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Nitrogen uptake and NH_4^+ regeneration by pelagic microplankton and marine snow from the North Atlantic

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ABSTRACT

Comparative rates of nitrogen uptake and NH_4^+ regeneration by plankton of <153 and <5 μm in size were determined in the Sargasso Sea and Gulf Stream, and by plankton associated with marine snow in the Gulf Stream during May 1982. Rates of total nitrogen uptake of Sargasso Sea phytoplankton exceeded those of the Gulf Stream phytoplankton by factors ranging from 1.8 to 5.6. Rates of microplankton NH_4^+ regeneration equaled or exceeded rates of NH_4^+ uptake in the Sargasso Sea, but in the Gulf Stream were negligible in all but one case. Significant rates of NH_4^+ regeneration were measured for Gulf Stream marine snow, and, in all but one case, exceeded those of NH_4^+ uptake. Rates of NO_3^- and urea uptake by the snow were less than half those of NH_4^+. Protozoan densities were enumerated on aliquots of the same snow particles and compared with previously reported bacterial estimates; enrichment factors of the cultivable ciliates and flagellates were 6500–9000 relative to ambient seawater. These organisms were also grazing and reproducing rapidly. Bacterial densities were also moderately enriched, but their productivity was lower than surrounding seawater bacteria. Thus, the large bacterivorous population associated with marine snow may have accounted for a substantial fraction of the observed NH_4^+ regeneration.

1. Introduction

Over the past decade the abundance of cyanobacteria, bacteria, and microeukaryotes, and their importance in oceanic carbon and nitrogen cycling have been recognized (Waterbury *et al.,* 1979; Johnson and Sieburth, 1979; Fenchel, 1982; Sherr and Sherr, 1983; Goldman and Caron, 1985). It is thought that microheterotrophic processes, rather than direct macrozooplankton grazing and release, are the primary regulators of nutrient cycling between autotrophs and heterotrophs in oceanic systems (Pomeroy, 1974; Sieburth *et al.,* 1978; Williams, 1981; Azam *et al.,* 1983; Caron *et al.,* 1985). Current estimates are that these small organisms supply over 80% of the nitrogen utilized by phytoplankton in oceanic euphotic zone waters (Eppley and Peterson, 1979; Harrison, 1978; Glibert, 1982; Probyn, 1987). In oceanic systems, where nutrient surpluses do not accumulate, efficient nutrient cycling necessitates a

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close coupling between the phytoplankton and the organisms responsible for NH_4^+ regeneration so that phytoplankton growth can be maintained (Goldman, 1984).

The most familiar example of temporal and spatial coupling of autotrophs and heterotrophs is marine snow. "Marine snow" refers to macroscopic detrital particles that host a rich community of bacteria, cyanobacteria, phytoplankton, protozoa, and other flagellates at densities many-fold those found in surrounding seawater (Silver *et al.*, 1978; Silver and Alldredge, 1981). They have been shown to have high rates of productivity and heterotrophy (Alldredge and Cox, 1982; Silver *et al.*, 1978; Trent *et al.*, 1978; Caron *et al.*, 1982; 1986). These communities also have been shown to be very high in nutrient concentrations; NH_4^+ in particular can be present in mM levels (Shanks and Trent, 1979). Clearly, the aggregates that can be conveniently sampled (using SCUBA techniques) are on the large side relative to the many microscopic aggregates that may exist, for perhaps only fleeting periods of time (Goldman, 1984). Consequently at best we can underestimate their importance in terms of nutrient cycling in oceanic systems.

The purpose of this paper is to describe a series of experiments conducted in the Sargasso Sea and Gulf Stream designed to assess the nutritional status, and the comparative rates of nitrogen uptake and NH_4^+ regeneration associated with plankton of different size fractions and with diver-collected marine snow particles. These data support the growing body of evidence that at times marine snow aggregates can be important sites of nitrogen cycling, and that this cycling of materials is likely mediated by protozoa rather than by bacteria.

2. Materials and methods

a. Station location and sampling techniques. Samples were collected over several day periods while on drifting stations in the Sargasso Sea and in the Gulf Stream on cruise 94 of R/V *Knorr* (May–June 1982). Stations were centered around 34°00'N 73°20'W and 35°00'N and 74°50'W.

Seawater samples were collected using acid-cleaned 30 l Niskin bottles. Depths for sampling were chosen from a light cast (Biospherical Instruments QSL-100 4-pi collector) and temperature profile (XBT trace). Typically the 60, 22, and 3% light depths were sampled. Samples were fractionated using Nitex netting to yield (depending on experiment) <153 μm, and <5 μm size fractions. All fractionation and sample withdrawal was done under reduced light.

Samples of marine snow were collected using sterile 10 or 50 ml syringes (without needles) by divers, as previously described (Alldredge *et al.*, 1986; Caron *et al.*, 1986). Collection of surrounding seawater was kept minimal by this technique, but no attempt was made to accurately measure the amount of dilution of the aggregate samples by the surrounding seawater. Caron *et al.* (1986) estimate that the dilution factor was probably less than an order of magnitude. All marine snow material from a single dive was pooled in a sterile polycarbonate flask, mixed by vigorous shaking, and dispersed

for individual experiments as described below. This sampling technique was necessary to perform the many analyses on snow samples that were carried out, but may have been destructive to individual particles. We suspect that sample handling may have resulted in an underestimate of physiological rates. Nevertheless, the abundances and rates we are presenting are large relative to the surrounding seawater. We were only able to collect sufficient marine snow for experimentation in the Gulf Stream. Macroscopic particles were smaller and less abundant in the Sargasso Sea.

b. Nutrient and biomass availability. The nitrogenous nutrient (NH_4^+, NO_3^-, urea) concentrations of seawater and marine snow samples were determined according to Strickland and Parsons (1972), with volumes scaled down to 5 ml. Determinations of NH_4^+ and urea were made immediately upon sample collection; samples for NO_3^- analysis were frozen for up to several days. Analyses of chlorophyll (chl) were done fluorometrically on samples filtered onto Whatman GF/F filters as described by Strickland and Parsons (1972). A post-cruise recalculation of the calibration factors revealed an error of a factor of two. The data reported here have been corrected for this error, but chl values reported from this study by Caron *et al.* (1986) and from a previous study (Glibert *et al.*, 1985a, b) are low by this amount. Particulate nitrogen (PN) and particulate carbon (PC) of seawater samples were determined using a Perkin-Elmer 240 elemental analyzer on samples filtered through precombusted GF/F filters. Particulate nitrogen concentrations of the marine snow samples were estimated from the N_2 pressure reading during mass spectrometric analysis. Dissolved inorganic carbon was determined using a Dohrmann PR-1 Analyzer.

c. Microscopy. Microscopic enumerations were as described in Caron *et al.* (1986). Direct counts were made of the total picoplankton (0.2–2.0 μm in size: Tpico), the photosynthetic picoplankton (Ppico), the photosynthetic and heterotrophic nanoplankton (2.0–20.0 μm: Pnano, Hnano), and the photosynthetic microplankton (20–200 μm: Pmicro). Hnano were counted using the procedures of Davis and Sieburth (1982) and Caron (1983). Bacterivorous protozoa were estimated using the Most Probable Number (MPN) culture technique of Davis *et al.* (1978) and described in more detail in Caron *et al.* (1986).

d. Rates of nitrogen uptake and regeneration. Incubation experiments were typically begun within an hour of the time of sampling. Nitrogen uptake experiments of the size-fractionated water samples were done in scrupulously cleaned 2.5 l polycarbonate bottles. Both trace (typically 0.03 μg atom N l^{-1}) and saturating (8 μg atom N l^{-1}) uptake experiments were conducted. Note that the now well-accepted term "trace" is used merely to differentiate the experiments with our lowest ^{15}N additions from those specifically made to saturate the uptake system; we do not imply that these trace experiments were free of all perturbation effects due to the ^{15}N additions (Glibert and

Goldman, 1981). Samples were incubated for 1–2 h periods in deck incubators maintained at ambient temperature with flowing seawater and at the *in situ* light intensity with layers of neutral density screening. Experiments were terminated by filtration onto GF/F filters precombusted for 2 h at 500°C. Filters were rinsed with a small quantity of filtered seawater; the period of intervening air exposure was kept to <1–2 sec.

Nitrogen uptake experiments on marine snow were conducted when sufficient material was collected. Aliquots of 25–40 ml of the aggregate slurry were incubated with 0.85–1.0 μg atom N l^{-1} $^{15}NH_4^+$, $^{15}NO_3^-$, or ^{15}N-urea for periods from 20 to 60 min. Particulate material was collected on GF/F filters as described above. Concentrations of nitrogenous nutrients of the slurry seawater were determined on filtered samples as above.

Rates of NH_4^+ regeneration were determined from size-fractioned seawater and marine snow samples using ^{15}N isotope dilution (Glibert *et al.*, 1982). For determination of the initial $^{15}NH_4^+$ enrichment in the seawater samples, aliquots of the $^{15}NH_4^+$ incubations were collected at time zero, filtered as above, and the filtrate collected for later distillation. For marine snow samples, due to the small amount of material available, the estimate of $^{15}NH_4^+$ enrichment at time zero was based on calculation from the ambient NH_4^+ concentration and the $^{15}NH_4^+$ added. For both the seawater and marine snow incubations, filtrates were collected on $^{15}NH_4^+$ incubations at the end of the stated incubation period for determination of the final $^{15}NH_4^+$ enrichment. Filtrates were distilled according to Glibert *et al.* (1982), and analyzed by mass spectrometry as described below. All estimates of NH_4^+ uptake were corrected for isotope dilution. This correction was negligible for the Gulf Stream samples, but was approximately a factor of 2 for the Sargasso Sea samples.

Determination of ^{15}N enrichment in the particulate material and in the distilled filtrates was by mass spectrometry as described in McCarthy *et al.* (1977). Typical precision is ±0.003 atom percent for samples as small as 1 μg atom N l^{-1} and ±0.001 atom percent for samples >3 μg atom N l^{-1} (McCarthy and Nevins, 1986a). Estimates of the total error associated with ^{15}N uptake and regeneration measurements in nutrient-poor water have been discussed by Glibert *et al.* (1982) and Glibert (1988). The largest source of error is in the determination of ambient substrate when the concentration is below the detection limit. This uncertainty is reduced considerably by direct measurements of the atom % enrichment of the NH_4^+ pool for each $^{15}NH_4^+$ uptake experiment. Using this protocol, replicated $^{15}NH_4^+$ uptake and regeneration experiments (unpub. data) yield rates that are within ±2–3%.

3. Results

a. Water column temperatures, nutrient and biomass availability and particulate composition of seawater samples. Water temperatures in the upper 100 m ranged between 19 and 22°C in the Sargasso Sea, and between 15.5 and 26°C in the Gulf Stream. In the Sargasso Sea, concentrations of NO_3^- and NH_4^+ remained virtually

Table 1. Mean biomass parameters (±std dev) for all seawater samples collected in the Sargasso Sea and Gulf Stream during May 1982. *n* is the number of samples analyzed.

Region	Depth (m)	% I_o	*n*	Chl ($\mu g \cdot l^{-1}$)	PC (μg at $\cdot l^{-1}$)	PN (μg at $\cdot l^{-1}$)	C:N atomic	PN:Chl
Sargasso Sea	12	60	9	0.08(0.02)	5.46(0.99)	0.55(0.05)	9.79(1.22)	6.79(1.48)
	36	22	6	0.10(0.02)	4.38(0.57)	0.43(0.07)	10.31(0.71)	4.45(0.90)
	70	3	9	0.28(0.09)	4.79(0.79)	0.54(0.08)	8.92(1.36)	1.54(0.91)
Gulf Stream	8	60	9	0.09(0.03)	5.33(1.60)	0.63(0.24)	8.79(1.28)	8.26(4.84)
	25	22	7	0.07(0.03)	4.42(1.09)	0.49(0.13)	8.98(0.46)	8.39(4.48)
	45	3	9	0.19(0.15)	5.50(3.97)	0.63(0.33)	8.47(1.25)	4.13(2.46)

undetectable in the bulk water samples on all sampling days at all depths sampled to 70 m and 100 m, respectively. Concentrations of NO_3^- increased to 0.55 μg atom N l^{-1} at 100 m and to 1.31 μg atom N l^{-1} at 150 m. Urea was sporadically present in concentrations up to 0.15 μg atom N l^{-1}, although generally it remained <0.03 μg atom N l^{-1}, our limit of detection for these analyses.

In the Gulf Stream the concentrations of NH_4^+, NO_3^-, and urea were nondetectable on May 28 and 29 in the upper 50 m, but at 100 m, NO_3^- concentrations increased to approx. 3.0 μg atom N l^{-1}. Between May 30 and June 1, ambient concentrations of NH_4^+ and urea in the upper 50 m increased to 0.07 (±0.04) and 0.15 (±0.14) μg atom N l^{-1}, respectively.

Concentrations of chl were similar in the Sargasso Sea and Gulf Stream, as were the general range of PC and PN values (Table 1). The average C:N atomic ratio for euphotic zone samples in the Sargasso was 9.59, and for the Gulf Stream was 8.73.

b. Nitrogen uptake and regeneration by plankton size fractions. In both the Sargasso Sea and the Gulf Stream, rates of uptake of NH_4^+ and urea were greater than the rates of uptake of NO_3^- (Tables 2, 3). The percent contribution of NO_3^- to the measurement of total uptake was <10% in all but one case. For both sites, there was generally no systematic difference between the rates of uptake at first light and at midday. For these two times of day, rates of total nitrogen uptake (sum of NH_4^+, NO_3^-, urea) of Sargasso Sea phytoplankton exceeded those of the Gulf Stream phytoplankton by factors ranging from 1.8 to 5.6 (Tables 2, 3).

The ratio Vsat:Vtrace has been suggested as an index of potential nitrogen deficiency (Glibert and McCarthy, 1984). This is based on the assumption that ratios elevated above 1.0 indicate that the ambient concentration of the nitrogen species in question is insufficient to support the maximal uptake capacity of the population; hence nitrogen deficiency can be inferred. However, a ratio of unity can also be the result of an enhancement in uptake upon addition of even the "trace" amount of ^{15}N. This latter situation is more likely to be the case for NH_4^+ than the other nitrogen forms (Horrigan and McCarthy, 1982). The mean Vsat:Vtrace ratios for all nitrogen forms for both the Sargasso Sea and Gulf Stream were elevated above 1.0, although in

Table 2. Specific uptake velocities (V h^{-1}) and uptake rates (μg atom N l^{-1} h^{-1}) at trace and saturating enrichment levels for Sargasso Sea seawater samples, <153 μm size class, collected 5/28/82, and incubated for ~2 h.

Time of collection	% I_o	Substrate	Vtrace	ρtrace	Vsat	ρsat	Vsat:Vtrace
0530	60	NH_4^+	0.0066	0.0035	0.0050	0.0027	0.8
	22		0.0056	0.0030	0.0058	0.0031	1.0
	3		0.0056	0.0026	0.0063	0.0029	1.1
1130	60	NH_4^+	0.0058	0.0032	0.0097	0.0053	1.7
	22		0.0054	0.0022	0.0076	0.0030	1.4
	3		0.0022	0.0012	0.0080	0.0044	3.6
0530	60	urea	0.0078	0.0041	0.0050	0.0027	0.6
	22		0.0066	0.0036	0.0047	0.0025	0.7
	3		0.0013	0.0006	0.0073	0.0034	5.6
1130	60	urea	0.0004	0.0002	0.0054	0.0030	13.5
	22		0.0018	0.0007	0.0052	0.0021	2.9
	3		0.0064	0.0035	0.0069	0.0038	1.1
0530	60	NO_3^-	0.0004	0.0002	0.0021	0.0011	5.3
	22		0.0003	0.0002	0.0010	0.0005	3.3
	3		0.0004	0.0002	0.0008	0.0004	2.0
1130	60	NO_3^-	0.0002	0.0001	0.0020	0.0011	10.0
	22		0.0004	0.0002	0.0022	0.0009	5.5
	3		0.0004	0.0002	0.0013	0.0007	3.3
0530	60	Σ		0.0078			
	22			0.0068			
	3			0.0034			
1130	60	Σ		0.0035			
	22			0.0032			
	3			0.0020			
0530	60	% as NO_3^-		2.6			
	22			2.9			
	3			5.9			
1130	60	% as NO_3^-		2.9			
	22			6.3			
	3			10.0			

the Sargasso the mean Vsat:Vtrace (NH_4^+) ratio was only 1.6 (Tables 2, 3). The mean ratios did not exceed 4.6 at either site. Occasionally a Vsat:Vtrace ratio <1.0 was observed for the reduced nitrogen substrates; we attribute this to a possible overestimation of substrate, leading to overestimation of the trace uptake rate (Eppley *et al.*, 1977; Fisher *et al.*, 1981).

Table 3. Specific uptake velocities (V h^{-1}) and rates (μg atom N l^{-1} h^{-1}) at trace and saturating enrichment levels for Gulf Stream seawater samples, <153 μm size class, collected 5/23/82, and incubated for ~2 h.

Time of collection	% I_o	Substrate	Vtrace	ρtrace	Vsat	ρsat	Vsat:Vtrace
0515	60	NH_4^+	0.0017	0.0007	0.0049	0.0020	2.9
	22		0.0072	0.0028	0.0030	0.0012	0.4
	3		0.0013	0.0005	0.0054	0.0019	4.2
1130	60	NH_4^+	0.0010	0.0004	0.0046	0.0017	4.6
	22		0.0011	0.0004	0.0044	0.0016	4.0
	3		0.0022	0.0009	0.0026	0.0010	1.2
0515	60	urea	0.0012	0.0005	0.0046	0.0018	3.8
	22		0.0008	0.0003	0.0028	0.0010	3.5
	3		0.0011	0.0004	0.0024	0.0009	2.2
1130	60	urea	0.0011	0.0004	0.0047	0.0017	4.3
	22		0.0012	0.0004	0.0026	0.0009	2.2
	3		0.0005	0.0002	0.0043	0.0018	8.6
0515	60	NO_3^-	0.0004	0.0002	0.0011	0.0004	2.8
	22		0.0001	<0.0001	0.0003	0.0001	3.0
	3		0.0002	<0.0001	0.0004	0.0001	2.0
1130	60	NO_3^-	0.0002	<0.0001	0.0005	0.0002	2.5
	22		0.0001	<0.0001	0.0002	<0.0001	2.0
	3		0.0001	<0.0001	0.0001	<0.0001	1.0
0515	60	Σ		0.0014			
	22			0.0031			
	3			0.0009			
1130	60	Σ		0.0008			
	22			0.0008			
	3			0.0011			
0515	60	% as NO_3^-		14.3			
	22			<3.2			
	3			<11.1			
1130	60	% as NO_3^-		<12.5			
	22			<12.5			
	3			<9.1			

Rates of NH_4^+ regeneration equaled or exceeded the rates of NH_4^+ uptake in the Sargasso Sea (Fig. 1). By necessity when conducting isotope dilution experiments, all size fractioning must be done prior to incubation. Thus, the reported rates for the <153 μm fraction also include any activity of the <5 μm fraction. From the comparison of the rates of the different size fractions (Fig. 1), it is apparent that much

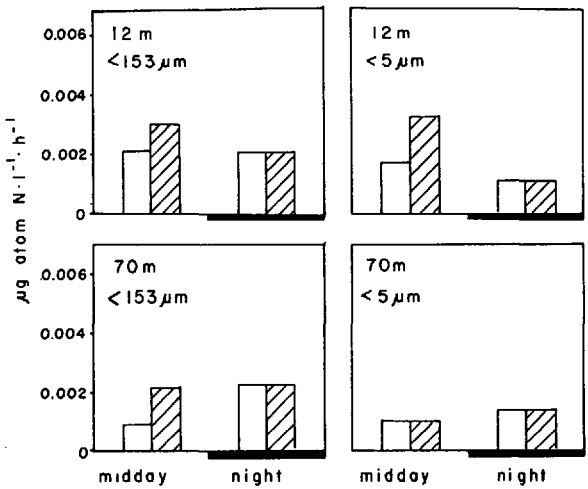

Figure 1. Comparison of NH_4^+ uptake (open bars) and regeneration (hatched bars) rates for the Sargasso Sea for depths and size fractions noted.

(48–100%) of the uptake and regeneration by Sargasso Sea plankton was in the <5 μm fraction. Differences in rates occurring at the two sampling depths were at most a factor of 2.

In the Gulf Stream, regeneration rates, with the exception of one measurement, were virtually undetectable (Fig. 2). Only the <5 μm sample from 8 m at night yielded a positive result. We cannot be certain why a comparable signal was not measured in the <153 μm fraction for the same depth and sampling time, but may have been due to a particle passing through the <5 μm screen or to an increase in dissolved organic matter (Furhman and Bell, 1985) during pre-filtration, either of which may have stimulated NH_4^+ regeneration. The rates of uptake measured at 8 m in the Gulf Stream in the <153 μm fraction exceeded those of the <5 μm fraction, suggesting greater activity by the >5 μm plankton.

c. Marine snow plankton composition and nitrogen flux. The most typical source of marine snow material collected in the Gulf Stream was discarded appendicularian houses. The direct counts of Ppico, Pnano, and Pmicro for these samples tended to range on the low side of the direct counts reported in the larger data set of Caron *et al.* (1986), of which this is a subset (Table 4). The data reported by Caron *et al.* (1986) represent Gulf Stream samples collected on 5 oceanographic cruises from August 1981–August 1983. Enrichment factors of the nanoplankton and picoplankton in marine snow were 14–54 relative to ambient seawater. The enrichment factor of Pmicro was >1200; this was typical of that found by Caron *et al.* (1986) for a wider

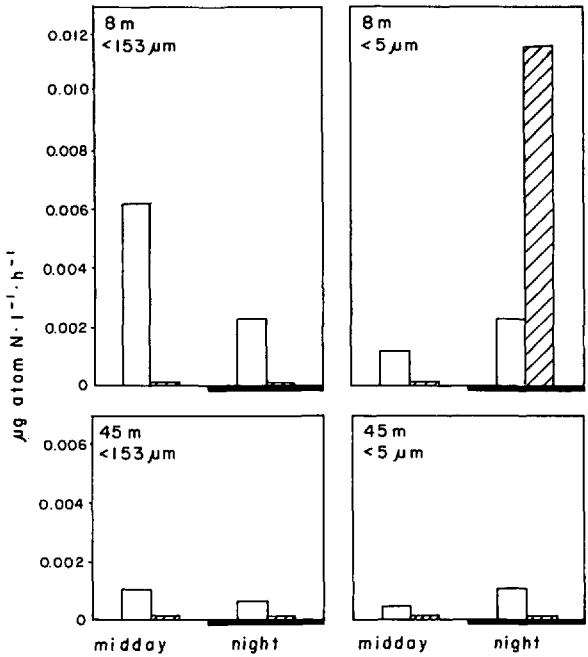

Figure 2. As for Figure 1, except for the Gulf Stream.

range of study sites, and was mostly the result of enrichment of *Oscillatoria* sp. (*Trichodesmium*).

Flagellates were the most abundant of the bacterivorous protozoa in these samples, based on the MPN technique (Table 4). Enrichment factors of cultivable ciliates and flagellates were 6500–9000, and were in line with the more general findings of Caron *et al.* (1986). Enrichment factors of amoebae were considerably less than the values reported by Caron *et al.* (1986).

Sufficient marine snow was collected on four occasions to allow measurements of nitrogen uptake and regeneration. These four samples varied widely in their NH_4^+ concentration, from <1 to >6 μg atom N l^{-1} (Table 5). In all marine snow samples, NO_3^- was undetectable, and in the two samples for which urea was determined, it too was undetectable. The specific uptake rates of NH_4^+ (V h^{-1}) were, on average, approximately an order of magnitude greater than the comparable rates determined for the surrounding seawater (Table 5). However, when expressed as ρ (μg atom N l^{-1} h^{-1}), NH_4^+ uptake rates exceeded those of the surrounding water by 600 – >1700 times. The rates of uptake of NO_3^- and urea were less than half those of NH_4^+. The regeneration rates of NH_4^+ in the marine snow samples exceeded those of NH_4^+ uptake for 3 of the 4 samples analyzed. In one particular sample, the rate of regeneration was sufficient to accumulate nearly 8 μg atom N l^{-1} of NH_4^+ by the end of the 1 h incubation period (Table 5).

Table 4. Average composition of marine show aggregates from the Gulf Stream. Data from this study are compared with the larger data set of Caron *et al.* (1986). Total number of aggregates sampled were 89 in this study and 282 in the Caron *et al.* (1986) study.

	This study		Caron *et al.* (1986)	
	Mean	Range	Mean	Range
Direct Counts				
Tpico ($\times 10^6$ ml^{-1})	9.0	0.91–28	11.4	0.91–250
Ppico ($\times 10^5$ ml^{-1})	2.9	0.07–11	6.6	0.07–34
Pnano ($\times 10^4$ ml^{-1})	0.8	0.16–2	4.6	0.16–13
Hnano ($\times 10^4$ ml^{-1})	1.5	0.44–4	4.6	0.44–18
Pmicro ($\times 10^3$ ml^{-1})	0.3	0.01–1	7.7	0.01–11
Enrichment Factors*				
Tpico	14		19	
Ppico	54		42	
Pnano	15		67	
Hnano	17		114	
Pmicro	1240†		1300	
MPN Estimates (ml^{-1})				
Ciliates	22	0.35–94	13	UN††–180
Flagellates	1110	180–>2300	3180	180–23100
Amoebae	45	0.35–180	53	0.37–180
Enrichment Factors*				
Ciliates	6520		9080	
Flagellates	8750		7640	
Amoebae	490		2280	

*Relative to ambient seawater
††UN indicates undetectable growth

4. Discussion

a. Comparative rates of nitrogen uptake by Sargasso Sea and Gulf Stream microplankton. The period of our cruise was almost 3 years after a similar occupation of the Sargasso Sea (18 June–16 July 1979: Glibert and McCarthy, 1984), and in between two cruises to warm core ring 82-B by the U.S. Warm Core Rings Program (e.g. McCarthy and Nevins, 1986b), on which several excursions to the Sargasso Sea were made. Thus, there are several data sets on nitrogen uptake to which ours are directly comparable. The uptake data we report here were low compared to the results reported by Glibert and McCarthy (1984) by a factor of approximately 2 for NH_4^+ and approximately 4.5 for NO_3^-. (No urea uptake data were reported by Glibert and McCarthy, 1984.) These factors are the same whether V or ρ are compared, as the mean PN concentrations for both data sets were nearly identical. The lower rates observed in this study can partially be attributed to ambient water temperatures which were about 4°C cooler than in the Glibert and McCarthy (1984) study.

Table 5. Ambient nitrogen nutrient concentrations (μg atom N l^{-1}), specific uptake velocities (h^{-1}) of nitrogenous nutrients, NH_4^+ uptake (μg atom N l^{-1} h^{-1}) and regeneration (μg atom N l^{-1} h^{-1}) rates associated with marine snow samples collected in the Gulf Stream on dates indicated.

	5/28/82	5/29/82	5/31/82	6/1/82
NH_4^+ conc.*	0.95	1.78	6.79	3.31
NO_3^- conc.	<0.03	<0.03	<0.03	<0.03
urea conc.	n.d.†	n.d.	<0.03	<0.03
V NH_4^+	0.011	0.016	0.027	0.023
V NO_3^-	0.005	0.007	0.004	0.006
V urea	n.d.	n.d.	0.004	0.006
ρNH_4^+	0.66	0.78	1.07	1.90
NH_4^+ regen.	8.24	1.19	1.34	0.87

*All concs. were determined on small volumes of surrounding seawater collected with snow particles

†n.d. indicates not determined

The considerable difference in the rates of utilization of NO_3^- between our study and that of Glibert and McCarthy (1984) (>4x) is consistent with the hypothesis that the contribution of "new" nitrogen (sensu Dugdale and Goering, 1967) can be quite variable, and potentially underestimated in oceanic gyres (Jenkins and Goldman, 1985). McCarthy and Nevins (1986b) report an unexpectedly high uptake of NO_3^- relative to other nitrogen sources for one occupation of a Sargasso Sea station approximately 21 days before our sampling. This observation was presumably due to a relatively late response to winter mixing or the influence of a cold-cold ring. Because we did not observe as high a percentage of NO_3^- uptake we suspect that the latter explanation is the more likely and that our sampling site was sufficiently removed from the influence of this ring. This degree of variability of NO_3^- uptake was also observed by Kanda *et al.* (1985) in a series of experiments in surface waters of the Pacific.

The percent contribution of NO_3^- to total nitrogen uptake has been termed the *f* ratio (Eppley and Peterson, 1979). Frequently the *f* ratio is reported using only determinations of NH_4^+ and NO_3^- uptake. This clearly inflates the value of *f* (McCarthy and Nevins, 1986b; Harrison *et al.*, 1987), and can compromise its usefulness as an index of nutritional status (Glibert and McCarthy, 1984). In our study the *f* ratio (calculated using the sum of NH_4^+, NO_3^-, and urea uptake) ranged between 2 and 14 and was only slightly greater on average for Gulf Stream plankton than for Sargasso Sea plankton.

Our data provide us with several approaches for addressing the nutritional status of the plankton in both of our study sites. The ratios Vsat:Vtrace, *f*, and C:N of the particulate material are all relative measures of nutritional sufficiency. The Vsat:Vtrace ratios for all substrates were moderately elevated, but not to a level that would strongly indicate nitrogen deficiency (experimental and physiological variability

make it difficult to precisely establish a value of Vsat:Vtrace which indicates a nitrogen deficient status, but values exceeding 5 have been found from clearly nitrogen deficient regions: Glibert, 1988). Likewise, the relatively low values of f would suggest little nitrogen deficiency: sufficient reduced nitrogen (NH_4^+ and urea) was apparently available to meet the nitrogen requirement of the phytoplankton. Finally, average C:N ratios were between 8.5 and 10, which are slightly greater than the Redfield ratio (Redfield *et al.*, 1963) of 6.6. Elevations in the C:N ratio may be attributable to the presence of significant amounts of detrital material, and factors of two or three elevation in the Redfield ratio are generally observed under nitrogen limitation (McCarthy and Nevins, 1986a). Thus, at neither of our two sites was there strong evidence that the phytoplankton were physiologically nitrogen stressed. This conclusion is in agreement with similar conclusions drawn (using a variety of approaches) for the subtropical waters of the Florida Strait (Morris *et al.*, 1971), Sargasso Sea (Glibert and McCarthy, 1984), oligotrophic waters off Hawaii (Laws *et al.*, 1984), and the Pacific Ocean (Kanda *et al.*, 1985).

The ratio of PN:chl has been used as an index of the quantity of particulate nitrogen that can be attributed to phytoplankton (McCarthy and Nevins, 1986a). Ratios near unity suggest that the particulate material is composed primarily of phytoplankton, whereas elevated ratios indicate a higher percentage of detritus and/or heterotrophs (McCarthy and Nevins, 1986a). We found that in the Sargasso Sea, the mean PN:chl ratio for the upper two sampling depths was 5.6, and for the lower depth, was 1.5 (Table 1). In the Gulf Stream, the ratio of PN:chl was typically higher than in the Sargasso, with mean values of 8.3 for the upper two sampling depths, and 4.1 for the lower depth (Table 1). Thus, the detrital or heterotrophic contribution to particulate nitrogen was large, and, at least for the Sargasso, quite comparable to the findings of McCarthy and Nevins (1986a).

b. Rates of pelagic nitrogen utilization relative to regeneration. The tight coupling between uptake and regeneration of NH_4^+ in many water bodies is now well recognized (Harrison, 1978; Glibert, 1982; Harrison and Harris, 1986; Probyn, 1987), and a similarly close coupling has been shown for uptake and release of amino acids (Fuhrman, 1987). At least in the upper water column of oceanic systems, NH_4^+ regeneration is thought to supply essentially all of the phytoplankton nitrogen requirement (Dugdale and Goering, 1967; Jenkins and Goldman, 1985). Thus, our finding that the measured short-term rates of uptake and regeneration of NH_4^+ in the Sargasso Sea were very similar was expected (Fig. 1). In contrast, our observation of virtually no measurable NH_4^+ regeneration (with the exception of one station) in the Gulf Stream in either the <153 or <5 μm size class was quite surprising (Fig. 2). Wheeler and Kirchman (1986) observed in the Gulf Stream in May 1984 that at one station the NH_4^+ uptake and regeneration rates were similar, but at another station, the uptake rate was approximately 3-fold greater than regeneration. Clearly in the

size-fractionated, water-column samples of the Gulf Stream we were missing the major source of nitrogen regeneration, since there was otherwise little evidence for nitrogen limitation.

c. Role of marine snow in nitrogen cycling. Marine snow aggregates have been well recognized as chemical enrichment sites, particularly of nitrogenous nutrients (Shanks and Trent, 1979; Alldredge, 1979; Alldredge and Cohen, 1987). The nutrient enrichment in marine snow, in particular that of NH_4^+, strongly suggests that this material is a site of nitrogen regeneration (Shanks and Trent, 1979). The values of NH_4^+ regeneration which we report (Table 5) indicate that there can be tremendous variability in rates associated with particles (we measured a ten-fold difference in four samples), and that these aggregates must have sustained high rates of regeneration for fairly long periods of time to accumulate the concentration of NH_4^+ that we observed in the water immediately surrounding the aggregates. It is noteworthy, also, that the highest concentrations of NH_4^+ in snow aggregates occurred on May 29–June 1, the period during which concentrations of NH_4^+ and urea were elevated, albeit modestly, in the surrounding seawater. While we have no direct evidence as to the age of the particles, it is clear from their NH_4^+ concentrations that they were not freshly formed. The uptake rates of NH_4^+ (μg atom N l^{-1} h^{-1}) also varied between samples, but not to the same degree as the regeneration rates.

Alldredge *et al.* (1986) have estimated the bacterial production associated with aliquots of these same Gulf Stream marine snow samples. They found that although bacteria were somewhat enriched on marine snow relative to surrounding seawater, as has been observed in previous studies (Silver and Alldredge, 1981; Caron *et al.*, 1982), thymidine incorporation rates by marine snow bacteria were lower than in free-living populations. They also observed that production by bacteria attached to these aggregates was generally only a small percentage of the total bacterial productivity in surface waters (3.5–6.0%). Microzooplankton were also enriched in the particles we sampled, and, based on the examination of food valuoles (Caron *et al.*, 1986) and on the observations of rapid population increases (Davoll and Silver, 1986), these protozoa were grazing and reproducing rapidly in the microenvironment of the particles. This large bacterivorous may have accounted for a substantial fraction of the NH_4^+ regeneration.

Were the rates of NH_4^+ regeneration associated with marine snow sufficient to compensate for the lack of regeneration in the size-fractionated seawater samples of the Gulf Stream? To obtain rates sufficient to equal the uptake rates found for surrounding seawater, we have calculated that marine snow aggregates would have had to have been present in concentrations between 0.1 and 1 l^{-1}. The lower estimate is comparable to the diver's direct estimates, as reported in Alldredge *et al.* (1986). Thus, in the Gulf Stream at that particular time, marine snow may have been an important site of NH_4^+ regeneration. It remains unclear whether the NH_4^+ regenerated within

the snow particles was in fact dispersed to the surrounding seawater and available for phytoplankton in a significant way, but modestly elevated concentrations of NH_4^+ were noted at that time in seawater. Also, the comparative extent to which larger zooplankton or physical processes contributed to nutrient availability at that time is unknown.

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