Limnol. Oceanogr., 27(4), 1982, 639–650

Isotope dilution models of uptake and remineralization of ammonium by marine plankton[1]

Patricia M. Glibert[2]
Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

Fredric Lipschultz
Division of Applied Sciences, Harvard University

James J. McCarthy and Mark A. Altabet
Museum of Comparative Zoology, Harvard University

Abstract

The utility of ^{15}N isotope dilution models for the calculation of uptake and remineralization of NH_4^+ by marine phytoplankton was examined in light of model limitations when applied to field data either when ambient NH_4^+ levels border on our limit of detection or when there is no statistically significant difference between ambient NH_4^+ at the beginning and end of an incubation. Through specific examples of field and laboratory data we show that the limitations are a function both of analytical error inherent in the methodology and of changes in rates of uptake and remineralization over the course of a given experiment. We propose modifications to the existing models of NH_4^+ uptake and remineralization which overcome some of these limitations. The results show that uptake rates have been traditionally underestimated by a factor of ≈2 in routine ^{15}N uptake methodology and that regeneration of NH_4^+ over relatively brief periods can supply the daily nitrogen requirements of the phytoplankton when there are no losses from the system.

Marine phytoplankton living in nitrogen-impoverished waters may be able to exploit a patchy nutrient distribution, since phytoplankton in both laboratory cultures and field assemblages can utilize NH_4^+ rapidly when it is delivered to the medium in pulse fashion. For example, N-deprived phytoplankton cells grown in NH_4^+-limited continuous cultures can assimilate this nutrient at rates much faster than their growth rate when exposed to uptake-saturating NH_4^+ concentrations for 5-min periods (McCarthy and Goldman 1979). Field assemblages can also utilize NH_4^+ rapidly and efficiently during the initial minutes of 2-h incubations following even trace additions of substrates (Glibert and Goldman 1981).

A corollary to the hypothesis of phytoplankton exploitation of nutrient microenvironments is that enough nitrogen to meet the phytoplankton demand must be made available by zooplankton excretion and bacterial remineralization. ^{15}N isotope dilution methodology has been used to measure rates of these processes in a freshwater lake (Alexander 1970), southern California waters (Harrison 1978), and Kaneohe Bay, Hawaii (Caperon et al. 1979). A similar methodology has been applied to NH_4^+ fluxes in the sediment (Blackburn 1979).

The analytical methodology in these studies of remineralization varies widely, as do the models used in the calculation of results. Alexander (1970; *see also* Dugdale 1965) and Harrison (1978; pers. comm.) assumed constant dilution with time to estimate remineralization rates, while Caperon et al. (1979) and Blackburn (1979) used linear differential equations to estimate uptake and remineralization rates of NH_4^+.

We will show that previous isotope models of uptake and remineralization have limitations when applied to field data, either when ambient NH_4^+ levels border on our limit of detection (≈0.03 μg-atom·liter^{-1}) or when there is no statistically significant difference between

[1] This work was supported by NSF grant OCE 77-26401 and OCE 80-22990 J. J. McCarthy.
[2] Present address: Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543.

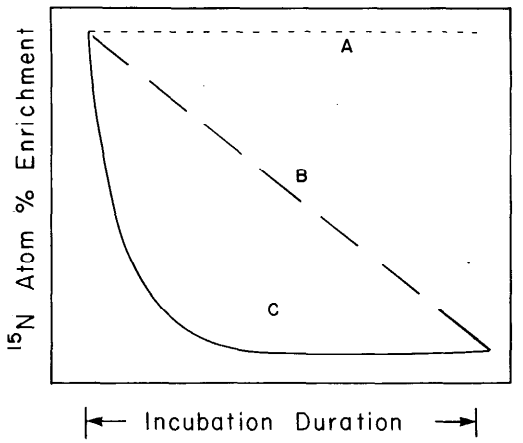

Fig. 1. Assumptions of changes in ^{15}N atom % enrichment during incubation in various calculation models of NH_4^+ uptake and remineralization. In case A, changes are assumed to be negligible; in case B, ^{15}N atom % enrichment is assumed to be diluted in linear fashion by $^{14}NH_4^+$; in case C, regeneration of $^{14}NH_4^+$ is assumed to result in exponential decrease in ^{15}N atom % enrichment with time.

ambient NH_4^+ at the beginning and end of an incubation. We propose modifications to the linear differential equation model which overcome some of the problems of estimating these rates when ambient NH_4^+ is not known very precisely and present four cases of field and laboratory data which show the magnitude of the corrections. Furthermore, we show that the limitations are due to both analytical error inherent in the methodology and to changes in rates of uptake and remineralization over the course of a given experiment. Finally we discuss the implications and importance of these corrections, as well as their limitations.

We thank J. Nevins and K. Cain for helpful discussions.

Models of NH_4^+ uptake and remineralization

Review of literature models—The use of ^{15}N as an isotopic tracer for the study of phytoplankton uptake of nitrogenous nutrients was introduced by Neess et al. (1962) and applied to marine systems by Dugdale and Goering (1967). Using general principles established for tracer methodology (Sheppard 1962) they proposed that rates of uptake of any particular form of nitrogen (V), as well as the flux (ρ) could be calculated by the formula (using NH_4^+ as an example):

$$V_{NH_4^+} = \frac{^{15}\text{N atom \% excess}}{^{15}\text{N atom \% enrichment} \times \text{time of incubation}}, \quad (1)$$

and

$$\rho NH_4^+ = V_{NH_4^+} \times PN \quad (2)$$

where ^{15}N atom % excess is the ^{15}N atom % of the particulate fraction minus ^{15}N atom % normal, ^{15}N atom % enrichment is the initial enrichment of NH_4^+ in the medium; and PN is the particulate nitrogen of the sample. The dimensions of $V_{NH_4^+}$ are reciprocal time and those of ρNH_4^+ are mass of N per volume of sample per time. Major assumptions of this model are that the ^{15}N atom % enrichment remains constant throughout the experiment (Fig. 1, curve A), and that V remains constant during the incubation.

A modification to the above method proposed (MacIsaac and Dugdale 1972) for calculating uptake rates at low substrate levels involves determining the rate of uptake after a larger amount of substrate is added and back-calculating with an independently determined half-saturation constant to estimate uptake rates at ambient levels of substrate. One difficulty with this method is that when ambient NH_4^+ concentrations are low, it may be difficult or impossible to relate the phytoplankton response to an uptake-saturating pulse of nutrient to a natural response at lower ambient concentrations (Conway and Harrison 1977; Dugdale 1977; McCarthy and Goldman 1979). We will not deal with this approach further here.

The first models proposed for the calculation of remineralization from ^{15}N isotope dilution assumed that during an incubation, the regeneration of N, in the form of $^{14}NH_4^+$, will dilute the ^{15}N:^{14}N isotopic ratio in a linear fashion (Fig. 1, curve B). Since neither of these models is fully described in the literature we will not discuss them further, but values de-

termined with them may not be accurate representations of true rates.

The two linear differential equation models for the simultaneous calculation of uptake and remineralization (Caperon et al. 1979; Blackburn 1979) are mathematically identical. They differ from the earlier models primarily in that they predict that the regeneration of $^{14}NH_4^+$ will result in an exponential decrease in ^{15}N atom % enrichment with time (Fig. 1, curve C). The complete derivations of these models have been given and will not be repeated here; however, it is important to review their assumptions and the final general form of the equations.

The linear differential equation models assume that remineralization and uptake rates are constant with time; that all remineralized NH_4^+ is unenriched in ^{15}N (i.e. the ^{15}N label of NH_4^+ incorporated during the experiment remains in the particulate fraction); and that isotopic discrimination by the uptake process is small relative to the changes discussed here. From these assumptions, the results of the models predict that ambient NH_4^+ will change linearly with time, and that the change in ^{15}N:^{14}N with time will be an exponential function depending on the initial ^{15}N atom % enrichment, the uptake and remineralization rates, and the initial and final ambient NH_4^+ concentrations. Given a change in ambient NH_4^+ pool size and the change in ^{15}N atom % enrichment, the uptake rate, i (for incorporation), and the remineralization rate, d (for dilution), can be determined by the simultaneous solution of

$$P_{(t)} = P_0 + (d - i)t \tag{3}$$

and

$$\frac{dR(t)}{dt} = -d \times \frac{R(t)}{P(t)} \tag{4}$$

where $P_{(t)}$ and P_0 are the ambient NH_4^+ concentrations at time t and time zero, $R(t)$ and $P(t)$ are the ^{15}N atom % enrichment and NH_4^+ pool size of the aqueous fraction as functions of time. Equation 4 can be integrated to give a logarithmic form, and, by including a correction for ^{15}N atom % normal, the equation becomes

$$\ln[R_{(t)} - {}^{15}n] = \ln(R_0 - {}^{15}n) - \left[\frac{d}{(d-i)}\right]\left[\ln \frac{P_{(t)}}{P_0}\right] \tag{5}$$

where ^{15}n refers to ^{15}N atom % normal ($\approx$0.365%). $R_{(t)}$ and R_0 are the ^{15}N atom % enrichments of the aqueous fraction at time t and 0. All variables except d and i are experimentally measured or determined. We will call this the Blackburn-Caperon model; we chose to use symbols equivalent with the Blackburn presentation for the sake of convenience.

Modified isotope dilution model—The linear differential equation models have heretofore been applied exclusively to conditions of high ambient NH_4^+. A major problem in applying them when ambient NH_4^+ is at or near the limit of detection is their dependence on Eq. 3—the mass balance of ambient NH_4^+ pool sizes. In Eq. 3 uptake is determined by merely correcting the initial ambient NH_4^+ for that generated during the course of the experiment and balancing that with the final measured ambient NH_4^+ concentration. The problem is that the measurement of NH_4^+ at very low levels is not too precise.

To overcome this dependence on the mass balance of NH_4^+ pool sizes in calculating uptake, we can determine uptake by the incorporation of ^{15}N into the particulate fraction and the application of Eq. 1 and 2. However, it is assumed in Eq. 1 and 2 that the initial atom % enrichment does not change during the course of the experiment, or that this change will be negligible (Fig. 1, curve A). Thus, by making the measurements necessary for the Blackburn-Caperon calculations, we cannot only estimate NH_4^+ remineralization but also improve our estimate of NH_4^+ uptake.

Estimates of NH_4^+ uptake may be improved when P_0, $P_{(t)}$, and $R_{(t)}$ are determined. These parameters not only improve our estimate of ambient NH_4^+, but also allow corrections to Eq. 1 and 2 for changing atom % enrichment. The esti-

mate of ambient NH_4^+ is improved because we now have a measure of ambient NH_4^+ for each experimental vessel. More commonly the ambient NH_4^+ concentration of a whole water sample is measured before it is dispensed into individual experimental vessels and the NH_4^+ concentration in the experimental vessel calculated by summing the measured amount of the whole water sample and the ^{15}N addition. Often there are delays between filling the incubation bottles and starting the experiment; during this period there can be changes in ambient NH_4^+ concentration, particularly if the sample has not been screened to remove small zooplankton (Sheldon and Sutcliffe 1978).

The estimate of NH_4^+ uptake is also improved from our better estimate of atom % enrichment. By directly measuring R_0 and $R_{(t)}$, we avoid the uncertainty in estimating the enrichment when ambient NH_4^+ is undetectable. The magnitude of this problem becomes apparent in the case of a sample with undetectable NH_4^+ and an addition of labeled NH_4^+ quantitatively equivalent to the minimum limit of detection; the isotope enrichment would lie between 50 and 100%, and the calculated rate of uptake would vary accordingly (Eppley et al. 1977; McCarthy 1980).

If the data fit a first-order rate law so that

$$R_{(t)} = R_0[\exp(-kt)] \tag{6}$$

or

$$\frac{dR_{(t)}}{dt} = -kR_{(t)} \tag{7}$$

where

$$k = \frac{\ln[R_{(t)}/R_0]}{t}, \tag{8}$$

then an exponential average between R_0 and $R_{(t)}$ can be calculated to yield $\bar{R}$.

$$\bar{R} = \frac{1}{t}R_0 \int_0^t [\exp(-kt)]\, dt \tag{9}$$

and

$$\bar{R} = \frac{R_0}{kt}[1 - \exp(-kt)]. \tag{10}$$

Given the first-order form of Eq. 6, the substitution of $\bar{R}$ instead of R_0 in Eq. 1 is the best estimator when V is assumed constant for a particular period. The new formulation for calculating uptake rates thus becomes

$$P_{NH_4^+} = \frac{^{15}\text{N atom \% excess}}{\bar{R} \times \text{time of incubation}} \times \text{PN} \tag{11}$$

where P (rho) is used to distinguish the corrected rate from ρ, the uptake rate uncorrected for changing R. The magnitude of this correction to P depends on the change in R over the period of the experiment. It is essential that this correction for changing R be applied whether or not NH_4^+ pool sizes change during an experiment.

For the sake of this discussion we assume that P and i represent the same processes, although, as we show later, the corrected P is not identical with parameter i when particulate uptake of NH_4^+ is not the sole loss from the NH_4^+ pool.

Having an improved estimate of uptake rates now allows us to re-evaluate the models for calculating remineralization rates. The application of Eq. 5 requires a statistically significant change in NH_4^+ pool sizes ($|P_{(t)} - P_0| > (t_{0.05})(\sigma_x)$ over an incubation. For if $|P_{(t)} - P_0| \approx 0$ then the ratio of $P_{(t)}$:P_0 is 1 and the logarithm is 0. When the ratios $P_{(t)}$:P_0 or $R_{(t)}$:R_0 are near 1, the effect of random analytical variations on the estimated dependent variables (i and d) increases dramatically. Thus two general cases must be differentiated when remineralization rates are determined, although the method of calculation will be similar.

First, if the NH_4^+ pool sizes are not significantly different ($P < 0.05$), then the rate of remineralization is indistinguishable from that of uptake; in other words, the system seems to be in steady state. In this situation Eq. 4 becomes

$$\frac{dR(t)}{dt} = \frac{-D \times R(t)}{\bar{P}} \tag{12}$$

where $\bar{P}$ is the average ambient NH_4^+ concentration over the course of the ex-

periment and $D/\bar{P}$ is identical to parameter k in Eq. 6. Thus

$$D = k \times \bar{P}. \quad (13)$$

For clarity we will use D to denote remineralization by this modification and will show that within analytical error under this condition, D does not equal i and that D does equal P. Under these circumstances our best estimator of D is P because of the smaller error associated with P.

In the second case, where the differences in pool sizes are statistically significant ($P < 0.05$), Eq. 5 should be applicable. However, when ambient NH_4^+ is low there are still difficulties in applying this equation: i will be overestimated. For this reason, we recommend Eq. 12 for the calculation of remineralization rates, and, as we show below, this rate is equivalent to that calculated from Eq. 5 (within error). We also use D to denote remineralization calculated under this situation by Eq. 12. The advantage of this form is that we can now use P (calculated from Eq. 11) as our better estimator of uptake. In contrast to the first general case, P and D are not in steady state.

Materials and methods

Field experiments—Samples were collected in the Sargasso Sea aboard *G. W. Pierce* (June–July 1979) and in the Chesapeake Bay aboard RV *Warfield* (May–June 1980).

Water was collected from the 100 or 60% light level, and incubations for uptake and remineralization were started within ≈1 h of sampling. Samples were prefiltered through either 130- or 10-μm Nitex, and trace concentrations (≈10% of ambient, or 0.05 μg-atom·liter^{-1} where ambient NH_4^+ was undetectable) of $^{15}NH_4^+$ (99% enriched preparation) were added. Samples were incubated on deck in 2.5-liter polycarbonate containers. Circulating surface seawater was used to maintain ambient temperature, and neutral density screens were used to simulate the 60% light level.

For the determination of NH_4^+ uptake, incubations were ended after ≈4 h by filtration with Reeve Angel 984H on the Sargasso Sea cruise and after ≈10 min to 2 h with Whatman GF/C (precombusted) filters on the Chesapeake cruise. Filters were washed with 50 ml of filtered seawater and dried.

Samples for ^{15}N atom % enrichment of the aqueous fraction were withdrawn from the same experimental vessel. Isotope was added, and a 300–500-ml subsample immediately filtered; the filtrate was kept for determination of ^{15}N enrichment in the nutrient pool. At the end of the experiment an aliquot of the filtrate was kept when the particulate fraction was collected. We were careful to retain the filtrate before the filter was washed to remove residual ^{15}N.

Laboratory experiment—We used a unialgal chemostat culture to test the validity of both the Blackburn-Caperon linear differential equation model and the modifications to the model described here. In this experiment all variables in the models could be independently measured or calibrated.

A culture of *Pavlova lutheri* was grown under PO_4^{3-}-limiting conditions at a steady state rate (μ) of 0.19·d^{-1}. Phosphate limitation was chosen rather than NH_4^+ limitation so that there would be measurable residual NH_4^+ in the medium, traces of $^{15}NH_4^+$ could be added without perturbing the system, and thus the possibility of enhanced NH_4^+ uptake with the isotope addition could be avoided. The inflowing medium was prepared with artificial seawater (McLachlan 1973) with PO_4^{3-} and NH_4^+ concentrations of 4 and 400 μg-atoms·liter^{-1}. Culturing protocols and apparatus have been described elsewhere (Goldman 1977; Goldman and McCarthy 1978). Culture temperature was 20°C.

The experiment was started by adding 0.75 μg-atom·liter^{-1} of $^{15}NH_4^+$ to the culture chamber. Ambient NH_4^+ in the culture was 9.9 μg-atoms·liter^{-1}. Fresh medium delivered during the experiment diluted the initial ^{15}N atom % in the NH_4^+ pool. The rate constant for dilution is analogous to d in the Blackburn-Cape-

Table 1. Comparison of calculated flux of NH_4^+ uptake as ρ (assuming constant ^{15}N atom % enrichment) and P (corrected for exponentially decreasing ^{15}N atom % enrichment) for selected samples from the Sargasso Sea. All samples were collected from the 60% light level. Calculated ^{15}N atom % enrichment was 100%, based on an $^{14}NH_4^+$ measurement on the whole water sample and not from the individual experimental bottle. Dimensions of P_0, $P_{(t)}$, and PN are μg-atoms·liter^{-1} and those of ρ and P are μg-atoms·liter^{-1}·h^{-1}.

Station location	P_0	$P_{(t)}$	Measured R_0	Measured $R_{(t)}$	Atom % excess	PN	Incubation (h)	ρ	P	P:ρ
31°56′N, 71°43′W	0.07	0.05	74.1	51.1	1.650	0.47	4.0	0.0019	0.0031	1.6
23°52′N, 76°33′W	0.08	0.05	63.0	39.1	1.643	0.43	4.4	0.0016	0.0032	2.0
27°50′N, 70°30′W	0.07	0.16	69.4	17.5	2.062	0.39	4.4	0.0019	0.0049	2.7
31°10′N, 65°30′W	0.08	0.03	64.0	54.7	1.187	0.53	4.0	0.0016	0.0027	1.7
34°32′N, 59°45′W	0.06	0.27	67.3	9.9	0.930	0.53	4.2	0.0012	0.0039	3.3
39°01′N, 64°15′W	0.05	0.24	90.0	9.1	1.284	0.64	4.3	0.0019	0.0054	2.8
40°30′N, 68°00′W	0.05	0.12	90.0	5.7	1.651	1.51	4.0	0.0175	0.0204	1.2

ron model. As for the field samples, immediately after inoculation an initial sample was withdrawn, filtered, and the filtrate kept for isotopic determination.

Incubations continued for ≈5 h; a sample was then withdrawn, filtered, and the filtrate retained as above. The filter (Whatman GF/C, precombusted) was rinsed with filtered seawater and dried.

Analytical methods—Analysis of ^{15}N in the particulate fraction was by mass spectrographic analysis (McCarthy et al. 1977).

For determination of ^{15}N in the aqueous fraction we collected the NH_4^+ by distillation, using modifications to the techniques of Harrison (1978) and Caperon et al. (1979). Just before distillation, a 200–300-ml sample was buffered with saturated MgO to raise the pH above 9.0. When ambient NH_4^+ was <5 μg-atoms·liter^{-1}, $^{14}NH_4^+$ was added to bring the final concentration to >10 μg-atoms·liter^{-1}. The sample was distilled under mild vacuum and 50 ml of condensate collected directly into 10 ml of 0.0024 N HCl; this volume was evaporated to <5 ml and stored until analysis. Immediately before analysis, the sample was transferred to a Reeve Angel 984H filter, dried, and analyzed by mass spectrography like the particulate samples. Recoveries of NH_4^+ were ≈95% and the range for replicates analyzed for ^{15}N:^{14}N was ±5%.

Ammonium in the sample water was determined before experiments began, and in the initial and final filtrates immediately after collection (Solórzano 1969). Particulate nitrogen (PN) was determined with a Coleman nitrogen analyzer.

Results

In Table 1 we give examples from the Sargasso Sea data illustrating the error that can result from calculating ρ without taking into account the change in ^{15}N atom % enrichment with time. The P:ρ ratio was fairly consistent for these examples; indeed, for 160 samples from the Sargasso Sea and Chesapeake Bay cruises the average P:ρ was 2.2 ± 1.6. The precision gained by measuring R_0 rather than calculating it from an analysis of whole water samples is clearly demonstrated. For each of these samples the initial analyses of NH_4^+ gave a value <0.03 μg-atom·liter^{-1}; had that value been assumed for each experimental vessel, the calculated atom % enrichment would have been 100%. The actual measured values of R_0 are very close to those calculated from individual measurements of P_0. The additional discrepancies between ρ values in Table 1 and those that would be calculated directly from Eq. 1 are due to the blank component.

The Blackburn-Caperon model for calculating NH_4^+ uptake rates ceases to be valid when ambient NH_4^+ is at or near our limit of detection or when changes in ambient NH_4^+ cannot be resolved with statistical confidence. Small errors in de-

Table 2. Selected examples from the Sargasso Sea comparing Blackburn-Caperon model of uptake (i) and remineralization (d) with modified model presented here (P and D) when ambient NH_4^+ borders on our limit of detection. Raw data for these calculations presented in Table 1. All rates are reported as μg-atoms·liter^{-1}·h^{-1}.

Example No.	Sample location	P	D	i	d
1	31°56′N, 71°43′W	0.0031	0.0056	0.0106	0.0056
2	27°50′N, 70°30′W	0.0049	0.0360	0.0143	0.0345

termining NH_4^+ can lead to large errors in i when uptake is calculated solely on the basis of mass balance of pool sizes (Eq. 3). Four examples of field and laboratory data will show the magnitude of these problems.

Example 1: ambient NH_4^+ borders on our limit of detection and there is no significant difference between P_0 and $P_{(t)}$ ($P < 0.05$). The analytical SD of the NH_4^+ analysis at this concentration level is ± 0.03 μg-atom·liter^{-1}. Since the pool size is constant the steady state Eq. 12 is appropriate and P should equal D if there are no other losses. As can be seen from data entry 1 in Table 2 there is essentially no difference in remineralization calculated by either the Blackburn model or this model (d vs. D). The differences between P and i are of the order of a factor of 3, however. The effect of not accounting for isotope dilution in calculating ρ can be seen in Table 1, data entry 2. The discrepancy between P and D derives from analytical uncertainty in P_0. Small differences in P_0, equal to the analytical SD will result in error in D of this magnitude; therefore, the best estimate of D in this case is P. Error of the sort seen in D is not as large in P because pool size does not enter into its formulation.

Example 2: ambient NH_4^+ borders on our limit of detection, and differences between P_0 and $P_{(t)}$ are significant ($P < 0.05$). Equation 12 is used for calculating remineralization, Eq. 11 for uptake, and comparisons are made to Eq. 5. As in example 1, the comparison between d and D for this example reveals relatively small differences (Table 2, data entry 2). Again, the comparison between P and i suggests that i overestimates NH_4^+ uptake by a factor of ≈ 3. We cannot explain why the magnitude of the overestimation in i is so consistent. The important difference between example 2 and example 1 is that P and D are not in steady state, and thus an equivalency between P and D is not expected.

Example 3: ambient NH_4^+ is relatively high (>1.0 μg-atom·liter^{-1}), and significant differences between P_0 and $P_{(t)}$ cannot be resolved. Again, steady state Eq. 12 is appropriate and P should equal D if there are no other losses. At a concentration of $\approx$1–10 μg-atoms·liter^{-1} NH_4^+, the analytical SD is ± 0.05 μg-atom·liter^{-1}, and when concentrations are >10 μg-atoms·liter^{-1} NH_4^+ the analytical SD is ± 0.23 μg-atom·liter^{-1}. The results of this comparison (Table 3) are very similar to example 1. The i values, again, tend to overestimate NH_4^+ uptake by a factor of $\approx$1.7–3.8. The effect of the isotope dilution correction on these data averaged $\approx$1.0–1.3 (ρ data not shown), which is reasonable since the change in R_0 to $R_{(t)}$ was not large. In contrast to example 1, the agreement between P and D is good considering the effect of analytical fluctuations in R when calculating D. Since $R_{(t)}$:$R_0 \approx 1$, the relative error due to these fluctuations is larger than in example 1.

Example 4: ambient NH_4^+ is relatively high, and differences between P_0 and $P_{(t)}$ are significant ($P < 0.05$). This situation was rarely encountered in our field experiments; however, our chemostat experiment was designed to fit these criteria. In this example Eq. 5 should be applicable and, since the only losses are due to uptake, then P should equal i. The results of NH_4^+ uptake and isotope dilution from this experiment were calculat-

Table 3. As Table 2, but from Chesapeake Bay when ambient NH_4^+ concentrations are detectable but P_0 and $P_{(t)}$ are not statistically different ($P < 0.05$). Station location for data entry 1 was in upper portion of the bay, and for data entry 2 was 19 km up the Potomac River. All rates are reported as μg-atoms·liter^{-1}·h^{-1}. P_0, $P_{(t)}$, and PN are reported as μg-atoms·liter^{-1}.

Measured										
P_0	$P_{(t)}$	R_0	$R_{(t)}$	Atom % excess	PN	Incubation (h)	P	D	i	d
4.39	4.02	5.92	5.79	0.069	10.38	0.80	0.153	0.115	0.587	0.124
7.22	6.66	8.33	7.32	0.212	32.20	0.75	1.165	1.196	2.000	1.254

ed by the three methods: the Blackburn-Caperon model (Eq. 5); the modified model presented here, in which P, the ρ corrected for isotope dilution, and Eq. 12 are used to calculate dilution; and the mass balance calculation of NH_4^+ uptake and NH_4^+ input to the system, since all components of the system were known independently. Results are presented in Table 4. The uptake data are also presented without the isotope dilution correction (ρ), for further evidence that this effect can be large (a factor of ≈1.4). A comparison between the Blackburn-Caperon model and the mass balance calculations clearly demonstrates the validity of the isotope dilution model and approximate equivalency of both P and i in a well defined, closed system when ambient NH_4^+ concentrations are relatively large and when differences between P_0 and $P_{(t)}$ can be determined with statistical confidence. The slight discrepancies between the various parameters are not surprising, considering the dependence of the parameters on logarithmic functions.

To this point we have considered only the effects of NH_4^+ pool size and analytical errors in P and R on the calculation of uptake and remineralization rates. However, there are also effects of variations in these rates with time, and thus, under certain circumstances, the assumption of constant uptake and constant remineralization may not be valid. Again, let us consider the cases of high and low ambient NH_4^+.

Figure 2 shows clearly that when ambient NH_4^+ is at our limit of detection and $P_0 \approx P_{(t)}$ the uptake rate of NH_4^+ is enhanced following even an addition quantitatively equivalent to our minimum limit of detection, and essentially all uptake is complete within ≈10 min. Furthermore, an equivalent enhancement of NH_4^+ remineralization is observed in the first few minutes of incubation, indicating that the two rates balanced even during the first few minutes. When P_0 is statistically different from $P_{(t)}$ (data not shown), remineralization occasionally exceeds uptake in the first few minutes, even though enhanced uptake is also observed; the rest of the time, the system is in approximate steady state at very low rates.

Figure 3 shows uptake and remineralization when ambient NH_4^+ is large (P_0 = 6.85 μg-atoms·liter^{-1}). In this situation a trace supplement does not result in an initially enhanced NH_4^+ uptake rate, and for the rest of the time, the rates of NH_4^+ uptake and remineralization are balanced at a fairly constant rate. However, an en-

Table 4. Results of chemostat mass balance experiment comparing Blackburn-Caperon model (i and d), modified model (P and D), and calculations of uptake and remineralization based on mass balances. All rates are reported as μg-atoms·liter^{-1}·h^{-1}. P_0, $P_{(t)}$, and PN are reported as μg-atoms·liter^{-1}.

Measured											Mass balance	
P_0	$P_{(t)}$	R_0	$R_{(t)}$	Atom % excess	PN	Incubation (h)	ρ	P	D	i	d	uptake	NH_4^+ input
9.91	10.73	6.48	2.98	0.168	191	4.8	1.04	1.48	1.67	1.65	1.82	1.51	1.86

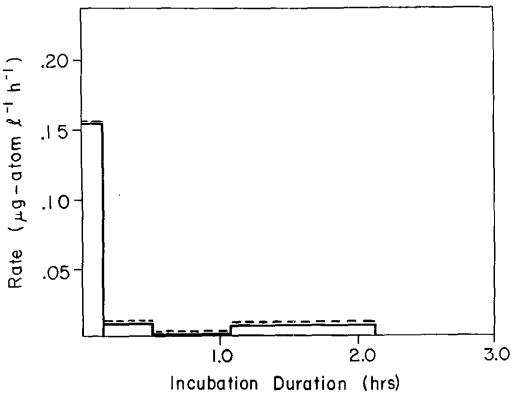

Fig. 2. Time-course of uptake (——) and remineralization (----) of NH_4^+ when ambient NH_4^+ is at our limit of detection and no difference exists between P_0 and $P_{(0}$ ($P < 0.05$). Both rates are in balance. Station was in open waters of lower Chesapeake Bay. Rates are calculated as time intervals (not as integrations from time of inoculation to time of termination).

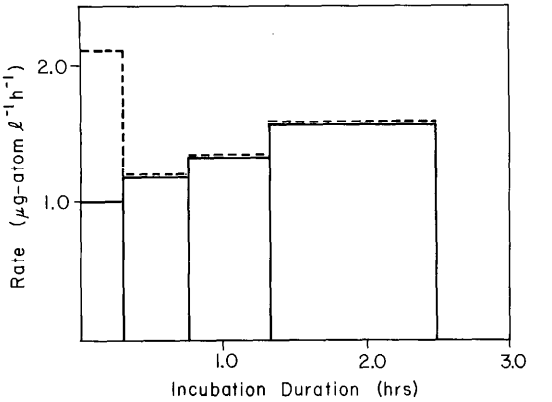

Fig. 3. As Fig. 2, but when ambient concentrations of NH_4^+ are detectable. Rates of uptake and remineralization are in balance except for initial period where rate of remineralization exceeds uptake. Station was 19 km up Potomac River (Chesapeake Bay). Rates calculated as in Fig. 2.

hancement of remineralization is observed in the first few minutes of incubation.

Discussion

Improved estimate of P *relative to* ρ— Quite recently it has been recognized that the assumption that ^{15}N atom % enrichment remains constant during an incubation may not be correct. Sharp et al. (1980) proposed this as a possible artifact by which their NH_4^+ uptake rates in the central North Pacific Ocean may have been underestimated. They had no data to determine the magnitude of this artifact but suggested that the resultant error would be small. On the other hand, Fisher et al. (1981), working with estuarine nitrogen dynamics, suggested that the assumption of constant ^{15}N atom % enrichment might lead to significantly large errors in their estimates of uptake; they also had no experimental data to estimate how large. Our data, from a variety of field conditions, indicate that NH_4^+ uptake by the particulate fraction is typically underestimated by a factor of ≈ 2 when constant ^{15}N atom % enrichment is assumed.

Recognition of the problem of correctly determining ambient NH_4^+ in the assay bottle is also not new. Murphy (1980) suggested that distillations and subsequent ^{15}N analyses of the NH_4^+ immediately after inoculation would give the most accurate calculation of ambient NH_4^+ and NH_4^+ uptake, but recognized the tediousness of the procedure. Fisher et al. (1981) recommended that NH_4^+ concentrations be monitored over the course of incubations to check for substrate exhaustion. As we have shown, however, monitoring NH_4^+ changes alone is not adequate without also monitoring the change in ^{15}N atom % enrichment because of our relative uncertainty in ambient NH_4^+ measurements, especially when concentrations border on our detection limit. Thus, in order to improve the accuracy of estimates of NH_4^+ uptake rates in waters of low ambient NH_4^+, ^{15}N atom % enrichment of the NH_4^+ pool must be determined.

It is conceivable, and perhaps likely, that uptake may be underestimated for still other reasons when incubations are long (> a few hours). During long incubations ^{15}N may be excreted and subsequently reincorporated by the phytoplankton, resulting in a further underestimation of uptake. If, for example, all the ^{15}N is completely recycled once, the under-

estimation would be by a factor of 2. In relatively short incubations this problem is minimized.

Error analysis of P *and* D—We have compared rates of remineralization and incorporation of NH_4^+ as measured by the isotope dilution of the NH_4^+ pool with uptake measured by the ^{15}N incorporation into the particulate fraction. The precision and accuracy of these estimates varies in each of the general cases. Where there is no statistically significant change in pool size over the incubation (i.e. steady state) Eq. 11 must be used to calculate incorporation and Eq. 12 to calculate remineralization. The precision of D can be calculated by using first-order terms of a Taylor series expansion of Eq. 12 and, assuming no cross-correlation,

$$\frac{\sigma_D^2}{D^2} = \frac{\sigma_P^2}{P^2} + \frac{\sigma_R^2}{[R \times \ln(R)]^2} \qquad (14)$$

where $\sigma_x^2{:}x^2$ is the relative variance of each term and R is the ratio $R_{(t)}{:}R_0$.

When pool size is large, the relative variance of P is small, but the value of R is close to 1 thus magnifying the variance in D. For data entry 1 in Table 3 the relative contribution due to R is 99.96% and σ_D is 0.127% on the estimated value of 0.115. When pool size is small, the effect of the ratio error diminishes since R is large, but the effect of error due to P increases. For data entry 2 in Table 2, the pool size variance contributes 63% of the total σ_D of 0.037.

We cannot explain why i is consistently overestimated in the field experiments and not to the same degree in the laboratory experiment, unless a systematic error existed in the field samples or there were other losses from the NH_4^+ pool, such as nitrification. The calculated values for i from examples 1 and 2 are unrealistically high in terms of our present view of uptake rates of NH_4^+ in oceanic waters.

Importance of time-course measurements—We have shown that an additional difficulty in applying these isotope dilution models may arise when the assumption of constant uptake and remineralization with time does not hold. In contrast to most data on sediment fluxes (Blackburn 1979) which usually show constant rates with time, data from oceanic systems show variable rates with time. Our data are consistent with others which suggest that relatively N-deprived laboratory cultures or natural assemblages can respond by rapid uptake of NH_4^+ when exposed for brief periods (McCarthy and Goldman 1979; Glibert and Goldman 1981). Furthermore, enhanced remineralization may be observed over relatively brief periods. This enhancement may be a result of increased excretion by microzooplankton stimulated by sample handling, passage through Nitex netting, and subsequent bottle confinement. Enhanced remineralization may also be observed in waters of high ambient NH_4^+, but a 10% error in isotope enrichments when enrichments are small may mask this effect. Also, the slight increase in pool size due to isotope addition is not enough to increase phytoplankton uptake rates much. For these reasons, time-courses must be done to determine the degree to which uptake and remineralization are constant with time. When the assumption of constancy is not valid, then, with time-course data, constant rates may be assumed at least for brief intervals.

Implications for data interpretations—Our data show that under a variety of conditions the fluxes of uptake and remineralization are in approximate steady state. Thus, regeneration of NH_4^+ over relatively brief periods can be sufficient to supply the N demands of the phytoplankton unless there are losses from the system, such as the sinking of fecal pellets.

These conclusions are not unlike those of others who have worked with isotope dilution methods (Alexander 1970; Brezonik 1972; Harrison 1978; Caperon et al. 1979). However, in light of our discussion of analytical variations and rate changes with time which show, under certain circumstances, enhanced uptake and remineralization following bottle confinement of a sample, we suggest that their data be re-examined. By similar rea-

soning the model proposed by Fisher et al. (1981) for calculating remineralization rate (R), in which

$$R = \frac{d[NH_4^+]}{dt} + U \qquad (15)$$

where U is the NH_4^+ uptake rate, can also not be valid. This equation is identical to Eq. 3 of the Blackburn-Caperon model (if rearranged); and, as both Blackburn (1979) and Caperon et al. (1979) point out, this equation should only be used in the context of the linear differential equation model. Furthermore, as we have stressed, without an isotope dilution measurement of ^{15}N atom % enrichment at time t, uptake rates will be underestimated. By understanding the limitations of these models we may better understand rates generated with the models.

In dealing with natural samples where ambient NH_4^+ is near the limit of detection it becomes imperative that uptake and remineralization be measured over time so that the processes can be fully understood. It is through measurements over time that short term physiological responses by phytoplankton, as well as the development of bottle effects such as nutrient depletion (Goldman et al. 1981), can be identified. We are still limited in our ability to determine the degree to which current methods simulate real world dynamics: to what extent is zooplankton excretion enhanced due to confinement, and to what extent can phytoplankton in nature respond with enhanced NH_4^+ uptake?

References

ALEXANDER, V. 1970. Relationships between turnover rates in the biological nitrogen cycle and algal productivity. Proc. Ind. Waste Conf. Purdue Univ. **25:** 1–7.

BLACKBURN, T. H. 1979. Method for measuring rates of NH_4^+ turnover in anoxic marine sediments, using a ^{15}N-NH_4^+ dilution technique. Appl. Environ. Microbiol. **37:** 760–765.

BREZONIK, P. L. 1972. Nitrogen: Sources and transformations in natural waters, p. 1–47. *In* H. E. Allen and J. R. Kramer [eds.], Nutrients in natural waters. Wiley.

CAPERON, J., D. SCHELL, J. HIROTA, AND E. LAWS. 1979. Ammonium excretion rates in Kaneohe Bay, Hawaii, measured by a ^{15}N isotope dilution technique. Mar. Biol. **54:** 33–40.

CONWAY, H. L., AND P. J. HARRISON. 1977. Marine diatoms grown in chemostats under silicate or ammonium limitation. 4. Transient response of *Chaetoceros debilis, Skeletonema costatum,* and *Thalassiosira gravida* to a single addition of the limiting nutrient. Mar. Biol. **43:** 33–43.

DUGDALE, R. C. 1977. Modeling, p. 789–806. *In* E. D. Goldberg [ed.], The sea: Ideas and observations on progress in the study of the seas. Wiley.

———, AND J. J. GOERING. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. **12:** 196–206.

DUGDALE, V. A. 1965. Inorganic nitrogen metabolism and phytoplankton primary productivity in a subarctic lake. Ph.D. thesis, Univ. Alaska. 93 p.

EPPLEY, R. W., J. H. SHARP, E. H. RENGER, M. J. PERRY, AND W. G. HARRISON. 1977. Nitrogen assimilation by phytoplankton and other microorganisms in the surface waters of the central North Pacific Ocean. Mar. Biol. **39:** 111–120.

FISHER, T. R., P. R. CARLSON, AND R. T. BARBER. 1981. Some problems in the interpretation of ammonium uptake kinetics. Mar. Biol. Lett. **2:** 33–44.

GLIBERT, P. M. AND J. C. GOLDMAN. 1981. Rapid ammonium uptake by marine phytoplankton. Mar. Biol. Lett. **2:** 25–31.

GOLDMAN, J. C. 1977. Steady state growth of phytoplankton in continuous culture: Comparison of internal and external nutrient equations. J. Phycol. **13:** 251–258.

———, AND J. J. MCCARTHY. 1978. Steady state growth and ammonium uptake of a fast growing marine diatom. Limnol. Oceanogr. **23:** 695–703.

———, C. D. TAYLOR, AND P. M. GLIBERT. 1981. Nonlinear time-course uptake of carbon and ammonium by marine phytoplankton. Mar. Ecol. Prog. Ser. **6:** 137–148.

HARRISON, W. G. 1978. Experimental measurement of nitrogen remineralization in coastal waters. Limnol. Oceanogr. **23:** 684–694.

MCCARTHY, J. J. 1980. Nitrogen and phytoplankton ecology, p. 191–233. *In* I. Morris [ed.], The physiological ecology of phytoplankton. Blackwell.

———, AND J. C. GOLDMAN. 1979. Nitrogenous nutrition of marine phytoplankton in nutrient depleted waters. Science **203:** 670–672.

———, W. R. TAYLOR, AND J. L. TAFT. 1977. Nitrogenous nutrition of the plankton in the Chesapeake Bay. 1. Nutrient availability and phytoplankton preferences. Limnol. Oceanogr. **22:** 996–1011.

MACISAAC, J. J., AND R. C. DUGDALE. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. Deep-Sea Res. **19:** 209–232.

MCLACHLAN, J. 1973. Growth media—marine, p. 25–51. *In* J. R. Stein [ed.], Handbook of phycological methods: Culture methods and growth measurements. Cambridge.

MURPHY, T. P. 1980. Ammonia and nitrate uptake

in the Lower Great Lakes. Can. J. Fish. Aquat. Sci. **37:** 1365–1372.

NEESS, J. C., R. C. DUGDALE, V. A. DUGDALE, AND J. J. GOERING. 1962. Nitrogen metabolism in lakes. 1. Measurements of nitrogen fixation with ^{15}N. Limnol. Oceanogr. **7:** 163–169.

SHARP, J. H., M. J. PERRY, E. H. RENGER, AND R. W. EPPLEY. 1980. Phytoplankton rate processes in the oligotrophic waters of the central North Pacific Ocean. J. Plankton Res. **2:** 335–353.

SHELDON, R. W., AND W. H. SUTCLIFFE, JR. 1978. Generation times of 3 h for Sargasso Sea microplankton determined by ATP analysis. Limnol. Oceanogr. **23:** 1051–1055.

SHEPPARD, C. W. 1962. Basic principles of the tracer method. Wiley.

SOLÓRZANO, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. **14:** 799–801.

Submitted: 7 July 1981
Accepted: 18 February 1982