Nitrogen Cycling in Coastal Marine Environments
Edited by T.H. Blackburn and J. Sørensen

CHAPTER 1

Primary Productivity and Pelagic Nitrogen Cycling

PATRICIA M. GLIBERT

1.1 INTRODUCTION

The importance of nitrogenous nutrients in marine phytoplankton production has long been recognized. Yet it is difficult to pinpoint precisely the origin of the concept that coastal and oceanic phytoplankton are nitrogen-limited. Several factors seem to have been instrumental in the formulation of this concept, which is now so deeply rooted in our current approach to the study of marine primary productivity. The first is that our perception of the role of nutrients as limiting elements has paralleled developments in analytical chemistry. For example, much of the early work on the role of nutrients in primary production focused on phosphate rather than nitrate due to the fact that methods were available for measuring phosphate routinely and more precisely than for the nitrogen species (Riley, 1946; see also reviews by Glibert and Garside, 1982; Nixon and Pilson, 1983). With improvements in the methodologies for determining nitrate and nitrate came the observation that nitrogen was the nutrient which became depleted more rapidly and more completely in surface seawater than other nutrients, and that ambient levels of nitrogen in surface waters were often inadequate to support a single doubling of phytoplankton biomass (Thomas, 1966, 1970; Ryther and Dunstan, 1971). A concurrent observation was the recognition that light alone could not be limiting productivity, as theoretical calculations suggested that the amount of light penetrating the euphotic zone should support up to an order of magnitude more productivity than was observed (Ryther, 1959; Vishniac, 1971). Nevertheless, the degree and manner in which nitrogen may be limiting phytoplankton growth in surface ocean waters is still not well understood.

The concept of nitrogen limitation in the sea was crystallized by the now classical work of Dugdale and Goering (1967), in which the distinction between 'regenerated production' and 'new production' was first drawn. Regenerated production is that resulting from the utilization of those nitrogen forms, primarily NH_4^+ and urea, which are regenerated *in situ* (e.g. from zooplankton excretion

and bacterial remineralization), whereas new production is that resulting from the utilization of those nitrogen forms, primarily NO_3^-, which represent allochthonous inputs of nitrogen to a particular system. The regeneration of nitrogenous nutrients is well known to be a major source of nitrogen for oceanic primary producers (McCarthy, 1972; Eppley *et al.*, 1973; McCarthy *et al.*, 1977; Harrison, 1978; Glibert, 1982), but it is the rate of supply of 'new' nitrogen which can ultimately determine the total amount of production of a particular system. Dugdale and Goering (1967) suggested that the amount of production associated with new nitrogen utilization would be equivalent to that available for export to higher trophic levels. Eppley and Peterson (1979) developed this distinction even further by suggesting the need for a balance to be maintained between the upward flux of NO_3^- into the euphotic zone and the downward flux of organic nitrogen out of the euphotic zone, if biomass and productivity are to be maintained at the near steady-state levels observed.

In systems for which the rate of supply of new or allochthonous sources of nitrogen is relatively high, the total production is generally higher than in systems for which this supply is small, assuming for the sake of argument that the influence of other factors, such as light availability in both types of systems, is constant (Eppley and Peterson, 1979; Malone *et al.*, 1983a). The same, however, cannot necessarily be said of the specific growth rates of phytoplankton in systems for which rates of allochthonous inputs of nitrogen are high versus those for which they are low. Thus, if phytoplankton growth is tightly coupled to zooplankton grazing and nutrient regeneration, high growth rates of phytoplankton need not be exclusively found in environments of high allochthonous nitrogen availability. The degree to which high growth rates are sustained for phytoplankton in oligotrophic waters is still the source of some controversy, however (Goldman *et al.*, 1979; Eppley, 1981a).

The significance of different forms of nitrogenous nutrients to phytoplankton production has mainly been addressed by the application of the stable isotope ^{15}N. Whereas during the late 1960s to mid-1970s, there was considerable emphasis placed on the determination of water column rates of phytoplankton nitrogen utilization (e.g. Dugdale and Goering, 1967; Goering *et al.*, 1970; Walsh and Dugdale, 1971; Eppley *et al.*, 1973), during the early to mid-1980s, the focus of attention has been, at least in part, on determining the degree to which phytoplankton nitrogen uptake is coupled to nitrogen regeneration, particularly on the small time and space scales which simulate as best as analytically possible the immediate environment of a phytoplankton cell (e.g. Conway *et al.*, 1976; Conway and Harrison, 1977; Harrison, 1978; McCarthy and Goldman, 1979; Collos, 1980; Glibert and Goldman, 1981; Goldman and Glibert, 1982; Wheeler *et al.*, 1982). In the discussion which follows, I will attempt to strike a balance between the quantification of the nitrogen nutritional status of the phytoplankton and the quantification of total water column nitrogen-based productivity. While techniques for the assessment of both of these quantities may

often be quite similar, they may be very sensitive to different biases depending on the time and space scale under investigation. Additionally, there are a number of methods not based on incubation techniques now being used to quantify various fluxes of allochthonous nitrogen on time and space scales which are not conveniently or accurately measured with tracers. In this review, emphasis will be placed on examining (1) current methods for determining primary productivity and nitrogen uptake and release, and their associated limitations on different time and space scales; (2) how data derived from short-term incubation experiments can be used to infer nitrogen nutritional status of the phytoplankton; and (3) the role of pelagic nitrogen remineralization—relative to allochthonous sources of nitrogen—in supplying nitrogen for phytoplankton uptake and production. While the perspective that is aimed for is one of balancing the time and space scales on which processes are measured, many comments and examples given in the chapter will inevitably reflect the author's own perspective.

1.2 METHODOLOGY

1.2.1 Tracer techniques

Our present knowledge of the rates of marine primary production and nitrogen uptake and cycling are largely based on the application of isotope tracer techniques. A rapid and widespread increase in the number of measurements made of primary productivity followed Steemann Nielsen's introduction in 1952 of the ^{14}C technique, and a similar increase in the numbers of measurements of nitrogen-based productivity followed the introduction of ^{15}N isotope techniques by Neess *et al.* in 1962.

The essence of the technique, as employed by most investigators, consists of first containing a sample in a glass or, preferably, polycarbonate bottle (Carpenter and Lively, 1980; Fitzwater *et al.*, 1982), adding the isotope tracer (either ^{14}C or ^{15}N), allowing the sample to incubate for a period ranging from several minutes to, in some cases, 24 or more hours, then collecting the particulate material by filtration and assaying for the amount of isotope incorporated into the particulate material during the course of the incubation. For ^{14}C, an alternative to filtration was suggested by Schindler *et al.* (1972), which involves acidification of an aliquot of the seawater sample, followed by bubbling to drive off the inorganic ^{14}C. The advantage of the acidification and bubbling technique relative to filtration is that the potential for loss of small phytoplankton and excretion products through the filter is avoided. However, an analogous methodology which eliminates the filtration step is not available for experiments involving ^{15}N tracers. For a more thorough treatment of the theory and inherent assumptions involved in the application of these isotope tracer techniques, the reader is referred to the comprehensive treatment by Sheppard (1962), and the review by Harrison (1983a). I will confine my comments here to several

methodological problems associated primarily with ^{15}N incubation studies, and how such problems may have biased our view of primary productivity and nitrogen cycling in oceanic waters. It appears that incubation techniques will continue to be the mainstay of our measurements of primary productivity and nitrogen cycling in the foreseeable future, and when applied with a number of cautions in mind, can provide valid and useful data. I will not deal with the recent tracer techniques which employ the stable isotope of carbon, ^{13}C (Slawyk *et al.*, 1977, 1979), or oxygen, ^{18}O (Grande *et al.*, 1984), nor the techniques employing analogues of NH_4^+ or NO_3^- (e.g. Wheeler, 1980; Balch, 1984), but many of the same concerns of incubation discussed below also apply to these techniques.

Venrick *et al.* (1977) first recognized that when phytoplankton samples were contained in incubation bottles for periods as short as 6 h, species composition changes often took place. Other technical problems were also recognized, including bottle size effects (Gieskes *et al.*, 1979), light response artifacts (Harris and Piccinin, 1977; Marra, 1978; Goldman and Dennett, 1984), and contamination from incubation containers, including trace metal toxicity (Carpenter and Lively, 1980; Fitzwater *et al.*, 1982). Indeed, Fitzwater *et al.* (1982) argued that trace metal effects could be so severe as to result in altered metabolism of many or most of the species, and they recommended a series of guidelines for collecting and incubating samples, which included such protocols as use of non-metal hydrowire and messengers, all plastic incubation ware, scrupulously cleaned, and metal-free ^{14}C stocks. In a subsequent comparison of the so-called 'standard' ^{14}C methodology with the clean-technique approach, as recommended by Fitzwater *et al.* (1982), no significant differences could be found in the rates of ^{14}C primary production at three stations near the island of Oahu, Hawaii (Marra and Heinemann, 1984). These latter findings, however, do not necessarily negate the need for clean techniques, as an investigator would not be able to ascertain prior to the execution of an experiment whether the organisms contained within the sample being measured were acutely sensitive to trace metals.

Another type of problem recently recognized as being associated with bottle incubations is that the rate at which label, whether it be ^{14}C or ^{15}N, is incorporated into particulate material may not necessarily remain constant with time. The time scales on which nonlinearities in the uptake of carbon or nitrogen can occur, range from the scale of minutes or less to hours or tens of hours; time course measurements are required to define whether such variations have occurred and their magnitude.

Observations of significant decreases in the maximum specific uptake rate of NH_4^+ over short incubation periods have been made for laboratory cultures (Conway and Harrison, 1977; Goldman and Glibert, 1982), and for natural phytoplankton populations (Glibert and Goldman, 1981; Wheeler *et al.*, 1982; Harrison, 1983b). The relative strength of this response for NH_4^+ has been noted to vary with the degree of nitrogen deficiency of the assemblage (cf. Eppley and Renger, 1974), although Goldman and Glibert (1982) noted that for some

laboratory-grown species the potential for enhanced NH_4^+ uptake was maintained even when growth rates were close to maximal.

Short-term (minutes) NH_4^+ uptake capability by phytoplankton has not been adequately determined for oligotrophic systems to date, as a serious analytical problem thwarts these measurements. That is, in order to obtain sufficient particulate material with which to satisfy the requirements of mass or emission spectrometers, one to several liters of seawater must be filtered, and the amount of time this takes at a vacuum that is sufficiently low to minimize cellular damage simply precludes any short-term measurements (McCarthy, 1980). While several investigators have attempted such measurements, the results are not conclusive, due either to the use of an emission spectrometer with inadequate resolution for the detection of the small quantities of isotope incorporated by phytoplankton in a few minutes time, or the incorrect subtraction of a time zero blank (i.e. the amount of uptake at the time the experiment is begun). The latter point, while seemingly trivial, has been the source of some confusion. The rationale for such a measurement is primarily to control for non-biological processes, such as adsorption, but since many phytoplankton species have the capability for rapidly utilizing NH_4^+, then from the instant an $^{15}NH_4^+$ tracer addition is made to an incubation vessel, they will begin to utilize it. Thus, if an investigator wishes to

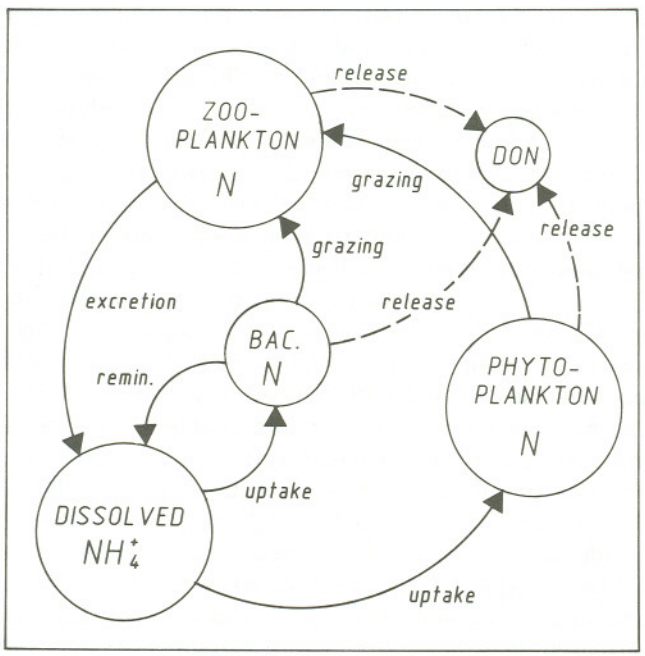

Figure 1.1. Schematic diagram of ammonium cycling between phytoplankton, bacteria and zooplankton

measure the tracer enrichment at time zero, it must be recognized that during the course of time that it takes to filter the sample, the uptake rates could change significantly. If it is, for example, several minutes before the time zero filtration is complete, the measured enrichment of the phytoplankton could be elevated relative to the true time zero value, and should that measured time zero value be subtracted from subsequent values along the time course, one could incorrectly conclude that rapid initial uptake did not occur. An alternative to time zero filtrations is the measurement of the $^{15}N/^{14}N$ ratio of a killed control; Glibert and Goldman (1981) have reported no elevation above background using this approach.

There are several possible causes of the nonlinearity in nitrogen uptake, in addition to the problems associated with incubation techniques previously mentioned, including depletion of the nitrogen substrate (Fisher *et al.*, 1981; Goldman *et al.*, 1981), as well as the progression towards isotopic equilibrium (Harrison, 1983a, b; Glibert *et al.*, 1985a). In studies of NH_4^+ uptake by natural phytoplankton, enrichment of the particulate nitrogen occurs concurrently with isotopic dilution of the NH_4^+ pool due to the regeneration of $^{14}NH_4^+$ by microzooplankton excretion and/or bacterial remineralization (Figure 1.1). Together these two processes lead to isotopic equilibration more rapidly than in systems in which one or the other process was occurring alone. In addition, increased ^{15}N enrichment of the phytoplankton leads to an increased probability of remineralization of the ^{15}N now contained in the particulate nitrogen fraction, reaching a maximum at isotopic equilibrium. The relative rates at which phytoplankton, bacteria and zooplankton accumulate ^{15}N are shown schematically in Figure 1.2. The ^{15}N label will accumulate first in the phytoplankton pool, then with a time lag representing the period during which phytoplankton become labelled, zooplankton will become labelled. The relative rate at which bacteria accumulate ^{15}N is still not well understood (shown as dashed line in Figure 1.2), and is complicated by the dual role of bacteria as consumers of NH_4^+ (Hattori and Wada, 1972; Laws *et al.*, 1985; Wheeler and Kirchman, 1986) and remineralizers (Fenchel and Harrison, 1976). The fundamental principles of isotope tracer work are compromised if a significant fraction of the tracer is remineralized back to the substrate pool. In field experimentation, attention must be paid to the time scale on which this might occur. Based on our current knowledge of potentially rapid recycling in at least certain systems, it is likely that isotopic equilibration might be attained within the time frame of a day-long incubation or less (Harrison, 1983a).

An additional source or error, specific to ^{15}N tracer experiments, concerns our inability to adequately measure the ambient concentrations of the inorganic forms of nitrogen in typical oligotrophic waters. While this problem may not be as formidable in many coastal situations, it remains quite clear that unless the ambient concentration of the substrate can be measured precisely, there is no way of accurately determining the rate of uptake of that substrate. The chemiluminescent technique for the determination of NO_3^- in seawater provides approxi-

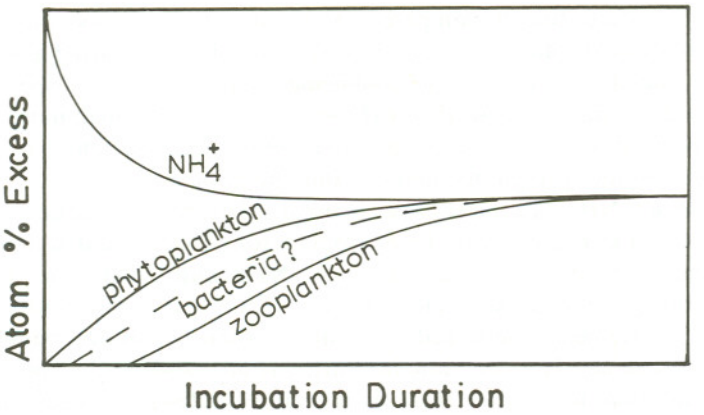

Figure 1.2. Schematic diagram of the time course changes in ^{15}N atom% excess in the dissolved NH_4^+ pool, and in the phytoplankton, bacterial, and zooplankton particulate pools. The shapes of the curves are intended to be relative only, showing the tendency towards isotope equilibration with time. The placement of the bacterial curve is speculative (see text)

mately an order of magnitude better precision than standard colorimetric methods (Garside, 1982), and there have been recent developments with regard to a comparably precise method for NH_4^+ (Brzezinski, 1987). The related problem for incubation experiments is that when the ambient concentration of substrate is low (i.e. at the detection limit), it is impossible to make an isotope addition that is simultaneously sufficiently large so as to provide a detectable signal, and at the same time sufficiently small so that the system is not perturbed. Dugdale and Goering (1967) suggested that additions of isotope which enriched the ambient by $\sim 10\%$ would not greatly perturb the system, but when the ambient is non-detectable (usually defined as $< 0.03\,\mu$mol/l for conventional methods), most investigators make additions that range from 0.03 to $0.10\,\mu$mol/l, which results in an incalculable enrichment lying between 50 and 100% (McCarthy, 1980).

In order to avoid several specific difficulties in the use of ^{15}N tracers for assessing nitrogen uptake, namely the potential perturbation of the sample upon tracer addition and the necessity of measuring uptake of all forms of nitrogen in determining total N uptake, DiTullio and Laws (1983) have proposed an alternative method to calculate N uptake based on the rate of ^{14}C uptake into cellular protein. The underlying assumption of the method is that both the N:C ratio of protein and the percentage of protein in nitrogen-limited cells remain fairly constant at ~ 0.3 by weight and $\sim 85\%$, respectively (DiTullio and Laws, 1983). This method does require the use of long (6–24 h) incubations, however. Additionally, the one data set in which ^{15}N uptake rates and ^{14}C uptake rates

into protein were directly compared for three stations (Laws *et al.*, 1985) is difficult to evaluate, due to the fact that recycling of ^{15}N occurred at one of the stations, and at another, bottle confinement artifacts were suspected from inadequate cooling of the incubator (Laws *et al.*, 1985). A much more rigorous comparison of this method is required before the usefulness of the ^{14}C incorporation into protein method can fully be evaluated.

The use of $^{15}NH_4^+$ as a tracer has also found applicability in the determination of the rates of nitrogen release by microplankton. This technique involves the measurement of the progressive dilution of $^{15}NH_4^+$ with $^{14}NH_4^+$ due to nitrogen regeneration by zooplankton and bacteria (Figure 1.1). Analogously, nitrification rates have been determined by monitoring the rate of isotope dilution of $^{15}NO_3^-$ (Koike and Hattori, 1978; McCarthy *et al.*, 1984). In the application of isotope dilution techniques there is a major trade-off between analytical resolution and potential perturbation of the sample, and various investigators have opted for different strategies. To optimize the analytical detection of ^{15}N isotope enrichment in the substrate pool, and to minimize the possibility of isotope depletion due to uptake during the course of an experiment, the isotope addition at the start of the experiment should be large relative to the ambient substrate pool. This has been the approach taken by Harrison (1978), Harrison *et al.*, (1983), Caperon *et al.* (1979), and others, in which the quantity of $^{15}NH_4^+$ added to the incubation vessel ranged from 1 to 10 μmol/l. On the other hand, if the rate of remineralization is relatively small, then a large isotope addition of $^{15}NH_4^+$ will not become diluted to a sufficient extent over the course of the incubation to be measured precisely. To overcome this problem, Glibert *et al.* (1982a) suggested use of a trace addition of $^{15}NH_4^+$ (~ 0.05 μmol/l or 10% of ambient). In this way, any remineralization of $^{14}NH_4^+$ will have a much larger impact on the isotope ratio. The trade-off, of course, is that the total amount of ^{15}N in both the dissolved and particulate fractions will be small when a trace isotope addition is made, so sensitivity of the isotope determination must be high. Additionally, the potential for nutrient depletion is greater, so incubation periods must be kept short.

The difference between these two approaches—use of near-saturating or trace enrichment levels—for the determination of remineralization rates will also influence the determination of uptake rates, as both processes occur simultaneously in any natural sample incubation. If a large (i.e. saturating) isotope addition is made, the degree to which the enrichment of the dissolved isotope becomes diluted over the course of the experiment will likely be relatively small, but the large addition may result in an overestimation of the *in situ* uptake rate, due to substrate saturation of uptake. If a small addition is made, the uptake rate would be perturbed to a lesser degree, but the rate of isotope dilution would be greater.

The degree of isotope dilution also directly affects the calculation of uptake. It is precisely this effect which necessitates that measurements of NH_4^+ uptake and

remineralization be made on the same experimental samples, rather than on parallel samples incubated for the same (or even more problematical) different periods of time. The original equation for the calculation of uptake as formulated by Dugdale and Goering (1967), using general principles established for tracer methodology (Sheppard, 1962) takes the form:

$$V = \frac{^{15}\text{N atom\% excess}}{^{15}\text{N atom\% enrichment} \times \text{incubation duration}} \tag{1}$$

where ^{15}N atom% excess is the ^{15}N atom% of the particulate fraction minus ^{15}N atom% normal (i.e. natural atmospheric enrichment of ^{15}N) and ^{15}N atom% enrichment is the initial enrichment of NH_4^+ in the medium. The dimensions of V reduce to reciprocal time (time^{-1}). Clearly, if isotope dilution due to remineralization is occurring over the course of the incubation, then the calculation of V must be corrected to account for the time-varying ^{15}N atom% enrichment in the denominator. Glibert *et al.* (1982a) suggested that regeneration of $^{14}NH_4^+$ will result in an exponential decrease in the ^{15}N atom% enrichment with time, and thus:

$$R_{(t)} = R_0[\exp(-kt)] \tag{2}$$

where $R_{(t)}$ and R_0 are the ^{15}N atom% enrichments at time t and zero, respectively, and k is solved for. Thus an exponential average between R_0 and $R_{(t)}$ can be calculated to yield $\bar{R}$:

$$\bar{R} = \frac{1}{t} R_0 \int_0^t [\exp(-kt)]\mathrm{dt} \tag{3}$$

and

$$\bar{R} = \frac{R_0}{kt}[1 - \exp(-kt)] \tag{4}$$

Then the corrected equation for the calculation of V becomes:

$$V = \frac{\text{atom\% excess}}{\bar{R} \times \text{incubation duration}} \tag{5}$$

Glibert *et al.* (1982a) also stressed that R_0 be measured directly, rather than calculated from the ^{15}N addition, as this value seriously influences the calculation of V when NH_4^+ is undetectable. These modifications together result in a significant improvement in the accuracy of V by as much as a factor of 2 in many cases (Glibert *et al.*, 1982a; Harrison and Harris, 1986). The impact of this correction to V, however, will be negligible under substrate-saturating conditions.

Laws (1984), in criticizing our technique for the calculation of $\bar{R}$, demonstrated mathematically that the dilution of R is more rapid than exponential when the rate of uptake is less than that of regeneration, and less rapid than exponential

when the rate of uptake is greater than regeneration during an experiment. While his alternative approach for calculating $\bar{R}$ is analytically correct, the effect on the correction to V in comparison to the approach taken by Glibert *et al.* (1982a) is quite small, a few percent in most cases (Glibert *et al.*, 1985a). The magnitude of the isotope dilution correction to V does depend on the change in R over the period of the experiment, which in turn depends on the amount of $^{15}NH_4^+$ addition. The correction to V does not depend on whether or not the ambient concentration of NH_4^+ changes during an experiment.

The methods for the calculation of remineralization rates from isotope dilution experiments have now been fairly well reviewed by Blackburn (1979), Caperon *et al.* (1979), Glibert *et al.* (1982a) and Harrison (1983a). In brief, they are based upon the simultaneous solution of the equations representing the change in ambient NH_4^+ concentration and the change in ^{15}N atom% enrichment:

$$P_{(t)} = P_0 + (d - i)t \tag{6}$$

and

$$\ln [R_{(t)} - {}^{15}n] = \ln (R_0 - {}^{15}n) - \left[\frac{d}{d-i}\right]\left[\ln \frac{P_{(t)}}{P_0}\right] \tag{7}$$

where $P_{(t)}$ and P_0 are the ambient NH_4^+ concentrations at time t and time zero, ^{15}n is the normal atmospheric enrichment of ^{15}N ($\sim 0.365\%$), i (for incorporation) is the uptake rate, and d (for dilution) is the remineralization rate. Glibert *et al.* (1982a) noted that, in a typical $^{15}NH_4^+$ remineralization experiment, one also obtains an uptake value based on the ^{15}N incorporation into the particulate material. The fact that this latter approach does not yield identical values of uptake as does the calculation of i above has been the source of some controversy (Laws, 1984; Glibert *et al.*, 1985a). If, on the one hand, there is loss of $^{15}NH_4^+$ from the system by processes such as adsorption or nitrification, then the correct approach for calculation of uptake must be ^{15}N accumulation into the particulates—the loss of $^{15}NH_4^+$ to other pathways is inconsequential to the calculation of uptake, but would be included as 'uptake' in the calculation of i (equations 6 and 7). If, on the other hand, a large fraction of ^{15}N is lost from the microplankton by processes subsequent to uptake, then the ^{15}N uptake method would underestimate total uptake. Resolution of this uncertainty awaits more extensive application of improved methods for measuring the ambient concentration of NH_4^+ and the execution of experiments under controlled laboratory conditions in which the flux of ^{15}N between phytoplankton, microzooplankton, and the dissolved NH_4^+ pool can be independently quantified.

1.2.2 Other techniques

Phytoplankton production in general appears to be less dependent on sources of new nitrogen as one moves from coastal regimes to oceanic regimes (Dugdale and Goering, 1967; Eppley *et al.*, 1973; Dugdale, 1976; Eppley and Peterson,

1979; Paasche, this volume). Estimates of the degree to which new nitrogen supports production in oceanic waters have been based primarily on comparisons of utilization rates of NH_4^+ and NO_3^- substrates in tracer incubation experiments. If we assume that the contribution of N_2 fixation to the 'new' nitrogen term is small (but see Martinez *et al.*, 1983), then uptake of NO_3^- by phytoplankton must be equaled by the upward flux of NO_3^- through the nitracline when steady-state biomass levels prevail. This flux in turn must equal, at steady state, the quantity of nitrogen that is exported from the euphotic zone via the passive sinking of particulate matter (Eppley and Peterson, 1979; Eppley *et al.*, 1983; Betzer *et al.*, 1984). However, precise and direct estimates of the flux of NO_3^- from deep waters to euphotic zone waters have been extremely difficult to obtain, yet they are important complementary measurements to those made by traditional incubation-tracer techniques.

Estimates of NO_3^- fluxes have been made from the vertical eddy diffusivity term (K_z) and the gradient of NO_3^- at the base of the euphotic zone ($\partial NO_3^-/\partial z$):

$$\text{nitrate flux} = K_z \frac{\partial [NO_3^-]}{\partial z} \tag{8}$$

Neither of these terms is known very precisely, however (Platt *et al.*, 1984). King and Devol (1979) and Eppley *et al.* (1979) estimated vertical coefficients for NO_3^- in the eastern tropical Pacific, and southern California coastal waters, respectively, from $^{15}NO_3^-$ uptake rates and the NO_3^- gradient at the top of the nitracline. This approach requires good resolution of sampling in the vertical for both $^{15}NO_3^-$ uptake and NO_3^- concentration, a factor limiting its application to many available data sets. Additionally, vertical transport of NO_3^- may be equaled or exceeded by horizontal fluxes, which are much more difficult to measure.

Another approach to the estimation of the contribution of new nitrogen to total production is based on the natural discrimination of ^{15}N relative to ^{14}N, caused primarily by differences in the kinetic behavior of the isotopes. This approach involves direct measurement of the natural abundance of ^{15}N in particulate organic nitrogen (PON). By convention, the natural abundance of ^{15}N in PON is reported as:

$$\delta^{15}\text{N per mil} = \left[\frac{^{15}N/^{14}N \text{ (sample)}}{^{15}N/^{14}N \text{ (standard)}} \right] \cdot 1000 \tag{9}$$

where the standard is atmospheric N_2, the $^{15}N/^{14}N$ ratio of which is assumed to stay constant (Sweeney *et al.*, 1978; Mariotti, 1983). Values ranging from +3 to +12 are typical for PON, whether collected by bottle or by net (Wada and Hattori, 1976; Sweeney and Kaplan, 1980; Saino and Hattori, 1980; Altabet and McCarthy, 1985). The obvious advantage of this approach is the elimination of the incubation step, with all its associated problems. One disadvantage of this approach, however, is that few laboratories are currently equipped with mass spectrometers with the required precision and sensitivity.

The degree of discrimination of ^{15}N relative to ^{14}N observed in particulate material is dependent on many factors, including species composition, growth rate (Wada and Hattori, 1978), and the cellular metabolic pathway or trophic level under investigation. For example, many primary producers tend to assimilate the lighter isotope of nitrogen (^{14}N) faster than the heavier isotope (^{15}N) when growing on NO_3^- (Bigeleisen and Wolfsberg, 1958; Wada and Hattori, 1978), and different kinetic discrimination effects have been shown to be associated with the active transport of NO_3^- across a cell membrane and the subsequent reduction of NO_3^- to NO_2^- (Miyazaki *et al.*, 1980). Also, the enrichment of ^{15}N in particulate material tends to increase with increasing trophic level. Additionally, isotopic fractionation may occur during oxidative degradation of particulate nitrogen (Saino and Hattori, 1980; Altabet and McCarthy, 1986). Isotope discrimination during uptake and degradation are the likely processes resulting in observed patterns of ^{15}N natural abundance with depth in the northeastern Indian Ocean, the Sargasso Sea and warm core rings; these patterns typically show lesser values above the nitracline than below, due to uptake of NO_3^- by phytoplankton at the base of the euphotic zone and degradation of PON below the euphotic zone (Saino and Hattori, 1980; Altabet and McCarthy, 1986).

One important aspect of the analysis of $\delta^{15}N$ in oceanic waters is that, due to practical constraints, one can only resolve the $\delta^{15}N$ of the total PON pool, and not the individual functional groups (i.e. phytoplankton, microzooplankton, detritus), due to the fact that these groups cannot be separated with conventional filtration techniques (Altabet and McCarthy, 1985). Larger macrozooplankton have, however, been analyzed independently from the microbial and detrital fractions (Mullin *et al.*, 1984). Thus, while fluxes of nitrogen to and from particulate intermediates may, in fact, have a discrimination associated with them, the analysis of the $\delta^{15}N$ of the total PON pool will not reflect this. Overall, this technique has been shown to be very useful in the analysis of vertical and horizontal patterns in $\delta^{15}N$, and thus in the transport of nitrogen into and out of the euphotic zone (Altabet and McCarthy, 1985, 1986). This technique also holds considerable promise for distinguishing allochthonous versus autochthonous sources of nitrogen in near-shore waters, due to the different nitrogen isotope composition of terrestrial inputs versus marine (Sweeney and Kaplan, 1980; Altabet and McCarthy, 1985). However, few such data are currently available for coastal or near-shore environments. Owens (1985) has recently presented data on $\delta^{15}N$ from the Tamar river estuary, and has described the factors which must be taken into account in interpreting $\delta^{15}N$ data from such a system.

Other approaches which have been brought to bear in the estimation of oceanic new production involve measurements of seasonal oxygen accumulation and utilization rates (Shulenberger and Reid, 1981; Jenkins, 1982; Jenkins and Goldman, 1985). These results represent longer time (months to decades) and space (hundreds of kilometers) averages than the techniques described above.

These techniques, however, are unlikely to have near-shore applications, and will not be discussed here.

1.3 NUTRITIONAL STATUS OF PHYTOPLANKTON

As stated above, the two general motivations for examining the uptake of nitrogen are (1) to establish the degree to which phytoplankton may be nutritionally stressed and (2) to estimate the integrated rates of nitrogen utilization. Sampling strategies and biases can differ in the two approaches, and care should be exercised in extrapolating data collected for one purpose to an interpretation of the second. This section describes some of the approaches that have been used for defining the nutritional status of an assemblage, relative to its nitrogen requirement, and the time scales on which they are appropriate.

There have been numerous attempts to define convenient indices of cellular nutritional status (Morris *et al.*, 1971a,b; Vincent, 1981; Dortch *et al.*, 1983, 1985; Glibert and McCarthy, 1984). The usefulness of these indices in a predictive fashion is often complicated due to the interaction of environmental effects, such as the prevailing light regime. Yet, from the point of view of describing the near-instantaneous physiological or nutritional status of an assemblage there are several approaches which appear promising. Glibert and McCarthy (1984) have advocated simultaneous measurement of several of these indices before any conclusions with respect to degree of nitrogen limitation are drawn, in order to minimize the biases associated with any one particular approach.

In one such approach the hyperbolic relationship between NH_4^+ uptake rate and substrate concentration, and the change in this relationship as a function of time, has been shown to be correlated with nutritional state (Wheeler *et al.*, 1982; Goldman and Glibert, 1983). For nutrient-stressed populations the magnitude of the ratio of NH_4^+ uptake rates at high (substrate-saturating) and low (at the limit of detection for wet chemistry) concentrations will increase significantly over the course of several hours, but a much smaller change will be observed for nutrient-replete phytoplankton. In practice, experimental errors make it difficult to establish precisely a value of V_{sat}/V_{trace} which indicates a deficient status, but values exceeding 5 have been found from clearly nitrogen-deficient regions (Glibert and McCarthy, 1984). This technique has been used in numerous field experiments covering the Caribbean Sea, the Sargasso Sea, Chesapeake Bay, and Gulf Stream warm core rings (Glibert and McCarthy, 1984; McCarthy and Nevins, 1986).

Another approach involves following the short-term course of incorporation of NH_4^+ and NO_3^- into protein (i.e. trichloroacetic acid (TCA) insoluble material). Although the function of nitrogen uptake by phytoplankton is the synthesis of new cellular material, the change in the composition with time of the newly synthesized material has been shown to reflect the nutritional state of the cells, and the molecular form of available nitrogen. Wheeler *et al.* (1982) observed that,

for a nitrogen-poor diatom assemblage in Chesapeake Bay, within minutes $> 80\%$ of the added $^{15}NH_4^+$ enrichment appeared in the protein fraction, but similar experiments in nutrient-replete waters revealed a much smaller percentage of the newly assimilated nitrogen in the form of the protein, even after a period of several hours.

The usefulness of the above indices depends on keeping incubation periods as short as possible. It is only with short incubation periods that one can assess the significance of membrane transport and initial assimilatory processes to nitrogen uptake capacity, and therefore nutritional status. But uptake rates determined over the course of minutes cannot be related to the total ambient water column uptake rates, nor to rates of phytoplankton growth. Thus, choice of an optimal incubation period requires careful consideration.

One approach for assessing nutritional status that does not depend on short-term measurements was suggested by Eppley and Peterson (1979) and Eppley (1981b), and involves the ratio of NO_3^- uptake to total nitrogen uptake (the '*f*' ratio). The application of this index to questions relating to phytoplankton nutritional status is based on the observation that, whereas the more reduced forms of nitrogen are typically the preferred forms for phytoplankton utilization (Harvey, 1940; Syrett, 1954; Morris and Syrett, 1963; Syrett and Morris, 1963; Dugdale and Goering, 1967; Eppley *et al.*, 1973; McCarthy *et al.*, 1977, 1982; Glibert *et al.*, 1982b), simultaneous utilization of NH_4^+ and NO_3^- may occur when insufficient NH_4^+ is available to meet the entire phytoplankton requirement for nitrogen. Thus, in conjunction with other supporting evidence for nitrogen limitation of an assemblage, a high *f* value could lend credence to such a conclusion. Regional comparisons of *f* ratios, as well as some additional problems associated with their measurement, are presented by Paasche (this volume).

Dortch *et al.* (1985) have provided evidence that the biochemical composition of phytoplankton populations may reflect their nutritional status. Indicators such as intracellular amino acid/protein, protein/DNA, RNA/DNA ratios, intracellular NO_3^- concentrations and NO_3^- reductase activities were used diagnostically in determining the degree of nutritional stress, as well as the degree to which NO_3^- served as a nitrogen source for growth for phytoplankton of Dabob Bay, Washington. Again, no single index provided data which were sufficient to draw generalizations concerning physiological or nutritional status (Dortch *et al.*, 1985), but when used together provide the investigator with yet another approach to addressing these important questions.

The application of these indices of nutritional status has thus provided considerably more insight into the nutritional status of natural phytoplankton assemblages than would be obtained by simply determining the nutrient concentrations and *in situ* rates of uptake. Yet, as Paasche (this volume) well summarizes, large-scale comparisons remain difficult due to experimental differ-

ences between different studies, as well as the species-specific and environmental effects discussed above.

1.4 PELAGIC UPTAKE AND MINERALIZATION OF NITROGEN

1.4.1 Rates of ammonium regeneration relative to uptake

Eppley (1981b) has proposed an analogous index to the *f* ratio for the assessment of nitrogen recycling based on ^{15}N uptake data: the ratio of regenerated nitrogen production to total nitrogen production. He further proposed that this index can be thought of as an index of grazing pressure on phytoplankton, assuming no allochthonous sources of NH_4^+ to the system in question. From an experimental point of view the contribution of urea is usually considered to be small. Inasmuch as this relationship is an index of how efficiently nitrogen is processed in a given system, any changes in this relationship with time might be expected to be correlated with changes in the contribution of various size classes of heterotrophs to the nutrition of the phytoplankton (i.e. regeneration). Direct comparisons in the field of rates of NH_4^+ remineralization by a range of size classes in concert with other measurements of size classes of zooplankton biomass and their grazing rate on phytoplankton, and bacterial abundance and activity are few. These measurements do constitute one of the primary objectives of the NSF-funded multi-investigator program 'Microbial Exchanges and Couplings in Coastal Atlantic Systems' (MECCAS) focusing on size- and time-dependent trophic relationships in the plume of Chesapeake Bay.

Most direct field measurements of NH_4^+ remineralization have been based on ^{15}N isotope dilution methodology. In general, it has been found that assimilative and regenerative fluxes are often in balance, and that microplankton of the size class $< 150\,\mu m$ are responsible for the bulk of the nitrogen regenerated in a wide range of water types (Harrison, 1978; Caperon *et al.*, 1979; Axler *et al.*, 1981; Glibert, 1982; Paasche and Kristiansen, 1982; Cochlan, 1986). In measurements of this type it is difficult, except on a size class basis, to determine the specific organisms that account for the nitrogen regeneration. Recent culture experiments involving a variety of techniques, including inhibitors of prokaryotic and eukaryotic activity, indicate that protozoans, rather than bacteria, are responsible for a major portion of this regeneration (Sherr *et al.*, 1983; Goldman *et al.*, 1985; Wheeler and Kirchman, 1986). This generalization will not hold true for all waters at all times of the year, and will depend on the nutritional and physiological state of the prokaryotes and eukaryotes.

One study (Harrison *et al.*, 1983) has attempted a more thorough mass balance of nitrogen fluxes, and a determination of the relative contributions of zooplankton, microplankton, and benthos to NH_4^+ regeneration in the Middle Atlantic Bight during summer. They found that 50–80% of the nitrogen

productivity (as the sum of NH_4^+ and NO_3^- uptake) was attributable to NH_4^+ regeneration, of which 30% was from macrozooplankton excretion, 63% from microzooplankton and bacterial regeneration, and 7% to benthic flux (Figure 1.3). These results lend further support to the hypothesis presented by Walsh *et al.* (1978) that a balance is maintained between nitrogen utilization, recycling, and export on the Atlantic Shelf at most times of the year.

The time scales over which such size- and time-dependent relationship are assessed will affect not only our view as to the degree of dependence on recycled nitrogen, but also the degree to which the appearance of steady state is maintained. Generalizations in this regard are made even more difficult by the fact that such few direct measurements of NH_4^+ remineralization have been made on regional bases, and even fewer time series or seasonal data are available. For example, during coastal phytoplankton blooms, which can often develop and dissipate quickly and episodically, bacteria and microzooplankton may respond on an equally rapid basis, maintaining a fairly tight coupling between uptake and remineralization of NH_4^+. On the other hand, macrozooplankton production

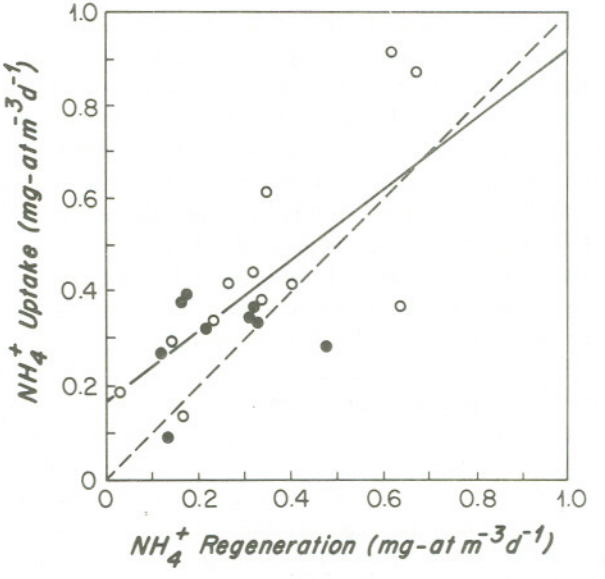

Figure 1.3. Relationship between NH_4^+ uptake and regeneration rates for samples collected in the Middle Atlantic Bight. Open circles are whole samples, closed circles are size-fractioned ($< 1\ \mu m$). Dotted line denotes 1:1 correspondence, solid line is least-squares linear regression for whole samples. Note that the uptake and regeneration rates were determined from parallel, rather than the same, incubation flasks. (From Harrison *et al.*, 1983.)

may lag behind phytoplankton production, rendering the importance of macrozooplankton excretion in phytoplankton nutrition more seasonally variable. Such time-dependent relationships have been inferred for the plume of the Hudson River, where, during February through April, large diatom blooms develop and dissipate frequently due to wind or storm events (Walsh *et al.*, 1978; Walsh, 1981; Malone *et al.*, 1983a), and macrozooplankton grazing and excretion remain low, whereas during the summer months phytoplankton biomass typically remains at much more constant levels, and macrozooplankton assume a larger role in nitrogen cycling (Malone and Chervin, 1979; Chervin *et al.*, 1981; Malone *et al.*, 1983b). Thus there appear to be strong seasonal differences in the correlation between size-dependent uptake and size-dependent remineralization, at least for coastal systems. Direct measurements of size-dependent nitrogen uptake and regeneration, in concert with measurements of both zooplankton and bacterial biomass and activity, are currently being made in the plume of the Chesapeake Bay estuary, and should allow us to assess in more detail than has previously been possible the time scales of trophic couplings and their response upon perturbation.

1.4.2 Rates of supply of 'new' versus 'regenerated' nitrogen

Malone (1980) suggested that often in coastal systems a size dependency of the relative utilization of new versus regenerated nitrogen could be found. He observed that netplankton ($> 20\,\mu$m) productivity was regulated by allochthonous NO_3^- influx, whereas nanoplankton ($< 20\,\mu$m) productivity was controlled by autochthonous NH_4^+ availability. The seasonal pattern of nitrogen utilization, on a size-class basis, that we found in Vineyard Sound, Massachusetts (Glibert *et al.*, 1982c) added credence to Malone's conclusion. Support for this hypothesis has also been provided for the southern Benguela upwelling system (Probyn, 1985). Malone based this conclusion not on a physiological basis, but rather on the observation that netplankton diatoms tend to bloom when environmental conditions, such as mixing or upwelling events, enhance the flux of NO_3^- into a system. However, the degree of phytoplankton dependence on allochthonous or autochthonous nitrogen depends not only on the availability of a particular nitrogen substrate, but also on the degree to which phytoplankton may discriminate between various forms of nitrogen available, and on the physiological or nutritional status of the cells.

In the oceanic realm it has been well demonstrated that phytoplankton depend almost exclusively on regenerated nitrogen for their nutrition (Dugdale, 1976; Eppley and Peterson, 1979; Glibert and McCarthy, 1984). Eppley and Peterson (1979), for example, estimate only a 10–20% contribution of NO_3^- to phytoplankton productivity, and, at least at certain times of year, this proportion may be less than 10%. It has been suggested (Horrigan and McCarthy, 1982; Dortch, 1982) that physiological differences in the strategy used by phyto-

plankton for the uptake of NH_4^+ versus NO_3^- may be related to temporal scales on which the two nitrogen substrates vary. Evidence for enhanced NH_4^+ uptake has been found numerous times (e.g. McCarthy and Goldman, 1979; Glibert and Goldman, 1981; Horrigan and McCarthy, 1982; Goldman and Glibert, 1982; Harrison, 1983b), yet a similar capacity for enhanced NO_3^- uptake is debatable (Horrigan and McCarthy, 1982; Dortch, 1982). Since NH_4^+ is typically supplied by local zooplankton or bacterial remineralization, whereas NO_3^- is supplied by physically driven transport, either via turbulent flux across the nitracline gradient or episodic pulses from water column mixing, there would be much less advantage for an enhanced uptake capacity of NO_3^- relative to that for NH_4^+. Energetic constraints would also contribute to differences observed in the strategy of uptake of these two substrates.

Whereas an extremely tight coupling of NH_4^+ uptake and remineralization has been noted for oceanic systems, the small percentage that NO_3^- contributes to

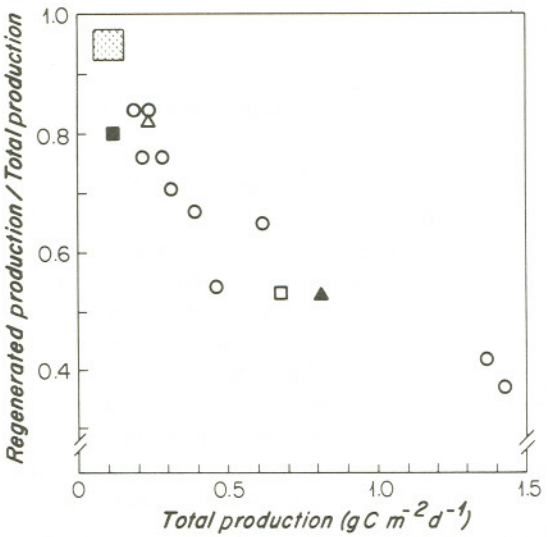

Figure 1.4. The ratio regenerated production/total production as a function of total production. Open circles are individual offshore stations in the Southern California Bight, the hatched rectangle represents several stations in the central North Pacific, solid square symbol is the Caribbean data of Carpenter and Price (1977), open square is offshore Monterey Bay (G. Knauer and R. Eppley, unpublished data). Other data are averages for several stations from MacIsaac and Dugdale (1972) and Dugdale (1976) as follows: solid triangle is eastern tropical Pacific; open triangle is eastern Mediterranean Sea. (From Eppley, 1981b.)

phytoplankton productivity is not only quite variable regionally and seasonally, but is of profound importance in balancing the marine nitrogen cycle. Eppley (1981b), in summarizing available data from ^{15}N uptake studies, demonstrated that the proportion of regenerated production to total production varies inversely with total production in terms of carbon (Figure 1.4). Evidence for regional variation in the uptake of NH_4^+, NO_3^-, and urea for the Pacific has been recently given by Kanda *et al.* (1985), in which they show, for fairly vast coverage of the North Pacific, that differences in utilization of the nitrogen substrates correlate with temperature and possibly light, but not with differences in nitrogen availability. Other examples of regional variability in the uptake rates of nitrogenous nutrients are easily found. Studies conducted as part of the Warm Core Ring Program have revealed that the utilization of different nitrogen forms within Gulf Stream warm core rings varies widely both with ambient nitrogen concentrations and physical events, and in a manner which differs from variations in nitrogen utilization in the Sargasso Sea (McCarthy and Nevins, 1986; Nelson *et al.*, 1984). For example, *f* ratios of ~ 0.70 have been reported for a warm core ring during fall (McCarthy and Nevins, 1986); values of this magnitude have never been reported for oligotrophic oceanic waters. Such high *f* values have been reported for the Antarctic Scotia Sea waters (Olson, 1980) for austral late-winter, where all-year NO_3^- concentrations of $\sim 20\,\mu$mol/l are found. Clearly, better regional data with respect to variability in uptake are required before the impact of varying environmental conditions is fully understood.

At the base of the euphotic zone, where turbulent mixing results in potentially large but episodic inputs of NO_3^- (Klein and Coste, 1984), phytoplankton may use proportionately more NO_3^- for their nutrition than phytoplankton residing closer to the surface (Dugdale and Goering, 1967). Unfortunately, estimates of the vertical eddy diffusive flux of NO_3^- remain imprecise (McCarthy and Carpenter, 1983; Platt *et al.*, 1984). Patterns in the ^{15}N natural abundance of PON have shown that not only are phytoplankton relatively non-nitrogen-deficient at the base of the euphotic zone, but that high concentrations of PON at this depth reflect active biological material (Altabet and McCarthy, 1986). The co-occurrence of maxima in PON and minima in $\delta^{15}N$ is consistent with an important contribution of NO_3^- to phytoplankton uptake in this region. The $\delta^{15}N$ minimum results from isotopic fractionation during uptake and assimilation of NO_3^- by phytoplankton near the top of the nitracline (Altabet and McCarthy, 1986).

Seasonally, it has been shown for the Sargasso Sea that the variability in the flux of particulate matter from the euphotic zone to the deep sea correlates with the seasonal pattern in primary productivity (Deuser and Ross, 1980). This relationship was based on a comparison of contemporary particle flux measurements with primary productivity data of nearly 20 years ago (Menzel and Ryther, 1960, 1961). This variability in particulate flux must be balanced by an equal and opposite flux of NO_3^-. The mechanism for this, on the seasonal scale, is winter

destratification of the water column. Altabet and Deuser (1985) have recently demonstrated that the seasonal changes in $\delta^{15}N$ of sediment-trap-collected organic material from the Sargasso Sea is correlated with the seasonal differences in total flux of particulate organic carbon out of the euphotic zone. This provides a long-term record of change in the contribution of NO_3^- to PON (Figure 1.5).

Seasonal shifts in the contribution of NO_3^- to phytoplankton utilization have also been documented in the Scotia Sea, where despite high ambient concentrations of NO_3^- year-round, during the austral summer there is a greater dependence on regenerated NH_4^+, whereas during the winter–spring there is a greater dependence on NO_3^- (Olson, 1980; Koike *et al.*, 1981; Glibert *et al.*, 1982b; Rönner *et al.*, 1983). The seasonal shift in *f* values coincides with a spring-to-summer increase in average concentration of NH_4^+ in the photic zone (Biggs and Bidigare, 1981; Glibert *et al.*, 1982b). At least part of this increase in ambient NH_4^+ may be due to the melting of pack ice during the summer (Biggs, 1978), but, as noted by Probyn and Painting (1985), NH_4^+ supplied by melting ice is a new rather than regenerated source of N. The same frustration in the interpretation of *f* ratios exists in many coastal data sets as well (see Paasche, this volume). A record of $\delta^{15}N$ for coastal regimes could substantially add to our understanding of dependence on allochthonous versus autochthonous sources of nitrogen.

Is it possible, then, to reconcile data demonstrating that oceanic phytoplankton nitrogen requirements are being met almost exclusively by nitrogen regenerated by microplankton with a known variability in the contribution of new nitrogen on regional, vertical, and seasonal scales? Several factors must be considered. First, from a very simplistic perspective, oligotrophic and coastal

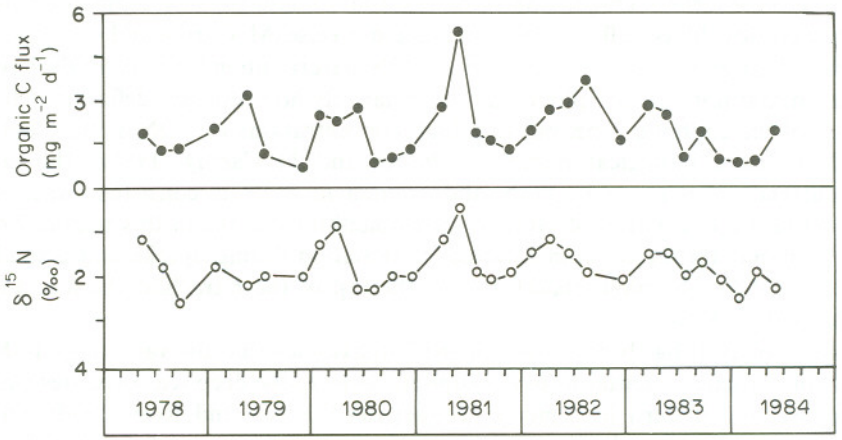

Figure 1.5. The 6-year record for organic carbon flux and the $\delta^{15}N$ of sinking particles at 3200 m depth in the Sargasso Sea. Each time point represents an integration over the previous 2 months. $\delta^{15}N$ values have been plotted on an inverse scale. (From Altabet and Deuser, 1985.)

waters world-wide have been severely undersampled, regionally and seasonally, with respect to nitrogen-based productivity. Also, due to the time-consuming nature of ^{15}N analyses relative to ^{14}C determinations, resolution with depth is poor for most studies. Secondly, the tendency (until very recently) for use of long-term incubations in the majority of ^{15}N studies in oceanic waters, with all of their associated problems, may have led to underestimates of rates of nitrogen utilization overall. A related problem is that where total daily and/or water column rates of uptake have been estimated from less than day-long incubations, there have been inconsistencies in the treatment of dark nitrogen uptake, which can account for as much as 50% of the total daily rate of uptake (Fisher *et al.*, 1982; Lipschultz *et al.*, 1985). Thirdly, direct measurements of nitrogen regeneration by microplankton—for the few areas for which such data are available—are based exclusively on short-term incubations, whereas, in contrast, estimates as to the relative contribution of new nitrogen to phytoplankton production for oceanic waters are based on a number of methods, several of which, including $\delta^{15}N$, are more appropriate for the estimation of fluxes on time scales approximating those of the residence time of the particulate nitrogen. In an incubation experiment, sources of new nitrogen are eliminated so, *a priori*, the uptake rates will not reflect this input term. Finally, the errors associated with each of these types of measurements alone (see Section 1.2) could result in many of these discrepancies. For example, errors in NH_4^+ remineralization measurements increase with decreasing resolution of the ambient concentration of NH_4^+, but the types and magnitudes of these errors are not fully known (Laws, 1984; Glibert *et al.*, 1985a). The resolution of these uncertainties is important for all of the field estimates of nitrogen utilization and regeneration, for our understanding of the dependence by phytoplankton on new versus regenerated nitrogen, and ultimately for our ability to balance the flux of nitrogen in the oceanic realm.

1.5 CONCLUSIONS

In summary, this review has attempted to provide a synthesis of currently available methods for directly assessing nitrogen flux through the water column and through microplankton, and a perspective on the limitations of time and space scales which can be adequately resolved by those methods. Extrapolations from short-term incubation measurements may not necessarily compare with long-term flux estimates. Yet we need not totally abandon short-term incubation procedures, in spite of the many recognized problems, as these data have provided us a means of determining physiological status and short-term cycling that other methods simply cannot provide.

The view that marine primary productivity is limited by nitrogen is far too simplistic. In an evolving field such as this, improvements in methodology, including the recognition of potential artifacts and errors, and the discovery of

new and promising relationships for defining nutritional status, have changed our perspective of nitrogen limitation. From the microscale, or short-term perspective, even phytoplankton residing in apparently nutrient-poor oceanic regimes appear to be deriving sufficient nitrogen from rapid turnover processes to meet their nutritional needs. We are far from a complete understanding of microscale processes, and two areas deserving of further effort include microscale food webs (Goldman, 1984) and the mechanisms of individual subcellular processes involved in uptake and metabolism of nitrogen (Wheeler, 1983).

Additional data are also needed on the diel scale. The degree of variability in photosynthesis, nitrogen uptake and release, and their interrelations on the diel scale for microplankton of different size and nutritional classes must be identified. This includes a better understanding of the role of dark uptake processes, as well as control by endogenous and exogenous periodicity. This information is vital to a general understanding of environmental regulation of, and variability in, primary productivity.

Finally, regional and seasonal patterns in allochthonous versus autochthonous nitrogen uptake by organisms of various size classes and taxa need to be more fully resolved. This may require a more complete accounting of the utilization and regeneration of what are now considered minor nitrogen forms, such as dissolved organic nitrogen, including urea and amino acids (Paul, 1983; Jackson and Williams, 1985). Additionally, collaboration between phytoplankton ecologists and geochemists will provide both new and complementary approaches (Altabet and McCarthy, 1985, 1986; Jenkins, 1982; Jenkins and Goldman, 1985) to the study of global nitrogen cycling.

ACKNOWLEDGEMENTS

I wish to thanks Drs E. Paasche, R. Eppley, T. H. Blackburn, J. McCarthy, M. Altabet and T. Kana for their helpful criticisms of this review. I am also grateful to Drs M. Altabet, F. Lipschultz and W. G. Harrison for access to unpublished materials.

Support for the preparation of this chapter was provided by Grant OCE-84-09465 from the National Science Foundation. This is contribution no. 5951 from the Woods Hole Oceanographic Institution.

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