mean night-time NO_3^- uptake was less than 7% of the mean daytime uptake (Fig. 5). In relatively NO_3^--low (usually, less than 2 μM) environment of the Yellow Sea, however, there was not an obvious diel periodicity of NO_3^- uptake (Fig. 2). The mean night-time NO_3^- uptake was from 0.3 to 8.0-fold of the mean daytime NO_3^- uptake in the YS.

In NO_3^--high WS, NO_3^- uptake was closely related

to light intensity (Fig. 6b), suggesting that light availability was one of the major factors controlling the magnitude of NO_3^- uptake. But, the dependence of NO_3^- uptake on light intensity may vary with ambient N concentrations; for example, over the spring bloom in Auke Bay, Alaska, Kanda *et al.* (1989) found little or no dark NO_3^- uptake during periods of elevated NO_3^- concentration (ca. 3 to 27 μM), but significant dark uptake and less dependence on irradiance after NO_3^- depletion (from undetectable to ca. 3 μM). Cochlan *et al.* (1991b) also reported that light-dependency of NO_3^- uptake decreased in N poor waters than in N replete waters. In NO_3^--low YS during this study, NO_3^- uptake was less dependent on light intensity, and instead showed a linear response on the variation of ambient NO_3^- concentration (Fig. 6a). Our observations confirmed that diel variation of NO_3^- uptake appears to be mainly determined by ambient N concentrations in NO_3^--low YS and by light in NO_3^--high WS. In addition to the ambient N concentrations, diel periodicity of N uptake in both environments could be influenced by several factors, such as phytoplankton biomass and its species composition, preconditioned light history of phytoplankton, variation in light intensity, and NH_4^+ concentration (Bates, 1976; Paasche *et al.*, 1984; Cochlan *et al.*, 1991a). As both V_{NO3} and V_{NO3}^{Chl} values showed similar patterns (Figs. 2 & 5), the differences in phytoplankton biomass do not seem to be responsible for the diel variation of NO_3^- uptake in our study. During time-course experiment conducted in the tidally influenced YS, however, the differences in species composition of the phytoplankton community may also influence on the diel variation of NO_3^- uptake. During this study, surface light intensities were similar in the YS and WS.

In contrast, NH_4^+ and urea uptake in the YS showed the obvious diel periodicity at the surface and 30% LPD, with maximal values around the noon and minimal values during the night-time, but did not show obvious diel patterns at 1% LPD (Fig. 2). Any significant relationships between NH_4^+ and urea uptake rates and their concentrations or other environmental parameters were not found (not shown), and diel variations of the uptake rates of the reduced N forms could not be explicitly explained by the available data during this study.

The magnitude of night-time N uptake and its significance

A review of available literature shows that night-time N uptake by phytoplankton over the natural light/dark cycle can vary greatly and be significant (Table 3). In the NO_3^--rich (range of 3-29 μM) environments, night-time NO_3^- uptake amounts to 20% (median) of the daytime uptake, ranging from negligible in the Scotia Sea (Olson, 1980) to 70% in an upwelled plume off the British Columbia coast (Cochlan *et al.*, 1991a). Night-time NO_3^- uptake in low NO_3^- (usually less than 3 μM) environments is also variable, but corresponds to much greater portion (100% median) of the daytime uptake, compared to that in the NO_3^--rich environments. The fraction in low NO_3^- environments ranges from 27% in the south-eastern Kattegat (Sahlsten *et al.*, 1988) to 800% in the Yellow Sea (this study). Also, night-time uptake of the reduced N forms (NH_4^+ and urea) seems to be substantial in both NO_3^--rich and poor environments, corresponding to 55 to 75% median and 58 to 69% median of the daytime uptake, respectively (Table 3). The fractions of night-time uptake of NH_4^+ and urea in the Yellow Sea during this study are similar to or greater than those reported in several marine environments (Table 3). In low NO_3^- environments diel periodicity of N uptake seems generally absent or substantially reduced, presumably due to the continued uptake during the night-time by N limitation.

Despite the considerable night-time N uptake (Table 3), a portion of our night-time N uptake in the Yellow Sea may also be attributed to marine bacteria. As Whatman GF/F filters (nominal pore size 0.7 μm) were used to collect particulate material after incubation with ^{15}N-labelled substrates in this study and all studies in Table 3, marine bacteria might contribute to night-time N uptake rates (Laws *et al.*, 1985; Wheeler and Kirchman, 1986; Tupas and Koike, 1990; Kirchman *et al.*, 1994; Hoch and Kirchman, 1995). Kirchman (1994) reported through the available literature review in marine and freshwater environments that the bacteria account for a large portion (30% median) of total NH_4^+ uptake. In the north Atlantic Ocean, Kirchman *et al.* (1994) observed that a small portion (< 10%) of NO_3^- uptake was due to bacteria. Wheeler and Kirchman (1986) also observed low NO_3^- uptake by bacteria in coastal waters of Georgia. Urea is known to be decomposed primarily by phytoplankton rather than bacteria in most marine and freshwater environments (Remsen *et al.*, 1972; Turley, 1985; Mitamura and Saijo, 1986; Shim *et al.*,

Table 3. Summary of literature values of the percentages of night-time N uptake rates (V_N) over the daytime N uptake rates (V_D), determined over natural light/dark cycles

Location	NO_3^- (μM)	V_N/V_D (%)			Reference
		NO_3^-	NH_4^+	Urea	
High NO_3^- environments					
Perru upwelling area	>8	25	62		Eppley *et al.* (1970)
Upwelling area off NW Africa		20			Collos & Slawyk (1976)
Scotia Sea	>25	0	25 or 85		Olson (1980)
Antarctic waters	21-29	10-30	50		Koike *et al.* (1986)
Oceanic subarctic Pacific		29.7	51		Wheeler *et al.* (1989)
Oceanic subarctic Pacific	5.8-17.0	2-10	62-92[b]	50-87[b]	Wheeler & Kokkinakis (1990)
NE Pacific Ocean	12	55			Cochlan *et al.* (1991a)
British Columbia coast (100 & 30% LPD)	6.3-12.9	15-16	30-36		
British Columbia coast (1% LPD)	8.6-11.5	70	120		
S Benguela upwelling system (inshore)	ca.5	11.9	54.5		Probyn *et al.* (1996)
Weddell Sea (≧14.5% LPD)	4.1-10.6	<7			*This study*
Weddell Sea (< 14.5% LPD)	3.0-12.7	28-48			
Median[a]		20	55	69	
Low NO_3^- environments					
Central N Pacific Gyre	<0.1	ca.100	ca.100	50	Sahlsten (1987)
SE Kattegat	0-8.53	27-54	34-83	40-82	Sahlsten *et al.* (1988)
Open Skagerrak	0.04-<1	100 or 200	50 or 100	100 or 200	Pettersson & Sahlsten (1990)
S Benguela upwelling system (offshore)	<2	65.5	170		Probyn *et al.* (1996)
Yellow Sea (100% LPD)	0.39-2.98	140	40	54	*This study*
Yellow Sea (30% LPD)	0.32-2.84	28	35	37	
Yellow Sea (1% LPD)	0.30-4.75	800	380	180	
Median[a]		100	75	58	

[a] Each study was considered as one datum point. When ranges were reported the median of the range was used
[b] The percentage of night-time uptake over the daily uptake

1994; Cho and Azam, 1995; Cho *et al.*, 1996) although Park *et al.* (1997) recently reported a significant urea decomposition by bacteria (on average 47.1%) in a hypertrophic freshwater pond. Here, we estimated the contribution of bacteria to the daytime and night-time N uptake rates measured in the Yellow Sea during this study as follows: if NO_3^-, NH_4^+, and urea were assumed to be utilized as the sole N source for bacterial growth of 5 fg N $cell^{-1}$ (Lee and Fuhrman, 1987), they would support bacterial turnover times of 4.2 to 39.1 d (for NO_3^-), 0.5 to 2.0 d (for NH_4^+), and 34.3 to 121.7 d (for urea), respectively. These turnover times are an order of magnitude shorter (for NO_3^- and NH_4^+) or 2 to 11-fold longer (for urea) than the actual turnover times (11.3 to 18.8 d; Table 3) of bacteria calculated from bacterial biomass and production, with 2 exceptional turnover times at 30% LPD during the night-time (15.1 d) and at 1% LPD during the daytime (39.1 d). Further, even if we assumed the potential bacterial uptake of 10% (for NO_3^-; Kirchman *et al.*, 1994), 30% (for NH_4^+; Kirchman, 1994) and 50% (for urea; Park *et al.*, 1997) reported in the literature, our general conclusion that bacterial N uptake was negligible and night-time phytoplankton N uptake did occur in the Yellow Sea would not be much affected.

Despite the importance of diel cycles in N uptake studies, it has been often ignored because diel study requires fairly intensive sampling in the field. Since the night-time N uptake by phytoplankton is substantial, particularly in NO_3^--low environments (Table 3), it should be considered for estimating daily N uptake rates. During this study, daily NO_3^- uptake rates were estimated to be 10.3 and 107.2 mg N m^{-2} d^{-1} in the Yellow Sea and the Weddell Sea, respectively, of which night-time NO_3^- uptake rate comprised 40.8% in the YS and 5.4% in the WS, respectively. Daily NH_4^+ and urea uptake rates in the Yellow Sea were estimated to be 99.3 and 1.2 mg N m^{-2} d^{-1}, respectively, of which night-time NH_4^+ and urea uptake rates comprised 33.2 (for NH_4^+) and 37.0% (for urea), respectively. Without considering night-time NO_3^- uptake, therefore, the daily NO_3^- uptake rates and thus new production could be significantly underestimated (up to 41%), especially in low NO_3^- environments. Similarly, if night-time NH_4^+ and urea uptake rates are ignored, daily uptake rates of the reduced N forms and regenerated production could be significantly underestimated in the study area. Further, without considering the night-time N uptake, slight underestimation (ca. 10%) of the *f*-ratio would result in the NO_3^--low Yellow Sea,

and significant overestimation of the *f*-ratios may occur in NO_3^--high environments, due to the relatively low rates of night-time NO_3^- uptake and high rates of night-time NH_4^+ and urea uptake compared to daytime (Table 3). To estimate the daily N uptake rates more accurately, although JGOFS (1994) recommended that N uptake measurements should be done at least twice a day, including both the daytime and the night-time, our results suggest that more frequent measurements be done on a diel scale, as indicated by the relatively high coefficient of variation values of N uptake rates during the diel cycles in the YS and the WS.

In summary, NO_3^- uptake by phytoplankton did not show any marked diel pattern in low NO_3^- environment, whereas it showed a strong diel periodicity in high NO_3^- environment. Diel variations of NO_3^- uptake seemed to be controlled by NO_3^- concentration in the Yellow Sea and light availability in the Weddell Sea. Our results suggest the need for diel studies to accurately estimate the daily N uptake rates and thus new and regenerated production, as night-time N uptake rates could comprise a significant portions (up to 41%) of the daily N uptake rates, particularly in low NO_3^- environment.

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