Vol. 82: 65–74, 1992 | MARINE ECOLOGY PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published May 14

NH_4^+ regeneration and grazing: interdependent processes in size-fractionated $^{15}NH_4^+$ experiments

Patricia M. Glibert[1], Carolyn A. Miller[1], Chris Garside[2], Michael R. Roman[1], George B. McManus[3]

[1] Horn Point Environmental Laboratory, University of Maryland Center for Environmental and Estuarine Studies, PO Box 775, Cambridge, Maryland 21613, USA

[2] Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine 04575, USA

[3] Marine Environmental Sciences Consortium, PO Box 369-370, Dauphin Island, Alabama 36528, USA

ABSTRACT: We measured size-dependent NH_4^+ regeneration by ^{15}N isotope dilution on a seasonal basis in the plume of the Chesapeake Bay estuary (USA) using several different approaches. In our short-term (1 h) size-fractionation experiments, the mean rate of NH_4^+ regeneration by total or by < 202 µm plankton increased from winter to summer as a linear function of temperature. On the other hand, the mean NH_4^+ regeneration rate by < 10 µm plankton was lowest in February, increased nearly 10-fold from February to April, then remained virtually unchanged through the end of summer. In all seasons during which experiments were conducted, we observed on occasion that the rate of NH_4^+ regeneration measured in the < 10 µm fraction exceeded the rate measured in the < 202 µm fraction by 8 to 1000 %. In other experiments in which size-fractionated plankton were maintained in large carboys for ≥ 24 h, we also found that NH_4^+ regeneration in the < 10 µm fraction exceeded that in the < 64 or < 202 µm fractions. In the third type of experiment conducted, we artificially manipulated the density of copepods in unfiltered seawater and measured NH_4^+ regeneration. In 5 out of 6 such experiments, we found that as the numbers of copepods were increased to ca 20 l^{-1}, the measured rate of NH_4^+ regeneration increased to a maximum level, then decreased when additional copepods were added. From all of these experiments, we hypothesize that total NH_4^+ regeneration in a natural food web can be significantly different from that measured in component size fractions, and that this effect is a nonlinear function of zooplankton density and/or multiple trophic interactions. These effects may, in addition, be a function of internal cycling of isotopically labelled substrate. This hypothesis implies that commonly used size-fractionation techniques may be insufficient or inappropriate for describing the true regeneration rates of different size classes in situ.

INTRODUCTION

One of our objectives in the MECCAS (Microbial Exchanges and Couplings in Coastal Atlantic Systems) Program was to determine the contribution of various heterotrophs, including large zooplankton (i.e. copepods), to NH_4^+ regeneration in the plume of the Chesapeake Bay, USA. The overall objective of the MECCAS program was to evaluate the relative roles and interactions of phytoplankton, zooplankton and bacteria in the utilization and recycling of carbon and nitrogen as the plume water exits the Chesapeake Bay and mixes with coastal water. In previous papers (Boicourt et al. 1987, Glibert et al. 1991), we have demonstrated that in the transition from the Bay to the coastal ocean, phytoplankton become progressively nutrient limited, a significant dependence on regenerated nitrogen develops, and increased heterotrophic regeneration of NH_4^+ occurs.

We chose to approach the question of size-dependent NH_4^+ regeneration using several different techniques, each of which employed the stable isotope of nitrogen, ^{15}N. Our techniques included standard size-fractionation protocols, as well as experimental manipulations of plankton densities. Our results demonstrated striking seasonal differences in the patterns of NH_4^+ regeneration in different size classes. However, the variability we observed within experi-

0171-8630/92/0082/0065/$ 03.00

mental treatments leads us to suggest that the potential for NH_4^+ regeneration by microzooplankton is different when these small heterotrophs are in the presence of their grazers than when the grazers are excluded. As a consequence, grazing by large heterotrophs on their smaller counterparts may be an important mechanism regulating the flow of regenerated nitrogen.

METHODS

Sampling. Four cruises to the Chesapeake Bay plume were made: February 17 to 28, 1985 (RV 'Oceanus'), June 8 to 27, 1985 (RV 'Gyre'), August 19 to September 7, 1985 (RV 'Gyre'), and April 6 to 29, 1986 (RV 'Gyre'). The overall sampling strategy and experimental procedures have been described by Glibert et al. (1991). Sampling involved alternation between hydrographic, nutrient, and biomass mapping, and measurements of plankton composition and metabolic processes while following a near-surface (1 to 2 m) drogue for several days. The data we report here were collected on several drogue studies per cruise. Additional experiments were occasionally performed using near-surface water in which we manipulated the composition of the plankton. These were conducted as time permitted, and thus were not done on each cruise. Experiments were not replicated between years; thus, we are assuming that each cruise is representative of its season.

Experiments. Three general types of experiments were performed. First, on virtually every sample collected, the rates of NH_4^+ uptake and regeneration were determined in size-fractionated samples. Rates were determined for the unfractionated (February), <202 µm (other seasons), and <10 µm (all seasons) plankton assemblages (Nitex screening).

Secondly, on occasion, rates of NH_4^+ uptake and regeneration were determined for unfiltered plankton assemblages which were altered by the addition of known numbers of copepods (20 to 80 l^{-1}). Copepods were collected from the surface mixed layer using a 64 µm net. Individual copepods were isolated microscopically with a Pasteur pipette, transferred to filtered seawater, then added to the experimental bottles. Thirdly, 2 experiments were conducted during the April cruise in which plankton assemblages of 3 size classes (unfractionated, <64 µm, and <10 µm) were contained in 20 l carboys and observed for 24 h.

In all 3 types of experiments, tracer and isotope dilution ^{15}N techniques were used to determine rates of NH_4^+ uptake and regeneration. Incubation experiments were started within 45 min of sample collection, and all experiments were done in acid-cleaned polycarbonate bottles. $^{15}NH_4^+$ additions were 0.03 to 0.05 µg-at. N l^{-1}. Immediately after the addition of tracer, an aliquot was filtered and the concentration and atom % enrichment of substrate were determined. In the case of the 'copepod addition' experiments, this step was done immediately prior to addition of copepods to avoid the potential stress of filtration on the copepods. In the case of the 'large carboy' experiments, aliquots were withdrawn from the carboys at intervals ranging from 1 to several hours, and dispensed into polycarbonate bottles for incubation. Incubations were 1 h in duration.

Filtration of sample through precombusted (1 h at 550 °C) Whatman GF/F filters was used to terminate ^{15}N incubations. The filtration was stopped before all the sample had passed through, the filtrate was collected into a polyethylene bottle, then the filtration was allowed to proceed. Before the last of the sample was drained, a small amount of filtered seawater was added to the filter tower to rinse the filter. Filters were then placed in plastic or precombusted aluminum foil envelopes, and dried overnight at 50 °C.

Zooplankton biomass and grazing. During the drogue studies, zooplankton were collected with a submersible pump and hose system that delivered water (400 l min^{-1}) to shipboard plankton nets (200 and 64 µm) and sensors of flow rate and temperature. Zooplankton nitrogen biomass was determined from preserved samples (Control Equipment CHN Analyzer) and corrected for preservation loss (Roman et al. 1988). The rates of zooplankton (>200 µm) grazing on heterotrophs were estimated from the uptake of [^{3}H-methyl]thymidine-labeled particles during 1 h *in situ* incubation experiments. Plexiglass 5 l chambers (General Oceanics) with 64 µm mesh covering the bottom opening were lowered to 3 m and then raised to the surface to gently concentrate zooplankton. A messenger triggered the closing of the bottle and released the radioactive tracer. After incubation, zooplankton were collected on 200 µm sieves and rinsed onto preweighed Nuclepore filters. Filters were treated, and isotopic counting was done, as described by Roman et al. (1990). The grazing impact of the zooplankton community (l filtered m^{-3} h^{-1}) was calculated as the product of the zooplankton biomass and their filtration rate [(mg zooplankton C)$^{-1}$ h^{-1}]. This was converted to the amount of nitrogen ingested (µg-at. N filtered l^{-1} h^{-1}) by multiplying the grazing rate times the particulate nitrogen (PN).

Methods for sample collection for the enumeration of flagellate abundance (both during the drogue studies and carboy experiments) are described more fully in McManus & Fuhrman (1990). Samples of 20 ml were preserved with 1 % (final concentration) filtered glutaradehyde and stored at 4 °C for 12 to 24 h before slides

were prepared. Subsamples of 10 ml were stained with 1.65 µg ml^{-1} of proflavin hemisulfate (Haas 1982), filtered onto 0.8 µm pore size Nuclepore filters, and mounted in Cargille's Type A immersion oil on glass slides. The slides were frozen at −20 °C until they could be counted on an Olympus BH2 epifluorescence microscope equipped with a 50 W mercury lamp.

Analytical. NH_4^+ concentrations were determined on a Technicon AutoAnalyzer, with precision of ± 0.05 µg-at. N l^{-1}. Analyses of PN were done using a Control Equipment CHN Analyzer. The ^{15}N atom % enrichment of the filtrates from the NH_4^+ experiments was determined by distillation and mass spectrometry as described by Glibert et al. (1982). Filters were prepared for mass spectrometery using a protocol described by Fiedler & Proksch (1975) and modified to suit our system. Mass spectrometry was as described in Glibert et al. (1991). The rates of NH_4^+ uptake and regeneration were calculated according to Glibert et al. (1982). These values are virtually identical to those derived from the Laws (1985) model (Glibert & Garside 1989).

RESULTS

Seasonal environmental conditions

The hydrographic conditions and prevailing environmental conditions for each of the 4 periods of study have been described in Malone & Ducklow (1990) and Glibert et al. (1991). Temperatures of the plume water ranged from 2.4 to 5.7 °C in February, 10.0 to 12.9 °C in April, 20.2 to 23.0 °C in June, and 25.3 to 27.5 °C in August. Ambient nitrogenous nutrients did not exceed a few tenths of a µg-at. N l^{-1} in the summer months, but ranged from the detection limit to several µg-at. N l^{-1} during winter and spring (Glibert et al. 1991).

Size-fractionated NH_4^+ regeneration

The mean rate (µg-at. N l^{-1} h^{-1}) of NH_4^+ regeneration by total or by <202 µm filtered plankton increased with season (Fig. 1A), and in fact, this seasonal change was described by a linear increase as a function of temperature (Glibert et al. 1991). In contrast, the mean rate of NH_4^+ regeneration by the <10 µm population was lowest in February, increased nearly 10-fold from February to April, and from April to August remained virtually unchanged.

The biomass (µg-at. N l^{-1}) of zooplankton >64 µm was higher in April and August than in February and June, and the grazing rate of zooplankton >200 µm was ca 4 to 10 times higher in August than during the other seasons (Fig. 1B, C). In contrast, abundance of bacterivorous flagellates varied with season, and on average actually decreased by a factor of about 2 from February to August for the drogue data (Fig. 1D; McManus & Fuhrman 1990). Uncertainty about biovolume:C and C:N conversion factors lead us to express the flagellate data as abundance rather than biomass (Borsheim & Bratbak 1987). However, since mean flagellate diameter also decreased from

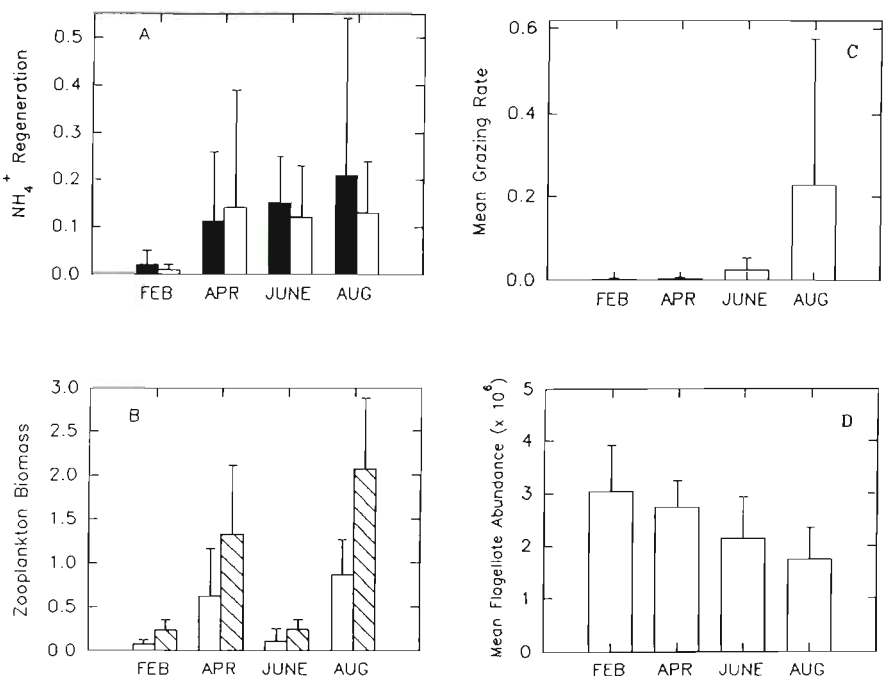

Fig. 1. (A) Mean NH_4^+ regeneration rates (µg-at. N l^{-1} h^{-1}) for the total (February) and <202 µm size fraction (other seasons), filled bars; and for the <10 µm fraction, open bars. (B) Biomass (µg-at. N l^{-1}) of zooplankton >202 µm (open bars) and >64 µm (hatched bars). (C) Mean grazing rate (µg-at. N l^{-1} h^{-1}) on heterotrophic particles by zooplankton >202 µm. (D) Mean flagellate abundance (10^6 flagellates l^{-1}). Error bars represent standard deviations

February to August, the observed decline would also be true for biomass.

The rates of NH_4^+ regeneration for the 2 size classes of plankton determined for one representative drogue study from each season are given in Fig. 2. For all available data from the drogue studies, there were frequent occasions (n = 1 in February, 5 in April, 7 in June, and 7 in August) during which the measured rate of NH_4^+ regeneration in the < 10 µm plankton fraction exceeded the rate measured in the < 202 µm fraction. This discrepancy ranged from ca 8 % to > 1000 % (Table 1). A similar comparison of the fraction of NH_4^+ uptake contributed by the < 10 µm samples revealed a total of 11 such observations throughout all seasons of study, and in no case did these discrepancies exceed 300 % (data not shown). There were no significant correlations between the periods during which such discrepancies occurred in NH_4^+ uptake and in regeneration.

Copepod-addition experiments

In 5 out of the 6 experiments conducted, we observed that as the numbers of copepods were artificially increased in the experimental bottles to ca 20 l^{-1}, the measured rate of NH_4^+ regeneration increased to a maximum level, then decreased when additional copepods were added (Fig. 3). In the sixth experiment (Stn 238, April 1986), the maximum rate of NH_4^+ regeneration was observed for the sample having no additional copepods, and as copepods were added, the NH_4^+ regeneration rate dropped to undetectable levels.

Large-carboy experiments

In both large (20 l) carboy experiments, plankton were contained and followed for 24 h. In the first experiment, the NH_4^+ regeneration rate for the unfractionated plankton exceeded that measured in the other size fractions for the sampling points taken at 3 and 14 h, then declined (Fig. 4A). However, with the exception of time 0, the measured NH_4^+ regeneration rate in the carboy containing the < 10 µm fraction consistently exceeded that measured in the carboy containing the 64 µm fraction, and thus by the end of the experiment, regeneration was greatest in the carboy containing the < 10 µm fraction. This is consistent with the findings of McManus & Fuhrman (1990) that in this particular experiment, significant growth of heterotrophic flagellates occurred in the carboy containing the < 10 µm population, but only after a 14 h lag.

During the second experiment, the NH_4^+ regeneration in the carboy containing the unfractionated plankton was virtually undetectable throughout the time course, and that measured in the carboy containing the

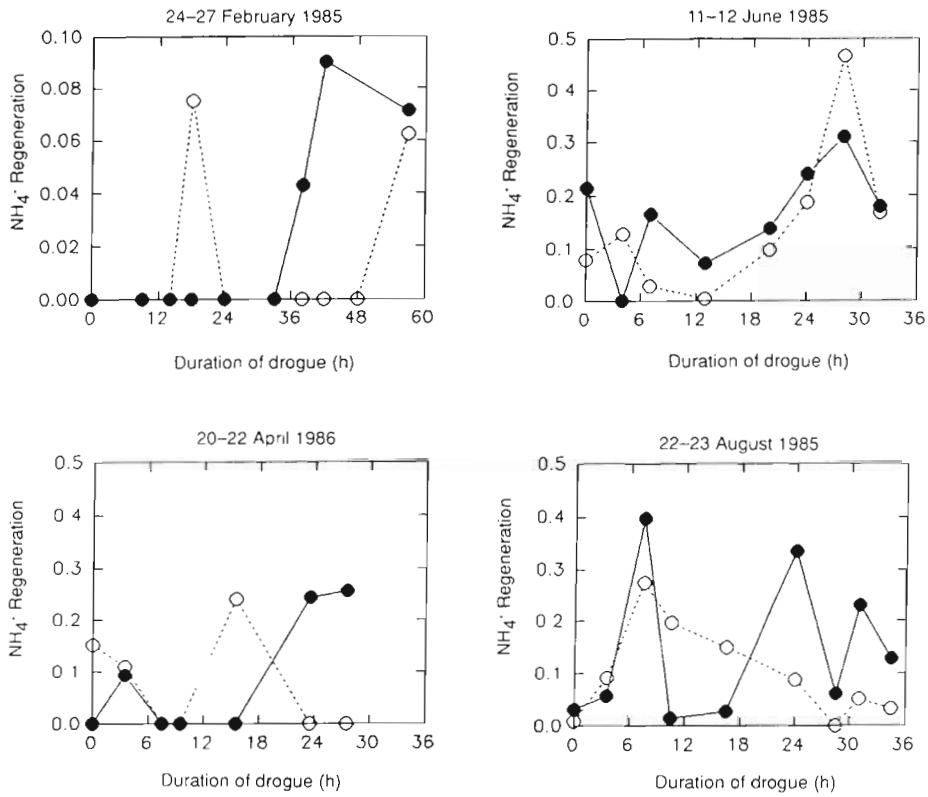

Fig. 2. Rates of NH_4^+ regeneration (µg-at. N l^{-1} h^{-1}) by 2 size classes of plankton for 1 representative drogue study from each month of study. ●: Rates determined for the total (February) and 202 µm (other seasons) size fraction; ○: rates determined for the < 10 µm size fraction

Table 1. Means and ranges of the percent contribution to NH_4^+ regeneration by the < 10 µm plankton fraction relative to the total fraction (February) or the < 202 µm fraction (other seasons) for the month of study indicated

Month	Mean %	Range
February	21	0 – >100[a]
April	86	0 – 119
June	147	7 – 1038
August	222	0 – 1369

[a] For one sampling period the rate of NH_4^+ regeneration in the < 10 µm fraction was 0.08 µg-at. N l^{-1} h^{-1}, while NH_4^+ regeneration in the comparable whole water sample was not detectable

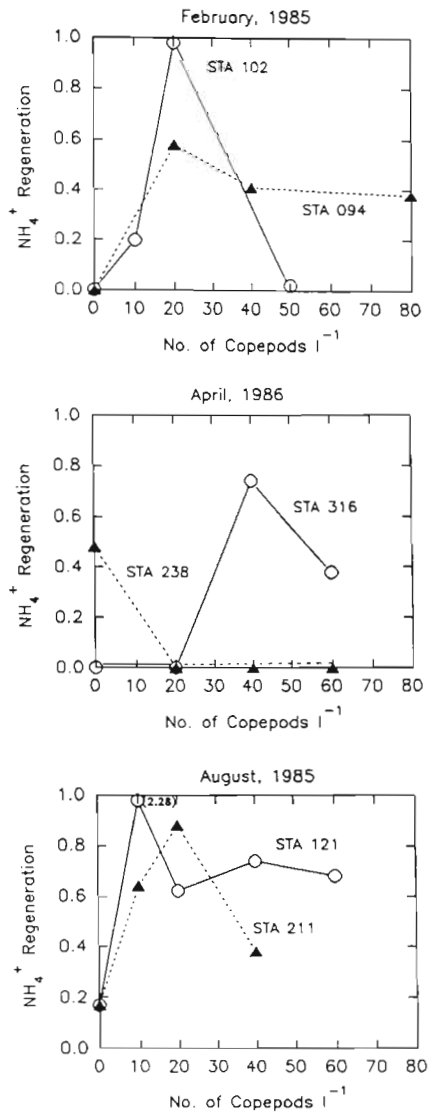

Fig. 3. Rates of NH_4^+ regeneration determined on plankton assemblages to which additional copepods were added

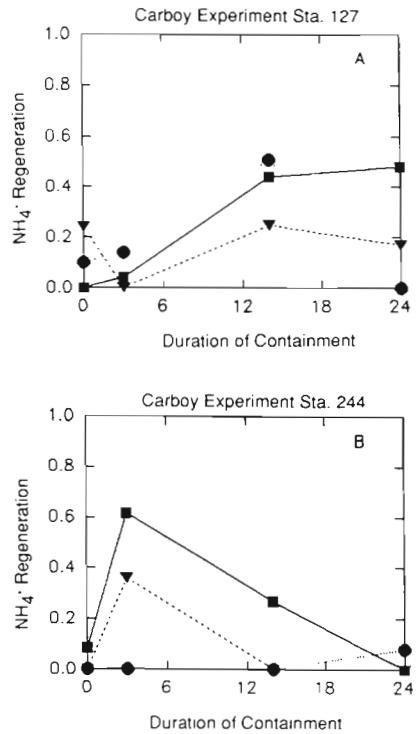

Fig. 4. (A, B) Rates of NH_4^+ regeneration for plankton assemblages as a function of duration of containment (h) in 20 l carboys for experiments conducted at 2 stations. ●: Carboy containing unfractionated plankton; ▼: carboy containing <64 µm plankton; ■: carboy containing < 10 µm plankton

< 10 µm fraction exceeded that measured in the carboy containing the <64 µm plankton for the first 14 h (Fig. 4B). The rates were extremely low for all fractions at the 24 h sampling interval. Growth of heterotrophic flagellates in all 3 carboys in this experiment was insignificant (McManus & Fuhrman 1990).

DISCUSSION

Throughout these experiments we observed that regeneration processes occurring in altered communities, such as those that have been size-fractionated, did not always represent a fraction of the total regeneration rate as measured in the whole (or < 202 µm) seawater. Our findings are similar to those of Hopkinson et al. (1989) in which respiration rates by organisms passing through a 1 µm screen exceeded, up to 180 %, the rates of respiration in 208 µm and 10 µm filtered samples. We discuss the extent of, and the potential processes contributing to, these altered rates in detail below. We also discuss seasonal patterns in these data for the purpose of comparison with other size-fractionation studies, in spite of our recognition of the inherent difficulties.

Average seasonal patterns

Seasonal differences in the average rates of NH_4^+ regeneration between the < 202 µm (or unfractionated) and the < 10 µm size classes are striking (Fig. 1A). The increase in NH_4^+ regeneration by the larger size class, representing the whole population in February, and the < 202 µm population in other seasons, paralleled the increase in temperature and also reflected the general increase in zooplankton biomass from winter to summer (Fig. 1B).

There was very little seasonal change in the rate of NH_4^+ regeneration by the < 10 µm fraction (Fig. 1A). There was approximately a 10-fold increase from February to April, when the water was 10 °C warmer, but with further warming there was no further increase in the rate of NH_4^+ regeneration. This pattern is consistent with the flagellate abundance (Fig. 1D). Flagellates did not increase over the seasonal period, and in fact decreased on average by a factor of 2 in the drogue studies. This relatively low flagellate biomass, and associated low variability, suggest that the flagellates were tightly controlled by grazers (McManus & Fuhrman 1990). The mean grazing rate on heterotrophs by copepods ([^{3}H]thymidine-labelled particles) was significantly higher in August than during any other season of study (Fig. 1C).

While the averaged data provide some insight into the overall changes occurring over a broad spectrum of differing temperatures, nutrients, and populations, the variability within each season was great, and the differences in responses between size fractions were unexpected. From each approach we took we observed that rates of NH_4^+ regeneration by the smaller size fraction were occasionally greater than the comparable measurements in the larger size fractions, and that as the density of larger heterotrophs increased, the rate of NH_4^+ regeneration by the < 10 µm fraction decreased. There are several possible explanations for these observations: experimental or analytical errors, real differences in rates of release when the plankton composition is artificially altered, and differences in the pathways of the flow of ^{15}N tracer with variable grazing.

Potential methodological artifacts in size-fractionated regeneration measurements

^{15}N uptake and isotope dilution techniques are subject to a host of potential artifacts (Dugdale & Wilkerson 1986, Glibert & Capone in press), primarily due to the facts that manipulations of the sample must occur, and the results of several independent chemical analyses are used in the final rate calculation (e.g. ambient concentrations of substrate, particulate nitrogen concentration, ^{15}N atom % of the particulate and dissolved fractions, etc.). We have carefully scrutinized the reported results and can find no reason to suspect analytical errors that could systematically bias the rates measured in the smaller size fractions.

To assess size-dependent differences in rates of NH_4^+ regeneration using substrate isotope dilution protocols, pre-filtration is necessary. The dilution of isotope is determined using the filtrate of a sample, not the filter, and it would be impossible to isolate 'size-fractionated' pools of regenerated NH_4^+ by filtration after incubation. The inherent assumption in size-fractionation measurements of NH_4^+ regeneration is that the metabolic processes measured after size-fractionation are the same as those occurring prior to fractionation.

Effects due to sample handling, such as increased release due to organismal stress upon filtration and confinement, have been recognized in previous studies (Glibert et al. 1982), and may well have been exaggerated in the smaller fractions assayed in these studies. At least some ciliates are susceptible to filtration stress (Gifford 1985, Nagata & Kirchman 1990), and even gentle gravity filtration can cause some dissolved free amine release (Kirchman et al. 1989). Contamination of NH_4^+ by the 10 µm netting itself is a possible artifact in these measurements; however, we experimentally determined the concentration of NH_4^+ before and after the 10 µm filtration, and only on rare occasions were differences observed. Netting was thoroughly cleaned between each use. Filtration of the sample through the 10 µm netting may also have resulted in some release of dissolved free amino acids. If this occurred, it would have increased the available organic nitrogen substrate for bacterial uptake, which in turn may have increased NH_4^+ regeneration. Nitrogen-sufficient bacteria are more likely to regenerate NH_4^+ than nitrogen-deficient bacteria (Goldman et al. 1987, Roman et al. 1988, Hopkinson et al. 1989).

Trophic alterations as a result of size-fractionation

Most bulk NH_4^+ regeneration measurements (in fact, most phytoplankton and microbial processes in general) are made with water prescreened through a 200 (± 20) µm mesh net, thereby excluding the larger zooplankton - adult copepods being the most abundant of those. The rationale is that in the measurement of phytoplankton processes, the impact of grazing by large zooplankton on these populations should be minimized. However, screening does not remove grazers that are similar in size to their prey, and multiple trophic interactions (Rassoulzadegan & Sheldon 1986) occur even in the small size classes.

Our results from both the carboy experiments conducted during April, and the size-fractionation experiments conducted at all 4 seasons in which NH_4^+ regeneration in the < 10 µm fraction occasionally exceeded that measured in a larger size fraction, are strongly suggestive that removal of grazing pressure by prescreening can result in potentially very different rates of NH_4^+ regeneration to those that are actually occurring when all components of the food web are present. In fact, the results from the short-term copepod-addition experiments (conducted in 3 seasons) and size-fractionation measurements (conducted in all 4 seasons) strongly suggest that the response to trophic alterations can be very rapid (i.e. within the incubation time of 1 h). In both the above sets of experiments, altering the natural assemblage by size fractionation, or by adding animals in somewhat higher than natural density, resulted in drastically different NH_4^+ regeneration rates. In the copepod-addition experiments, additions of relatively few copepods greatly enhanced NH_4^+ regeneration rates; additions of more copepods did not. In the size-fractionation experiments, the < 10 µm fraction occasionally regenerated more NH_4^+ than did larger size fractions, implying that microzooplankton grazing on < 10 µm heterotrophs controlled NH_4^+ regeneration by that fraction on those occasions.

If we assume, for example, a simple 3-consumer linear food 'chain', the interactions among consumers and their impacts on NH_4^+ regeneration become readily apparent (Fig. 5). In this simple model, we assume that no food limitation is occurring at any trophic level, and that changes in NH_4^+ regeneration within a size class are only a function of the density of heterotrophs within that size fraction. Thus, with relatively few copepods present (from prescreening, etc.) the rate of NH_4^+ regeneration can be relatively high, with microzooplanktonic ciliates, and bacteria, primarily responsible. In this example, grazing by ciliates on flagellates would keep their contribution to total NH_4^+ regeneration in check. On the other hand, when many copepods are present, the overall rate of NH_4^+ regeneration may be reduced relative to the condition with few copepods, and this may be primarily due to the reduction in the 10 to 200 µm microzooplankton population. Thus, if the microzooplankton population not being grazed were bacterivorous, the relative grazing pressure on bacteria would remain high (e.g. Bjørnsen et al. 1988, Ducklow & Taylor 1990, Stoecker & Capuzzo 1990), whereas if the food source preferred by the macrozooplankton were a bactivorous microzooplankter, or phytoplankter for that matter, the relative control on the bacterial population may be quite different (Rassoulzadegan & Sheldon 1986, Sherr et al. 1986). The degree to which a simplistic scheme such as we have depicted in Fig. 5 may be occurring is debatable. There is no doubt, however, that population dynamics such as these must influence our measurements of NH_4^+ regeneration when we artificially separate one group from another.

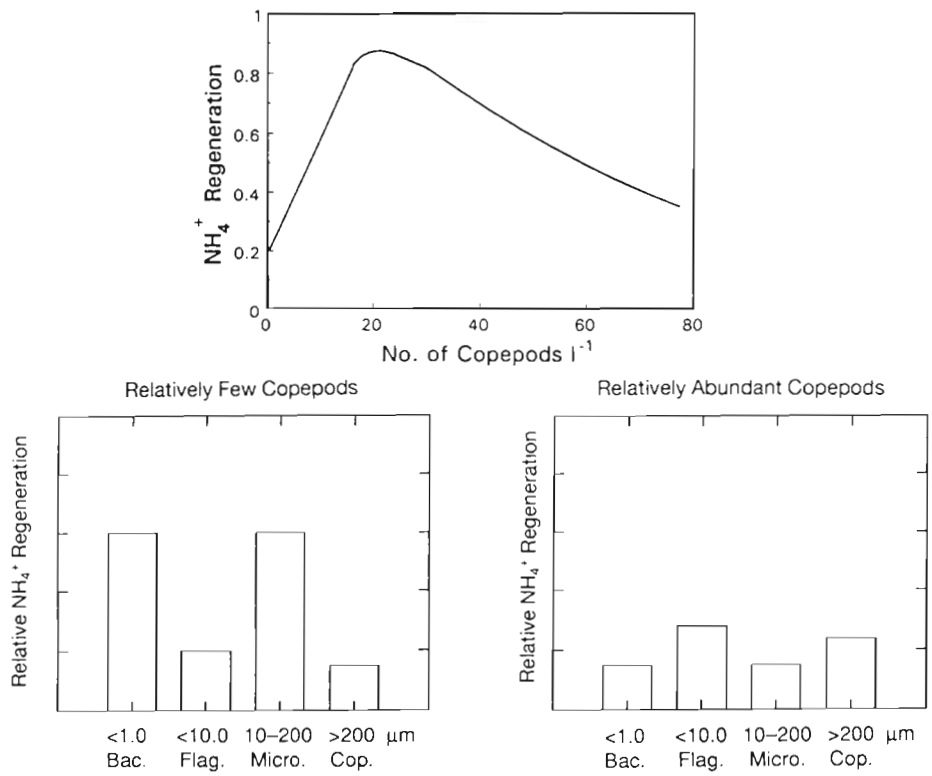

Fig. 5. Schematic representation of the effect of increasing numbers of copepods l^{-1} on NH_4^+ regeneration rates (upper panel), and proposed contributions of plankton populations within a simplified 'food chain' having relatively few copepods (10 to 20 l^{-1}) and more abundant copepods (> 40 l^{-1}). Bac.: bacteria; Flag.: flagellates; Micro: microzooplankton; Cop.: copepods

Such trophic interactions may also help to explain the observations in oceanic waters that virtually all the measured NH_4^+ regeneration is by organisms < 35 µm, and in some cases < 5 µm (Harrison 1978, Glibert 1982, Glibert et al. 1988). It has been argued by Goldman (1984), for example, that the reported high percentage of NH_4^+ regeneration measured in small size fractions cannot be in balance with the assimilation of NH_4^+ on time scales much beyond those of the experiment, and that high nutrient conversion efficiencies of protozoa (Goldman et al. 1985) are inconsistent with high regeneration rates in small size fractions.

In our experiments, measurements of regeneration and grazing were not conducted in the same experimental bottles, but grazing estimates were made on comparable plankton assemblages. We did not observe a consistent direct relationship between the measured grazing rate on heterotrophic particles by copepods and the frequency or magnitude of the discrepancy between size fractionation results (data not shown). Such a relationship would not necessarily be expected because of the short time interval of the incubations (1 h) and the fact that NH_4^+ regeneration or excretion is not only a function of grazing, but also growth and nutritional state (e.g. Park et al. 1986, Roche-Mayzaud et al. 1991).

Potential differences in the flow of ^{15}N tracer with variable grazing

Grazing is a necessary, but may not always be a sufficient, explanation for our observations in which NH_4^+ regeneration was frequently greater in the < 10 µm fraction compared to larger fractions and, in particular, for observations in which total rate of NH_4^+ regeneration declined as the abundance of copepods increased. In the copepod-addition experiments, the regeneration rates were too large to be attributable solely to the copepods, even after the rate of NH_4^+ regeneration in the 'control' bottles was subtracted. For example, if the NH_4^+ regeneration rates observed here in bottles containing 20 copepods l^{-1} were due totally to copepods, the rates would be ca 10-fold higher than expected from physiological data. An excretion rate of 0.5 µg-at. N h^{-1} (20 copepods)$^{-1}$ represents a release of 480 % body nitrogen copepod^{-1} d^{-1}, compared to the more commonly accepted release of 30 to 50 % body nitrogen copepod^{-1} d^{-1} (Roman 1983). Since the control bottles measured the rate of regeneration in unfiltered seawater, the effect of added copepods must incorporate the sum of all possible processes affecting the ^{15}N tracer.

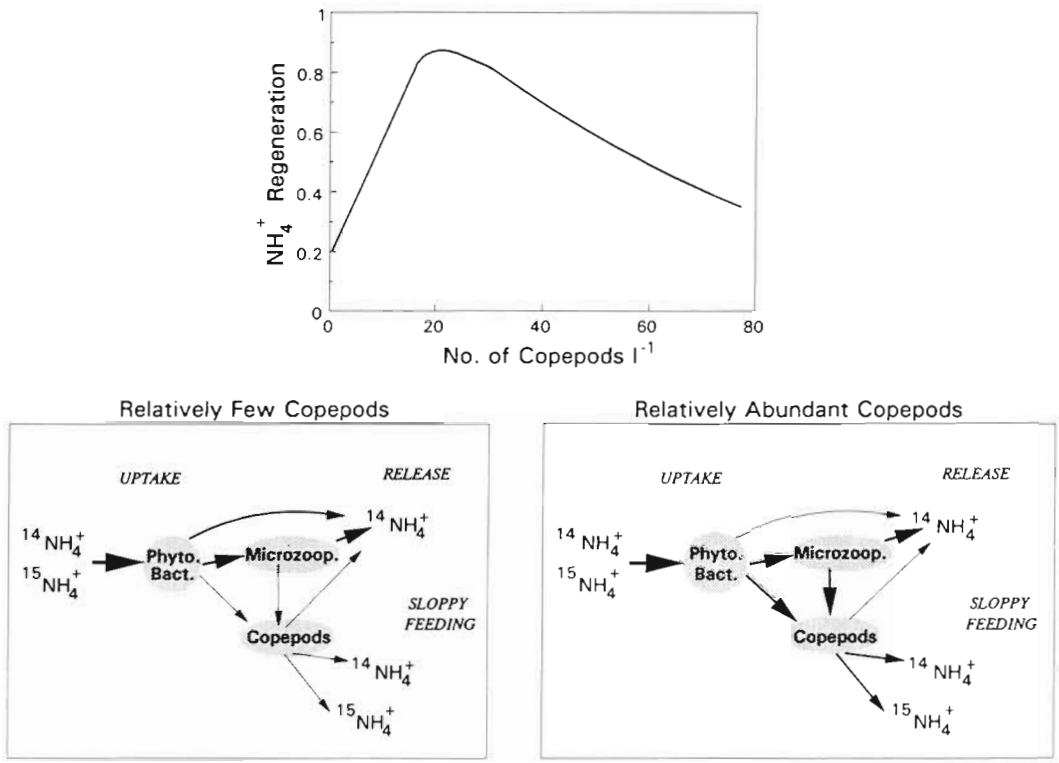

Fig. 6. Schematic representation of the effect of increasing numbers of copepods l^{-1} on NH_4^+ regeneration rates (upper panel), and the proposed pathways by which both ^{15}N-labelled and unlabelled NH_4^+ might be released to the NH_4^+ pool when relatively few copepods (10 to 20 l^{-1}) and more abundant copepods (> 40 l^{-1}) are present. Thicker arrows represent larger flux

Copepods can be 'sloppy feeders' (Dagg 1974, Lampert 1978, Eppley et al. 1981). Through sloppy feeding, internal pools of phytoplankton are disrupted. This process results in the release of internal NH_4^+, which may be in the form of either $^{14}NH_4^+$ or $^{15}NH_4^+$. We hypothesize that at relatively low copepod densities and grazing rates, direct release of $^{14}NH_4^+$ from excretion would dominate and isotope dilution of the $^{15}NH_4^+$ substrate would be observed. However, at higher copepod densities and grazing rates, the release of internally labelled phytoplankton NH_4^+ pools would potentially be higher. Thus more ^{15}N could be recycled to the NH_4^+ pool, giving the appearance of a lower NH_4^+ regeneration rate, as there would be less isotope dilution in the NH_4^+ substrate (Fig. 6). Even in an experiment of short duration, the relative effects of these differing processes are difficult to quantify because reincorporation of released NH_4^+ is occurring.

CONCLUSIONS

Seasonal differences existed in the mean rates of NH_4^+ regeneration by size fraction, which generally reflected seasonally changing temperatures and population differences. However, the variability within individual size-fractionation and plankton-community manipulated experiments leads us to believe that total nitrogen regeneration in a natural food web can be significantly different from that in the component size fractions, and this effect is a nonlinear function of macrozooplankton density. New protocols may have to be developed to estimate size-dependent microbial processes which minimize trophic disruptions.

Acknowledgements. This research was supported by NSF grants OCE84-09465 (P.M.G.), OCE84-07033 (C.G.), OCE84-06700 (M.R.R.), OCE84-06712 (J. Fuhrman and G.B.M.) and OCE87-016909 as part of the MECCAS program. This is Contribution No. 2282 from the Center for Environmental and Estuarine Studies, University of Maryland, and No. 92001 from the Bigelow Laboratory for Ocean Sciences. We thank Sue Banahan, Robert Ray, Jean Garside, Gary Tilton, Gary Newhart, Anne Gauzens, Kathy Ashton, Joe Montoya, Robert Allen and others who assisted with the collection and analysis of the data presented here. We also thank our other MECCAS collaborators who have assisted in numerous ways. We are grateful for the cooperation of the captains and the crews of the research vessels RV 'Oceanus' and RV 'Gyre' This manuscript benefited from the helpful comments of the anonymous reviewers.

LITERATURE CITED

Bjørnsen, P. K., Riemann, B., Horsted, S. J., Neilsen, T G., Pock-Stein, J. (1988). Trophic interactions between nanoflagellates and bacterioplankton in manipulated seawater enclosures. Limnol. Oceanogr. 33: 409–420

Boicourt, W. C., Chao, S.-Y., Ducklow, H. W., Glibert, P. M., Malone, T C., Roman, M. R., Sanford, L. P., Fuhrman, J. A., Garside, C., Garvine, R. W. (1987). Physics and microbial ecology of a buoyant estuarine plume on the continental shelf. EOS (Trans. Am. geophys. Un.) 68: 666–668

Borsheim, K. Y., Bratbak, G. (1987). Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar. Ecol. Prog. Ser. 36: 171–175

Dagg, M. J. (1974). Loss of prey body contents during feeding by an aquatic predator. Ecology 55: 903–906

Ducklow, H. W., Taylor, A. H. (1990). Modelling session summary. In: Reid, P. C., Turley, C. M., Burkill, P. H. (eds.) Protozoa and their role in marine processes. Springer, Berlin, p. 431–442

Dugdale, R. C., Wilkerson, F. P. (1986). The use of ^{15}N to measure nitrogen uptake in eutrophic oceans; experimental considerations. Limnol. Oceanogr. 31: 673–689

Eppley, R. W., Horrigan, S. G., Fuhrman, J. A., Brooks, E. R., Price, C. C., Sellner, K. (1981). Origins of dissolved organic matter in southern California coastal waters: experiments on the role of zooplankton. Mar. Ecol. Prog. Ser. 6: 149–159

Fiedler, R., Proksch, G. (1975). The determination of nitrogen-15 by emission and mass spectrometry in biochemical analysis: a review. Anal. chim. Acta 78: 1–62

Gifford, D. J (1985). Laboratory culture marine planktonic oligotrichs (Ciliphora, Oligotrichida). Mar. Ecol. Prog. Ser. 23: 257–267

Glibert, P. M. (1982). Regional studies of daily, seasonal, and size fraction variability in ammonium remineralization. Mar. Biol. 70: 209–222

Glibert, P. M., Capone, D. G. (in press). Mineralization and assimilation in aquatic, sediment, and wetland systems. In: Knowles, R., Blackburn, T. H. (eds.) Nitrogen isotope techniques in plant, soil, and aquatic systems. Academic Press, New York

Glibert, P. M., Dennett, M. R., Caron, D. A. (1988). Nitrogen uptake and NH_4^+ regeneration by pelagic microplankton and marine snow from the North Atlantic. J. mar. Res. 46: 837–852

Glibert, P. M., Garside, C. (1989). Discussion on 'Spring recycling rates of ammonium in turbid continental shelf waters off the southeastern United States.' Contin. Shelf Res. 9: 197–200

Glibert, P. M., Garside, C., Fuhrman, J. A., Roman, M. R. (1991). Time-dependent coupling of inorganic and organic nitrogen uptake and regeneration in the plume of the Chesapeake Bay estuary and its regulation by large heterotrophs. Limnol. Oceanogr. 36: 895–909

Glibert, P. M., Lipschultz, F., McCarthy, J. J., Altabet, M. A. (1982). Isotope dilution models of uptake and remineralization of ammonium by marine plankton. Limnol. Oceanogr. 27: 639–650

Goldman, J. C. (1984). Oceanic nutrient cycles. In Fasham, M. J. R. (ed.) Flows of energy and materials in marine ecosystems. NATO Conf. Ser. IV. Marine sciences, Vol. 13. Plenum, New York, p. 137–170

Goldman, J. C., Caron, D. A., Andersen, O. K., Dennett, M. R. (1985). Nutrient cycling in a microflagellate food chain. I. Nitrogen dynamics. Mar. Ecol. Prog. Ser. 24: 231–242

Goldman, J. C., Caron, D. A., Dennett, M. R. (1987). Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. Limnol. Oceanogr. 32: 1239–1252

Haas, L. W. (1982). Improved epifluorescence microscopy for observing planktonic micro-organisms. Annls Inst. océanogr., Paris 58(S): 261–266

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

Hopkinson, C. S., Jr, Sherr, B., Weibe, W. J (1989). Size fractionated metabolism of coastal microbial plankton. Mar. Ecol. Prog. Ser. 51: 155–166

Kirchman, D., Soto, Y., van Wanbeck, F., Bianchi, M. (1989). Bacterial production in the Rhone River plume: effects of mixing on relationships among microbial assemblages. Mar. Ecol. Prog. Ser. 53: 267–275

Lampert, W. (1978). Release of dissolved organic carbon by grazing zooplankton. Limnol. Oceanogr. 23: 831–835

Laws, E. (1985). Analytic models of NH_4^+ uptake and regeneration experiments. Limnol. Oceanogr. 30: 1340–1350

Malone, T. C., Ducklow, H. W. (1990). Microbial biomass in the coastal plume of Chesapeake Bay: phytoplankton-bacterioplankton relationships. Limnol. Oceanogr. 35: 296–312

McManus, G. B., Fuhrman, J. A. (1990). Mesoscale and seasonal variability of heterotrophic nanoflagellate abundance in an estuarine outflow plume. Mar. Ecol. Prog. Ser. 61: 207–213

Nagata, T., Kirchman, D. L. (1990). Filtration-induced release of dissolved free amino acids: application to cultures of marine protozoa. Mar. Ecol. Prog. Ser. 68: 1–5

Park, Y C., Carpenter, E. J., Falkowski, P. G. (1986). Ammonium excretion and glutamate dehydrogenase activity of zooplankton in Great South Bay, New York. J. Plankton Res. 8: 489–503

Rassoulzadegan, F., Sheldon, R. W. (1986). Predator-prey interactions of nanozooplankton and bacteria in an oligotrophic marine environment. Limnol. Oceanogr. 31: 1010–1021

Roche-Mayzaud, O., Mayzaud, P., Biggs, D. C. (1991). Medium-term acclimation of feeding and of digestive and metabolic enzyme activity in the neritic copepod *Acartia clausii* I. Evidence from laboratory experiments. Mar. Ecol. Prog. Ser. 69: 25–40

Roman, M. R. (1983). Nitrogenous nutrition of marine invertebrates. In: Carpenter, E. J., Capone, D. G (eds.) Nitrogen in the marine environment. Academic Press, New York, p. 347–384

Roman, M. R., Ducklow, H. W., Fuhramn, J. A., Garside, C., Glibert, P. M., Malone, T. C., McManus, G. B. (1988). Production, consumption, and nutrient cycling in a laboratory mesocosm. Mar. Ecol. Prog. Ser. 42: 39–52

Roman, M. R., Furnas, M. J., Mullin, M. M. (1990). Zooplankton abundance and grazing at Davies Reef, Great Barrier Reef, Australia. Mar. Biol. 105: 73–82

Sherr, B. F., Sherr, E. B., Fallon, R. D., Newell, S. Y (1986). Small aloricate ciliates as a major component of the marine heterotrophic nanoplankton. Limnol. Oceanogr. 31: 177–183

Stoecker, D. K., Capuzzo, J. (1990). Predation on protozoa: its importance to zooplankton. J. Plankton Res. 9: 901–915

This article was submitted to the editor

Manuscript first received: October 30, 1991
Revised version accepted: March 17, 1992