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Time course of uptake of inorganic and organic nitrogen by phytoplankton in the Strait of Georgia: comparison of frontal and stratified communities

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ABSTRACT: In both frontal and stratified water of the Strait of Georgia, changes in dissolved nitrogen concentrations provided evidence for the simultaneous uptake of ammonium, nitrate and urea by a summer phytoplankton community. Chlorophyll *a* specific uptake rates of ammonium and urea were ca 2 and 2.4 times greater in stratified water than in frontal water, whereas chlorophyll *a* specific nitrate uptake rates were ca 1.6 times greater in frontal water. Ammonium and urea regeneration rates, calculated using a mass balance approach, were similar in frontal water, but urea regeneration rates were 2 to 5 times greater in the stratified water during the first 12 h of the experiment. Increases in particulate nitrogen could not be accounted for by corresponding decreases in total concentration of dissolved inorganic nitrogen and urea, or by ^{15}N accumulation in the particulates. In frontal water the change in total dissolved inorganic nitrogen and urea consistently overestimated the change in particulate nitrogen, and in stratified water the change in total dissolved inorganic nitrogen and urea consistently underestimated the change in particulate nitrogen. These data suggest that the plankton community in frontal water was losing nitrogen in the form of dissolved organic nitrogen. By contrast, the plankton community in stratified water took up nitrogen compounds which were not measured as part of the total dissolved inorganic and urea nitrogen, but were most likely dissolved organic nitrogen compounds. Results stress the importance of determining uptake rates of all 3 nitrogen substrates (NH_4^+, NO_3^- and urea) using ^{15}N isotopes and by simultaneously measuring the change in concentration of these compounds throughout the incubation period.

INTRODUCTION

Shallow sea fronts, areas of high primary productivity (Pingree et al. 1975, Parsons et al. 1981, 1983, Holligan et al. 1984), are located at the boundary between mixed and stratified water. These regions are characterized by having high phytoplankton biomass in surface water with measurable concentrations of dissolved nitrate, and a shallow pycnocline which extends to the surface at the frontal boundary (e.g. Simpson & Pingree 1978). In the Strait of Georgia, a coastal basin off the west coast of Canada, several tidally-induced frontal regions have been described (Parsons et al. 1981).

The nitrogen dynamics of frontal regions have received little attention. Floodgate et al. (1981) found elevated rates of urea decomposition in frontal water, relative to mixed and stratified water, which were concomitant with higher dissolved urea concentrations. Furthermore, high rates of carbon and nitrate uptake have been observed in the proximity of a front by Parsons et al. (1984). Recently Holligan et al. (1984) calculated that ammonium excretion by zooplankton could account for >50 % of the potential phytoplankton requirements on the stratified side of a front. Clearly, additional information is required to understand and describe the nitrogen cycling between dissolved and particulate components in these areas.

A surface transect normal to a frontal boundary progresses from high concentrations of dissolved nitrate on the mixed side to nitrogen-deplete stratified water, and thus represents a gradient of nitrogen availability and phytoplankton nutritional states. Moreover, most of the nitrogen demands of phytoplankton in nitrogen-impoverished water are supplied by ammonium and urea from regenerative processes, whereas in nitrogen-

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rich areas nitrogen compounds appear to be utilized at rates proportional to their availability (e.g. Dugdale & Goering 1967, McCarthy et al. 1977). Experiments using laboratory cultures of phytoplankton have demonstrated that the preconditioning nitrogen substrate affects the response of phytoplankton to the additions of different forms of nitrogen (Horrigan & McCarthy 1981, 1982, Dortch & Conway 1984). Additionally, in nitrogen-starved phytoplankton, the ability to take up nitrate may be lost and must be induced (Dortch et al. 1982, review by Collos 1983, Parslow et al. 1984b). These observations suggest that phytoplankton communities in frontal and stratified water may differ in their response to perturbations of nitrogen by their preference for, and uptake rates of, different nitrogen substrates.

Previous nitrogen uptake experiments have involved single end-point measurements of accumulated ^{15}N-labelled substrates in particulate matter over long time intervals (Goldman et al. 1981, review by Harrison 1983). In theory these experiments provide important information concerning daily rates of nitrogen utilization as they invariably take into account diel patterns of uptake (providing they are of 24 h duration). The existence of uptake periodicity has been reported for cyclostat cultures of *Skeletonema costatum* (Eppley et al. 1971b) and *Chaetoceros* sp. (Malone et al. 1975), and natural phytoplankton communities (e.g. Goering et al. 1964, Eppley et al. 1970, 1971a, McCarthy & Eppley 1972, MacIsaac 1978, Fisher et al. 1982).

The experiments presented in this paper were designed to examine the time course of nitrogen uptake by phytoplankton from nitrate-deplete stratified water and nitrate-replete frontal water over a 24 h cycle. In conjunction, we examined the response of the phytoplankton to additions of ammonium, nitrate, and urea as a function of their nutritional past history. From measurements of nitrogen uptake rate using ^{15}N isotope incorporation, and changes in the concentration of dissolved nitrogen over time, we have calculated regeneration rates of ammonium and urea. We believe that this is the first publication of estimates of urea regeneration rates by intact plankton communities. Finally, we report discrepancies between measured particulate nitrogen concentrations and theoretical values based on changes in the concentrations of dissolved inorganic nitrogen and urea. These results are discussed within the current concepts of nitrogen cycling in marine planktonic ecosystems.

METHODS

Three 24 h time course experiments were conducted in the Strait of Georgia, B. C., Canada aboard the C. S. S. 'Vector' (July 1984); station locations for Time

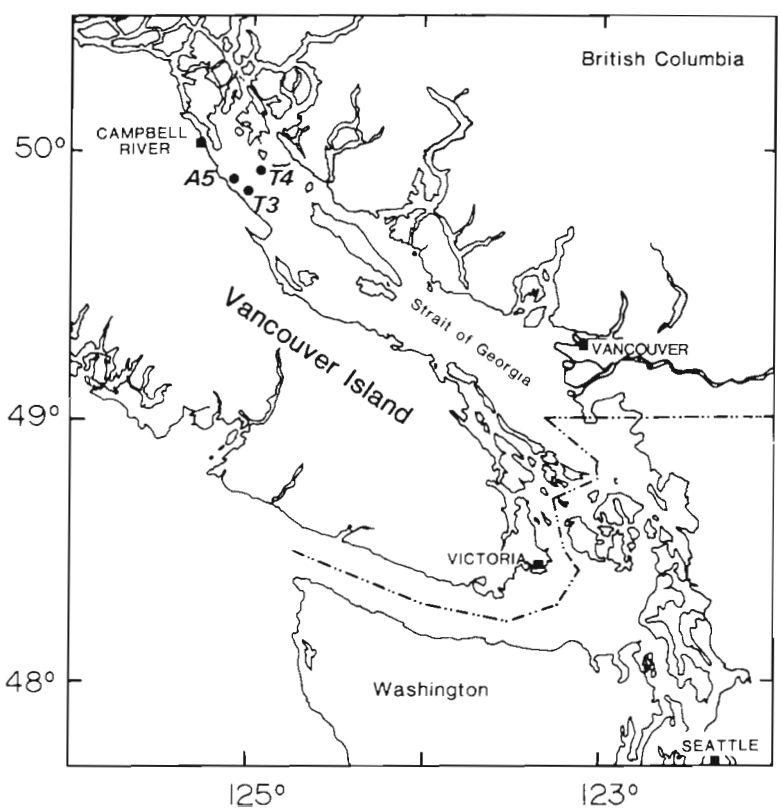


Fig. 1. Station locations for Time Course 1 (T3, frontal station), Time Course 2 (A5, frontal station) and Time Course 3 (T4, stratified station)

Courses 1, 2 and 3 are shown in Fig. 1. At approximately 0900 h water samples were collected from a depth corresponding to 50 % of the surface irradiance using 5 l PVC Niskin bottles and transferred into a 20 l Nalgene® carboy. Subsamples for nutrient analyses were then filtered through combusted (460 °C for 4 h) Whatman GF/F filters using an acid-washed syringe and a 25 mm Millipore Swinex® filter holder. Samples were gently filtered into acid-washed polyethylene bottles and were analyzed immediately for dissolved inorganic nitrogen (DIN) as ammonium (NH_4^+) and nitrate ($NO_3^- + NO_2^-$) and for urea concentrations. Alternatively, filtered samples were stored frozen (−20 °C) and analyzed within 24 h. NH_4^+ and $NO_3^- + NO_2^-$ were measured with a Technicon Autoanalyzer® II following the procedures outlined in Slawyk & MacIsaac (1972) and Wood et al. (1967), respectively. Urea was determined by the diacetyl monoxime thiosemicarbizide technique described by Price & Harrison (unpubl.). Duplicate samples for chlorophyll *a* (chl *a*) (coefficient of variation, CV, = 4.4 ± 4.1 %; 5 data pairs) were collected on Whatman GF/F filters and stored frozen in a desiccator. Chl *a* was extracted in 90 % acetone and analyzed by *in vitro* fluorometry (Strickland & Parsons 1972) using a Turner Designs model 10 fluorometer. Particulate organic carbon and nitrogen (POC & PON) (CV = 5.2 ± 4.8 % and 3.8 ± 4.1 %; 7 data pairs), collected on combusted Whatman GF/F filters, were stored similarly and analyzed later with a Perkin Elmer model 240 elemental analyzer. Vertical profiles of temperature and salinity were obtained from continuous profiles, run prior to bottle sampling, using an Interocean CTD system and *in vivo* fluorescence was measured simultaneously with a Turner model 111 fluorometer. Incident solar irradiance (P.A.R.) was monitored continuously with a Lambda Instruments LI-185 light meter equipped with a LI-190S Surface Quantum Sensor, and subsurface light measurements were determined with a LI-192S Underwater Quantum Sensor. Phytoplankton species samples (250 ml) were preserved in Lugol's solution and 10 ml subsamples were settled and counted on an inverted microscope; 100 ml subsamples were examined for microzooplankton.

Within 1 h of collection, water was transferred into 500 ml Wheaton glass bottles (clear: light bottles, or darkened with black tape: dark bottles) with teflon-lined caps and saturating additions of either $^{15}NH_4Cl$, $Na^{15}NO_3$ or $CO(^{15}NH_2)_2$ (all 99 at % ^{15}N) were added. In Time Course 1, 2 µg-at Nl^{-1} of $^{15}NO_3^-$ or $CO(^{15}NH_2)_2$ was added and in Time Courses 2 and 3, 6 µg-at Nl^{-1} of $^{15}NH_4^+$, $^{15}NO_3^-$ or $CO(^{15}NH_2)_2$ was added. The precision (± 1 SD) of our nutrient determinations for the time-zero samples was ± 0.09 µg-at Nl^{-1} (n = 5) for NH_4^+, ± 0.07 µg-at Nl^{-1} (n = 5) for NO_3^- and ± 0.02 µg-at Nl^{-1} (n = 4) for urea. Light and dark bottle uptake rates of each nitrogen substrate were measured over the time course and all sample bottles were mixed hourly. Time-zero samples for dissolved nitrogen were withdrawn immediately and analyzed for NH_4^+, NO_3^- and urea concentrations in all bottles. Incubations were conducted under natural light in clear Plexiglas® deck incubators, cooled with surface seawater and covered with neutral density screening to simulate the irradiance at the 50 % light depth. At 3 h intervals particulate matter, from duplicate samples, was collected by filtration (<125 mm Hg) onto combusted Whatman GF/F filters and stored frozen in a desiccator. Samples for dissolved nitrogen concentrations were taken concurrently and those for chl *a* and POC & PON every 6 h. It is important to note that samples were taken from randomly selected incubation bottles and that chl *a* and POC & PON samples were taken from bottles distinct from those analyzed for ^{15}N atom % excess in particulate matter and dissolved nitrogen concentrations.

Nitrogen in the particulate samples was converted to N_2 (g) by the micro-Dumas dry combustion technique as described by La Roche (1983) and then analyzed for ^{15}N enrichment with a JASCO model N-150 emission spectrometer (Fiedler & Proksch 1975). Nitrogen uptake rates were calculated according to the equations of Dugdale & Goering (1967) and are presented as nitrogen-specific (h^{-1}) and absolute (µg-at $N\,l^{-1}\,h^{-1}$) rates. The ratio of nitrogen uptake in the dark bottle (continuous darkness) to that in the light bottle (exposed to the natural light cycle) ($V_D : V_L$) is also reported. Ammonium and urea regeneration rates (d) have been determined using the approach of Fisher et al. (1981). These rates were calculated using the Blackburn-Caperon equation (Blackburn 1979, Caperon et al. 1979) $d = \triangle P/t + i$, where $\triangle P$ = change in concentration of NH_4^+ or urea (µg-at Nl^{-1}) over time interval t (h), and i = nitrogen uptake rate (µg-at $N\,l^{-1}\,h^{-1}$) calculated from ^{15}N accumulation in the particulate matter (Dugdale & Goering 1967). Disappearance uptake rates (V^d) have been calculated from the change in concentration of dissolved nitrogen per unit time ($\triangle P/t$) and, like the nitrogen-specific and absolute ^{15}N uptake rates (V^i), are reported for the time intervals over which they have been calculated.

RESULTS

The vertical profiles of temperature, relative *in vivo* chl *a* fluorescence, and NO_3^- concentration for the frontal water stations (Time Course 1 [T3]; and Time Course 2 [A5]) showed almost identical trends with depth; thus only the synoptic profile of Time Course 2

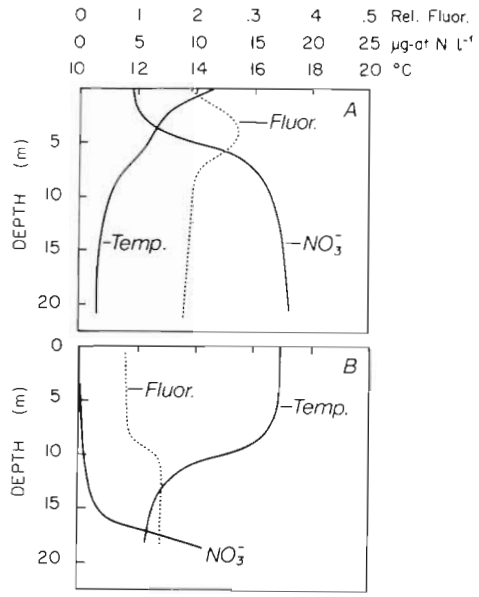


Fig. 2. Depth profiles of temperature, *in vivo* fluorescence, and NO_3^- concentration. (A) Frontal station A5, Time Course 2. (B) Stratified station T4, Time Course 3

is presented (Fig. 2A). Throughout the Results and Discussion, reference to frontal water pertains to Time Course 2 unless specified otherwise. We will refer to the results of Time Course 1 only briefly because of the paucity of data. The diagnostic features of the frontal water were the shallow thermocline and high fluorescence at the depth of the nitracline (3 to 7 m). Time Course 3 was conducted in stratified water at Station T4 and the depth profile (Fig. 2B) demonstrated a subsurface fluorescence maximum (ca 10 m) which was overlain by nitrate-depleted mixed water. A summary of the initial biomass data and environmental conditions for each station is given in Table 1.

The species composition of the phytoplankton community in the frontal and stratified water was very different (Table 2). In the frontal water large chain-forming diatoms of the genus *Chaetoceros* formed aggregates (≤ 1 mm) which contained some pennate diatoms belonging to *Navicula* spp. and *Nitzschia* spp. The size of the diatom flocs prevented us from screening the water samples through Nitex® netting (in order to minimize macrozooplankton predation during incubations), and therefore, to remain consistent, none of the water samples were screened. Small flagellates (< 5 µm) were the most common phytoplankton in the stratified water. *Chaetoceros* spp., *C. socialis* and *Skeletonema costatum* were the most abundant of the diatoms whereas dinoflagellates were almost exclusively *Gymnodinium* spp. Water samples were not originally taken for zooplankton species enumeration, however the abundance of these organisms, as seen in the phytoplankton samples, suggested that they could have been important grazers and nitrogen remineralizers. As a first approximation we have determined the numbers and types of these organisms in our samples (Table 2).

Table 1. Initial environmental conditions of seawater collected for time course experiments

Station and location	Description	Time course experiment	Date	Starting time of incubation (local time)	Sample depth (m)	Dissolved nutrient concentration (NH_4^+ NO_3^- Urea[a]) (µg-at N l^{-1})			Chlorophyll *a* (µg l^{-1})	PON (µg-at N l^{-1})	POC (µg-at C l^{-1})
T3 49°50′42″ N 125°00′54″ W	Frontal	1	24 Jul 1984	0730	2	–	2.99	0.18	6.55	14.8	106 C:N = 7.2*
A5 49°53′02″ N 125°05′48″ W	Frontal	2	28 Jul 1984	1000	3	0.27	4.55	0.60	2.12	7.28	47.3 C:N = 6.5
T4 49°55′30″ N 124°55′30″ W	Stratified	3	29 Jul 1984	0800	3	0.19	<.05	0.33	0.39	3.57	31.4 C:N = 8.8

* By atoms

Table 2. Plankton community composition in frontal and stratified water

Station	Phytoplankton (10^6 cells l^{-1})			Zooplankton (l^{-1})			
	Diatoms	Dinoflagellates	Flagellates	Tintinnids	Calanoid copepods	Ciliates excl. tintinnids	Others[1]
Frontal A5	2.3	0.023	1.6	470	50	730	280
Stratified T4	0.43	0.049	1.6	180	60	140	300

[1] 50 to 60% were *Noctiluca* sp., the remainder were *Oikopleura* sp. and unidentified zooplankton

During the time course experiments we measured changes in the concentration of dissolved NH_4^+, NO_3^- and urea and followed the incorporation of ^{15}N-labelled nitrogen into the particulate matter. Both approaches yield different information concerning nitrogen utilization by the phytoplankton. Changes in dissolved nitrogen concentration represent net community flux of that nutrient and encompass regenerative and uptake processes. By contrast, ^{15}N-isotope accumulation gives a measure of the gross uptake by the phytoplankton providing there is no recycling of ^{15}N, and ^{15}N enrichment remains constant. Results from Time Course 2 (frontal water) and Time Course 3 (stratified water) experiments are shown in Fig. 3 and 4, respectively. Data from Time Course 2 demonstrate multiple nitrogen substrate utilization by phytoplankton; specifically for NH_4^+, NO_3^- and urea (Fig. 3C, E) and NO_3^- and urea (Fig. 3G). The high ambient NO_3^- concentration in the frontal waters enabled us to determine the disappearance uptake rates of NO_3^- in the NH_4^+ and urea-spiked samples. Disappearance uptake rates for nitrate were similar in the presence (V^d_{0-6h} = 0.521 µg-at N l^{-1} h^{-1}) and absence (V^d_{0-9h} = 0.567 µg-at N l^{-1} h^{-1}) of urea but were reduced in the NH_4^+ spiked samples (V^d_{0-9h} = 0.267 µg-at N l^{-1} h^{-1}). The ^{15}N-urea atom % accumulation rate was constant over the first 15 h, but prior to the end of the dark period it increased and remained linear until the end of the incubation (Fig. 3F). The increase in urea uptake rate coincided with the depletion of external NO_3^-, moreover the change in urea concentration was minimal (Fig. 3G) over the first 6 h, when NO_3^- concentrations were high (4.55 to 1.4 µg-at N l^{-1}) and NO_3^- was being taken up. ^{15}N-NO_3^- and ^{15}N-NH_4^+ incorporation was non-linear with time, however substrate exhaustion did not occur until the 21 to 24 h time interval.

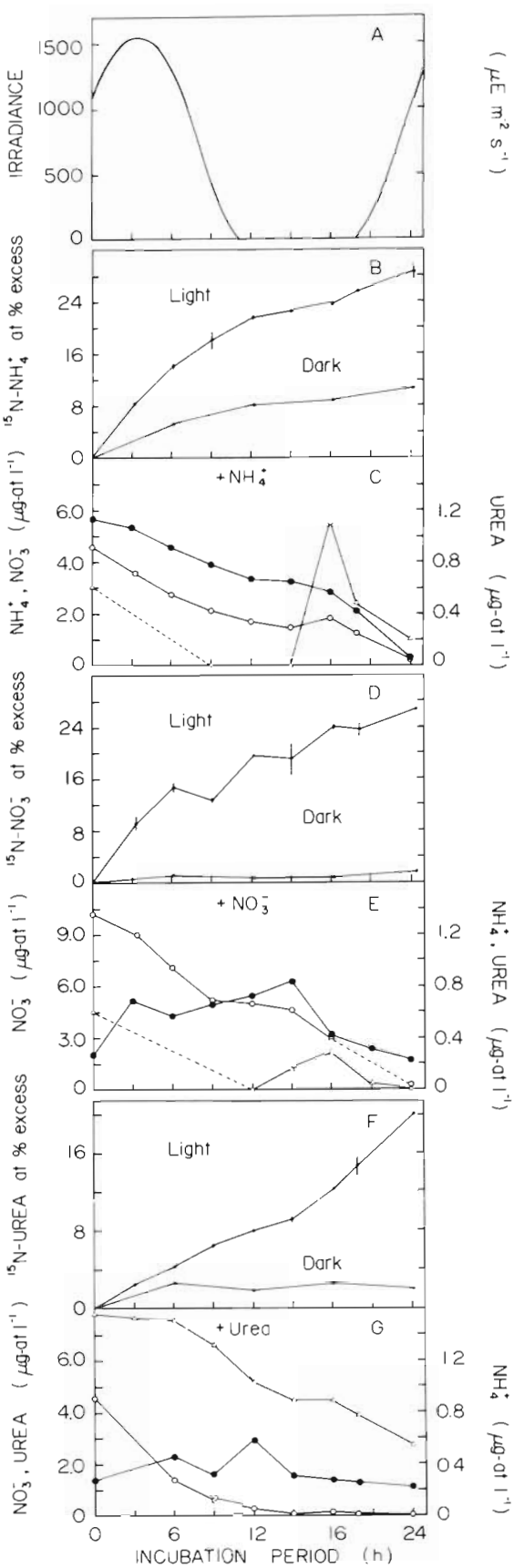
Time Course 1 was conducted in phytoplankton-rich water and NO_3^- depletion occurred in less than 3 h. Nitrogen-specific uptake rate of NO_3^- (V^n_{0-3h} = 0.070 h^{-1}) was the highest of any nitrogen substrate measured in all time course experiments. The disappearance uptake rate over the same time interval (V^d_{0-3h}) was 1.35 µg-at N l^{-1} h^{-1}, and using a time averaged particulate nitrogen, calculated from the amount of NO_3^- taken up and the initial measured particulate nitrogen, the nitrogen-specific uptake rate (V^d_{0-3h} = 0.081 h^{-1}) was in fair agreement with the rate determined by ^{15}N uptake. As a consequence of substrate exhaustion both techniques yielded rates which were underestimates. Simultaneous uptake of NO_3^- and urea was evident in the urea-spiked samples and the maximum disappearance rate of urea (V^d_{3-8h} = 0.314 µg-at N l^{-1} h^{-1}) was less than the NO_3^- rate (V^d_{0-6h} = 0.441 µg-at N l^{-1} h^{-1}).

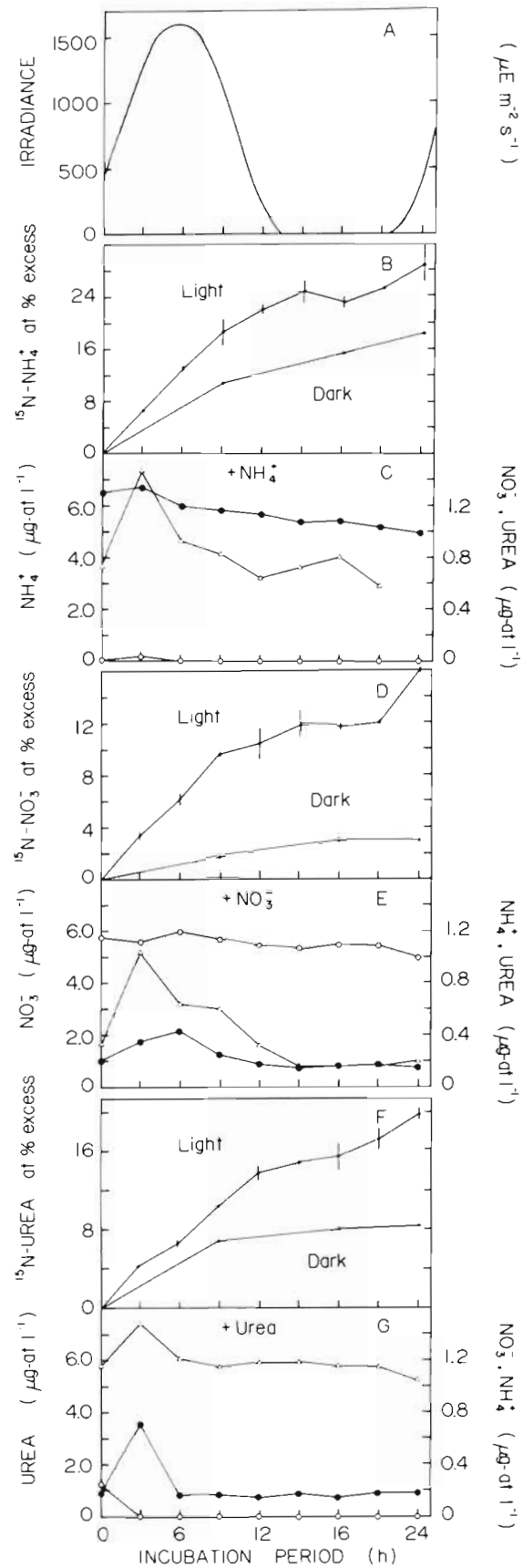
The pattern of ^{15}N uptake by the phytoplankton in the stratified water was similar in the NH_4^+, NO_3^- and urea-spiked samples (Fig. 4B, D, F). Uptake was linear over the first 9 to 12 h and was subsequently reduced during the dark period and increased again in the early morning. Substrate depletion did not occur in these experiments and utilization of nitrogen was minimal in the NH_4^+, NO_3^- and urea-spiked samples (23, 18 and 10 %, respectively).

Clear indications of urea regeneration, and to a lesser extent NH_4^+ regeneration, were evident from increases in their concentrations over the time course in all 3 experiments (Fig. 4C, E, G). Furthermore, the pattern of NH_4^+ and urea production in the samples suggested that there was a peridocity in uptake and/or regeneration processes. Similar results were seen in Time Course 2 (Fig. 3C, E, G) particularly for urea production over the 15 to 21 h time interval. Results from both frontal and stratified time course experiments demonstrate that simultaneous utilization of 2 or more nitrogen substrates occurs even when the concentration of one of the nitrogen substrates (NH_4^+, NO_3^- or urea) is in excess of the other(s) and indicates that such a phenomenon may naturally occur in these communities. The pattern of ^{15}N-labelled NH_4^+, NO_3^- and urea uptake rates suggests the existence of a diel periodicity in nitrogen uptake in both frontal and stratified water (Fig. 5). The decrease in uptake of NH_4^+ and NO_3^- from 21 to 24 h in Time Course 2 was due to substrate exhaustion (see Fig. 3C, E). In the frontal community, uptake rates of NO_3^- were greatest throughout the time course, and there was significant dark uptake of NO_3^-. In comparison, NH_4^+ uptake rates were highest in the stratified community and NO_3^- and urea uptake rates were similar but of a lesser magnitude. Additionally, in both time courses there was a tendency for nitrogen uptake rates to increase prior to the onset of the light period and this was most evident in the urea-spiked samples.

Uptake rates normalized per unit chl *a* showed that NH_4^+ and urea uptake were on average 2 and 2.4 times higher in the stratified water, whereas NO_3^- uptake rates were on average 1.6 times higher in frontal water (Table 3). Chl *a* specific uptake rates for each nutrient, when compared between stations, were most similar over the dark period (12 to 18 h) and the greatest disparity was found over the first 6 h.

The ^{15}N uptake rate, disappearance uptake rate and the rate of change of the PON calculated from the difference between measured values are presented in Fig. 6. All rates were calculated over 6, 12, 18, and 24 h time intervals, and this approach has been taken, rather than calculating the rates over successive 6 h intervals, to minimize fluctuations due to sample variability. Comparison of data from the frontal station indicates that in the NH_4^+ and urea-spiked samples the rate


A
IRRADIANCE
(μE m⁻² s⁻¹)
B
Light
Dark
¹⁵N-NH₄⁺ at % excess
+ NH₄⁺
C
NH₄⁺, NO₃⁻ (μg-at l⁻¹)
UREA (μg-at l⁻¹)
D
¹⁵N-NO₃⁻ at % excess
+ NO₃⁻
E
NO₃⁻ (μg-at l⁻¹)
NH₄⁺, UREA (μg-at l⁻¹)
F
¹⁵N-UREA at % excess
+ Urea
G
NO₃⁻, UREA (μg-at l⁻¹)
NH₄⁺ (μg-at l⁻¹)
INCUBATION PERIOD (h)


A
IRRADIANCE
(μE m⁻² s⁻¹)
B
Light
Dark
¹⁵N-NH₄⁺ at % excess
+ NH₄⁺
C
NH₄⁺ (μg-at l⁻¹)
NO₃⁻, UREA (μg-at l⁻¹)
D
¹⁵N-NO₃⁻ at % excess
+ NO₃⁻
E
NO₃⁻ (μg-at l⁻¹)
NH₄⁺, UREA (μg-at l⁻¹)
F
¹⁵N-UREA at % excess
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G
UREA (μg-at l⁻¹)
NO₃⁻, NH₄⁺ (μg-at l⁻¹)
INCUBATION PERIOD (h)

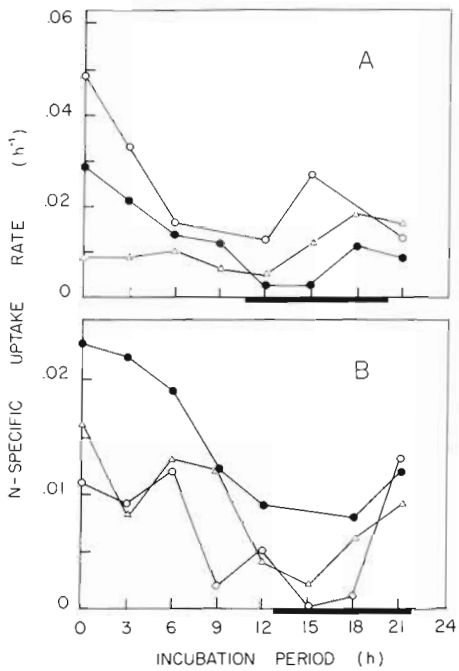

Fig. 5. Nitrogen-specific uptake rates of NH_4^+ (•), NO_3^- (○) and urea (△) in (A) frontal and (B) stratified water. Rates determined for 3 or 6 h intervals; each point indicates a rate calculated over the time interval between it and the next point on the curve. Shaded area on the abscissa delimits the dark period

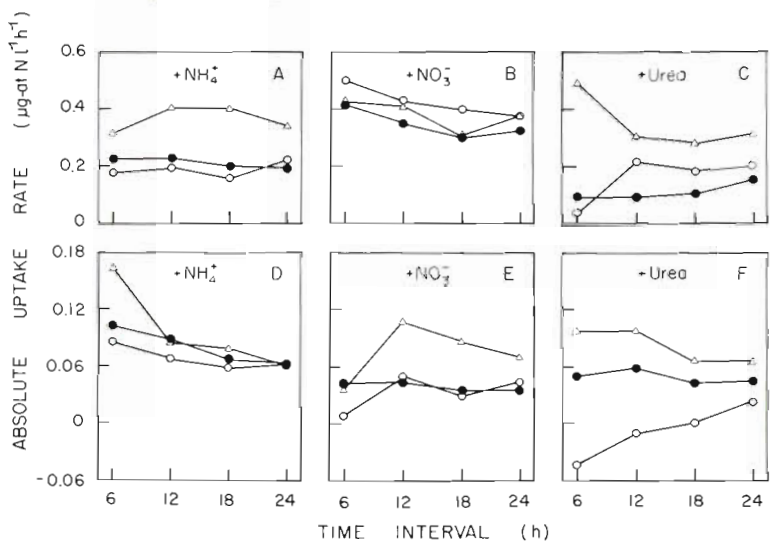

Fig. 6. Nitrogen uptake rates determined by ^{15}N atom % excess accumulation in the particulates (•), change in dissolved nitrogen concentration (○) and by change in the particulate nitrogen concentration (△) over 6, 12, 18 and 24 h time intervals. (A) NH_4^+, (B) NO_3^-, and (C) urea-spiked samples in frontal water and (D) NH_4^+, (E) NO_3^- and (F) urea-spiked samples in stratified water

Fig. 3. (Opposite, left). Time course measurements at frontal station (A5), Time Course 2. (A) Daily incident irradiance under which experiment was conducted. (B, D, F) ^{15}N atom % excess in particulate matter for light and dark bottle incubations following addition of 6 μg-at Nl^{-1} of (B) NH_4^+, (D) NO_3^- and (F) urea (error bars represent the range of duplicates). (C, E, G) Corresponding measurements of dissolved NH_4^+ (•), NO_3^- (○) and urea (△) in (C) NH_4^+, (E) NO_3^-, and (G) urea-spiked samples. Dashed line indicates no measurements of dissolved urea at 3 and 6 h

Fig. 4. (Opposite, right). As Fig. 3 except at stratified station (T4), Time Course 3

of change of the particulate nitrogen is greater than the accumulation of ^{15}N or the disappearance of either nutrient (Fig. 6A, C). In the NO_3^- spiked samples (Fig. 6B) the rate of nitrate uptake as determined by the disappearance of NO_3^-, the incorporation of ^{15}N-NO_3^- and the change in PON are similar. A general feature

Table 3. Chlorophyll *a* specific uptake rates of NH_4^+, NO_3^- and urea in frontal (A5) and stratified (T4) water. The dark period occurs during the 12 to 18 h time interval

Nitrogen substrate	Time interval (h)	Chl *a* specific N-uptake rate [μg-at N (μg chl *a*)$^{-1}$ h^{-1}]	
		Frontal stn	Stratified stn
NH_4^+	0 – 6	0.091	0.261
	6 – 12	0.060	0.133
	12 – 18	0.025	0.030
	18 – 24	0.028	0.047
NO_3^-	0 – 6	0.162	0.098
	6 – 12	0.075	0.082
	12 – 18	0.042	0.019
	18 – 24	0.068	0.039
Urea	0 – 6	0.040	0.127
	6 – 12	0.028	0.125
	12 – 18	0.026	0.019
	18 – 24	0.050	0.053

of the data from the stratified station is the more rapid change in PON than the ^{15}N uptake or disappearance uptake rates (Fig. 6D, E, F). Furthermore, the disappearance rates of NH_4^+ and urea are consistently less than the ^{15}N uptake rates.

The ratio of dark to light ^{15}N uptake rate (V_D:V_L) for NH_4^+, NO_3^- and urea is given in Table 4. At both stations, NH_4^+ dark uptake rates were a significant fraction of the light uptake rates throughout the entire time course. The V_D:V_L for NH_4^+ in frontal water was

Table 4. Ratio of dark to light uptake rates ($V_D : V_L$) of NH_4^+, NO_3^- and urea for frontal and stratified water

Station	Time interval (h)	NH_4^+ $V_D : V_L$	NO_3^- $V_D : V_L$	Urea $V_D : V_L$
Frontal T3	0 – 6	–	–	0.81
	6 – 11	–	–	0.37
	11 – 18	–	–	0
	18 – 24	–	–	–
Frontal A5	0 – 6	0.37	0.08	0.60
	6 – 12	0.39	0	0
	12 – 18	0.37	0	0
	18 – 24	0.39	0	0
Stratified T4	0 – 9	0.58	0.18	0.66
	9 – 18	1.02	0.60	0.24
	18 – 24	0.52	0	0.06

constant (38 %) and was less than the ratio in stratified water (52 to 102 %). Initial dark rates of urea uptake were 60 to 81 % of the light rates in all 3 time course experiments. Dark urea uptake in the stratified water was always a measurable fraction of the light rate and appeared less light dependent than in both frontal stations. The light dependency of NO_3^- uptake was more similar to that of urea than ammonium in both stratified and frontal water.

The regeneration rates of NH_4^+ and urea in the frontal water were similar (Table 5). Note that the change in substrate concentration was much greater than the ^{15}N uptake rate over the 18 to 24 and 6 to 12 h time periods for NH_4^+ and urea, respectively. As a result, negative rates of regeneration have been calculated. A consistent pattern was seen for the calculated values of NH_4^+ and urea regeneration rates in stratified water. The disappearance rates of both nutrients surpassed the ^{15}N uptake rates in the 12 to 18 and 18 to 24 h time intervals. Urea regeneration rates were approximately 5 and 2 times greater than the corresponding NH_4^+ regeneration rates for the first two 6 h intervals.

We have calculated the change in the PON over 6, 12, 18 and 24 h time intervals for each set of nitrogen-spiked samples (Table 6). By way of comparison the change in the total DIN and urea ($\triangle P_T$) over the same time interval and the amount of nitrogen accumulated in particulate matter (ΣV_T^1) as determined by ^{15}N atom percent excess data are reported. The results indicate that the frontal and stratified communities were very different. The change in $\triangle P_T$ in the frontal water samples consistently overestimated the change in the PON for all 3 nutrients. However, the opposite is true in the stratified water samples where the change in PON was always greater than $\triangle P_T$. Summation of the ^{15}N accumulation in the particulate matter over time indicates that this nitrogen contribution cannot account for the change in the particulate nitrogen except in the NO_3^- spiked time course in frontal water and the NH_4^+ spiked time course in stratified water.

Table 5. Regeneration rates of NH_4^+ and urea in frontal and stratified water

Station	Nitrogen addition	Time interval (h)	Change in concentration of added nitrogen[1] (μg-at $N\,l^{-1}\,h^{-1}$)	^{15}N-uptake rate (μg-at $N\,l^{-1}\,h^{-1}$)	Regeneration rate[2] (μg-at $N\,l^{-1}\,h^{-1}$)
Frontal A5	NH_4^+	0 – 6	.177	.224	.047
		6 – 12	.206	.232	.026
		12 – 18	.086	.141	.055
		18 – 24	.420	.174	−.246*
	Urea	0 – 6	.040	.094	.054
		6 – 12	.398	.093	−.305*
		12 – 18	.116	.132	.016
		18 – 24	.287	.308	.021
Stratified T4	NH_4^+	0 – 6	.085	.103	.018
		6 – 12	.052	.073	.021
		12 – 18	.039	.025	−.014*
		18 – 24	.074	.053	−.021*
	Urea	0 – 6	−.044	.050	.094
		6 – 12	.023	.066	.043
		12 – 18	.026	.014	−.012*
		18 – 24	.088	.050	−.033*

[1] Equivalent to V^d; negative value indicates an increase in substrate concentration over the incubation period
[2] Regenerative fluxes were calculated using a mass balance approach; see 'Methods'
* Indicates that disappearance of dissolved nutrient was greater than uptake rates calculated from ^{15}N

Table 6. Changes over time in measured DIN and urea concentration ($\triangle P_T$), particulate nitrogen ($\triangle$PON), and amount of ^{15}N-nitrogen accumulated in the particulate matter (ΣV_T^+) in frontal and stratified water

Station	Nitrogen addition	Time interval (h)	$^1\triangle P_T$ (μg-at N l^{-1})	$^2\triangle$PON (μg-at N l^{-1})	$^3\Sigma V_T^+$ (μg-at N l^{-1})
Frontal A5	NH_4^+	0 – 6	3.51	1.94	1.38
		0 – 12	5.79	4.86	2.76
		0 – 18	5.59	7.24	3.62
		0 – 24	10.10	8.19	4.67
	NO_3^-	0 – 6	3.41	2.66	2.58
		0 – 12	5.36	4.99	4.27
		0 – 18	7.74	5.58	5.46
		0 – 24	10.58	9.18	7.82
	Urea	0 – 6	3.21	2.97	0.57
		0 – 12	6.56	3.65	1.13
		0 – 18	7.70	5.13	1.93
		0 – 24	9.62	7.60	3.79
Stratified T4	NH_4^+	0 – 6	−0.01	0.98	0.62
		0 – 12	0.58	1.01	1.06
		0 – 18	0.66	1.42	1.21
		0 – 24	1.51	1.47	1.53
	NO_3^-	0 – 6	−1.08	0.21	0.23
		0 – 12	0.01	1.28	0.51
		0 – 18	0.16	1.55	0.61
		0 – 24	0.65	1.66	0.85
	Urea	0 – 6	−0.24	0.57	0.29
		0 – 12	−0.08	1.15	0.69
		0 – 18	0.08	1.19	0.78
		0 – 24	0.59	1.60	1.08

[1] Calculated from the change in concentration of DIN and urea; negative values indicate net production
[2] Measured change in particulate nitrogen
[3] Amount of nitrogen accumulating in the particulate matter, calculated from the ^{15}N atom % excess

DISCUSSION

Experimental considerations

In these experiments, saturating additions of each nitrogen compound (NH_4^+, NO_3^- and urea) were required to ensure that substrate exhaustion did not occur during the time course. We chose this approach rather than collecting water samples at various times and determining *in situ* rates of nitrogen uptake, in order to eliminate potential complicating factors such as diel migration of phytoplankton (Blasco 1978), surface water advection and problems associated with adding tracer amounts of ^{15}N-substrate (Goldman et al. 1981, Glibert et al. 1982b). Therefore our rates of nitrogen uptake are potential rates (with the exception of NO_3^- uptake in frontal stations) as they will only be realized under conditions where the nitrogen substrate concentration is elevated to a level sufficient to saturate the uptake system. Empirical observations, such as deep water injection (Walsh et al. 1977), soliton enrichment (Holligan et al. 1985), diel migratory behavior (Cullen & Horrigan 1981), phytoplankton sinking (Bienfang et al. 1982) and patchy excretion (Lehman & Scavia 1982) plus theoretical considerations (McCarthy & Goldman 1979, Parslow et al. in press) lend credence to this approach. More importantly we have been able to derive additional information concerning the physiological state of, and the nitrogen cycling within, the plankton community of these 2 types of coastal ecosystems.

Simultaneous uptake of nitrogen compounds

Simultaneous utilization of NH_4^+ and NO_3^- is well documented (Collos & Lewin 1974, Eppley & Renger 1974, Bienfang 1975, Conover 1975, Caperon & Ziemann 1976, Conway 1977, Maestrini et al. 1982) and our results not only demonstrate dual nitrogen substrate utilization but that NH_4^+, NO_3^- and urea may be taken up concurrently. As pointed out by Collos

(unpubl.) multiple nitrogen substrate utilization will result in a reduction of the nitrogen-specific uptake rate of the ^{15}N-labelled compound compared to the nitrogen-specific uptake rate determined when only the ^{15}N-labelled compound is being taken up. We have calculated our absolute uptake rates using the final PON, determined at the end of an incubation, which gives an accurate measure of the uptake rate of the ^{15}N-labelled nutrient into the phytoplankton and avoids potential artifacts caused by the incorporation of non-^{15}N-labelled nitrogen. Recently Maestrini et al. (1982) demonstrated that microalgae of oyster ponds took up NH_4^+ and NO_3^- at the same rate once the NH_4^+ concentration had decreased to ca 7 µg-at $N\,l^{-1}$. Our results from the frontal community demonstrated the similarity of NH_4^+ and NO_3^- disappearance uptake rates in the NH_4^+ spiked samples. However, the NO_3^- disappearance uptake rate was reduced by 50 % in the NH_4^+ spiked samples as compared to the NO_3^- spiked samples. Similar NH_4^+ suppression of NO_3^- uptake has been reported for both laboratory (e.g. Conway 1977, Cresswell & Syrett 1979) and natural phytoplankton assemblages (e.g. Blasco & Conway 1982). NO_3^- and urea uptake interactions from 2 time course experiments in frontal water are contradictory. In Time Course 1 there was a 70 % reduction in the NO_3^- disappearance uptake rate in the presence of urea, but the NO_3^- disappearance uptake rate was unaffected or slightly enhanced in the presence of urea in Time Course 2. The reasons for this discrepancy are not apparent, nonetheless variation in phytoplankton community structure, relative growth rates and internal nitrogen status may be important differences between the 2 stations. These variables have been identified as affecting uptake interactions among nitrogen compounds (Dortch & Conway 1984). In Time Course 2 the apparent slow disappearance uptake rate of urea, over the first 6 h, may be explained by regeneration of urea over this period. Alternatively, McCarthy & Eppley (1972) have reported NO_3^- inhibition of urea uptake in natural seawater samples. Irrespective of the concentration of NH_4^+, NO_3^- or urea ($\leq$ 6 µg-at $N\,l^{-1}$), phytoplankton in both the frontal and stratified water are capable of utilizing low concentrations of regenerated nitrogen (NH_4^+ and urea).

Variations in nitrogen uptake rate

Our sampling intervals were long relative to phytoplankton rapid uptake responses seen in the laboratory (Conway et al. 1976, Parslow et al. 1984a, b) and the field (e.g. Glibert & Goldman 1981) and thus we were unable to detect short term variations in uptake rate. Enhanced uptake of NH_4^+ and urea by NO_3^--sufficient phytoplankton has been reported (Horrigan & McCarthy 1981, 1982, Parslow et al. 1984b) but in the light of the slower long term rates of NH_4^+ and urea uptake, relative to NO_3^- uptake in the frontal station, it appears unlikely that such a process occurred on time scales shorter than our measurements. On long time scales, changes in uptake rate due to diel periodicity were evident in our results. Bottle confinement effects have been shown to lead to serious underestimates of rate processes (Venrick et al. 1977) but the constant rates of chl *a* and POC & PON synthesis indicate no such artifacts in our experiments. Olson & Chisholm (1983) have shown that cell division patterns of nitrogen-limited phytoplankton cultures may be entrained by NH_4^+ pulses. Although our samples were spiked with saturating additions of each nitrogen compound, we have evidence from an earlier cruise in the Strait of Georgia (August 1983) of uptake periodicity at ambient concentrations of dissolved nitrogen (Parslow et al. unpubl.). Uptake periodicity was also evident in nitrogen-sufficient frontal water.

Effects of light/dark regime on nitrogen uptake

The constancy of V_D:V_L for NH_4^+ in frontal water, when NH_4^+ uptake rates of phytoplankton exposed to the natural light/dark cycle were periodic, suggests that NH_4^+ uptake is circadian; in absence of the light/dark cycle the rhythm is free running (see Chisholm 1981). This conclusion is supported by Goering et al. (1964) who found rhythmic variation in both NH_4^+ and NO_3^- uptake by natural communities under continuous light. Our results for NO_3^- and urea demonstrate their dependency of uptake on light and in this respect both nutrients are comparable. The light dependence of uptake of both nutrients is well established (MacIsaac & Dugdale 1972, Mitamura & Saijo 1975, 1980, Webb & Haas 1975, Harvey & Caperon 1976, Nelson & Conway 1979). Other reports have shown that nitrogen-deprived phytoplankton have higher dark uptake rates of nitrogen than nitrogen-replete phytoplankton (e.g. Syrett 1962, Eppley & Coatsworth 1968, Malone et al. 1975, Rees & Syrett 1979). In our results dark nitrogen uptake rates normalized to chl *a* were highest in the nitrogen-depleted stratified water in agreement with these observations; also, relative to the frontal community, dark uptake rates were a greater proportion of the light rates for NH_4^+, NO_3^- and urea in stratified water. The higher chl *a* specific uptake rates of NH_4^+ and urea in stratified water and of NO_3^- in frontal water are consistent with the way we envisage nitrogen supply to these areas. Specifically, regenerated nitrogen (NH_4^+ and urea) has been shown to supply most of the phytoplankton nitrogen demand in nitrogen-depleted

waters and as the concentration of ambient NO_3^- increases so does the relative importance of NO_3^- for the phytoplankton nitrogen ration (e.g. McCarthy et al. 1977, Harrison 1980, Glibert et al. 1982a, Cochlan in press).

NH_4^+ and urea regeneration

Our estimates of NH_4^+ regeneration are in agreement with previously published rates for coastal waters (0.01 to 0.31 µg-at $N\,l^{-1}$) (Caperon et al. 1979, Cochlan 1982, Glibert 1982, Glibert et al. 1982b, Paasche & Kristiansen 1982, La Roche 1983). The method we have used to calculate regeneration rates is inferior by comparison to the isotope dilution method (Blackburn 1979, Caperon et al. 1979). However, unlike experiments employing trace additions of ^{15}N, the concentration of regenerated nitrogen was small relative to the added ^{15}N substrates, and thus the ^{15}N enrichment factor remained constant over the incubation. The rates of urea regeneration are of similar magnitude to the NH_4^+ regeneration rates and are comparable in both communities. These experiments have enabled us to quantify urea regeneration by an intact plankton community and therefore these results are an improvement over previous attempts which have quantified urea production by species, or size fractioned assemblages, of zooplankton. The patterns of NH_4^+ and urea production over the time course experiments indicate a periodicity which is not a result of reduced uptake rates. This corroborates data of Caperon et al. (1979) and Glibert (1982) who reported higher rates of NH_4^+ regeneration at night and early morning. Additionally, Collos & Lewin (1974) and Hattori (1982) have shown diel variations in dissolved NH_4^+ concentration in coastal waters. Unlike our dissolved nitrogen concentration measurements our calculated regeneration rates do not show this same periodicity since the time scales over which they were calculated are much greater than these physiological processes.

Regeneration of nitrogen has long been recognized as a possible artifact in determining ^{15}N uptake rates (Dugdale & Goering 1967) and recently it has been shown that these rates may be underestimated by a factor of ca 2 when a constant ^{15}N atom % enrichment is assumed (Glibert et al. 1982b). We have not corrected our uptake rates for isotope dilution, as we will now show regeneration of NH_4^+ and urea, in these experiments, has little effect on uptake rates calculated with the initial enrichment, however, such a process may dramatically effect the disappearance uptake rates.

Initial additions of nitrogen were 6.0 µg-at l^{-1}, correcting for background and purity of the substrate this represents 5.92 µg-at $^{15}N\ l^{-1}$. When no regeneration occurs and ^{15}N is conserved;

$$P_t = P_o - V^i\,t \quad (1)$$

and

$$\frac{P_o - P_t}{t} = V_i \quad (2)$$

where P_o and P_t = initial and final concentrations of dissolved nitrogen; V^i = ^{15}N uptake rate; t = time; and disappearance uptake rate equals ^{15}N uptake rate. It is obvious that large additions of ^{15}N to samples cause the isotope enrichment factor (R) to be relatively insensitive to additions of regenerated nitrogen. For example, if R changes from 0.9394 to 0.8500 and we assume that the pulse of regenerated ^{14}N (0.66 µg-at $N\ l^{-1}$) is added immediately after time zero, disappearance uptake rates will be underestimated by 62 % while ^{15}N uptake rates will decrease by only 10 %. Therefore regenerative processes are of lesser consequence to ^{15}N-uptake rate calculations when the concentration of ^{15}N is large; if the total concentration of dissolved nitrogen becomes low, for example toward the end of a time-course experiment, regeneration of ^{14}N will have a greater effect on ^{15}N uptake rate calculations.

With this line of reasoning and as discussed earlier, we have interpreted discrepancies between the disappearance uptake rate and ^{15}N uptake rate as indicative of regeneration. In the NH_4^+ and urea-spiked samples from stratified water, regeneration is evident; however, the disappearance uptake rates are in closer agreement with the ^{15}N uptake rates at the end of the time course compared with the beginning (Fig. 6). In frontal water the discrepancies between disappearance uptake rates of NH_4^+ and $^{15}NH_4^+$ uptake rates may be adequately explained by regeneration; the exception is the final 6 h period where changes in dissolved concentrations exceed ^{15}N incorporation rates. The urea disappearance rates are greater than ^{15}N incorporation rates after the first 6 h, and the very fast disappearance rates from 6 to 12 h make the 0 to 18 and 0 to 24 h rates high as well.

Particulate nitrogen balance

In a 2-compartment system consisting of DIN + urea and PON, regardless of the flux rates between the 2 pools, changes in the concentration of 1 component should be reflected by corresponding changes in the other. Using this approach, nitrogen will be conserved providing the system is closed. Additionally, by including NH_4^+, NO_3^- and urea as part of the dissolved nitrogen pool we are able to account for circumstances when regenerated nitrogen differs from the assimilated form. The corollary of this is that the regenerated nitrogen is in the form of NH_4^+ and/or urea. In summary, this relation may be expressed as:

$$PON_o + P_{T_o} = PON_t + P_{T_t} \quad (3)$$

and

$$\triangle PON = \triangle P_T \quad (4)$$

where PON_o and PON_t = initial and final particulate nitrogen concentrations; P_{T_o} and P_{T_t} = initial and final DIN + urea concentrations; $\triangle PON = PON_t - PON_o$; $\triangle P_T = P_{T_o} - P_{T_t}$. Deviations from this model are instructive as they provide information concerning nitrogen cycling and its transformation in aquatic systems. From our data it is apparent that additions and losses of nitrogen are occuring and that this trend is consistent within the frontal and stratified communities.

The discrepancies between $\triangle PON$ and $\triangle P_T$ (Table 6) in frontal water indicate that nitrogen is being lost from the system. Given our precision and accuracy for determining the concentration of the different dissolved nitrogen fractions (see 'Methods') we argue that nitrogen losses are occurring from the PON compartment. Changes in PON for the $^{15}NO_3^-$ spiked samples from frontal water, as predicted by ^{15}N uptake (ΣV_T^i), are not significantly different from the measured values ($\triangle PON$) (paired t-test, $p > 0.05$). Thus the incorporation of ^{15}N into particulate matter accounts for the increase in PON. The discrepancies between $\triangle PON$ and ΣV_T^i in the NH_4^+ and urea-spiked samples are, in part, a consequence of the simultaneous uptake of unlabelled NO_3^- and its contribution to PON. Further statistical analysis of the data from the $^{15}NO_3^-$ spiked samples showed that the hypotheses $\triangle P_T = \Sigma V_T^i$ and $\triangle P_T = \triangle PON$ must be rejected ($p < 0.05$ and $p < 0.05$). Therefore, because $\triangle P_T > \triangle PON$ and ΣV_T^i, we are forced to conclude that the lost PON is labelled with ^{15}N and that it has the same isotopic composition as the dissolved NO_3^-. Furthermore, our results suggest that nitrogen losses from the PON pool can be most easily explained by excretion or grazing losses to a dissolved organic pool (DON). The alternative explanations, that PON or DON is lost directly via methodological artifacts, are untenable for the following reasons. The effective retention size of Whatman GF/F filters (0.7 μm) is sufficient to have caught all of the large chain-forming diatoms which dominated the frontal water. Phytoplankton samples collected on 0.2 μm Nuclepore® filters and examined with a Zeiss epifluorescence microscope showed that there were no chlorophyll-containing organisms less than 2 μm and that bacterioplankton were greater than 1 μm. Secondly, the low filtration pressure differential (< 125 mm Hg) would have minimized cell lysis on the filter, and in the stratified water, dominated by soft-bodied flagellates, loss of nitrogen was not seen. Feeding zooplankton have been shown to contribute to the dissolved organic carbon pool by loss of phytoplankton cell contents during handling and feeding (Lampert 1978). The high zooplankton biomass in the frontal and stratified stations suggests that such processes may have contributed to the loss of PON, as DON, during our incubations, although it is not clear why similar losses were not seen in the stratified station. Additionally, active excretion of DON by phytoplankton has been reported (Newell et al. 1972, Mague et al 1980). The interpretation of our results is consistent with the analysis by Laws (1984) that losses of ^{15}N seen in these data and from previously published results could be attributed to losses of $DO^{15}N$, at least for experiments lasting 6 h.

In stratified water the differences between $\triangle PON$ and $\triangle P_T$ are exactly opposite to the results from the frontal station. Anomalously high PON values indicate the phytoplankton must be utilizing additional nitrogen sources other than those we accounted for in the initial mass balance. These compounds are most probably DON as the nitrogen fixing microorganisms were absent from our samples. In the $^{15}NH_4^+$ spiked samples ΣV_T^i is in good agreement with $\triangle PON$ suggesting that

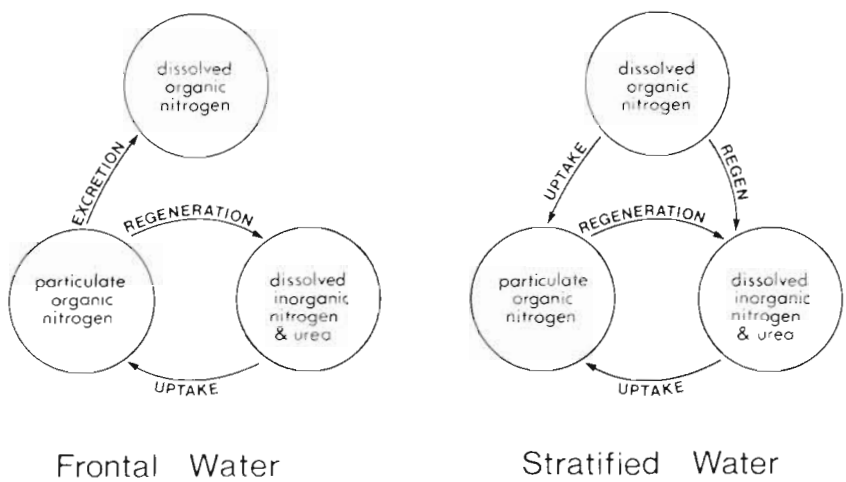

Fig. 7. Schematic diagram of nitrogen cycling in the euphotic zone of frontal and stratified water. Arrows indicate major pathways of nitrogen transformation between the various pools; we have excluded other pathways since they were not found to be dominant in these experiments. The dissolved organic nitrogen pool includes amino acids, proteins and other nitrogen containing macromolecules. Excretion may involve active and passive processes

no additional nitrogen was required to account for the increase in PON; also, at the end of the experiment, $\triangle P_T$ is the same as $\triangle$PON. Clearly this is not true for the $^{15}NO_3^-$ and ^{15}N-urea-spiked samples but reason(s) for this difference are not apparent.

Characterization of seawater DON remains an enigma and current estimates suggest that free and combined amino acids and humic acids can account for only 50 % of the DON pool (Sharp 1983). Wheeler et al. (1974) and Geesey & Morita (1979) have shown that these types of compounds can be utilized by marine phytoplankton and bacteria, respectively. Similarly, Hollibaugh (1978) has reported degradation of several amino acids in natural seawater samples incubated in the dark. Support for *in situ* DON utilization is scarce, but indirect evidence from depth profiles in the Indian Ocean (Fraga 1966) and work by Armstrong et al. (1966) showed surface depletion of DON relative to deep samples. More significantly, Fisher & Cowdell (1982) have reported 8 diatom clones which were able to utilize at least some natural DON. We have schematically depicted simplified nitrogen cycles in frontal and stratified water in Fig. 7. Our results, however, do not enable us to distinguish between direct utilization of DON or indirect utilization via regenerated NH_4^+ and/or urea in stratified water.

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