Marine Biology 115, 501–508 (1993)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Application of a ^{15}N tracer method to the study of dissolved organic nitrogen uptake during spring and summer in Chesapeake Bay

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Received: 17 July 1992 / Accepted: 28 October 1992

Abstract. The dissolved organic nitrogen (DON) pool in marine waters contains a diverse mixture of compounds. It is therefore difficult to accurately estimate planktonic uptake of DON using the limited number of radiolabeled compounds commercially available. We describe a method to estimate DON uptake rates using ^{15}N-labeled DON recently released from phytoplankton. To make ^{15}N-labeled DON, we incubated surface water with $^{15}NH_4^+$ and then isolated the DON, including any recently released $DO^{15}N$, with ion retardation resin. This DON was then added to a freshly collected water sample from the same environment to quantify the rate of DON uptake. The technique was applied to investigate rates of DON uptake relative to inorganic nitrogen in the mesohaline Chesapeake Bay during May 1990 and August 1991. The May experiment took place after the spring bloom, and rates of DON uptake [ranging from 0.31 to 0.53 μg-atom (μg-at) $Nl^{-1}h^{-1}$] often exceeded rates of NH_4^+ and NO_3^- uptake combined. The rates of DON uptake at this time were higher than estimated bacterial productivity and were not correlated with bacterial abundance or bacterial productivity. They were, however, correlated with rates of NO_3^- uptake. In May, we estimate that only 7 to 32% of DON uptake was a result of urea utilization. In contrast, in August, when regenerated nutrients predominate in Chesapeake Bay, rates of DON uptake (ranging from 0.14 to 0.51 μg-atom $Nl^{-1}h^{-1}$) were an average of 50% of the observed rates of NH_4^+ uptake. Consistent with the May experiment, rates of DON uptake were not correlated with bacterial production. A sizable fraction of DON uptake, however, appeared to be due to urea utilization; rates of urea uptake, measured independently, were equivalent to an average of 74% of the measured rates of DON uptake. These findings suggest that, during both periods of study, at least a fraction of the measured DON uptake may have been due to utilization by phytoplankton.

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Introduction

Traditionally the dissolved organic nitrogen (DON) pool in marine waters has been considered largely refractory and therefore unimportant to planktonic nutrition. A comprehensive understanding of the importance of the DON pool to planktonic nutrition, however, still eludes us, in large part due to methodological problems. The DON pool is inherently difficult to study as a nitrogen source because it contains a large number of compounds and the exact composition of the pool is unknown and probably changes over small space and time scales (Gardner and Stephens 1978, Sharp 1983, Antia et al. 1991). For this reason, measurement of DON uptake rates to date have largely been limited to a few compounds for which there are commercially available ^{15}N, ^{14}C, or ^{3}H-labeled tracers (e.g. amino acids and urea; McCarthy et al. 1977, Flynn and Butler 1986, Fuhrman 1990, Keil and Kirchman 1991). The isolated studies investigating uptake of other organic nitrogen compounds such as purines (Douglas 1983), pyrimidines (Knutsen 1972), amines (Neilson and Larsson 1980, Wheeler and Hellebust 1981), and combined amino acids (Coffin 1989) have shown that, while some phytoplankton and bacteria can utilize these compounds, the rates of uptake appear quite low (reviewed by Paul 1983 and Antia et al. 1991). Given the large number of compounds which make up the DON pool, however, DON could represent a significant source of nitrogen for the planktonic community even when uptake rates of individual components are low (Jackson and Williams 1985).

In the present paper we describe a ^{15}N-tracer approach to the study of DON utilization by plankton in which ^{15}N-labeled DON tracer is produced by the plankton community present in the study environment. The basic premise of our method is that phytoplankton produce intracellular DON which can be released through passive release, viral lysis, or heterotrophic grazing. We have previously described a method for measuring DON release which involves incubating phytoplankton with ^{15}N-labeled dissolved inorganic nitrogen (DIN) and

measuring the subsequent production of $DO^{15}N$ (Bronk and Glibert 1991). Here, we extend that method by collecting the labeled $DO^{15}N$ and adding it back to a freshly collected water sample to measure the rate of its incorporation by plankton (i.e., the rate of uptake of recently released DON). The technique was applied to measure DON uptake in Chesapeake Bay during late spring and summer.

Materials and methods

Overview of field experiments

Experiments were conducted during 16 to 18 May 1990 and 21 to 23 August 1991 in the mesohaline central channel of Chesapeake Bay. We were anchored on station during each experiment, and water was collected with a Niskin bottle from just below the surface for the measurement of nitrogen uptake every 3 h (May) or every 6 h (August).

Preparation and isolation of $DO^{15}N$ tracer

During both experiments, $DO^{15}N$ tracer was prepared by adding 5 μg-atom (μg-at) N l^{-1} $^{15}NH_4^+$ (99% enriched) to unfiltered surface water several hours before DON uptake experiments were performed. During the May experiment, $DO^{15}N$ tracer was made only once, and then used for numerous uptake experiments throughout a 24-h period. During the August experiment, $DO^{15}N$ tracer was made just prior to each uptake experiment throughout a 41-h period. Water was incubated with $^{15}NH_4^+$ for 1 h in May 1990 and for 2.5 h in August 1991. At the end of the incubation, the water was filtered (<125 mm Hg in May and <50 mm Hg in August) through a 0.2-μm Nuclepore filter and the filtrate was retained. $DO^{15}N$ was separated from the $^{15}NH_4^+$ by passing the filtrate through ion retardation resin, which removes inorganic ions, as outlined in Bronk and Glibert (1991). The eluate was then neutralized to pH 7 to 8 and boiled to reduce its volume and to reduce salinity changes when the $DO^{15}N$ tracer was added to the freshly collected water sample. This concentrated DON solution, now salt-free, was retained as the $DO^{15}N$ tracer; the concentration of NH_4^+ within the $DO^{15}N$ tracer was below the limit of detection (<0.03 μg-at N l^{-1}). An aliquot of the tracer was frozen in an acid-washed polyethylene bottle for later analysis of the atom % enrichment (atom %) of the $DO^{15}N$ as well as the concentrations of DON, NH_4^+, and NO_3^- (described below).

When isolating DON using ion retardation resin, the resin must be regenerated between each sample. During the course of our studies, we improved this procedure. In May, 1 *M* NaOH was used to regenerate the resin as outlined in Bronk and Glibert (1991). In August, however, we regenerated the resin using successive washes of 1 *M* HCl (180 ml) and 0.5 *M* NaOH contained in 0.5 *M* NaCl (250 ml). The columns were then shaken with 0.86 *M* NaCl followed by a NaCl rinse (60 ml), then thoroughly rinsed with distilled water (500 ml). This regeneration protocol created a more neutral environment within the column and eliminated the need to neutralize sample eluates.

Measurements of nitrogen uptake and availability

To begin the $DO^{15}N$ uptake incubations, in May, 10 ml of $DO^{15}N$ tracer was added to 1 liter of freshly collected near-surface water; this addition represented an increase in DON concentration within the sample of 1 to 6%. In May, the atom % of the DON tracer before it was added to a fresh water sample was 27.0. In August, 40 ml of $DO^{15}N$ tracer was added to 250 ml of freshly collected near-surface water; this addition represented an increase in DON concentration within the sample of 20 to 37%. In August, the atom % of the DON tracer before it was added to a fresh water sample was 1.3 to 2.9. A larger $DO^{15}N$ tracer addition was made to a smaller water sample in August to increase the final atom % of the $DO^{15}N$. During both May and August, the final atom % of the DON tracer varied over the diel experiments due to the fluctuations in the ambient DON pool. Time-courses of DON uptake were not performed due to the difficulty of making large quantities of the tracer. In parallel, uptake rates of NH_4^+, NO_3^-, and NO_2^- and urea (August only) were determined using ^{15}N techniques (Glibert et al. 1991). All ^{15}N uptake samples were incubated for 1 h under simulated in situ conditions in on-deck incubators. At the end of the incubations, samples were filtered through precombusted (450 °C for 2 h) Whatman GF/F filters and frozen until they were dried and stored in ampoules for mass spectrometric analysis (Glibert et al. 1991). A separate filter was taken for analysis of particulate nitrogen (PN; Control Equipment CHN analyzer) at the end of the experiment.

Nutrient analyses, except for dissolved primary amines (DPA) were performed on GF/F filtrate. Concentrations of DON were measured using the persulfate oxidation method of Valderrama (1981), concentrations of NH_4^+ and urea were measured manually (Parsons et al. 1984), and concentrations of NO_3^- and NO_2^- were measured with a Technicon AutoAnalyzer according to Parsons et al. (1984). Concentrations of DPA were measured as described in Parsons et al. (1984) on samples which were filtered through 0.2-μm Nuclepore filters and frozen in muffled (500 °C for 2 h) scintillation vials with solid polyethylene caps. Concentrations of DPA were corrected for fluorescence due to NH_4^+. A glycine standard was used in the DPA measurements and all DPA concentrations are presented in glycine equivalents. Concentrations of chlorophyll *a* (chl) and phaeopigment were measured fluorometrically as described in Parsons et al. (1984).

Rates of NO_3^-, NO_2^-, and urea uptake were calculated as described by Dugdale and Goering (1967), and the rate of NH_4^+ uptake was calculated as described by Glibert et al. (1982). The rate of DON uptake was calculated using the equation derived by Dugdale and Goering (1967) and modified as follows:

$$\text{DON Uptake Rate} = \frac{\text{atom\% excess PN}}{\text{atom\% excess DON} \times \text{time}} \times [\text{PN}], \quad (1)$$

where atom % excess in the atom % of the PN or DON tracer minus the ^{15}N atom % of the N_2 standard (e.g. atom % normal as measured on our mass spectrometer), time is the duration of incubation, and [PN] is the concentration of PN. The atom % excess of the DON was corrected for dilution of the $DO^{15}N$ by unlabeled DON present in the freshly collected water sample at the time of the addition. We assumed that the unlabeled DON in the sample had the atom % of the N_2 standard. This correction is analogous to the correction made for the addition of carrier nitrogen described in Bronk and Glibert (1991).

Error analysis

Uptake rates were not measured in duplicate due to the large amount of time required in sample processing and limitations in the amount of $DO^{15}N$ tracer. To estimate the variability associated with the final rate measurements, a propagation of error analysis was performed as described in Bevington (1969) which was found to provide a conservative estimate of the variance. The variances of the nutrient concentrations and atom % enrichments were calculated from replicates when available. When nutrient samples were not replicated due to limitations of sample volume, an estimate of the variance was calculated as described in Walpole (1970) using the variability associated with the regression line of nutrient standards which were used to calculate nutrient concentrations.

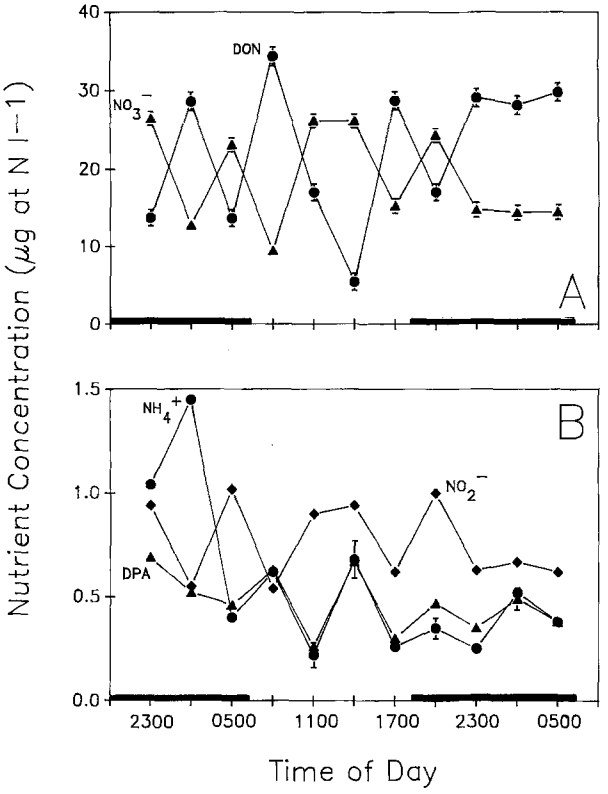

Fig. 1. Concentrations of (A) dissolved organic nitrogen (DON) and NO_3^- and (B) NH_4^+, NO_2^-, and dissolved primary amines (DPA) as a function of time of day in the mesohaline Chesapeake Bay beginning on 16 May 1990. All concentrations plotted with error bars indicating standard deviations. Where error bars are not shown, standard deviations are smaller than the size of the symbol. Heavy bars *on* *x*-axis indicate hours of darkness

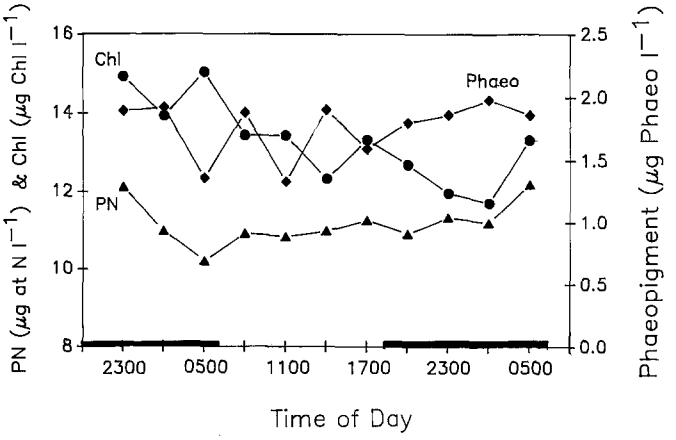

Fig. 2. Concentrations of particulate nitrogen (PN), chlorophyll *a* (chl), and phaeopigments (phaeo) as a function of time of day beginning on 16 May 1990. Heavy bars on *x*-axis indicate hours of darkness

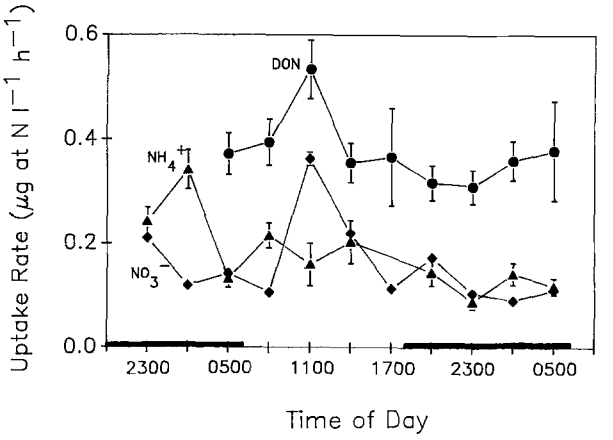

Fig. 3. Uptake rates of dissolved organic nitrogen (DON), NO_3^-, and NH_4^+ in the mesohaline Chesapeake Bay as a function of time of day beginning on 16 May 1990. All rates plotted with error bars indicating standard deviations. Where error bars are not shown, standard deviations are smaller than the size of the symbols. Heavy bars on *x*-axis indicate hours of darkness

Bacterial abundance and productivity

Bacterial productivity was measured with short-term (0.5 h) ^{3}H-thymidine incorporation (Malone et al. 1991). Measurements of bacterial productivity were made every 3 h during the May experiment and every 6 h during the first 24 h of the August experiment. Bacterial abundance was measured with acridine orange on samples preserved with glutaraldehyde as described by Hobbie et al. (1977) during the May experiment only.

Results

May experiment

During the 24-h diel study in May, concentrations of DON and NO_3^- varied dramatically, ranging from 5.4 to 34.4 μg-at N l^{-1} (Fig. 1 A), and were negatively correlated ($r^2=0.89$; $p=0.01$). Changes in concentrations of DON and NO_3^- of this magnitude are probably the result of changes in water masses fluctuating due to the tidal cycle, though DON and NO_3^- concentrations were not significantly correlated with changes in surface salinity. Concentrations of NO_2^- ranged from 0.54 to 1.02 μg-at N l^{-1} (Fig. 1 B) and were positively correlated to NO_3^- concentrations ($r^2=0.89$; $p=0.01$). Concentrations of DPA ranged from 0.26 to 0.69 μg-at N l^{-1} (Fig. 1 B) and, with the exception of the second time point, were positively correlated to NH_4^+ concentrations ($r^2=0.82$, $p=0.01$; Fig. 1 B).

Concentrations of chl varied from 11.7 to 15.0 μg chl l^{-1} (Fig. 2), and the concentrations of phaeopigment and PN ranged from 1.3 to 2.0 μg l^{-1} and from 10.2 to 12.2 μg-at N l^{-1}, respectively (Fig. 2). Ratios of PN:chl were 0.7 to 1.0 μg-at N μg chl^{-1}, suggesting a community composed primarily of living phytoplankton (McCarthy and Nevins 1986).

Rates of DON uptake ranged from 0.31 to 0.53 μg-at N l^{-1}; the highest rate was measured at 1100 and the lowest rate at 2300 (Fig. 3). Rates of NO_3^- uptake were the lowest of the three uptake rates measured, ranging from 0.09 to 0.36 μg-at N l^{-1} (Fig. 3). Uptake rates of DON were often higher than uptake rates of NH_4^+ and NO_3^- combined, and rates of DON uptake were significantly correlated to rates of NO_3^- uptake ($r^2=0.57$; $p=0.02$; Fig. 3).

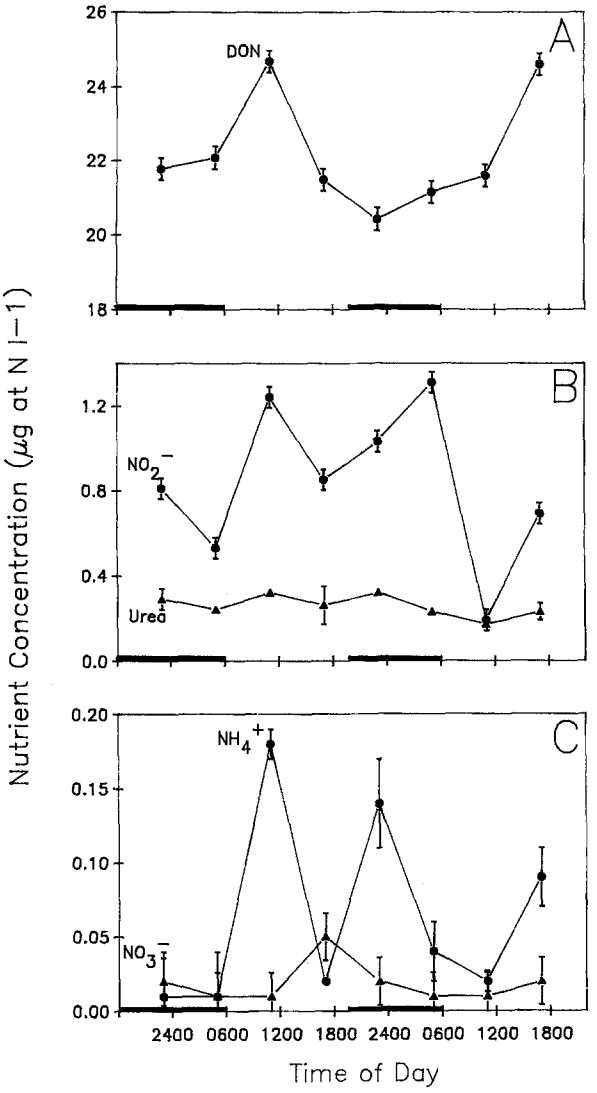

Fig. 4. Concentrations of (A) dissolved organic nitrogen (DON), (B) NO_2^- and urea, and (C) NH_4^+ and NO_3^- as a function of time of day in the mesohaline Chesapeake Bay beginning on 21 August 1991. All concentrations plotted with error bars indicating standard deviations. Where error bars are not shown, standard deviations are smaller than the size of the symbol. Heavy bars on *x*-axis indicate hours of darkness

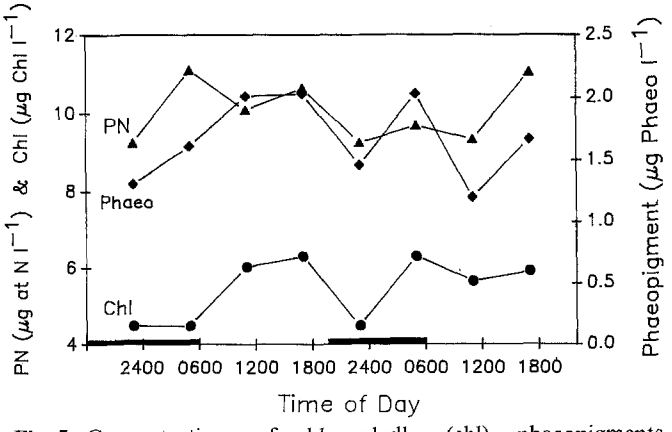

Fig. 5. Concentrations of chlorophyll *a* (chl), phaeopigments (phaeo), and particulate nitrogen (PN) as a function of time of day in mesohaline Chesapeake Bay beginning on 21 August 1991. Heavy bars on *x*-axis indicate hours of darkness

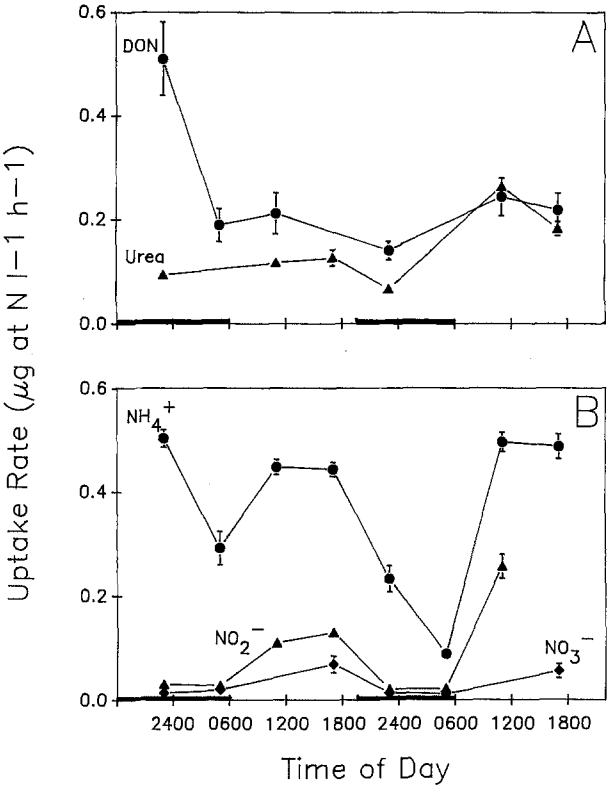

Fig. 6. Uptake rates of (A) dissolved organic nitrogen (DON) and urea and (B) NH_4^+, NO_2^-, and NO_2^- as a function of time of day in the mesohaline Chesapeake Bay beginning on 21 August 1991. All rates plotted with error bars indicating standard deviations. Where error bars are not shown, standard deviations are smaller than the size of the symbol. Heavy bars on *x*-axis indicate hours of darkness

Primary productivity, measured during a 24-h incubation begun on 17 May 1990, was 0.19 ± 0.05 μg-at N l^{-1} h^{-1} when converted to nitrogen units using the mean C:N ratio, for the May study, of 8.5 (Malone unpublished data). Bacterial productivity ranged from 0.027 to 0.033 μg-at N l^{-1} h^{-1} (Peele unpublished data) when converted to nitrogen units using a C:N ratio of 5 (Goldman et al. 1987), and did not show a diel pattern. Bacterial abundance ranged from 1.9 to 4.1×10^9 cells l^{-1} and was positively correlated to the concentration of DPA ($r^2 = 0.78$; $p = 0.01$). There was no significant correlation between bacterial abundance or bacterial productivity and rates of DON uptake.

August experiment

During the 41-h diel study in August, concentrations of DON were found to vary much less than in the May experiment, ranging from 20.4 to 24.7 μg-at N l^{-1} (Fig. 4A). Concentrations of NO_2^- ranged from 0.19 to 1.31 μg-at N l^{-1}, and urea concentrations ranged from 0.17 to 0.32 μg-at N l^{-1} (Fig. 4B). Concentrations of NO_3^- and NH_4^+ were often near the limit of detection (≤ 0.03 μg-at N l^{-1}; Fig. 4C).

Concentrations of chl were ca. two to three times lower than observed in May, while concentrations of

phaeopigment and PN were similar to those measured in May (Fig. 5). Ratios of PN:chl were 1.5 to 2.5 μg-at N μg chl^{-1}, suggesting that there was more heterotrophic or detrital nitrogen present in August relative to May.

Rates of DON uptake were also comparable to those observed in May, and ranged from 0.14 to 0.51 μg-at N l^{-1} h^{-1} (Fig. 6A). Urea uptake rates ranged from 0.09 to 0.26 μg-at N l^{-1} h^{-1} (Fig. 6A), and with the exception of the first time point, were equivalent to an average of 74% of the measured rates of DON uptake. Consistent with May, rates of NO_3^- uptake were the lowest of the uptake rates measured. The highest rates of both NO_3^- and NO_2^- uptake were measured during the day and the lowest at night (Fig. 6B). In contrast to May, rates of NH_4^+ uptake were generally the highest uptake rates measured in August, ranging from 0.08 to 0.50 μg-at N l^{-1} h^{-1}; rates of NH_4^+ uptake were an average of 30% higher than rates of DON uptake (Fig. 6B).

Primary productivity, measured during a 24-h incubation begun on 22 August 1991, averaged 0.19 ± 0.01 μg-at N l^{-1} h^{-1} when converted to nitrogen units using the mean C:N ratio, for the August experiment, of 7.0 (Malone unpublished data). Bacterial productivity ranged from 0.08 to 0.11 μg-at N l^{-1} h^{-1} (Shiah unpublished data) when converted to nitrogen untis using a C:N ratio of 5 (Goldman et al. 1987). Bacterial productivity was not correlated to uptake rates of DON or urea.

Discussion

Advantages and limitations of the DO^{15}N method

Measuring the rate of DON uptake using DO^{15}N, produced as outlined above, allows us to estimate rates of uptake of recently released DON. The tracer which is produced contains labeled DON compounds which are the actual release products of the planktonic community in the environment at that time. Compared to uptake rates measured using defined substrates, rates of DON uptake measured using the DO^{15}N techniques are not biased by assumptions of the availability of various DON compounds. Furthermore, uptake rates measured using defined substrates have been shown to result in lower uptake rates than studies using naturally produced organic compounds at comparable concentrations (Smith and Wiebe 1976, Wiebe and Smith 1977). In addition, many uptake studies using defined substrates use DON compounds labeled with ^{14}C or ^{3}H; while informative, these studies can not directly address utilization of DON as a nitrogen source.

There has been extensive use of a similar technique for measuring the uptake of DOC with ^{14}C (Lancelot 1979, Bell and Sakshaug 1980, Smith 1982, Malone et al. 1991). Many of the uncertainties of measuring DOC uptake with ^{14}C are also encountered when measuring DON uptake with ^{15}N. The DO^{15}N method has uncertainties with regard to which DON compounds are labeled, what fraction of the DON pool is labile, what effect the addition of $^{15}NH_4^+$ and filtration has on the production of DO^{15}N, and whether or not DO^{15}N is taken up during the initial incubation used to prepare the label. We discuss each of these uncertainties below.

First, when the DON tracer is produced, we cannot be certain which DON compounds are labeled, nor do we know which compounds are taken up. Our measurements of DON uptake, therefore, are made under the assumption that the extracellular DON pool is uniformly labeled. In this respect, the DON uptake rates we calculate approximate maximum or potential rates of DON utilization. If the assumption of uniform labeling is not met, and the ^{15}N-labeled DON which is released is not representative of the ambient DON pool, the atom % enrichment of the initial tracer would be underestimated, and, consequently, the calculated DON uptake rate would be overestimated (Eq. 1). Also, as is true for all tracer experiments, the calculated uptake rate is affected by the initial enrichment of the tracer. The initial enrichment varied in our experiments because a constant quantity of DO^{15}N tracer was added to ambient seawater containing a variable concentration of DON. Whereas this addition perturbed the total concentration of DON by a maximum of 37% (in August), concentrations of individual components of the DON pool may have been affected to a greater or lesser extent. In further experiments the kinetic effects of variable enrichment levels of DO^{15}N should be investigated.

The extent to which we overestimate uptake rates of DON is also a function of the percentage of the DON pool which originates from phytoplankton, relative to other sources (i.e., allochthonous inputs or heterotrophic excretion). For example, if only 50% of the ambient DON at a sample site is due to phytoplankton release, the DON release rates we calculate would be overestimated by 50%. Though we can not say for certain which fraction of the ambient DON pool was due to phytoplankton release in the studies reported here, it is likely that the rates of DON uptake which we calculated were overestimated to some degree due to the dilution of the DON tracer by non-phytoplankton derived DON.

Second, by adding saturating $^{15}NH_4^+$ concentrations to label the DON and filtering the cells when making the DO^{15}N tracer, we may be affecting both the composition and quantity of released DON relative to ambient conditions. This was likely occurring during the May experiment when an elevated vacuum pressure was used during label preparation, and the ^{15}N atom % of the DON pool was extremely high, suggesting cell breakage.

Finally, measured rates of DON uptake may also be affected by uptake of DO^{15}N which occurs when the tracer is being produced. If close coupling between DON release and DON uptake occurs, the most nutritionally desirable DON compounds would be rapidly taken up and so removed from the tracer. If the DO^{15}N tracer is composed of nutritionally inferior DON compounds, the resulting DON uptake rates would be underestimates. Rates of DON uptake in August would probably be more affected by this underestimate, relative to May, as primary and bacterial productivity were at a seasonal maximum and inorganic nitrogen concentrations were at a minimum in August (Malone et al. 1988).

Rates of DON uptake and prevailing environmental conditions

We chose to conduct the initial diel experiment in May, after the spring bloom, when we anticipated relatively high rates of DON release and, therefore, a high potential for uptake of recently released DON. Nitrogen nutrient inputs to Chesapeake Bay peak during the spring, driven largely by flow of the Susquehanna River, and the resulting phytoplankton biomass is at a maximum. For comparison, we also measured rates of DON uptake during late summer when concentrations of DIN are low, nitrogen input is dominated by benthic flux of NH_4^+, and primary and secondary productivity are at seasonal maxima (Malone et al. 1988). Here we discuss rates of DON uptake relative to rates of DIN uptake, and relative to estimates of uptake of individual components of DON. We also speculate about the role of phytoplankton in the uptake of DON.

In May, rates of DON uptake often exceeded the combined uptake rates of NH_4^+ and NO_3^-. We suggest that the high DON uptake rates we measured may not be representative of the entire spring but possibly reflect a brief episode when the supply of recently released DON was high resulting from the decline of the spring bloom. A series of cruises to the experimental site during the months of April and May allowed us to document the decline of the bloom. Between 17 April and 23 May 1990 primary productivity at the sample site decreased by over 30% and chl concentrations decreased almost three-fold (Malone et al. unpublished data). Measurements of DON release were also made at the same site on 23 May 1990 and ranged from 0.05 to 0.18 μg-at N l^{-1} at 09:00 hrs depending on the length of incubation (Bronk and Glibert 1991). Flynn and Butler (1986) have noted that planktonic uptake of dissolved free amino acids (DFAA) increases as levels of NO_3^- decline in spring, and Larsson and Hagström (1982) have documented increased rates of DOC release during the decline of a spring bloom in the Baltic.

Uptake rates of individual components of DON were not measured on the same date in May as the results reported here. Uptake rates of urea (McCarthy et al. 1977, Glibert et al. unpublished data) and DFAA (Bell 1990), however, have been measured previously in Chesapeake Bay. Rates of urea uptake at this site during May of 1989 ranged from 0.05 to 0.15 μg-at N l^{-1} h^{-1} (Glibert et al. unpublished data). Urea uptake rates were measured 5 d after the May study and ranged from 0.04 to 0.10 μg-at N l^{-1} h^{-1}. These rates suggest that an estimated 7 to 32% of the total DON uptake rates measured may have been due to uptake of urea. Rates of DFAA uptake in the mesohaline Chesapeake Bay during May ranged from 25 to 180 mg C m^{-3} d^{-1} or 0.03 to 0.21 μg-at N l^{-1} h^{-1} assuming a DFAA C:N ratio of three (Bell 1990). These rates suggest that an estimated 5 to 67% of the DON uptake we measured could have been due to DFAA uptake.

In contrast to the May experiment, we believe our study in August is more typical of uptake rates which persist throughout summer. The nitrogen requirements of the planktonic community in Chesapeake Bay in the summer are met largely with regenerated nutrients, such as NH_4^+ (McCarthy et al. 1977), and the results from our August study are consistent with this pattern. Concentrations of DIN were very low, and uptake rates of NH_4^+ were the highest measured. We also observed NH_4^+ regeneration rates as high as 0.34 μg-at N l^{-1} h^{-1} (data not shown). With the dependence of the system on regenerated nutrients, we had anticipated relatively high rates of DON uptake, and we did find that DON uptake rates were higher than uptake rates measured for NO_2^- and NO_3^-.

In August, most of the DON uptake we measured appeared to be due to utilization of urea. Rates of urea uptake, measured independently, were equivalent to an average of 74% of the rate of DON uptake. We are not aware of comparable rates of DFAA uptake in the mesohaline Chesapeake Bay during August.

Organisms responsible for DON uptake

Heterotrophic bacteria are usually considered the primary users of DON, with the exception of urea (Billen 1984, Wheeler and Kirchman 1986). During both of our study periods, however, we suggest that phytoplankton may have been responsible for at least some fraction of the DON uptake for the following reasons. First, there was no correlation between rates of DON uptake and bacterial abundance or productivity. Second, rates of DON uptake were 10 ± 2 (May) and 2 ± 1 (August) times higher than bacterial productivity, suggesting another sink for DON in addition to bacteria. However, if our DON uptake rates are overestimated as discussed above, the difference between DON uptake and bacterial productivity would not be as large. Finally, for May, DON uptake rates were positively correlated to uptake rates of NO_3^-, a nitrogen form used predominantly by phytoplankton (Glibert and Garside 1991). For August, when the rate of urea uptake was subtracted from the rate of DON uptake, yielding a closer approximation of non-phytoplankton DON uptake, rates of DON uptake more closely approximated rates of bacterial productivity.

Laboratory studies have shown that phytoplankton have the ability to use many DON compounds as nitrogen sources (Antia et al. 1975, Neilson and Larsson 1980, reviewed by Antia et al. 1991), and this ability is highly variable both between and within species (Flynn and Butler 1986). In addition to direct uptake of amino acids, cell-surface L-amino acid oxidases have recently been discovered in several species of marine phytoplankton (Palenik and Morel 1990). These oxidases oxidize amino acids and primary amines to H_2O_2, alpha keto acids, and an amino group; the amino group is subsequently taken up as NH_4^+. There are other cell surface oxidases in phytoplankton such as asparaginase, which cleaves an amino group from asparagine, thereby making NH_4^+ available for uptake (Paul and Cooksey 1979), and phosphatases which convert organic phosphate to inorganic phosphate which can then utilized by phytoplankton (Chrost et al. 1986). A similar mechanism could be invoked to cleave

nitrogen from larger organic molecules. The method we outline here measures uptake of ^{15}N-labeled nitrogen, not the uptake of an entire nitrogen-containing compound, and so we would measure uptake due to oxidases if it were occurring.

Conclusions

These studies suggest that, at certain times of the year, DON supplies a significant fraction of the nitrogen utilized by the planktonic community. While analytical biases may lead to overestimates of the uptake rates determined using DO^{15}N techniques, sharp seasonal differences were noted in our field studies. In May, we sampled during what we believe to be an episodic event when uptake rates of recently released DON exceeded those of the inorganic nitrogen forms due to increased availability of recently released DON present from the decline of the spring bloom. The lack of correlations between rates of DON uptake and bacterial abundance or bacterial productivity, the ten-fold difference in rates of DON uptake above bacterial productivity, and the correlation between rates of NO_3^- and DON uptake suggest that phytoplankton were likely using at least a fraction of the recently released DON. Rates of DON uptake which appear to be more typical of the season studied were measured in late August when the highest measured uptake rates were for NH_4^+. In August, rates of DON uptake exceeded uptake of NO_2^- and NO_3^- and a large fraction of DON uptake appeared to be due to urea utilization.

Acknowledgements. We thank S. Banahan for assistance throughout the development of the method. We are indebted to S. Kelly, S. Pike, and T. Malone for providing the chl, phaeopigment, and primary productivity data and E. Peele and F. Shiah for providing the bacterial production and abundance data for the May and August experiments, respectively. We are grateful to A. Lewitus, H. Ducklow, D. Capone, T. Fisher, B. Ward and two anonymous reviewers for reading earlier versions of this manuscript. We also thank the Captains and crews of the R. V. "Ridgely Warfield" and the R. V. "Cape Henlopen". This work was supported by grant OCE-8817585 and BSR-88114272 to P. M. Glibert. Additional funding was also supplied by the Foundation for the Advancement of Research in the Medical Sciences to D. A. Bronk. This is contribution no. 2 369 from the Center for Environmental and Estuarine Studies.

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Communicated by J. Grassle, New Brunswick