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Ammonium regeneration: its contribution to phytoplankton nitrogen requirements in a eutrophic environment

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Abstract

Ammonium regeneration, nutrient uptake, bacterial activity and primary production were measured from March to August 1980 in Bedford Basin, Nova Scotia, Canada, a eutrophic environment. Rates of regeneration and nutrient uptake were determined using ^{15}N isotope dilution and tracer methodology. Although primary production, nutrient uptake and ammonium regeneration were significantly intercorrelated, no relationship was detected between these parameters and heterotrophic activity. The average contribution of ammonium to total nitrogen (ammonium + nitrate) uptake was similar in the spring and in the summer (approximately 60%). On a seasonal average basis, 36% of the phytoplankton ammonium uptake could be supplied by rapid remineralization processes. In spite of the high average contribution of NH_4 regeneration to phytoplankton ammonia uptake, there is indirect evidence suggesting that other NH_4 sources may occasionally be important.

Introduction

Nutrient regeneration is the dominant process controlling primary production in oligotrophic oceans where nutrients limit phytoplankton growth (Dugdale and Goering, 1967; Eppley *et al.*, 1973).

It is now becoming evident that nutrient regeneration also contributes to a significant fraction of the primary production in coastal areas (Eppley and Peterson, 1979; Eppley *et al.*, 1979; Harrison, 1980). The ammonia rapidly recycled in the euphotic zone is an important source of regenerated nitrogen and ammonia uptake by phytoplankton is often taken as a measure of regenerated production (Olson, 1980). In fact, a close coupling between direct measurements of NH_4 regeneration and NH_4 uptake rates has been documented in several field studies (Harrison, 1978; Axler, 1981; Glibert, 1982).

High rates of respiration and nutrient excretion by zooplankton (Conover and Mayzaud, 1975), as well as excretion rates associated with high bacterial biomass and heterotrophic activity (Palumbo and Ferguson, 1978; Billen *et al.*, 1980; Fuhrman *et al.*, 1980), probably account for most of the water column nutrient regeneration in coastal areas. However, the partitioning of regeneration among different groups of organisms is not well documented. Harris' (1959) early experiments in coastal waters indicated that zooplankton excretion could provide 77% of the nutrients required for primary production. More recent studies have suggested much lower contributions, ranging from 5 to 30% (Seki *et al.*, 1974; Smith, 1978; Whiteledge, 1978). Laboratory experiments (Johannes, 1965, 1968) and field studies using size fractionation techniques (Harrison, 1978; Caperon *et al.*, 1979; Glibert, 1982) suggested that microplankton ($< 100\,\mu m$) may provide most of the regenerated nutrients.

Bedford Basin, Nova Scotia, is a eutrophic coastal environment where nutrients are abundant year round (i.e. summer average concentrations of NO_3 and NH_4 are around 0.70 and 1.10 $\mu mol\,l^{-1}$ respectively) (Krauel, 1969; Taguchi *et al.*, 1975; Coté, 1979). The large daily fluctuations (3 fold) in primary production observed throughout the summer in Bedford Basin are not correlated with the variations in ambient nutrient concentrations (Coté, 1979; Harrison and Platt, 1980). The present study investigates the relationship between primary production and nitrogen fluxes. The following hypotheses were tested: (1) Rapid *in-situ* NH_4 regeneration changes proportionally with NO_3 uptake (new production), NH_4 uptake (regenerated production), and CO_2 uptake (primary production); (2) Bacterial metabolic activity, measured by glutamic acid uptake, contributes significantly to nutrient remineralization and is therefore correlated with NH_4 regeneration rates.

Materials and methods

Sampling

Samples were collected weekly between 09.00 and 10.30 hrs in Bedford Basin from February 29 to May 15 (spring period) and June 26 to August 18, 1980 (summer period). Salinity and temperature profiles were recorded at 2-m intervals to a depth of 20 m using a model Beckman RS-5 conductivity-temperature probe. Water samples for nutrient analysis were collected from 0, 5, 10 and 15 m using a submersible pump, and were stored in opaque plastic bottles for later nutrient analyses. An additional 20 l of water were collected at the 5-m depth using a Niskin bottle, and stored in a dark plastic carboy for later use in tracer experiments.

Analytical methods

Particulate matter collected on Reeve-Angel 984-H glass fiber filters was analysed for chlorophyll *a* by fluorometry (Strickland and Parsons, 1972) and for particulate organic matter (PON and POC) with a Hewlett-Packard 185 B CHN analyser (Sharp, 1974). Ammonium was determined colorimetrically (Solorzano, 1969) approximately one hour after collection and other nutrients were determined with a Technicon autoanalyser a few weeks later (Strickland and Parsons, 1972). Fig. 1 B shows the relationship between NH_4 concentrations determined from fresh and frozen water samples. The contamination of frozen NH_4 samples observed by Carpenter *et al.* (1972) was not found in my data. Acridine orange direct cell counts (Hobbie *et al.*, 1977) were used to enumerate bacteria.

Tracer methods

Uptake experiments began within one hour of water collection. All the incubations were carried out for 4 h under simulated *in-situ* conditions at 50% light attenuation except for the heterotrophic activity incubations, which were conducted in the dark. Samples were not prefiltered.

Photosynthetic rate was determined by $[^{14}C]$-CO_2 tracer uptake (Strickland and Parsons, 1972). Five μCi of ^{14}C-$NaHCO_3$ were added to a 250-ml water sample and incubated in glass bottles. At the end of the incubations the particulate matter was collected by filtration onto Reeve-Angel 984-H glass fiber filters. The incorporation of ^{14}C was measured by liquid scintillation spectrometry. An estimate of heterotrophic activity was obtained by measuring ^{14}C-glutamic acid uptake (Griffiths *et al.*, 1977).

Nutrient uptake and regeneration experiments were measured using the techniques described by Harrison (in press). Nutrient uptake was measured by inoculating duplicate 1-l water samples with 0.1 μmol of either 95 at% ^{15}N-NO_3 or 99 at% ^{15}N-NH_4. NH_4 regeneration was determined from the time-dependent dilution of the dissolved NH_4 pool initially enriched with 1 μmol of 99 at% ^{15}N-NH_4. Calculations were based on time zero and time final differences of ^{15}N-NH_4 isotopic ratio. Samples were run in triplicate.

^{15}N analysis

The filters for nutrient uptake were dried at 80 °C for 48 h and ground in a mortar with a small amount of CuO. The samples were then stored in vacutainer tubes and kept in a desiccator until they were analysed. Ammonium present in the regeneration samples was concentrated by distillation (Harrison, 1978). NH_4 recovery averaged 60 to 80%. A microdiffusion technique (Blackburn, 1979) was used to transfer the NH_4 samples into small capillary tubes. The capillaries were then placed in discharge tubes containing CaO and CuO. The tubes were attached to a vacuum line and sealed with a torch after evacuation to approximately 2×10^{-4} torrs. The sealed particulate or dissolved NH_4 samples were converted to dinitrogen gas by Dumas combustion at 550 °C for 2 h and analysed by emission spectrometry (Harrison, in press). The precision of isotope analyses was good in general. (The coefficient of variation was 4% for particulate nitrogen and 6 to 10% for NH_4 distillates).

Calculation of uptake and regeneration rates

Nutrient uptake rates were calculated according to Dugdale and Goering (1967). Their method assumes linear substrate uptake and constant specific activity during the incubation period. A linear differential equation model (Blackburn, 1979; Caperon *et al.*, 1979) was used to calculate regeneration rates. This model assumes that regeneration and uptake rates of NH_4 are constant during the incubation period and that ^{15}N-NH_4 is not recycled.

Nutrient uptake rates may be overestimated when the amount of added substrate is large compared to ambient substrate concentrations (Eppley *et al.*, 1973, 1977). In my study, the ammonia concentrations were usually well above the detection level and the ratio of isotope added to ambient nutrient concentration was less than 0.2 in 90% of the nutrient uptake experiments. However, for the NH_4 regeneration experiments, the amount of isotope added represented a 100% NH_4 enrichment on average. A 24-h time-course experiment was performed to verify the linearity of substrate uptake and regeneration with time and measure the enhancement of uptake rate due to NH_4 enrichment. The time-course for ^{15}N assimilation in the particulate matter and the linear decrease in ammonia concentration observed during the regeneration experiment are shown in Fig. 1 A and 1 B, respectively. The time courses of nitrogen (NO_3 and NH_4) uptake were linear for 5 h, after which essentially no ^{15}N was incorporated, thus suggesting nutrient depletion (Fig. 1 C). CO_2 uptake and heterotrophic activity were linear for at least 5 h and un-

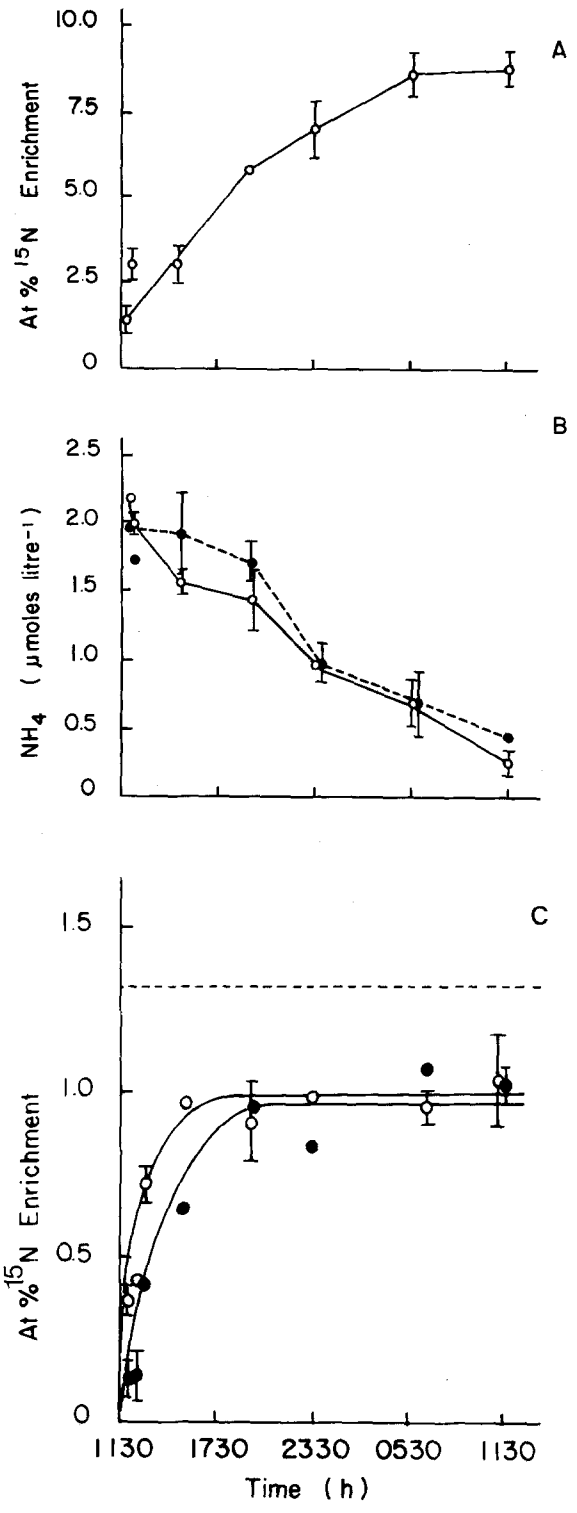

Fig. 1. (a) Time-course of ^{15}N enrichment in PON during NH_4 regeneration experiments. Maximum possible ^{15}N-enrichment is 11.66 at%. One μmol ^{15}N-NH_4 litre^{-1} was added to the sample. (b) Decrease in total NH_4 concentration during NH_4 regeneration experiments for fresh NH_4 (dark circles) and frozen NH_4 (open circles). Error bars represent the range of duplicate measurements. No significant difference is observed between the two types of measurements. (c) Time-course of ^{15}N enrichment in particulate organic nitrogen (PON) for NO_3 (dark circles) and NH_4 (open circles). The dotted line indicates the maximum possible enrichment that could occur in the particulate matter (1.3 at% ^{15}N). The lines were fitted to the data visually

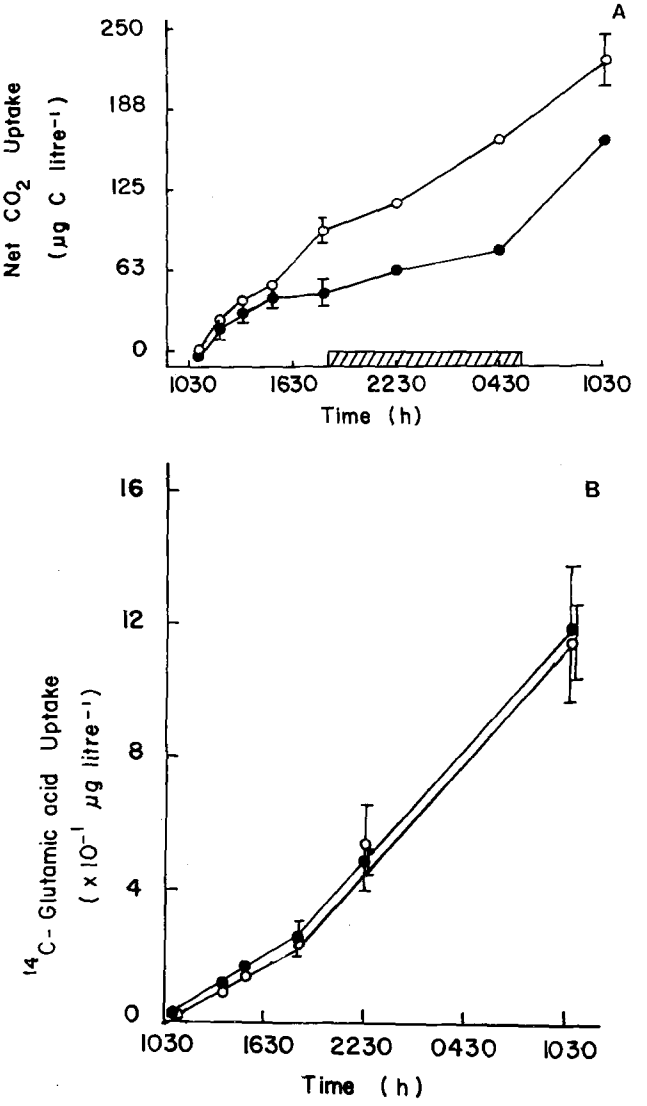

Fig. 2. (a) Net CO_2 uptake (light-dark bottles) for natural water samples (dark circles) and samples enriched with 1 μmol of NH_4 (open circles). Error bars represent the range of duplicate measurements. The shaded area on the abscissa delimits the dark period. (b) Time-course of ^{14}C-glutamic acid uptake with natural water samples (dark circles) and samples enriched with 1 μmol of NH_4 (open circles). Error bars represent the range of triplicate measurements

affected by enrichment with 1 μmol of NH_4 over the same time period (Fig. 2 A and B).

Assuming that ^{15}N labelled NH_4 is not recycled during short-term experiments (Blackburn, 1979; Caperon *et al.*, 1979), Glibert *et al.* (1982) have shown that isotope dilution due to high regeneration rates can result in a significant underestimate of uptake rates. In my experiments, a 15% difference was observed between uptake rates calculated in the conventional way (Dugdale and Goering, 1967) and uptake rates corrected for isotope dilution effects (Glibert *et al.*, 1982). The time-course experiment suggested that a 4-h incubation should minimize many of the technical problems. It reduced the chances of substrate

exhaustion during the nutrient uptake incubation and minimized the enhancement of primary production as well as problems related to isotope dilution.

Results

Figure 3 illustrates the variations in several of the biological parameters measured during the sampling period. Simple correlation analysis indicated a strong correlation between temperature (Fig. 3 A) and assimilation number (mgC mgChl a^{-1} h^{-1}; Fig. 3 I), glutamic acid uptake (Fig. 3 L) and bacterial abundance (Fig. 3 K). The high nitrate concentration characteristic of the winter months was rapidly depleted at the onset of the spring bloom (Fig. 3 B). After the bloom, NO_3 and NH_4 concentrations as well as NO_3 and NH_4 uptake rates covaried until the end of the sampling period (Fig. 4). Similarly, rate measurements of NO_3 and NH_4 uptake, NH_4 regeneration and CO_2 uptake were all significantly intercorrelated after the initial NO_3 depletion at the 5 m depth. However, no relationship could be detected between any of these parameters and glutamic acid uptake (Table 1). NH_4 uptake was most highly correlated with NH_4 regeneration, however not in a 1:1 relationship (Fig. 5). Although glutamic acid uptake was not correlated with the other rate measurements, NH_4 regeneration seemed to be most important when phytoplankton and bacterial biomass were high (Fig. 3 F, K). The high regeneration rate observed on July 31 is noteworthy because it corresponds to a peak in bacterial numbers and in primary production and to a minimum in nutrient concentrations. Microscopic observation of the preserved sample showed that on that date, most of the bacteria were associated with particulate matter. However, large variations in replicate bacterial cell counts complicated the statistical interpretation of this parameter.

Significant differences in several environmental and biological variables were observed between the spring and summer sampling periods (Table 2). Total nitrogen con-

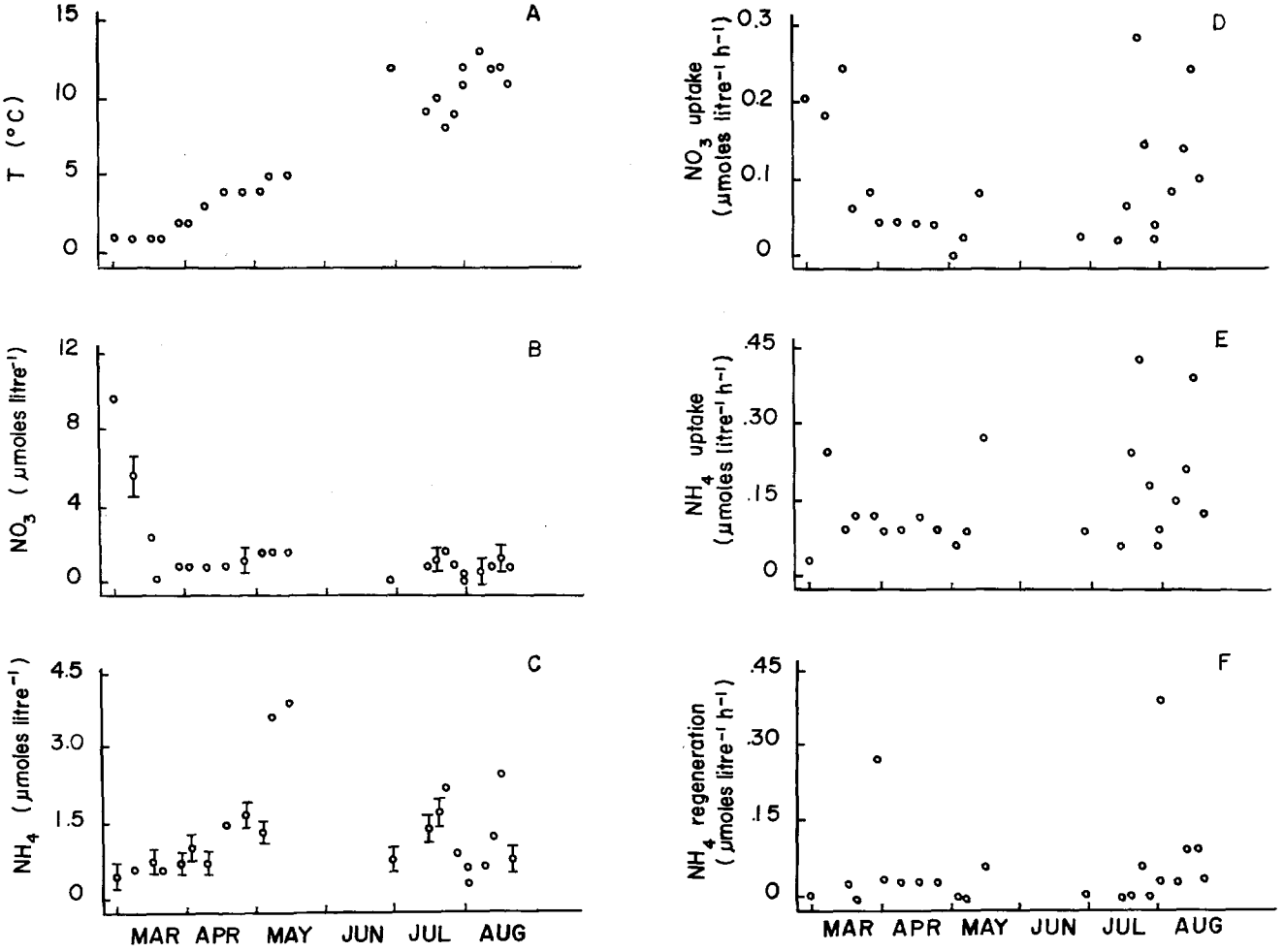

Fig. 3. Variations in some of the chemical and biological variables measured during the sampling period. All variables were measured at 5 m. Error bars represent the range of replicate measurements. Variations in (A) temperature, (B) NO_3 concentration, (C) NH_4 concentration, (D) NO_3 uptake, (E) NH_4 uptake, (F) NH_4 regeneration, (G) chlorophyll *a*, (H) CO_2 uptake, (I) assimilation number, (J) particulate organic nitrogen, (K) bacterial abundance, (L) ^{14}C-glutamic acid uptake

Table 1. Correlation matrix of uptake and regeneration rates for data after the spring bloom. The few experiments where more than 80% of the substrate uptake occurred were omitted. $NO_3 = NO_3$ uptake (μmol l^{-1} h^{-1}), $NH_4 0.1 = NH_4$ uptake (μmol l^{-1} h^{-1}) with the addition of 0.1 μmol of labelled substrate, $NH_4 1.0 = NH_4$ uptake (μmol l^{-1} h^{-1}) with the addition of 1.0 μmol l^{-1} of substrate, $NH_4 R = NH_4$ regeneration (μmol l^{-1} h^{-1}), $CO_2 = CO_2$ uptake (μg C l^{-1} h^{-1}), *GLU* = Glutamic acid uptake ($\times 10^{-1}$ μg C l^{-1} h^{-1}). All the correlations are statistically significant ($P < 0.05$, d.f. = 17) except for the correlations with glutamic acid uptake

	NO_3	$NH_4 0.1$	$NH_4 1.0$	TOTALN	$NH_4 R$	CO_2	GLU
NO_3	1						
$NH_4 0.1$	0.92	1					
$NH_4 1.0$	0.87	0.90	1				
TOTALN	0.97	0.98	0.91	1			
$NH_4 R$	0.68	0.74	0.66	0.73	1		
CO_2	0.77	0.86	0.91	0.84	0.68	1	
GLU	0.23	0.20	0.41	0.22	0.08	0.20	1

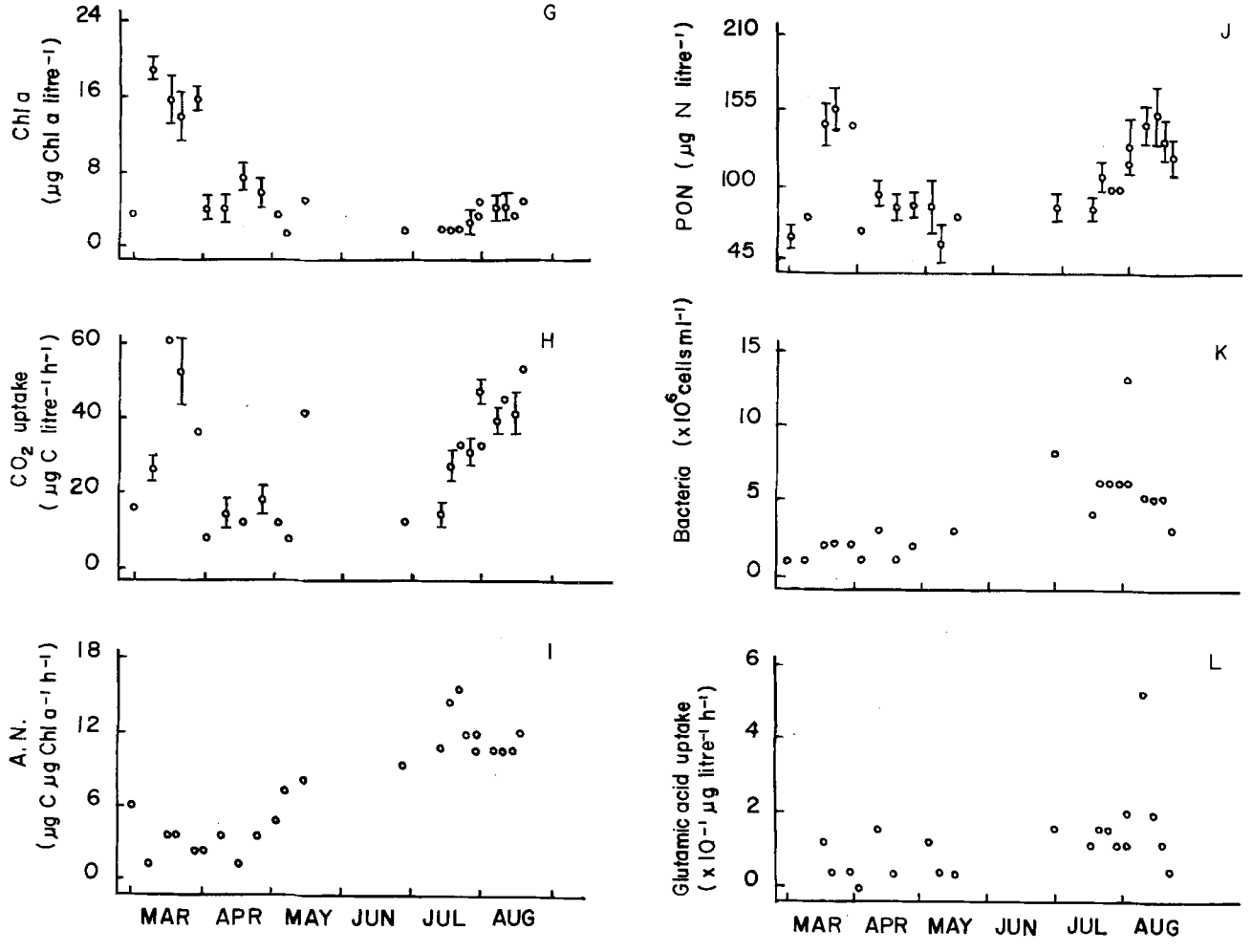

Fig. 3. (continued)

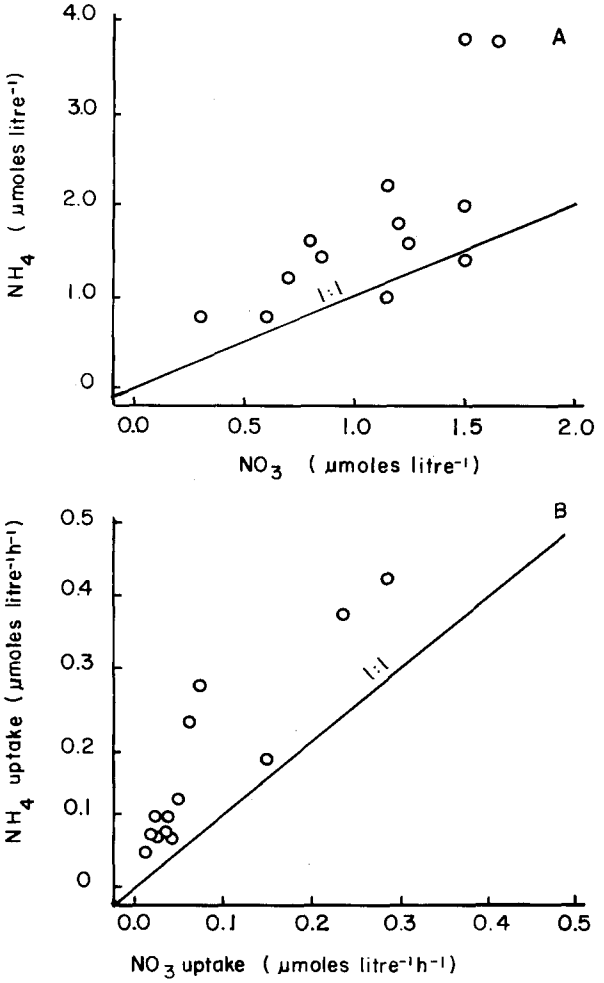

Fig. 4. Relationship between (A) NH_4 and NO_3 concentrations and (B) NH_4 and NO_3 uptake rates for the data collected after the onset of water column stratification. The data from experiments where more than 80% of the substrate was taken up was omitted. The solid line represents the 1:1 relationship between the two parameters

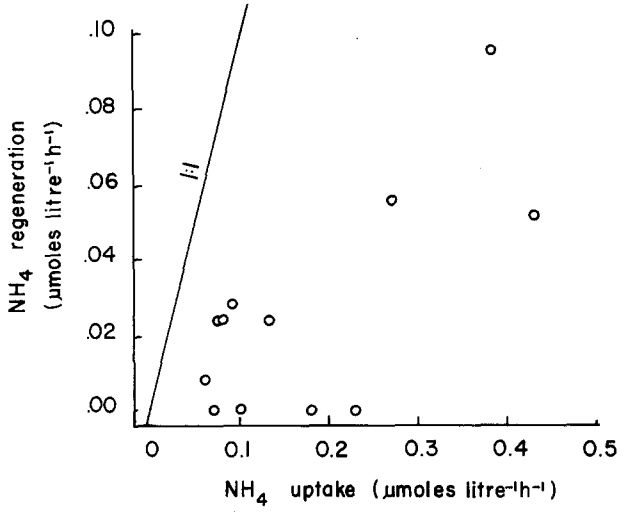

Fig. 5. Relationship between NH_4 uptake and regeneration for the data collected after the onset of the water column stratification. The data, where more than 80% of the substrate added was assimilated, was omitted. The solid line represents the 1:1 relationship

Table 2. Comparison of summer and spring mean values of selected variables. P represents the level of significance of a one tail *t*-test. Temperature is in °C, light is in langley h^{-1}, PON is in μg N l^{-1}, NO_3, NH_4, $[NO_3+NH_4]$ are in μmol l^{-1} h^{-1}, NO_3, NH_4, regeneration rate and total nitrogen uptake are in μmol l^{-1} h^{-1}, Chl *a* is in μg Chl *a* l^{-1}, C:Chl *a* is in μg C: μg Chl *a*, CO_2 uptake is in μg C l^{-1} h^{-1}, A.N. is in μg C (μg Chl a^{-1}) h^{-1}, C:N uptake is mol:mol ratio, bacterial counts are in cell ml^{-1}, heterotrophic activity is in $\mu g \times 10^{-1}$ l^{-1} h^{-1}

Variables	Spring		Summer		P
	Mean	SD	Mean	SD	
Temperature	2.77	1.48	10.85	1.68	0.00
Light	1.52	0.83	2.35	0.60	0.01
PON	93.9	33	115.5	23.9	0.08
NO_3	2.26	2.76	0.72	0.48	0.08
NH_4	1.43	1.17	1.13	0.66	0.23
$[NH_4+NO_3]$	3.68	2.72	1.85	1.11	0.05
NO_3 uptake	0.085	0.077	0.105	0.089	0.50
NH_4 uptake	0.115	0.071	0.182	0.123	0.06
Total nitrogen uptake	0.20	0.11	0.29	0.21	0.12
Chlorophyll *a*	7.8	5.9	2.87	1.21	0.01
C : Chl *a*	36.6		115.0		0.01
CO_2 uptake	25.24	17.24	33.23	13.07	0.20
Assimilation number	4.01	2.05	11.73	1.82	0.00
C/N uptake	10.99	5.32	13.67	9.53	0.21
Bacterial counts	1.67	0.70	6.23	2.69	0.00
Heterotrophic activity	0.75	0.54	1.22	0.76	0.03
Regenerate rate	0.041	0.08	0.066	0.12	0.28

centrations (NO_3+NH_4) and chlorophyll *a* were lower in the summer while C:CHl *a*, assimilation number, PON, bacterial numbers and heterotrophic activity were higher. Note, on the other hand, that no significant differences were observed in the means of CO_2 uptake, NO_3 uptake, NH_4 uptake and regeneration and C/N uptake ratios.

Discussion

There exists a large record of the seasonal variations in physical, chemical and biological parameters for Bedford Basin. My data agrees very well with the recent work on yearly cycles (Harrison and Platt, 1981) and summer daily variations (Coté, 1979) of biological parameters related to phytoplankton production.

However, heterotrophic activity, NH_4 regeneration and nutrient uptake had not been measured simultaneously in this study area. My initial hypotheses were tested using data from the experiments performed after the water column became stratified (April 11). The correlation matrix presented in Table 1 supports the hypothesis that NO_3 uptake, NH_4 uptake, as well as NH_4 regeneration, changed proportionally with total primary production. However, the suggestion that bacterial activity as measured by glutamic acid uptake is correlated with NH_4 regeneration rates is not supported.

There are now several field studies suggesting a tight coupling between NH_4 regeneration and NH_4 assimilation by phytoplankton. While Caperon *et al.* (1979) and Paasche and Kristiansen (1982) measured low regeneration to assimilation ratios in some coastal embayments, Harrison (1978), Axler (1981) and Glibert (1982) observed that regeneration rates were generally equal to or greater than NH_4 uptake rates. Harrison (1978) found in two coastal areas (Southern California Bight, USA and Saanich Inlet, British Columbia, Canada) that NH_4 production and NH_4 assimilation were often in a one to one balance. More recently, Glibert (1982) also found that, even on time scales of a few minutes, all of the NH_4 assimilated by phytoplankton could be supplied by rapid NH_4 regeneration. Caperon *et al.* (1979) observed in Kaneohe Bay, Hawaii, USA an enhancement of NH_4 production at night that could balance the uptake of NH_4 on a daily basis. Additionally, Axler's (1981) study of a subalpine lake suggested that a large proportion of regenerated nutrients was available to the phytoplankton during the night. Glibert (1982) observed diel variations in regeneration to uptake ratios in the Sargasso sea. On the other hand, she also measured NH_4 regeneration rates equal to NH_4 uptake rates in short-term experiments performed in daylight hours. Results from remineralization studies are difficult to compare when experimental conditions (i.e. incubation time, amount of isotope added, time of study, etc.) differ considerably (Harrison, in press). For example, the Saanich Inlet measurements were made from columns of seawater contained in polyethylene cylindrical enclosures. In this type of experimental system where seawater is confined, physical processes replenishing nutrients are decreased and NH_4 regeneration may become a relatively more important mechanism of nutrient renewal. This can help to explain the high regeneration:assimilation (R:A) ratio measured in this coastal embayment.

Table 3. Estimated contribution of bacterial activity and microplankton regeneration to the nitrogen requirements of phytoplankton using mean values for spring (February 29 – May 15), summer (June 26 – August 18) and post-bloom (average) data set. A C/N composition ratio of 6.6 was used to convert the mean CO_2 uptake rate to nitrogen uptake rate. All the rates are expressed in μmol l^{-1} h^{-1} and ratios as percentages. (A) Contribution of $(NO_3 + NH_4)$ uptake to the total nitrogen requirements and contribution of NH_4 uptake to $(NO_3 + NH_4)$ uptake. (B) Contribution of NH_4 regeneration to NH_4 uptake. (C) Contribution of bacterial activity to NH_4 regeneration

	Spring	Summer	Average
(A)			
Nitrogen demand set by CO_2 assimilation	0.319	0.420	0.370
Nitrogen uptake measured by NO_3 and NH_4 assimilation	0.200	0.287	0.240
Nitrogen assimilation/ nitrogen demand (%)	62.7%	68.3%	65.5%
NH_4 assimilation/ $(NO_3 + NH_4)$ assimilation	58.0%	63.0%	60.5%
(B)			
NH_4 assimilation	0.115	0.182	0.147
NH_4 regeneration	0.041	0.066	0.056
NH_4 regeneration/ NH_4 assimilation	36.0%	36.0%	36.0%
NH_4 regenerated during 4 h/ ambient $[NH_4]$	11.5%	23.0%	17.5%
% of NH_4 regenerated assimilated by phytoplankton	38.0%	39.5%	38.7%
(C)			
Bacterial nitrogen excretion	0.0057	0.0135	0.0092
Bacterial nitrogen excretion/ NH_4 regeneration (%)	13.9%	18.5%	16.4%
Maximum bacterial nitrogen excretion*	0.0106	0.0191	0.0132
Max bacterial N excretion/ NH_4 regeneration	25.9%	26.2%	26.0%

* Maximum bacterial excretion was calculated from the upper value of a 95% confidence interval of the mean respiration rate of glutamic acid (see "Discussion" for more details)

The average seasonal importance of NH_4 and NO_3 uptake, NH_4 regeneration and bacterial activity to the nitrogen requirements of phytoplankton were estimated and are presented in Table 3. Phytoplankton nitrogen requirements were estimated from photosynthetic measurements, assuming an C:N assimilation ratio of 6.6 (Table 3 A; Redfield *et al.*, 1963). Individual POC:PON ratios or the C:N ratios derived from the slopes of the linear regressions of POC and PON on Chl *a* supported this assumption. Physical conditions were very different during the spring and the summer sampling periods. The increase in POC/Chl *a* possibly indicates a change in the physiological state of the phytoplankton (Healy and Hendzel, 1980) or a shift in species composition (Malone, 1977). However, the average estimates of the relative contribution of NH_4 uptake and regeneration to the phytoplankton nitrogen requirements were similar for both periods. NH_4 assimilation rates accounted for 60% of the phytoplankton total nitrogen $(NH_4 + NO_3)$ uptake on the average. Except in regions of strong upwelling (Whiteledge, 1978), NH_4 is often more important than NO_3 as a source of nitrogen for phytoplankton (see Harrison, 1980). The results of my study are no exception to this more general observation.

The seasonal average of NH_4 regeneration to assimilation observed in my study was relatively high (36%; Table 3 B). However, the contribution of regeneration to NH_4 uptake was very small on several days during the sampling period (Fig. 6). On these dates, phytoplankton probably utilised the ammonia that had accumulated in the euphotic zone as a result of processes other than rapid *in-situ* NH_4 regeneration. Although alternative mechanisms of NH_4 supply cannot be identified from my data, one can speculate on the basis of indirect evidence that the deeper water of Bedford Basin may be a reservoir of NH_4, supplying nutrients to the euphotic zone. Fig. 7 shows the strong correlation between nitrate and NH_4 concentrations observed at 4 depths. The ammonia concentrations mea-

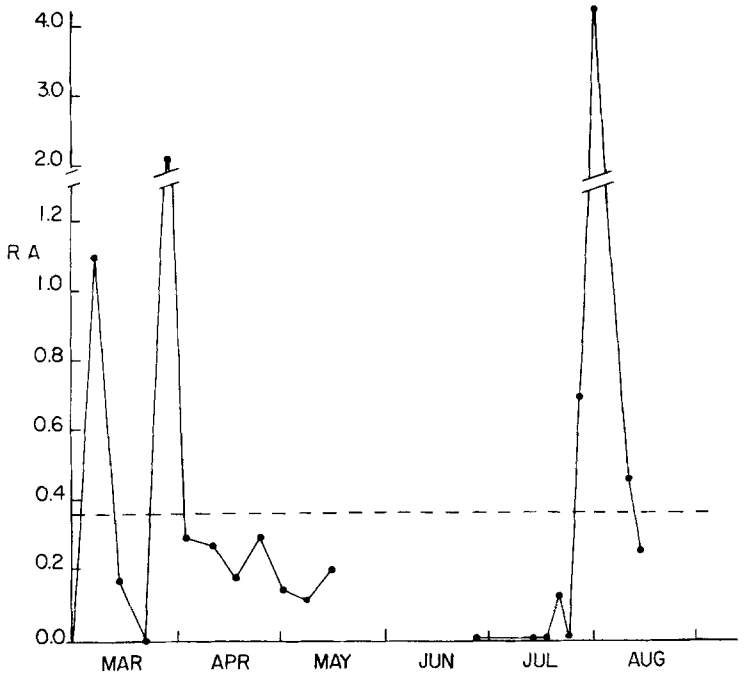

Fig. 6. Variations in the regeneration to assimilation ratio of NH_4 (R:A) with time. The dotted line represents the seasonal average of R:A (36%)

sured at 10 and 15 m (i.e. below the euphotic zone) are in general higher than nitrate concentrations found at the same depths. The high ammonia concentrations found at depth could result from benthic regeneration processes (Sorensen, 1978; Blackburn, 1979) or may simply result from an increase in R:A below the euphotic zone (Harrison, 1978). Although my data suggest that sources of NH_4 other than *in-situ* regeneration are important, further work is needed in order to identify the mechanisms involved. The variability of the R:A suggested that in Bedford Basin, new and regenerated production as defined by Eppley and Peterson (1979) cannot be identified only on the basis of ammonia and nitrate uptake rates.

Aside from examining the possible role of microheterotrophs, my study made no attempt to identify the organisms responsible for NH_4 regeneration. However, copepod excretion rates measured previously in the same study area (Conover and Mayzaud, 1975) suggested that these organisms could account for only about 5% of the NH_4 regeneration rates I observed. Size fractionation studies of nutrient regeneration have generally been consistent in suggesting that the microplankton provide most of the remineralized NH_4 (Harrison, 1978; Caperon *et al.*, 1979; Axler, 1981; Glibert, 1982; Paasche and Kristiansen, 1982).

A maximum production of NH_4 by microheterotrophs was estimated from glutamic acid respiration measurements using the following assumptions:

(1) Observed substrate respiration rates were maximal.

(2) All the amino acids present in seawater and utilised by microheterotrophs are assimilated at comparable rates. This assumption is supported by several workers who found that turnover rates of several amino acids were similar (Crawford *et al.*, 1974; Wright, 1974; Hollibaugh *et al.*, 1980).

(3) Respiration and ammonium excretion are equatable. If it is assumed that all the nitrogen in the metabolized amino acids is excreted, then each mole of amino acid will yield on average 1.5 mol NH_4.

(4) Several studies of amino acid abundance in seawater showed that the composition of the amino acid pool is relatively constant and glutamic acid represents 5 to 13% of the total pool (Siegel and Degens, 1966; Riley and Segar, 1970; Clark, 1972; Daumas, 1976; Williams *et al.*, 1976; Billens *et al.*, 1980; Dawson and Gocke, 1980).

Using these assumptions and estimates of heterotrophic activity, the maximum estimated contribution of bacteria to NH_4 regeneration was 25% with an average 16% for spring and summer (Table 3 C).

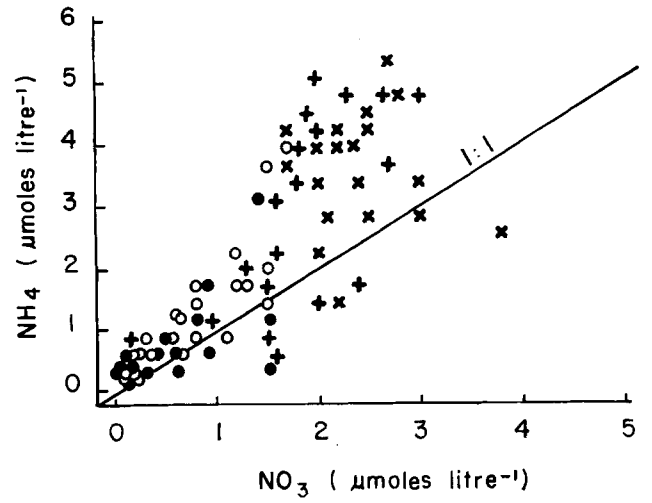

Fig. 7. Relationship between NH_4 and NO_3 concentrations for the data collected after the spring bloom. ● = surface, ○ = 5 m, + = 10 m, x = 15 m. The solid line represents the 1:1 relationship

Glutamic acid uptake was related with temperature and bacterial numbers only. Thus my results indicated that this measure of bacterial activity from short-term incubations was not useful in predicting regeneration fluxes in this environment.

Although the observations made in this study contradict the general idea that bacterioplankton is responsible for a large fraction of the nutrient recycling, other conflicting results have been observed in recent works. For example, Hollibaugh *et al.* (1980) suggested, on the basis of indirect evidence, that nitrogen excretion by microheterotrophs could provide a significant fraction of the total microplankton NH_4 regeneration. However, other workers (Fuhrman *et al.*, 1980) failed to demonstrate a direct relationship between heterotrophic activity and processes related to primary production. They observed that thymidine uptake rates in a coastal area were significantly correlated with Chl *a* and other measures of phytoplankton standing stock but not with primary production. These conflicts may be resolved by giving more attention to the methodology. A time lag may exist between the assimilation of the organic molecule and the excretion of metabolites (Iturriaga and Zsolnay, 1981). For example, Horrigan and McCarthy (1981) recently observed preferential incorporation of nitrogen over carbon during ^{14}C labelled urea uptake of phytoplankton cultures. This type of selectivity for a particular element of an organic molecule may also occur in substrate assimilation by heterotrophs. Although there is evidence that most amino acids have similar turnover rates (Billen *et al.*, 1980), it is not clear that the use of a single substrate is appropriate for estimating bacterial nitrogen excretion in coastal eutrophic waters (Fuhrman *et al.*, 1980; Hollibaugh *et al.*, 1980). Furthermore, the relative importance of the bacterial contribution to the total community nutrient regeneration may vary seasonally (Taguchi and Platt, 1977). However, the results obtained from the methodology described in this paper indicated that bacterial NH_4 remineralization was apparently unimportant in the near-surface water during the time of my study.

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