UPTAKE OF NEW AND REGENERATED FORMS OF NITROGEN IN PRIMARY PRODUCTIVITY[1]

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ABSTRACT

The use of ^{15}N-labeled compounds to obtain specific uptake rates for the various nitrogen sources available to the phytoplankton makes it possible to separate the fractions of primary productivity corresponding to new and regenerated nitrogen in the euphotic zone of the ocean. Measurements of nitrate uptake as a fraction of ammonia plus nitrate uptake have been obtained from the northwest Atlantic and the northeast Pacific oceans. Mean values range from 8.3 to 39.5%, the former being characteristic of subtropical regions and the latter of northern temperate regions or coastal and inland waters.

Nitrogen fixation is also a source of new nitrogen. Rates of nitrogen fixation are found to be as high or higher than nitrate uptake, in some cases suggesting an important role for nitrogen-fixing phytoplankton.

The role of zooplankton in regenerating nitrogen as ammonia in the Sargasso Sea is examined theoretically. Probably only about 10% of the daily ammonia uptake by phytoplankton is contributed by the zooplankton living in the upper 100 m.

INTRODUCTION

Marine primary production can be measured by a number of techniques, the ^{14}C method of Steemann Nielsen (1952) being perhaps the most popular due to its great sensitivity. However, measurements of primary production alone are not enough to assess the capacity of a region to support production at higher levels in the food chain. The reasons for this were considered by Riley (1963) and are easily grasped by considering the path of a major nutrient element. A simplified cycle of nitrogen in the euphotic zone is shown in Fig. 1, where it can be seen that the nitrogen available to the phytoplankton is in one of two categories: 1) newly incorporated nitrogen as NO_3^--N, or N_2, and 2) recycled nitrogen in the form of NH_4^+-N or dissolved organic-N. Clearly, the rate of export of organic nitrogen from the production system cannot exceed the rate at which new nitrogen becomes available to replace it if the phytoplankton is to maintain itself.

This kind of analysis can be made for any major element contained in the phytoplankton; however, nitrogen is a logical choice since it is a major structural component of cells and is reasonably constant in its ratio to carbon and phosphorus. Measurements of population growth using nitrogen may, in fact, show less scatter than would those using carbon or phosphorus, because the latter two elements are not only structural components but are continuously turned over in the energetic processes of organisms. Theoretically, nitrogen should provide an inherently satisfactory and fundamental measure of production in ecosystems. Nitrogen is also often thought to limit production in the sea in the Liebig sense, another reason for studying its flow through the marine ecosystem.

We propose for the time being to call the primary production associated with ammonia (regenerated nitrogen) "regenerated production" and all primary production associated with newly available nitrogen, for example, NO_3-N and N_2-N, "new production."

The use of ^{15}N-labeled compounds makes it possible to measure the fractions of new nitrogen and regenerated nitrogen associated with the primary production in the sea. Preliminary data suitable for analysis along these lines have been obtained from
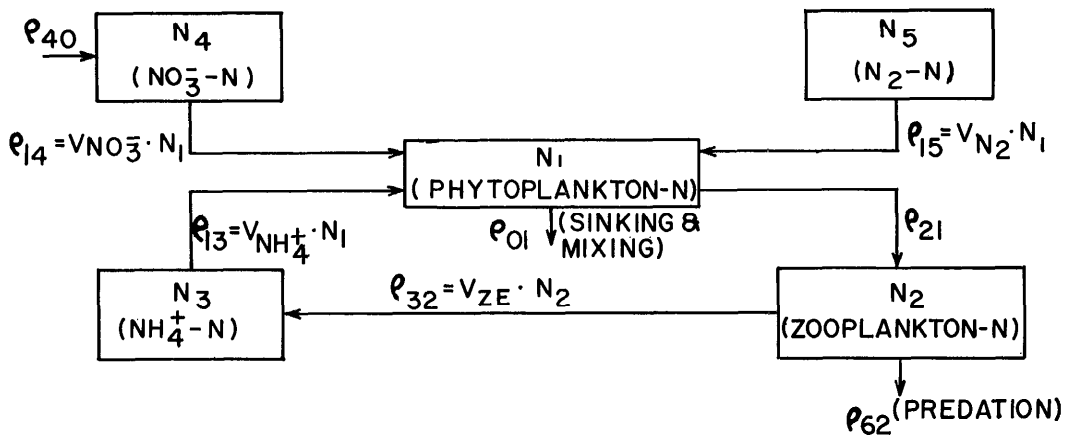
FIG. 1.   Circulation of inorganic nitrogen through the euphotic zone ecosystem.

the Sargasso Sea at Bermuda and *Chain* cruises 15 and 25, and from the Gulf of Maine (*Atlantis II*, cruise 2), the Arabian Sea (*Anton Bruun*, cruise 4A), and the northeast Pacific Ocean (*Acona*, cruises 1, 3, and 7).

METHODS

Tracer techniques

The ^{15}N method of measuring uptake of inorganic forms of nitrogen has been described by Neess et al. (1962). This procedure involves 1) addition of ^{15}N-labeled nitrogen compounds to seawater, 2) incubation under conditions chosen for the experiment, 3) filtration of the water through glass filters (Hurlbut 984II ultrafilter) to capture the particulate matter, 4) conversion of nitrogen compounds in the particulate matter to gaseous nitrogen by a Dumas method (Barsdate and Dugdale 1965), and 5) determination of the nitrogen isotope ratio of the gas with a mass spectrometer and comparison of the ratio with standards to determine if any ^{15}N has been incorporated into the particulate fraction during incubation. We have controlled a number of experiments in each region visited by adding formalin to light- and dark-incubated seawater with the result that all uptake of ^{15}N-labeled compounds ceases. The results of the experiments reported here can therefore safely be inter-preted to indicate biologically-mediated processes.

Sample conversion and mass spectrometry have been carried out in the laboratory or on shipboard, using a Bendix Time-of-Flight Model 17-210 mass spectrometer. The precision of the mass spectrometer is about 0.01 atom per cent for replicate samples containing the natural abundance of [15]N(0.370 at.% [15]N). A conversion system and the mass spectrometer were installed on the RV *Chain* for cruise 25 and on the RV *Acona* for cruise 7 to permit immediate analysis of experiments.

Carbon uptake was measured by the ^{14}C technique of Steemann Nielsen (1952). In the *Atlantis II* studies, light and dark bottles were incubated under the same experimental conditions selected for the nitrogen observations.

Following mass spectrometry, the variables, $V_{NO_3^-}$ and $V_{NH_4^+}$ are obtained directly from the following computations using the notation in Fig. 1 suggested by Sheppard (1962).

$$V_{NO_3^-} = \frac{\rho_{14}}{N_1} = \frac{da_1/dt}{a_4 - a_1}, \qquad (1)$$

where ρ_{14} = the rate of transport of nitrate from the labeled compartment, 4, into the initially unlabeled compartment, 1, the particulate fraction in this case. N_1 = the concentration of nitrogen in the particulate
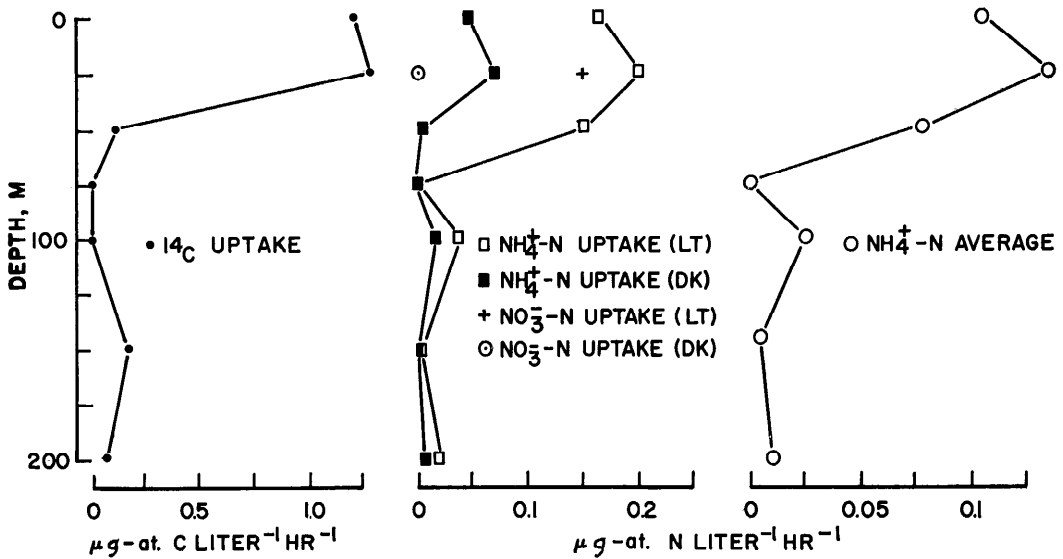
FIG. 2.   Uptake of ^{15}N-labeled NH_4^+ and ^{14}C at station 1 of cruise 2 of the RV *Atlantis II* in vertical series.

nitrogen fraction. $a_4 =$ the atom per cent ^{15}N in compartment 4. $a_1 =$ the atom per cent ^{15}N in compartment 1. $V_{NO_3^-}$ is essentially a growth rate in terms of nitrogen and has units of μg-at. N taken up (μg-at. N)$^{-1}$ (unit time)$^{-1}$ or simply unit time^{-1}.

Rearranging equation (1):

$$\rho_{14} = V_{NO_3^-} \times N_1. \qquad (2)$$

The computations for $V_{NH_4^+}$ follow in similar fashion.

The measurement of inorganic nitrogen uptake is influenced by the varying amount of nitrogen detritus. The effect is to dilute the living fraction of particulate nitrogen and thus to produce an underestimate of V in the following manner:

$$V_{cells} = V_{measured} \times \frac{N_{1\ cells} + N_{1\ detritus}}{N_{1\ cells}}$$

$$= V_{measured} \times \frac{N_{1\ total}}{N_{1\ cells}}. \qquad (3)$$

All V's measured on a particular water sample are in error by the same factor, so the effect of detritus disappears when ratios of V's are obtained, for example:

$$\frac{V_{NO_3^-\ cells}}{V_{NH_4^+\ cells}} = \frac{V_{NO_3^-\ measured} \times \dfrac{N_{1\ total}}{N_{1\ cells}}}{V_{NH_4^+\ measured} \times \dfrac{N_{1\ total}}{N_{1\ cells}}};$$

thus,

$$\frac{V_{NO_3^-\ cells}}{V_{NH_4^+\ cells}} = \frac{V_{NO_3^-\ measured}}{V_{NH_4^+\ measured}}. \qquad (4)$$

The effect of detritus also cancels when the transport rates are computed:

$$\rho_{14\ cells} = V_{NO_3^-\ cells} \times N_{1\ cells}$$

from equation (3).

$$= V_{NO_3^-\ measured} \times \frac{N_{1\ total}}{N_{1\ cells}} \times N_{1\ cells}$$

$$= V_{NO_3^-\ measured} \times N_{1\ total}.$$

The addition of tracer nitrate and ammonia to raw seawater will result in increased rates of uptake of nitrogen from those sources unless the pre-existing levels are already high. We have, therefore, limited the addition of the labeled compound to 10% of that already present in the water. Until recently, nitrate analyses could not be obtained in less than 24 hr.
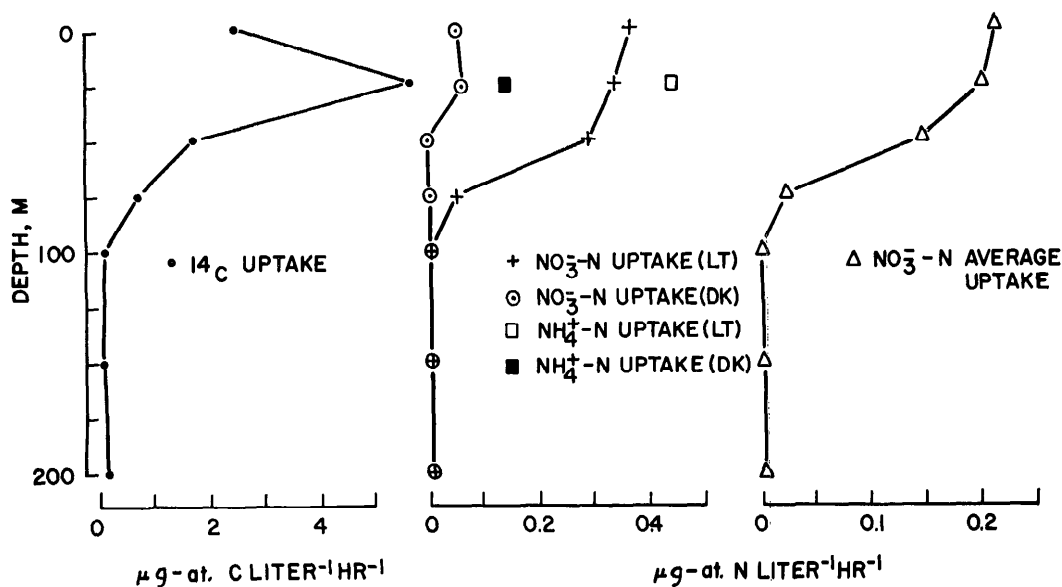
FIG. 3. Uptake of ^{15}N-labeled NO_3^- and ^{14}C at station 5 of cruise 2 of the RV *Atlantis II* in vertical series.

Hence, additions of the isotope were made from estimates of the concentration likely to be found in the water. With this technique, we were able to hold the enrichment tolerances to 5–20% for most of the data reported here. As a first approximation, we assume that our estimates of nitrate and ammonia uptake may be on the order of 5–20% high as a result of this error.

Another source of error is in dilution of the initial isotope enrichment during incubation resulting in an underestimate of the amount of nitrogen uptake. We have observed a dilution of the isotope ratio in the ammonia fraction in a number of experiments in which the ammonia was distilled from subsamples obtained periodically during incubation. We have not corrected for this effect in our uptake data, so the uptake values given may underestimate the actual uptake rates by as much as 10%. The amount of nitrate dilution, if any, is unknown. However, it is our impression that it is probably low because our incubation periods were short (12 hr or less), and we have failed to detect measurable rates of nitrification in surface seawater incubated for up to one week with ^{15}N-labeled ammonia (unpublished).

Incubation and sampling procedures

In the Bermuda experiments, 12-liter carboys were filled with seawater collected at a depth of 15 or 20 m, 28 km southeast of Bermuda. After addition of [15]NH_4^+ or [15]NO_3^-, the carboys were incubated for 24 hr in a seawater-cooled Plexiglas tank exposed to natural light.

The water of the *Chain* 25 experiments was collected from a depth of 25 m. Incubation of the carboys was carried out in a constant temperature incubator reproducing *in situ* sea surface temperatures. The light level in the incubator was 9,000 lux.

A vertical series of ammonia and nitrate uptake measurements was conducted in the Gulf of Maine in April, 1963. The conditions of incubation of the nitrogen uptake carboys on this cruise were similar to those of the *Chain* 25 cruise with one exception—the light level in the incubator was about 5,000 lux.

The water for the Arabian Sea experiments was collected from depths of 15 or 25 m. The carboys were incubated in front of a fluorescent light bank that supplied a light level of about 5,000 lux. On the RV *Acona* cruises, samples were cooled with
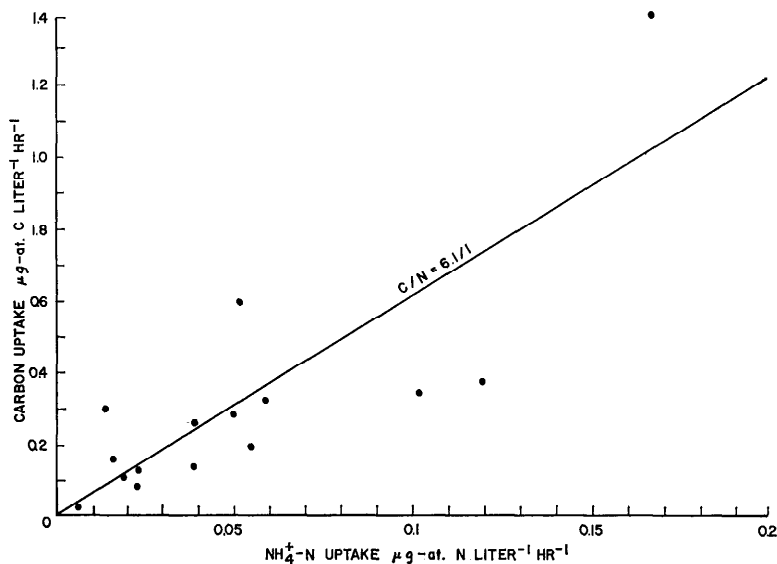
FIG. 4.   Uptake of ^{15}N-labeled NH_4^+ vs. uptake of ^{14}C on cruise 4A of the RV *Anton Bruun*.

running seawater and illuminated with fluorescent lights supplying a level of about 5,000 lux.

Chemical analysis

On *Chain* cruises 15 and 25 and at Bermuda, concentrations of ammonia-nitrogen were determined by the vacuum distillation technique of Riley (1953). The ammonia-nitrogen values used for calculations in the Arabian Sea data were obtained by isotope dilution. This involves 1) addition of a known amount of [15]NH_4^+ to seawater, 2) distillation of the free ammonia from filtered samples of this water buffered to pH 9.2, 3) conversion of the ammonia to gaseous nitrogen by alkaline hypobromite, 4) measurement of the nitrogen isotope ratio, and 5) calculation of ammonia concentration.

In all experiments, nitrate-nitrogen was determined by the method originally described by Mullin and Riley (1955) or by the modifications later suggested by Strickland and Parsons (1960).

RESULTS AND DISCUSSION

Photosynthesis and nitrogen uptake

Ammonia and nitrate uptake in the light, ρ_L; dark uptake, ρ_D; average uptake, $\bar{\rho} = (\rho_L + \rho_D)/2$; and ^{14}C-measured primary production are plotted in Figs. 2 and 3 for stations 1 and 5 of cruise 2 of the RV *Atlantis II*. It is evident that both the light and dark ammonia uptake curves follow the same pattern shown for the uptake of carbon, suggesting that both dark and light uptake are related to the activities of photosynthetic organisms. The similarity between the curves for average ammonia and nitrate uptake with those for carbon uptake suggest that $\bar{\rho}$ may provide a relative estimate of nitrogen uptake by photosynthetic organisms in the euphotic zone. Thus, we use $\bar{\rho}$ in computing carbon : nitrogen uptake ratios taking into account, however, the daylight uptake of carbon and the continuous uptake of nitrogen implied by the use of $\bar{\rho}$.

In Fig. 4, ammonia uptake is plotted against carbon uptake. These data were collected during cruise 4A of the RV *Anton Bruun*. The regression line, a least square fit, passes through the origin with a mean ratio of carbon uptake to nitrogen uptake, 6.1 : 1, similar to the expected ratio of about 7 : 1 (Fleming 1940). Nitrate data are not available; however, the effect of including nitrate uptake would be to decrease the C : N ratio by perhaps 10–40%.

TABLE 1. *Light and dark uptake of ammonia and nitrate*

		$V_{NH_3}hr^{-1}$	$\dfrac{V_{NH_4+L}}{V_{NH_4+D}}$	$V_{NO_3}hr^{-1}$	$\dfrac{V_{NO_3-L}}{V_{NO_3-D}}$
Temper-	$L*$	0.0391(3)†	3.8	0.0267(7)	9.9
ate	$D‡$	0.0101(8)		0.0027(6)	
Tropical	L	0.0103(50)	1.7	0.0026(25)	3.3
	D	0.0062(24)		0.0008(17)	

* L = light incubation.
† (X) = mean of X number of samples.
‡ D = dark incubation.

The data for light and dark uptake of ammonia and nitrate are summarized in Table 1. The ratio of light-to-dark uptake is greater than one for both forms of nitrogen. The small number of measurements available for temperate waters makes it impossible to draw definite conclusions about geographical differences; however, there is a suggestion that the ratio of light-to-dark uptake of ammonia and nitrate is lower in tropical waters than in temperate waters.

The uptake of nitrogen is not a direct photosynthetic process but an indirectly related one with a variable degree of coupling to photosynthesis. The data given here are consistent with earlier observations and conclusions regarding the uptake of nitrogen in the dark. The assimilation of inorganic nitrogen compounds in the dark by nitrogen-deficient cells led Harvey (1963) to suggest that phytoplankton that become nutrient deficient during the day may make up for this deficiency in the dark. Harris and Riley (1956) suggest that phytoplankton assimilate nitrogen and other nutrients continuously from media containing low concentrations, while carbon uptake proceeds only in the light, thereby maintaining the average carbon-to-nitrogen ratio in the organic matter.

Nitrogen uptake at Bermuda

The primary productivity at station S, 28 km southeast of Bermuda in the Sargasso Sea, has been studied intensively by Menzel and Ryther (1960). A significant feature is the annual winter breakdown of the seasonal thermocline with subsequent circula-

TABLE 2. *Uptake of ammonia and nitrate at station S in the Sargasso Sea near Bermuda*

Date	$*\bar{V}_{NH_4}+hr^{-1}$	$\bar{V}_{NO_3}-hr^{-1}$	Per cent NO_3^- uptake $\dfrac{\bar{V}_{NO_3-} \times 100}{\bar{V}_{NO_3-} + \bar{V}_{NH_4+}}$
24 Sep 1962	0.01320	0.00050	3.6
10 Oct 1962	0.01225	0.00020	1.6
6 Nov 1962	0.01055	0.00005	0.5
22 Nov 1962	0.00705	0.00075	9.6
18 Dec 1962	0.01100	0.00100	8.3
7 Jan 1963	0.00560	0.00105	15.8
17 Jan 1963	0.00750	0.00175	18.9
Mean	0.0114	0.00076	8.3

* Uptake in a single undarkened carboy, divided by 24 (the hours of incubation under natural light).

tion down to the permanent thermocline. Nutrients are mixed upward and a spring bloom of diatoms occurs from January through March or April. Following formation of the seasonal thermocline, primary production declines and remains low for the remainder of the year. The minimum primary productivity for 1957, 1958, and 1959 occurred in November.

The results of a series of nitrogen uptake measurements made on water collected from a depth of 15 m at station S over the period 24 September 1962 to 17 January 1963 are presented in Table 2. Samples were incubated for 24 hr under natural light as described above. Darkened carboys were not used, and total uptake was divided by 24 to obtain an average hourly uptake rate. The low percentage of nitrate uptake in the early fall arise from low nitrate uptake values while the increases observed later result from increased nitrate uptake combined with decreased ammonia uptake. The mean nitrate uptake is 8.3% for the entire period.

The course of events related to the variation in nitrate uptake is shown in Fig. 5. To emphasize the relationship to primary production, nitrogen uptake has been converted to equivalent carbon using 7.0 as a factor. As new production (nitrate uptake) falls to undetectable levels, the particulate nitrogen (phytoplankton) and the regenerated production (ammonia uptake) fall rapidly. This period is associated with the
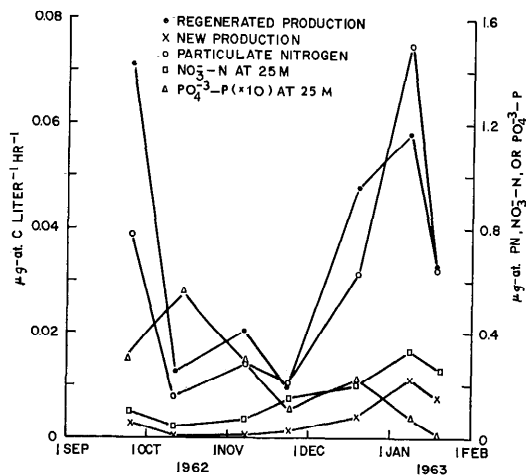
FIG. 5. Seasonal changes in nitrogen uptake at station S near Bermuda expressed in terms of carbon as new and regenerated primary productivity.

FIG. 6. Comparison of primary productivity measured on *Chain* cruise 25 by the ^{14}C method and new productivity measured by the ^{15}N method.

minimum 25-m nitrate concentration. As the nitrate concentration at 25 m rises steadily, new production increases and is accompanied by large increases in particulate nitrogen and regenerated production. The subsequent decline in all of these parameters appears to be associated with the decline in 25-m nitrate concentration, suggesting the possibility that nitrogen is limiting primary production. Phosphate-phosphorus does not show a clear relationship to the concentration of nitrate or the other parameters plotted in Fig. 5. Thus, the role of nitrate as a limiting nutrient appears to be reasonably well established for the winter diatom bloom in the Sargasso Sea near Bermuda, both from the results shown in Fig. 5 and from the work of Steele and Menzel (1962). Searching for a nutrient factor to be used in predicting conditions for maximum primary production in the mixed layer, they found that phosphate showed little change over the winter; however, nitrate showed distinct seasonal peaks varying from year to year. Using a nutrient factor based on nitrate, and knowing the depth of the mixed layer, they were able to compute chlorophyll concen-
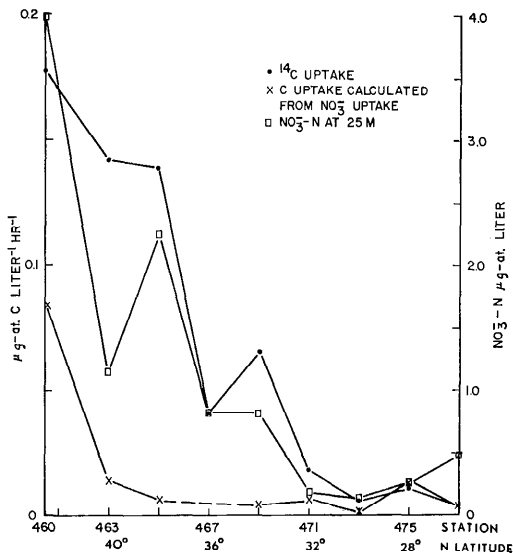
trations that agreed well with those measured during three winter blooms.

The increase in particulate nitrogen occurring in December can be used to compute the growth rate for the particulate nitrogen fraction, which can then be compared to the nitrate uptake results if the assumption is made that there is no net loss from the 15-m layer by sinking or other processes and that the zooplankton population remains constant. The latter assumption is probably valid because Menzel and Ryther (1961) observed lag periods of the order of months before the zooplankton responded to the increased winter production. On this basis, a value of $V_{NO_3^-}$ can be computed using the expression:

$$N_{1t} = N_{1i}e^{tV_{NO_3^-}},$$

where: N_{1t} = particulate nitrogen at time t, and N_{1i} = initial particulate nitrogen.

The value so obtained, 0.00183/hr, agrees with ^{15}N-measured $V_{NO_3^-}$ at the beginning and at end of the period, 0.00100 and 0.00105.

The contribution of zooplankton excretion to primary productivity in the Bermuda

TABLE 3. *Comparison of primary production measured with ^{14}C and estimates made from nitrate uptake measurements on RV* Chain *cruise 25*

Station	Location (N lat)	(W long)	(1) Total ^{14}C uptake μg-at. C liter^{-1} hr^{-1}	(2) % NO$_3^-$ uptake $\frac{NO_3^-}{NH_4^+ + NO_3^-} \times 100$	(3) New production $\frac{(1) \times (2)}{100}$ μg-at. C liter^{-1} hr^{-1}	(4) New production NO$_3^-$ uptake $\times 7$ μg-at. C liter^{-1} hr^{-1}
460	42°24'	65°34'	0.178	30.4	0.054*	0.085
463	40°00'	65°00'	0.142	17.3	0.024†	0.014
465	38°00'	65°00'	0.139	21.0	0.029	0.006
467	36°00'	65°00'	0.041	—	—	—
469	34°00'	65°00'	0.066	—	—	0.004
471	31°55'	65°00'	0.018	13.9	0.002	0.006
473	30°00'	65°00'	0.006	16.7	0.001	0.001
475	28°00'	65°00'	0.012	72.5	0.009	0.014
477	26°00'	65°00'	0.003	30.2	0.001	0.004

* Dark uptake estimated at 10% of light value.

† Particulate nitrogen value measured; all other particulate nitrogen values were obtained by extrapolating between measurements at previous and succeeding stations.

region can be examined theoretically. If the system of Fig. 1 is assumed to be in steady state and other ammonia sources can be ignored, it can be seen that ammonia uptake is equal to zooplankton ammonia excretion. Then:

$$V_{ZE} \times N_2 = V_{NH_4^+} \times N_1 , \text{ and}$$

$$V_{NH_4^+} = V_{ZE} \times \frac{N_2}{N_1}.$$

Beers (1962) has measured the ammonia excretion rate of *Sagitta hispida* and gets a value of $V_{ZE} = 0.006$ μg-at. NH$_4^+$-N excreted per μg-at. org-N animal^{-1} hr^{-1}. The excretion coefficient can be computed independently from the respiration rates measured for Bermuda zooplankton by Menzel and Ryther (1961). They give a mean value of 0.125 g C respired per g zooplankton dry wt per day, close to the mean of 0.103 quoted by them for other investigations. Raymont (1963) suggests 11.1–12% for the nitrogen content of copepods and 10.9% for *Sagitta* on a dry weight basis. Making the assumption that nitrogen is excreted in proportion to the carbon respired and in the average ratio they occur in the phytoplankton (7 : 1) and using an average nitrogen content of 11.3%, $V_{ZE} = 0.0078$/hr, in close agreement with Beers' measured value of 0.006/hr for *S. hispida*. Steemann Nielsen (1962) showed that, with

certain reasonable assumptions, the total zooplankton population below a square meter of sea in the Bermuda region may be about twice the size of the phytoplankton population in the euphotic zone when both populations are measured in terms of carbon. Applying Menzel and Ryther's (1961) figure of 13.5% for the fraction of the population to be found in the upper 100 m, the ratio N$_2$: N$_1$ for 0–100 m is $(2N_1 \times 0.135)/N_1 = 0.27$. Then the expected $V_{NH_4^+} = 0.27 \times 0.0078 = 0.0021$/hr, an order of magnitude lower than the mean observed $V_{NH_4^+}$ in Table 1, 0.0114. Apparently only 10% of the observed ammonia uptake is supplied by zooplankton excretion, although diurnal vertical migrations may increase this amount (Beers and Kelly 1965).

Ammonia and nitrate are added to the sea surface by precipitation and constitute a source of nitrogen for new production. The contribution of ammonia in the Bermuda region can be computed from the data of Menzel and Spaeth (1962), who found between 1,407 and 189 μg-at. of N falling upon a square meter of sea surface per month. Using 1,000 μg-at. m^{-2} as the monthly contribution and assuming its distribution through a euphotic zone 100 m deep, the mean hourly rate of ammonia delivery is 1.39×10^{-5} μg-at. of N/liter. The daily ammonia uptake rate computed from Table

TABLE 4. *Mean per cent nitrate uptake for all available Pacific and Atlantic Ocean data*

Cruise	Area	Dates	Mean per cent NO_3^- uptake
Acona 1	NE Pacific coast, Seattle–Juneau	Sep 1964	20.3 (5)*
Acona 3	NE Pacific coast, Juneau–Cape Spencer	Nov 1964	27.6 (3)
Acona 7	N Pacific, Vancouver–Honolulu	Feb 1965	8.7 (8)
Chain 25	NW Atlantic, Georges Bank–Caribbean	Mar 1962	28.8 (8)
	Bermuda, station S	Sep 1962 to Jan 1963	8.3 (7)
Atlantis II 2	NW Atlantic, Gulf of Maine	Apr 1963	39.5 (2)

* (x) = number of samples.

2 gives a value of 5.7×10^{-3} μg-at. liter^{-1} hr^{-1}. Clearly, ammonia from atmospheric sources plays an insignificant role in the daily ammonia requirement.

The mean rate of nitrate uptake at 15 m in the Bermuda region can be computed from $V_{NO_3^-} = 0.00076$ (Table 2) and taking a figure of 0.5 μg-at. N liter^{-1} for the concentration of particulate nitrogen (Goering and Dugdale, unpublished). The value so obtained, 3.8×10^{-4} μg-at. N liter^{-1} hr^{-1}, is an order of magnitude higher than the value computed for ammonia uptake supplied by rainfall.

Nitrate is also contained in precipitation at Bermuda. The concentration of nitrate may equal or exceed that of ammonia (Goering, unpublished). The contribution of nitrogen from nitrate and ammonia in rain may then be estimated to be on the order of 20% or less of the new production computed from the nitrate uptake data.

Chain 25

Measurements of nitrate and ammonia uptake in water from 25 m were made on a section along 65° W long from Georges Bank to the Caribbean Sea in May 1962. The per cent nitrate uptake is shown in column 2 of Table 3. New primary production (column 3) is computed from the product of this ratio and the ^{14}C-measured primary productivity. By an alternative method, the new production has been calculated directly by multiplying the nitrate uptake rate by 7 as in the Bermuda data (column 4). A comparison of the two methods can be made from columns 3 and 4 and the agreement appears to be good. Significant disparities between expected and observed nitrogen and carbon uptake ratios should

be revealed here. The per cent nitrate uptake at station 471 near Bermuda compares well with the value given above for that region.

A comparison of ^{14}C primary production with new primary production (column 4, Table 3) along this section is shown in Fig. 6. New primary production gives a picture of uniformly low rates throughout the Sargasso Sea while ^{14}C primary production declines more gradually until the two methods give about the same values. The new fraction rises at the southern end, probably as a result of the trade winds and increased nitrate concentration at the 25-m level. The significance of the steady decline of regenerated production toward the south cannot be assessed but is presumably due to a decline in bacterial activity or some other regeneration pathway whose specific contribution we are unable to measure.

Northwest Pacific Ocean

Ammonia and nitrate uptake measurements have been made on Alaskan cruises 1, 3, and 7 of the RV *Acona*. Water was collected from a depth of 10 m. Table 4 summarizes all available data from which per cent nitrate uptake can be computed. RV *Acona* cruises 1 and 3 were conducted in coastal or inside waters between Seattle and Juneau and show relatively high proportions of nitrate uptake comparable to those shown for the Gulf of Maine on RV *Atlantis II* cruise 2. RV *Acona* cruise 7, from Vancouver, British Columbia, to Honolulu, Hawaii, was carried out in late winter; values obtained were low, comparable to the mean winter Bermuda data given in Table 2.

SUMMARY AND CONCLUSIONS

The ability to fractionate primary production into components by observing the rates of flow of various nitrogen components, using ^{15}N-labeled compounds, may lead to the solution of some important problems. We now have some first estimates for the fraction of primary production that is available for consumption at higher trophic levels based on nitrate and ammonia uptake measurements. As we accumulate more of these data for more regions of the sea and at different seasons, it should be possible to correct existing ^{14}C primary production measurements to give a more accurate picture of the potential for production of fish in a given region. This is true especially in the tropics where so little is known about recycling of nutrients in relation to primary production. Perhaps the most important point requiring confirmation is the finding that those tropical regions that appear from ^{14}C measurements to have low primary productivity have a lower proportion of new production than do regions of higher ^{14}C-measured primary productivity. Thus, it appears that rich seas are richer and poor seas are poorer than measurements of photosynthetic carbon fixation would suggest.

It is important to distinguish clearly between the relative importance of ammonia and nitrate as sources of nitrogen for the cell and as sources of nitrogen for the population. Vaccaro (1963) has suggested that in coastal water off New England in summer, ammonia is likely to be a more important source of nitrogen than is nitrate, and the uptake data given in this paper support his view. Thomas (1966) has obtained similar data from the tropical Pacific Ocean and arrives at essentially the same conclusion as Vaccaro (1963). The algal cells present do depend primarily on ammonia as a source of nitrogen. However, because ammonia is the result of short-term regeneration of nitrogen, only new sources such as nitrate from deep water or nitrogen fixation allow increases in population size or in production passed on to higher trophic levels. These points are embodied in Fig. 1. Under quasi-steady-state conditions, ammonia can circulate indefinitely if the phytoplankton population incurs no losses whatever, that is, $\rho_{21} = \rho_{32} = \rho_{13}$. The primary production system is real, so losses are incurred primarily through sinking and mixing, ρ_{01}, and by predation by zooplankton, ρ_{62}. The sum of these losses, $\rho_{01} + \rho_{62}$, must at least be balanced by nitrate uptake, ρ_{14}, or by nitrogen fixation, ρ_{15}, or by any other possible source of nonregenerated nitrogen. Thus, for the phytoplankton cell in the sea, ammonia is an important nitrogen source serving to maintain the cell in a healthy state and providing much of the nitrogen used in reproduction when nitrate levels are low, but nitrate and nitrogen fixation are the most important parameters with respect to nitrogen limitation of primary productivity.

The potential importance of nitrogen fixation is indicated by comparing rates of it with rates of nitrate uptake, because both enter the cycle as new nitrogen. Dugdale, Goering, and Ryther (1964) have presented data from which rates of nitrogen fixation associated with Trichodesmium sp. can be computed. These rates vary from undetectable to $V_{N_2} = 0.0116$/hr. The rapid increase in particulate nitrogen observed during the winter of 1962–1963 in the Sargasso Sea at Bermuda (Fig. 5) was apparently supported by nitrate uptake rates of about $V_{NO_3^-} = 0.001$/hr. Nitrogen fixation can quite obviously support growth of Trichodesmium sp. at rates that are competitive to those of other phytoplankton whose growth is supported by nitrate.

Certain assumptions have been made and should be clearly stated; for example, the oxidation of ammonia to nitrate has been ignored. Although nitrification clearly occurs in the sea, indications are that the rates are low compared with those considered here. If nitrification rates are eventually shown to be sufficiently higher than has been assumed, the assumption that nitrate is a nonregenerated nutrient form in the euphotic zone would have to be modified. Also, the regenerated fraction of available nitrogen is not entirely measured by the ammonia uptake, the contribution of dissolved organic nitrogen being of po-

tential importance. The uptake of ^{15}N-labeled urea and glycine has been detected (Goering and Dugdale, unpublished); however, absolute rates cannot be computed until the concentration of these compounds in seawater is measured concurrently.

The question of gross vs. net uptake is an important one that cannot be resolved with the information currently available. Appropriate laboratory investigations with pure cultures of algae do not appear to have been made. However, we have looked carefully at the time-course of uptake of ammonia in raw seawater from Bermuda stations, measuring the isotope ratios in both the seawater and the particulate fraction (unpublished). Uptake rates were found to be linear with time for the first 24–36 hr, suggesting that if exchange occurs, release from the cell to the medium proceeds at a much slower rate than does uptake from the medium into cells. A high net-to-gross uptake ratio (close to unity) is thereby implied.

Problems of interpretation and technique remain to be solved. However, the measurement of primary production with ^{15}N and the analysis of marine production systems in terms of nitrogen have sufficient advantages to warrant the additional effort required to place this approach on a firm experimental basis.

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