THE EFFECT OF ORGANIC CARBON AND NITROGEN ADDITIONS ON INORGANIC NITROGEN UPTAKE BY PHYTOPLANKTON AND BACTERIA

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ABSTRACT

This study examines the effects of enrichment with organic carbon and nitrogen on inorganic nitrogen partitioning between phyto- and bacterio- plankton. Strongly preferential uptake of ammonium over nitrate was observed by both the phytoplanktonic and bacterial fractions, with RPI_{NH4} values typically between 1 and 5. The bacterial fraction (<0.8μm) was found to be responsible for as much as 48-75% of community uptake of ammonium; while the netplanktonic fraction was observed to take up approximately 50% of intact community uptake of nitrate. The addition of amino acids appeared to mediate bacterial competition for ammonium, indicating their preference for DON as a nitrogen source and allowing increased ammonium uptake by the nanoplanktonic fraction. The effect of glucose enrichment was complicated by the presence of protozoans, which appeared to be indirectly responsible for decreased ammonium uptake due to depletion of this substrate in the presence of added glucose. The nanoplankton appeared to be responsible for the least nitrogen uptake with respect to biomass, indicating that they may have been subject to competition pressure from both the bacterial and netplanktonic fractions.

INTRODUCTION

Primary production in the oceans can be divided into new and regenerated production (Dugdale and Goering 1967). New production depends on allochthonous inputs of nitrogen (predominantly nitrate) introduced into the euphotic zone via advection and turbulent diffusion. Regenerated production is based on reduced forms of nitrogen (ammonium and urea), which are regenerated in the upper layer of the water column via decomposition and excretion (Dugdale & Goering 1967). The ratio of new to total nitrogen assimilation is known as the f-ratio (Eppley & Peterson 1979) and has important implications for the carbon flux of the system. The f-ratio provides an index of the quantitative importance of nitrate uptake relative to total nitrogen uptake, giving an indication of the proportion of nitrogen throughput which is derived from sources external to the system (new nitrogen). This is indicative of the potential for phytoplankton population increase, and the amount of primary production available for export to higher trophic levels (Dugdale & Goering 1967; Eppley and Peterson 1979), and sedimentation out of the euphotic zone.

Downward flux of organic particles from the euphotic zone is largely a function of particle size, with nanoplankton and picoplankton sinking considerably slower than netplankton (Bienfang 1980). Malone (1980) suggested that ammonium (NH_4^+) tended to support production by cells in the nanoplankton size range (<20μm), while netplanktonic (>20μm) production was based mainly on nitrate (NO_3^-). Several authors subsequently found a relationship between phytoplankton size and the favoured species of nitrogen assimilated (e.g. Nalewakjo & Garside 1983; Probyn 1985; Probyn and Painting 1985; Wheeler & Kirchman 1986; Harrison & Wood 1988).

The availability of NH_4^+ and NO_3^- may therefore potentially affect the size distribution of the phytoplankton population, influencing the flow of organic material through the food chain, and rates of flux out of the upper layers of the ocean.

The importance of ammonium to primary production:

Nitrogen is considered to be the most important element limiting primary production in the open ocean (Dugdale & Goering 1967; Fuhrman *et al.* 1988), with NO_3^- and NH_4^+ assuming major significance (e.g. Brown *et al.* 1972; Bienfang 1975). The growth of phytoplankton may often be supported primarily by NH_4^+ supplied by excretion (Kiefer & Atkinson 1984). Phytoplankton exhibit strongly selective uptake of ammonium when it is available, and the presence of ammonium is thought to inhibit and/or reduce nitrate assimilation (e.g. Bienfang 1975; McCarthy *et al.* 1977; Price *et al.* 1985; Wheeler & Kirchman 1986). This suppression effect is triggered by NH_4^+ concentrations as low as 0.5-1.0 μg-at $N.l^{-1}$ (Eppley *et al.* 1969; Goering *et al.* 1970; McCarthy & Eppley 1972). Due to the higher affinity for NH_4^+ as compared with NO_3^-, rates of ammonium assimilation may still be high in regions of high nitrate concentration (e.g. McCarthy *et al.* 1975; Probyn 1985).

The qualitative preference of phytoplankton for different nitrogen species can be expressed as a relative preference index (RPI: McCarthy *et al.* 1977). This index incorporates aspects of the relative uptake rate and concentration of a particular nutrient, compared with alternative available nutrients, giving an indication of the physiological preference of phytoplankton for that particular nutrient. An RPI value greater than unity indicates relative preference for a given nutrient, while a value less than unity indicates relative discrimination against that nutrient. McCarthy *et al.* (1977) report RPI values of NH_4^+-N in Chesapeake Bay as always >1 and seldom <2, and found a

negative correlation between RPI values of NO_3^--N and the total ambient nitrogen, indicating that nitrate uptake increases as availability of the preferred nitrogen species decreases. Preferential uptake of ammonium has been observed even when nitrate comprised >90% of total nitrogen available (McCarthy *et al.* 1977; Probyn & Painting 1985), and it appears that NO_3^- uptake is a function of NH_4^+ availability as well as of NO_3^- concentration (McCarthy *et al.* 1977).

Fuhrman *et al.* (1988) found ammonium pool turnover rates during summer to be as high as $116\%.h^{-1}$ (Long Island Sound, N.Y.), and suggested that ammonium in the dissolved phase is rapidly taken up upon its release into the water, indicating a tight coupling between regeneration and uptake. This is supported by the finding of Glibert *et al.* (1982a) that both laboratory cultures and field assemblages are able to utilize NH_4^+ rapidly when it is delivered to the medium in pulse fashion.

Organisms are able to directly utilize NH_4^+ in amino acid synthesis whereas NO_3^- must first undergo a stepwise reduction from nitrate -> nitrite -> hyponitrite -> hydroxylamine -> ammonium (Bienfang 1975; Billen 1984). This reduction pathway requires an energy expenditure of 77.4 $kcal.mole^{-1}$ NO_3- at 25°C (Goering *et al.* 1970), and is facilitated by a substrate induced NO_3^- reductase enzyme system (Eppley & Coatsworth 1968; Eppley *et al.* 1969; Eppley & Rogers 1970). Suppression of this system (and potentially the nitrate permease system) appears to occur in the presence of NH_4^+ (Eppley *et al.* 1969; Bienfang 1975; Billen 1984), giving rise to the observed inhibition of nitrate assimilation, and acting as a potential energy saving mechanism. Nitrate uptake and reduction may be uncoupled under transient conditions (Dortch *et al.* 1979), and given the complexity of nitrogen uptake and assimilation physiology, it is difficult to attribute the observed inhibition of nitrate uptake by ammonium to any one

mechanism (Dortch 1990). Nevertheless, it is evident that ammonium is an important nitrogen source for primary production.

The role of bacteria as competitors for ammonium:

Historically, dissolved inorganic nitrogen (DIN) uptake was considered to be due to phytoplankton, and uptake of dissolved organic nitrogen (DON) to bacterioplankton (e.g. Wright & Hobbie 1965; Dugdale & Goering 1967; Billen 1984). However, more recent studies have shown that bacteria frequently assimilate ammonium as a major nitrogen source. Various authors have investigated ammonium assimilation by bacteria and found that bacteria may be responsible for 30-90% of ammonium uptake (Laws *et al.* 1985; Wheeler & Kirchman 1986; Fuhrman *et al.* 1988; Tupas & Koike 1990). Tupas and Koike (1990) found concurrent uptake of both ammonium and DON which contradicts the concept of ammonium uptake ocurring only under DON limited conditions. Several authors have noted the effectiveness of bacteria as competitors with phytoplankton for ammonium (e.g Eppley *et al.* 1977; Horstmann & Hoppe 1981). In addition, phytoplankton generally lack uptake mechanisms which would enable them to compete successfully with bacteria for organic nutrients (Wright & Hobbie, 1965). Recent work indicates that little if any nitrate uptake is due to bacterial activity (Wheeler & Kirchman 1986). Brown *et al.* (1972) found that several strains of bacteria were capable of growth on nitrate, but that bacterial uptake of NO_3^- was completely suppressed in the presence of amino acids and ammonium. It thus seems likely that competition between phytoplankton and bacteria for NH_4^+ will shift phytoplankton nitrogen assimilation from NH_4^+ to NO_3^- by means of competition pressure, and by decreasing ambient NH_4^+ concentrations to a level where the suppression effect is no longer operative. Little research has been carried out

investigating the circumstances under which such competition occurs, and its impact on primary production.

The importance of amino acids:

Amino acids are the primary nitrogen source of bacteria, and may affect their nitrogen metabolism (Billen 1984). It has been found that the presence of amino acids tends to inhibit and suppress ammonium uptake and transport (Jayakamur & Barnes 1987; Fuhrman *et al.* 1988; Kirchman *et al.* 1989) and also the related enzyme systems (Brown *et al.* 1972; Magasanik 1982). Heterotrophic bacteria have developed efficient amino acid transport systems with high substrate affinity (Billen 1984). The strong selection for amino acids has a sound energetic basis as they are directly utilizable metabolically (Goldman *et al.* 1987) and are a source of both nitrogen and carbon (Brown *et al.* 1972; Billen 1984; Fuhrman & Bell 1985; Fuhrman & Ferguson 1986; Fuhrman 1987). They are also essential as structural components of protein, and are involved in many metabolic processes (Keller *et al.* 1982; Ferguson & Sunda 1984; Goldman *et al.* 1987; Jorgensen 1987). The flux of dissolved free amino acids (DFAA) may be a quantitatively important route of carbon and nitrogen transfer through the ecosystem (Hollibaugh *et al.* 1980).

Bacterial protein comprises approximately 50% of bacterial biomass (Parsons *et al.* 1977; Hagstrom *et al* . 1984), and recent studies indicate that amino acids make up a large fraction of bacterial biomass production (Ferguson & Sunda 1984). This is supported by the fact that amino acid uptake appears to be tightly coupled with protein synthesis (Kirchman & Hodson 1984). Bacteria utilize extracellular amino acids for protein synthesis in preference to *de novo* synthesis of amino acids (Kirchman *et al.* 1985), responding rapidly to increased DON concentrations by decreasing rates of *de*

novo amino acid synthesis (Kirchman & Hodson 1986). This would represent large savings in the energy cost of growth. Protein synthesis in the absence of amino acids may account for as much as 60% of bacterial energy budgets (Stouthamer 1973).

Several authors have recorded amino acid uptake by some species of phytoplankton (e.g. Stephens & North 1971; Wheeler *et al.* 1974). However, this seems to occur only under extremely nitrogen limited conditions and growth is slower than on equivalent concentrations of nitrate (Wheeler *et al.* 1974). Williams (1970) and Keller *et al.* (1982) demonstrated that amino acid uptake was largely due to bacterioplankton rather than phytoplankton. It would therefore seem that, while some phytoplankton may, if stressed, utilize amino acids, this process plays a minor role in primary production.

Dissolved free amino acids in the ocean:

Amino acids, though present at very low concentrations in the ocean are among the most labile of the nitrogenous carbon compounds (Lee & Cronin 1984), and have rapid turnover rates (Lee & Bada 1977; Ferguson & Sunda 1984; Fuhrman 1987). In seawater, dissolved organic carbon (DOC) forms the major organic matter fraction (Williams 1975; Keller *et al.* 1982), with DON comprising 20-80% of total nitrogen (Billen 1984). However, a large fraction of the DOC and DON in the ocean is refractory, has a long turnover time, and is not readily utilized by bacteria; while approximately 10% comprises labile organic matter which is rapidly assimilated and supports nearly all of bacterial production (Billen 1984).

Although concentrations of total organic nitrogen vary widely from estuarine and coastal, to open ocean environments, ambient DFAA concentrations remain fairly

uniform regardless of distance from the coast or the season (Riley & Segar 1970; Andrews & Williams 1971; Crawford *et al.* 1974; Williams *et al.* 1976; Lee & Bada 1977; Billen 1984). Numerous studies have indicated the importance of DFAA in bacterial production (e.g. Andrews & Williams 1971; Crawford *et al.* 1974; Ferguson & Sunda 1984) and, according to Sharp (1983) and Henrichs *et al.* (1984), DFAA and dissolved combined amino acids (DCAA) may comprise up to 50% of DON in the sea. Lee & Bada (1977) found DCAA concentrations in near surface waters to be 10-15 times those of DFAA, and concentrations of DFAA are typically much less than of DCAA (e.g. Ferguson & Sunda 1984; Fuhrman & Ferguson 1986). However, DCAA are composed of high molecular weight proteins and must be hydrolysed before uptake can occur, and can therefore not be directly assimilated by bacteria (Law 1980). Coffin (1989), however, reported DCAA to comprise 51% of bacterial amino acid nitrogen demand, but found DFAA to be taken up more efficiently than DCAA. In terms of energetic cost, DFAA logically represent the more desirable nitrogen source for growth.

Despite low oceanic concentrations (average literature value, 40 $nmol.l^{-1}$: e.g. Riley & Segar 1970; Pocklington 1971; Clark *et al.* 1972; Lee & Bada 1977; Palumbo *et al.* 1983), DFAA may be directly assimilated (Goldman *et al.* 1987), their turnover rates are high (Lee & Bada 1977; Ferguson & Sunda 1984; Fuhrman 1987), and they are considered to be of major nutritional importance in marine ecosystems (Hollibaugh *et al.* 1980; Hammer & Kattner 1986).

Bacterial competition for ammonium:

Pelagic marine DFAA concentrations are low (e.g. Mopper & Lindroth 1982; Carlucci *et al.* 1984; Ferguson & Sunda 1984; Fuhrman & Bell 1985; Fuhrman & Ferguson

1986). It is suggested that DFAA availability in the ocean may be limiting and that under such circumstances additional nitrogen sources must be exploited to supply bacterial nitrogen requirements for growth (Williams *et al.* 1976; Ferguson & Sunda 1984; Hagstrom *et al.* 1984; Jorgensen & Sondergaard 1984; Laws *et al.* 1985; Fuhrman & Ferguson 1986; Kirchman *et al.* 1986; Wheeler & Kirchman 1986). It is indicated that bacterial utilization of ammonium is dependant on the contribution of amino acids to their nitrogen requirements. Where amino acids are limiting bacteria are expected to assimilate ammonium to fulfil their nitrogen requirements. Carbon and nitrogen are incorporated stoichiometrically, and the bacterial C:N ratio ($C:N_b$, approximately 5:1 : Nagata 1986; Goldman *et al.* 1987) is thus maintained. The balance between nitrogen mineralization and incorporation is dependent on the ratio of the C:N ratio of the substrate ($C:N_s$) to $C:N_b$ (Billen 1984), and ammonium uptake by bacteria is subject to carbon-limitation. Where the $C:N_s$ is less than that of the bacterial population ($C:N_b$), it is expected that nitrogen will be excreted in the form of NH_4^+ (Goldman *et al.* 1987). Kirchman *et al.* (1989) found ammonium regeneration to occur when >80% of bacterial nitrogen requirements were supplied by amino acids, while ammonium was taken up when amino acids supplied <80% of the nitrogen required for growth. Caron *et al.* (1988) suggest that the $C:N_s$ must be <10 for nitrogen regeneration by bacteria to occur, and this is unlikely to be the case in an oceanic environment. Tupas and Koike (1990), however, found NH_4^+ regeneration to occur while the $C:N_s$ was between 12 and 18, indicating that the $C:N_s$ is not a conclusive determinant of bacterial regeneration of NH_4^+. Hollibaugh *et al.* (1980) found catabolism of amino acids by bacteria to account for about 60% of measured ammonium production. However, although bacteria were classically viewed as mineralizers of organic nitrogen, several authors (e.g. Johannes 1965; Williams 1981; La Roche 1983; Palumbo *et al.* 1983) question the significance of this process, and

suggest that bacteria are inefficient mineralizers, directly contributing only a minor fraction of regenerated nutrients. To date little is known of the conditions under which ammonium is assimilated by bacteria to fulfil their nitrogen requirements, or the extent to which this influences primary productivity and the ratio of new to total production.

In examining the relative uptake of ammonium and nitrate by the microplanktonic assemblage (<200μm), the central question which this study addresses is:-
What are the effects of organic additions (amino acids and glucose) on ammonium and nitrate assimilation by heterotrophic bacteria and phytoplankton?

To examine this problem a series of hypotheses have been erected:-

1. Amino acids

Additions of amino acids are expected to inhibit NH_4^+ uptake by bacteria. This would increase NH_4^+ availability to phytoplankton, thus enhancing ammonium uptake, and inhibiting nitrate uptake by the phytoplanktonic assemblage resulting in a decreased f-ratio.

2. Glucose

Glucose additions are expected to stimulate bacterial uptake of NH_4^+ if DOC limited, thus enhancing NO_3^- uptake due to reduced NH_4^+ availability to phytoplankton. The ratio of new to regenerated production would thus be increased.

3. Amino Acids and Glucose

It is suggested that simultaneous addition of amino acids and glucose will potentially result in increased bacterial utilization of NH_4^+ thus decreasing availability of NH_4^+ to phytoplankton and enhancing NO_3^- uptake. Bacterial excretion of NH_4^+ is expected to cease in the presence of an abundance of carbon supplied by the glucose, further limiting ammonium availability.

METHODS

Preparation:

Sea water was collected approximately 4 km offshore in Table Bay, Cape Town, at a location removed from any kelp beds; harbours; and storm water or sewage outlets. The incubation water was collected in 20*l* plastic buckets which had been washed with 10M HCl then rinsed with de-ionised water. Immediately prior to collection each bucket was rinsed three times in the sea at the collection site. Incubation water was then collected from the other side of the boat to avoid any potential contamination arising from this rinsing. The water was immediately transported back to the laboratory and transferred into eight 20*l* polyethylene incubation bags. Any toxic polymers which might potentially affect the incubations were minimized by leaching the bags with 2 µm filtered seawater for 24 hours then rinsing with distilled water prior to collection of the water. The prepared incubation bags were then allowed to stand for 24 hours to acclimatise to laboratory conditions.

Experimental procedure:

All incubations were carried out at the Sea Fisheries Research Institute in the algal culture laboratory (Sea Point branch). Incubations were performed under constant illumination (provided by a bank of 40W cool-white fluorescent tubes) and temperature ($15^{o}C$). Air was bubbled through the water after passing through activated carbon and then cottonwool to remove contaminant particles. The bubbling of air provided oxygen, CO_2, constant mixing, and a positive pressure within the bags to limit any potential airborne contamination.

At the start of the incubation time course, the incubation bags were innoculated variously with ^{15}N-labelled ammonium ($^{15}N\text{-}NH_4^+$), 0.131 $\mu mol.l^{-1}$; ^{15}N-labelled nitrate ($^{15}N\text{-}NO_3^-$), 1 $\mu molN.l^{-1}$; glucose, 20 $\mu mol.l^{-1}$; and an amino acid mixture, 1 $\mu molN.l^{-1}$ (see Table 1). The amino acid mixture was made up to resemble the composition of algal protein (see Table 2).

Sampling:

Subsamples were taken immediately (T_0), and again at approximately 2 hrs (T_1), 6 hrs (T_2), 12 hrs (T_3), and 24 hrs (T_4) after the start of the experiment (see Table 3).

Samples taken at T_0 to determine ammonium, nitrate, amino acid and aqueous $^{15}N\text{-}NH_4^+$ concentrations were passed through pre-combusted Whatman GF/F glass fibre filters (47mm diameter) at <5mmHg, prior to processing in the relevant manner as described below.

Table 1: The terminology used to identify the different treatments, nutrient additions and ^{15}N tracers used to examine nutrient uptake.

TREATMENT	^{15}N TRACER	NUTRIENT ADDITION	
		AMINO ACIDS	GLUCOSE
CONTROL 1	$^{15}N\text{-}NH_4+$	♦	♦
^{15}NH4+AA	$^{15}N\text{-}NH_4+$	*	♦
^{15}NH4+GLU	$^{15}N\text{-}NH_4+$	♦	*
^{15}NH4+AA+GLU	$^{15}N\text{-}NH_4+$	*	*
CONTROL 2	$^{15}N\text{-}NO_3^-$	♦	♦
^{15}NO3+AA	$^{15}N\text{-}NO_3^-$	*	♦
^{15}NO3+GLU	$^{15}N\text{-}NO_3^-$	♦	*
^{15}NO3+AA+GLU	$^{15}N\text{-}NO_3^-$	*	*

The addition of a particular nutrient is indicated by a * in that column, while the lack of a nutrient addition is denoted by a ♦.

Table 2: The relative composition of amino acids in the amino acid mixture.

l-amino acids	% composition	formula	µg.ml-1
serine	21	$C_3H_7NO_3$	22.07
glycine	17	H_2NCH_2COOH	12.76
threonine	16	$C_4H_9NO_3$	19.06
valine	15	$C_5H_{11}NO_2$	12.59
alanine	5	$C_3H_7NO_2$	4.45
phenylalanine	5	$C_9H_{11}NO_2$	8.26
glutamic acid	5	$C_5H_9NO_4$	7.36
aspartic acid	4	$C_4H_7NO_4$	5.32
arginine	3	$C_6H_{14}N_4O_2$	1.58
histidine	3	$C_6H_{10}ClN_3O_2$	2.10
leucine	3	$C_6H_{13}NO_2$	3.97
lysine	1	$C_6H_{15}ClN_2O_2$	0.91
proline	1	$C_5H_9NO_2$	1.15
tyrosine	1	$C_9H_{11}NO_3$	1.81
isoleucine	0.5	$C_6H_{13}NO_2$	0.66

Table 3: Time periods and the corresponding incubation intervals for the $^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}NO_3^-$ spiked incubations.

TIME PERIOD	TIME (h)	
	$^{15}N\text{-}NH_4^+$ spiked incubations	$^{15}N\text{-}NO_3^-$ spiked incubations
T_0	0	0
T_1	2	3
T_2	6	7
T_3	12	14
T_4	24	26

At subsequent sampling periods (T_1, T_2, T_3 and T_4), the experiment was terminated by filtration. Samples were fractionated in order to enable distinctions to be drawn between three size fractions; the netplanktonic fraction (20-200μm), the nanoplanktonic fraction (0.8-20μm) and the picoplanktonic fraction (<0.8μm).

Post-fractionation was selected over pre-fractionation as it was felt that the inherent error introduced by fractionation was minimized this way. The most serious constraint imposed by pre-fractionation is the disruption of important trophic interactions. Since this study investigates the effects of competition between several fractions, these interactions are vital, and pre-fractionation was therefore not suitable to the purposes of the study. In addition, pre-fractionation resulting in the inevitable rupturing of cells would release unknown quantities of nutrients into the incubation chambers at the start of the experiment, obscuring the effect of the selected nutrient additions. This would be greatly complicated by the fact that the degree of enrichment varies with the severity of the filtration process required to produce the different size fractions. Potential effects on the viability of the cells in these size fractions was also considered to be undesirable. Post-fractionation has inherent error associated with it due to the rupturing of cells during filtration which causes leakage of nutrients into the filtrate, and resulting in potential underestimation of particulate nutrient content, and overestimation of aqueous substrate concentration. While post-fractionation is not a trouble-free option, it was felt that any error associated with this process were less severe than those of pre-fractionation, given their *a postiori* nature as opposed to the *a priori* nature of pre-fractionation error.

In order to achieve post-fractionation, four litres were removed from each incubation chamber at each sampling time. Of this, two litres were passed through a 0.8μm

Nuclepore filter at less than 100mm Hg (the filtrate thus representing the picoplanktonic fraction), one litre through a 20μm mesh (representing the nanoplanktonic and picoplanktonic fractions) and one litre left intact (representing all three size fractions). It was thus possible at a later stage to differentiate between the data for the three size fractions by means of simple subtraction. Aliquots (50ml) of <0.8μm filtrate were preserved with 2% formalin (by final volume). These were subsequently (within two weeks) examined by means of epifluorescent microscopy after staining with DAPI (Porter & Feig 1980) to determine the proportion of autotrophic organisms present in this fraction. Kirchman *et al.* (1989) found the picoplanktonic fraction (<0.8μm after gravity filtration) to comprise largely heterotrophic cells (>95%), although filtration under 120 mm Hg increased the proportion of autotrophic cells in the filtrate by nearly an order of magnitude. In this study the cells were found to be predominantly heterotrophic (about 90%), with the autotrophic component comprising exclusively cyanobacterial cells. The picoplanktonic fraction therefore can therefore be regarded as essentially representing an heterotrophic bacterial assemblage.

Subsequently the particulate content of the <0.8μm filtrate (2*l*), the <20μm filtrate (1*l*) and the unfiltered sample (1*l*) were retained on individual pre-combusted Whatman GF/F filters (47mm). The filters were wrapped in foil and frozen for particulate ^{15}N analysis. The filtrate was retained and samples collected for determination of aqueous $^{15}N\text{-}NH_4^+$ concentrations (900ml; bags 1-4), amino acid concentrations (15ml), and substrate nutrient concentrations (NH_4^+: 3x5ml, bags 1-4; NO_3^-: 15ml, bags 5-8). Kirchman *et al.* (1989) reported a 50% retention of the heterotrophic bacterial assemblage on Whatman GF/F filters. In order to determine the proportion of cells retained by Whatman GF/F filtration in this study several aliquots of 0.8μm filtrate pre- and post GF/F filtration were preserved and examined by epifluorescent microscopy as

above. It was found that only between 8 and 10% of cells passed through the Whatman GF/F filters. Correction was not made for this.

Analyses:

Inorganic nitrogen:

Triplicate samples for ammonium determination (5ml aliquots) were frozen immediately in 10x160mm glass test tubes sealed with parafilm. Samples were analysed within a week according to Grasshoff (1976) scaled down for 5ml sample volumes. Thorough mixing following reagent additions is particularly important in ammonium analysis (McCarthy *et al.* 1977) and may present a problem when analysing small volume samples. This was facilitated by the use of a vortex mixer. Samples were left to stand overnight at room temperature to allow colour development. Full colour development occurs within approximately 10 hours and stability is good for approximately 40 hours (Probyn pers. comm.). Samples for nitrate analysis (15ml) were placed in 25ml glass scintillation vials and frozen immediately for later analysis on a Technicon II autoanalyser according to standard autoanalytical techniques modified by Mostert (1983).

Amino acid analysis:

Samples for amino acid analysis (15ml) were frozen immediately in 25ml glass scintillation vials for later analysis. Analysis was carried out by means of precolumn derivatization using 9-fluorenylmethyl chloroformate (FMOC-Cl) and reversed phase HPLC (high performance liquid chromatography) separation. This technique eliminates the need for desalination at the sample preparation stage, thereby greatly reducing the time required for sample preparation and analysis, and the potential

associated error (Lee and Bada 1975; Lee and Cronin 1982; Mopper and Lindroth 1982; Lee *et al.* 1983; Garside *et al.* 1988). The technique requires very small amounts of sample (of the order of 1 ml), and yields a characteristically stable derivative formation and complete amino acid reaction, while allowing detection of as little as femtomolar quantities of amino acids (Einarsson *et al.* 1983). The detection limits are, however, dependent on constraints such as the purity of solvents used and the type of detector employed. In this study, detection limits were inhibited by solvent impurities (methanol) and thus examination of amino acid concentrations are limited to those generating a peak area of greater than 500 000 (pico- to nano- molar quantities).

A mass of 0.388mg of FMOC-Cl (Fluka Chemicals) was dissolved in 100 ml acetone (Uvasol, Merck) to give a 15mM solution. A 1M boric acid solution adjusted to a pH of 6.2 with 1M sodium hydroxide gave a borate buffer solution. This was then adjusted to a pH of 7.7 by dilution (1:5) with Milli-Q water. In an acid washed, methanol rinsed glass test-tube, 0.25ml of borate buffer and 1.25ml of FMOC-Cl was added to 0.4ml of sample. This was immediately mixed and allowed to stand approximately 30 seconds before extraction with 1ml of pentane. The sample was extracted twice for approximately three minutes to ensure removal of all excess FMOC-Cl. While it may remove some of the less polar amino acid derivatives, rapid and efficient extraction is of major importance in reducing the formation of hydrolysis product caused reaction of the fluorochrome with water. If formed this gives rise to a large water peak which will swamp amino acid peaks in its proximity. A vortex mixer was used throughout the derivatization procedure to ensure thorough mixing. Chromatographic separations and analyses were achieved using a composite HPLC system linked to a Beckman pump unit. A 100µl Rheodyne injection loop was used coupled to a 7cm Altex Ultrasphere XL-ODS column with 0.3µm packing. A

Perkin-Elmer 150 Xenon Power Supply unit provided the power for a Perkin-Elmer 650-10S Fluorescence Spectrophotometer which was set at 260nm excitation and 320nm emission wavelengths, linked to a Hewlett-Packard 3390A Integrator which provided traces of the eluted peaks and printouts of the peak areas. In order to convert peak areas to actual concentrations of the various amino acids, an amino acid mixture was made up to several standards (0.1μM, 0.5μM and 1μM). Standard peaks were identified and the peak areas regressed against the standard concentrations, with the y-intercept forced through the origin. Sample peak areas were converted to concentrations (expressed as $\mu mol.l^{-1}$) using the corresponding regression data for the equivalent standard peaks.

Particulate nitrogen and nitrogen uptake:

The determination of ^{15}N was carried out according to the Kjeldahl-Rittenberg procedure (Fiedler and Proksch 1975). All glassware used was acid washed in 10N HCl and rinsed with distilled water and then high purity Milli-Q water to minimize contamination.

Initially the Kjeldahl method was employed to convert organically bound nitrogen to ammonium. Particulate matter retained on Whatman GF/F glass fibre filters was covered with 5ml of digestion solution, containing sulphuric acid with SeO_2 as catalyst, and digested at 375^{o}C for six hours. High purity Milli-Q water was then added and the digested samples frozen overnight. An excess of 50% sodium hydroxide was then added to the sample, rendering the sample alkaline and allowing the ammonium to be driven off and recovered by means of steam-distillation. The resultant ammonium gas was subsequently entrapped in a hydrochloric acid solution. Just less than 50ml of distillate was collected and then made up to 50ml by addition of

Milli-Q water. Particulate nitrogen was measured concurrently with nitrogen uptake using a 0.2ml aliquot of this distillate. This was made up to 5ml with high purity Milli-Q water for determination of total nitrogen (in the form of ammonium) according to Grasshoff (1976). To the remainder of the sample a few drops of Tashiros indicator was added to test the pH of the sample. Any alkaline samples were acidified by the addition of hydrochloric acid solution, this was necessary to prevent loss of nitrogen as ammonia gas. Samples were then evaporated under a stream of air over moderate heat to a volume of approximately 0.2ml. A contamination of 0.611 μmolN per sample was calculated from blank samples processed concurrently with experiment samples. This correction factor was included in all calculations.

By means of the Rittenberg technique, the ammonium in the sample was subsequently converted to nitrogen gas by the addition of sodium hypobromite, after all impurities had been driven out by repeated freezing and thawing of the sample under vacuum. The vacuum was produced by a Model G-II vacuum system consisting of an oil rotary pump ($150l.min^{-1}$) and an oil diffusion pump ($250l.sec^{-1}$), and monitored by means of a Pirani gauge ($3x10^{-3}$ Torr). Nitrogen gas was trapped in a pyrex tube, and activated by application of a high voltage charge, before admission to the mass spectrometer for determination of the 14N:15N ratio. The spectrometer used in this study was a Jasco ^{15}N-analyser, Model N-150.

Nitrate uptake rates were determined according to a model derived from Dugdale and Goering (1967):

$$\rho = (PE \times PN)/(R \times T) \qquad (1)$$

where ρ = uptake rate ($\mu molN.l^{-1}.h^{-1}$); PE = percent ^{15}N enrichment of the particulate fraction in excess of natural abundance (%); PN = particulate nitrogen concentration ($\mu mol.l^{-1}$); T = duration of the incubation (h); and R = aqueous ^{15}N enrichment at T_0 (%): R = amount of added $^{15}N\text{-}NO_3^-$ ($\mu molN.l^{-1}$) divided by the total amount of measured $^{15}N\text{-}NO_3^-$ ($\mu molN.l^{-1}$) including the added $^{15}N\text{-}NO_3^-$.

In all calculations correction was made for the natural abundance of ^{15}N in seawater (0.365%) and for measured contamination during digestion-distillation (0.611 μmolN). The measured atom % of ^{15}N in the samples were corrected for machine error (emission spectrometer) by means of a calibration curve calculated by Dr T.A. Probyn, by standard analysis, for the emission spectrometer used in this study.

Ammonium uptake rates were calculated similarly using the Dugdale and Goering model in which correction was made for isotope dilution according to Glibert *et al.* (1982a):

$$\rho = (PE \times PN)/(R \times T) \qquad (2)$$

where ρ = uptake rate ($\mu molN.l^{-1}.h^{-1}$); PE = percent ^{15}N enrichment of the particulate fraction in excess of natural abundance (%); PN = particulate nitrogen concentration

($\mu mol.l^{-1}$); T = duration of the incubation (h); and R = exponential average aqueous enrichment (%), calculated as follows:

$$R = (R_0/kT)(1-e^{-kT}) \quad (3)$$

where $k = (-\ln R_t/R_0)/T$, and R_0 and R_t are measured aqueous ^{15}N enrichments at the start and finish of the incubation (%).

Aqueous ammonium:

To satisfy the mass requirements for atomic spectrometry, 1 ml of NH_4^+ standard stock solution (10 $\mu mol.ml^{-1}$) was added to 900ml of Whatman GF/F filtrate in a 1 litre borosilicate Schott bottle. This was then shaken and frozen for later analysis. Aqueous ammonium was recovered from alkaline solution by diffusion based on release of ammonium from a basic solution, and retention by an acidic environment. Excess MgO (muffled at 550°C for approximately 4 hours) was added to the thawed sample giving a pH above 9. Above this was suspended a folded Whatman GF/F glass fibre filter (25mm) wetted with 0.05ml of 5N HCl. The bottle was tightly resealed and left to stand for about 3 weeks at room temperature to allow recovery of the ammonium on the filter. This technique usually results in an excess of 50% of the ammonium recovered (Probyn 1987). At the end of this recovery period, the filters were carefully removed, placed in 5ml Milli-Q water and shaken. An aliquot was removed for determination of ammonium content (Grasshoff 1976) and the remainder stained and evaporated as described above. Regeneration of ^{15}N was determined by atomic emission spectroscopy according to the Rittenberg oxidation procedure (Fiedler and Proksch 1975) described above. Ammonium regeneration rates were calculated

using a modification of the Blackburn-Caperon model (Blackburn 1979; Caperon *et al.* 1979):

$$r = [\ln(R_t/R_0)]/[\ln(S_t/S_0)] \times (S_0-S_t)/T \qquad (4)$$

where r = regeneration of ammonium ($\mu molN.l^{-1}.h^{-1}$); R_t and R_0 are measured aqueous ammonium enrichments at the start and finish of the incubation (%); S_t and S_0 are the measured ammonium substrate concentrations at the beginning and end of the incubation ($\mu molN.l^{-1}$); and T is the duration of the incubation (h). Since ammonium concentrations decreased to zero before the end of the experiment, Laws' equation (1984) was used to calculate ammonium regeneration over the time intervals ending with ammonium depletion:

$$r = \{\ln(R_0/R_t)S_0\}/T \quad (5)$$

Relative preference indices:

RPI values were calculated for nitrate and ammonium according to McCarthy *et al.* (1977); e.g. for nitrate:

$$RPI_{NO3^-} = \{\rho NO_3^-/(\rho NO_3^- + \rho NH_4^+)\}/ \{S_{NO3^-}/(S_{NO3^-} + S_{NH4^+})\} \qquad (6)$$

where ρNO_3^- = absolute nitrate uptake ($\mu molN.l^{-1}.h^{-1}$); ρNH_4^+ = absolute ammonium uptake ($\mu molN.l^{-1}.h^{-1}$); S_{NO3^-} = nitrate substrate concentration ($\mu molN.l^{-1}$) and S_{NH4^+} = ammonium substrate concentration ($\mu molN.l^{-1}$).

F-ratios:

F-ratios (new versus total production; Eppley and Peterson 1979) were calculated for all treatments using equation 7 below. Due to rapid ammonium depletion, f-ratios are calculated only for uptake over the first time interval (T_0-T_1):

$$\text{f-ratio} = \{\rho NO_3^- / (\rho NO_3^- + \rho NH_4^+)\} \qquad (7)$$

ρNO_3^- and ρNH_4^+ represent absolute uptake of nitrate and ammonium respectively ($\mu molN.l^{-1}.h^{-1}$).

RESULTS

Figures presented within the body of the text represent tabulated data which are presented in appendices to this document. Time course values for ammonium and nitrate are presented in Appendix 1. Biomass parameters, for the three size fractions with time, expressed as particulate nitrogen (PN), are given in appendix 2. Absolute uptake rates of NH_4^+ and NO_3^- are presented in Appendix 3, and atom % excess (a%e) values are presented in Appendix 4. Appendix 5 shows particulate nitrogen values, and absolute uptake rates of nitrate, ammonium and total inorganic nitrogen at T_1. RPI values for NO_3^- and NH_4^+ at T_1 are given in Appendix 6; and f-ratio values for the three size fractions at T_1 in Appendix 7. Ammonium regeneration rates are presented in Appendix 8. Total amino acid concentrations with time are given in Appendix 9.

In order to simplify the terminology used to describe the treatments for the different incubations, I will be using the following terms hereafter. Control 1 ($^{15}N\text{-}NH_4^+$ added); control 2 ($^{15}N\text{-}NO_3^-$ added); NH_4+AA ($^{15}N\text{-}NH_4^+$ plus added amino acids); NH_4+GLU ($^{15}N\text{-}NH_4^+$ plus added glucose); NH_4+AA+GLU ($^{15}N\text{-}NH_4^+$ plus added amino acids and glucose); NO_3+AA ($^{15}N\text{-}NO_3^-$ plus amino acids); NO_3+GLU ($^{15}N\text{-}NO_3^-$ plus added glucose); and NO_3+AA+GLU ($^{15}N\text{-}NO_3^-$ plus amino acids and glucose) (see Table 1). Time periods are referred to as T_0 (start of incubation); T_1 (approximately 2 hrs after the start of the incubation); T_2 (approximately 6 hrs); T_3 (approximately 12 hrs) and T_4 (approximately 24 hrs) (see Table 3).

Substrate concentration with time:

Ammonium concentration:

The substrate concentrations for $^{15}N\text{-}NH_4^+$ spiked incubations with time are shown in figure 1A. All data points represent a mean of three replicate aliquots taken from a single sample after filtration through a Whatman GF/F filter. Variation between replicates was typically of the order of 1%, with a maximum variation of 3% occurring in fewer than 5% of samples. The initial ammonium concentrations ranged from 0.549 to 0.667 $\mu mol.l^{-1}$. Given an addition of 0.131 $\mu mol.l^{-1}$ of $^{15}N\text{-}NH_4^+$, the ^{15}N enrichment is calculated to have comprised about 20% of the available ammonium. This is consistent with measured enrichments of ^{15}N of between 16% and 19% calculated from aqueous enrichment at T_0. NH_4^+ concentrations for control 1 and the three $^{15}N\text{-}NH_4^+$ spiked treatments at T_0 are similar. In the control and ^{15}N+AA incubation the NH_4^+ substrate concentration remains insignificantly changed till T_1 (0.667 and 0.549 $\mu molN.l^{-1}$ to 0.564 and 0.528 $\mu molN.l^{-1}$ for the CONTROL 1 and NH_4+AA incubations at T_0 and T_1 respectively), after which

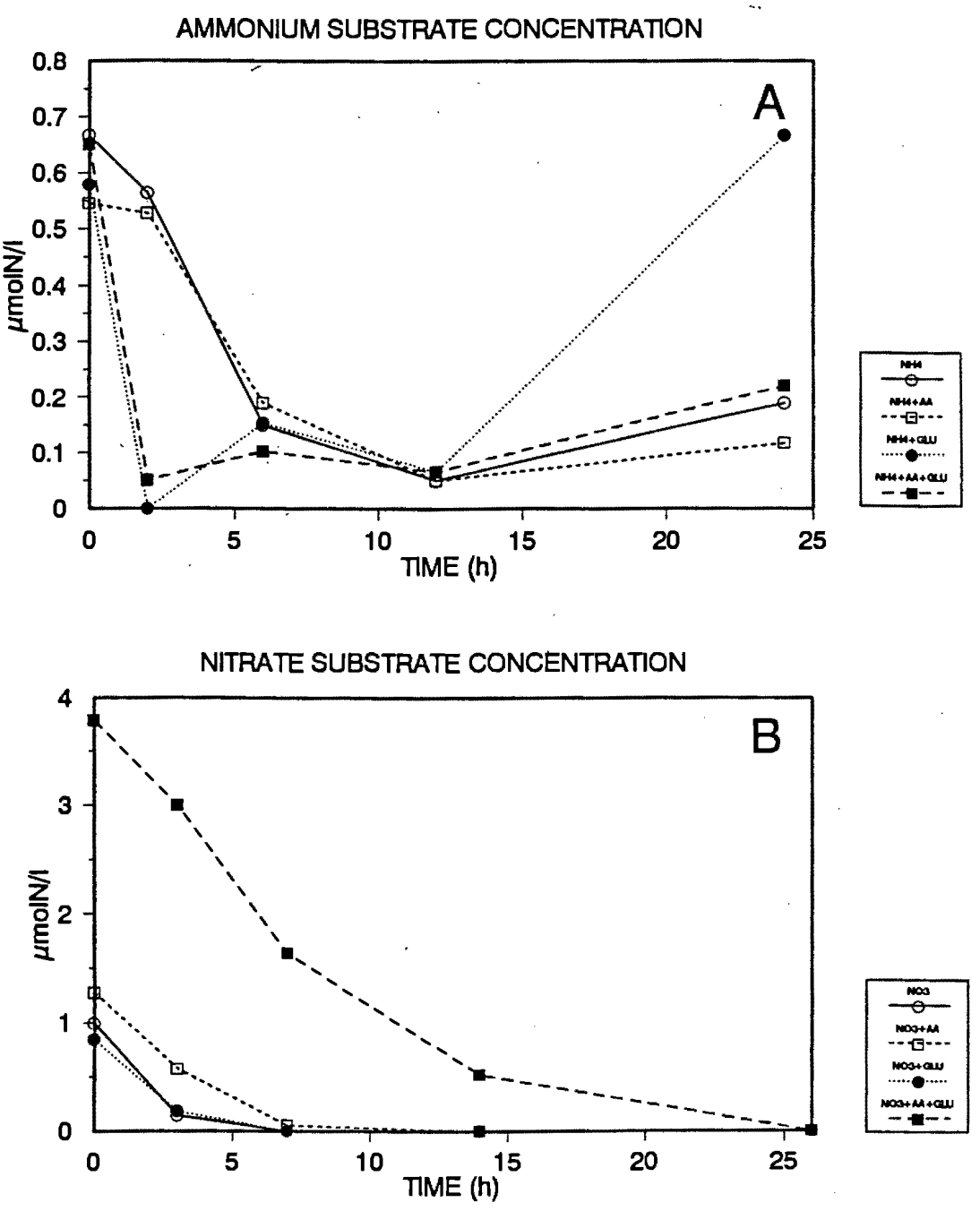

Figure 1: Inorganic nitrogen substrate concentration (µmolN/l) with time

there is a rapid decline by T_2 (0.150 and 0.190 $\mu molN.l^{-1}$ respectively). In the NH_4+GLU and NH_4+AA+GLU incubations there is a rapid decline in NH_4^+ concentration from 0.579 and 0.651 $\mu molN.l^{-1}$ respectively, effectively reaching zero concentration by T_2 (0.000 and 0.051 $\mu molN.l^{-1}$). NH_4^+ concentrations remain low in all incubations (0.051-0.067 $\mu molN.l^{-1}$) at T_3 after which there seems to be a slight increase in the CONTROL 1 (0.190 $\mu molN.l^{-1}$), NH_4+AA (0.118 $\mu molN.l^{-1}$) and NH_4+AA+GLU (0.221 $\mu molN.l^{-1}$) bags. An abrupt increase of ammonium concentration is seen in the NH_4+GLU bag from T_3 to T_4 where NH_4^+ concentrations increase from 0.067 $\mu molN.l^{-1}$, to a level similar to the concentration at the start of the experiment (0.667 $\mu molN.l^{-1}$). The possibility of process error cannot be ignored here.

Nitrate concentration:

The time course values for NO_3^- concentrations are shown in figure 1B. It must be noted that, given an addition of 1.03 $\mu mol.l^{-1}$ of ^{15}N-NO_3^-, added ^{15}N-NO_3^- constitutes 100% of the available nitrate in the control 2 and NO_3+GLU bags, approximately 80% in the NO_3+AA incubation, and about 27% in the NO_3+AA+GLU incubation. It appears that a highly active assemblage may have been present, which stripped the water of nitrate prior to the start of the experiment during the 24 hour acclimation period, during which the assemblages were exposed to continual illumination. The NO_3+AA+GLU bag, however, showed an initial NO_3^- concentration of 3.79 $\mu molN.l^{-1}$, which was elevated relative to the CONTROL 2, NO_3+AA and NO_3+GLU bags which exhibited similar initial NO_3^- concentrations (between 0.84 and 1.28 $\mu molN.l^{-1}$) and subsequent nutrient decline. The reasons for this discrepancy are not entirely clear. However, this bag was situated at the end of the row of incubations and may thus have been exposed to lower irradiance levels and/or

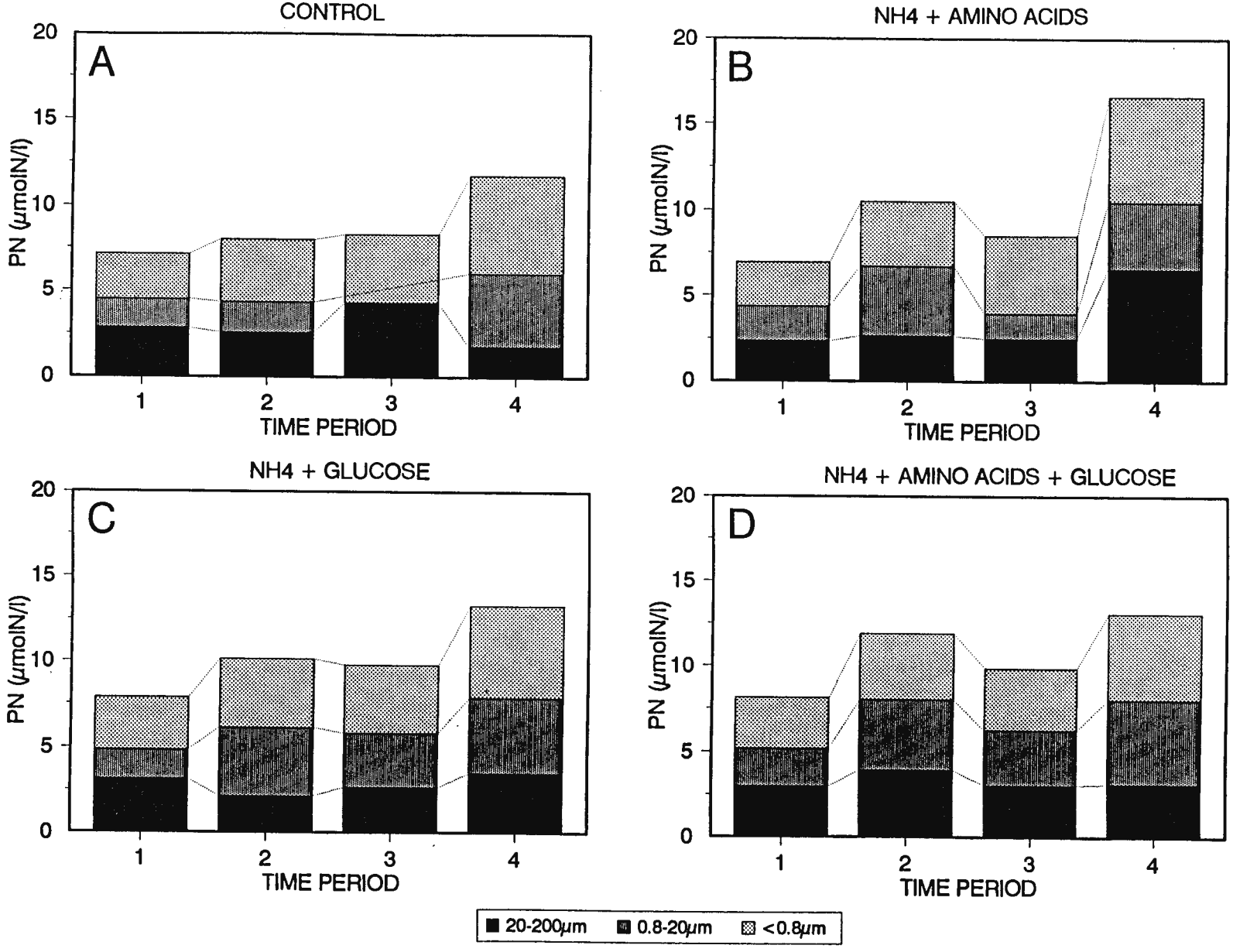

Figure 2: Biomass expressed in terms of particulate nitrogen (µmolN/l) for the 15N-NH4 spiked incubations.

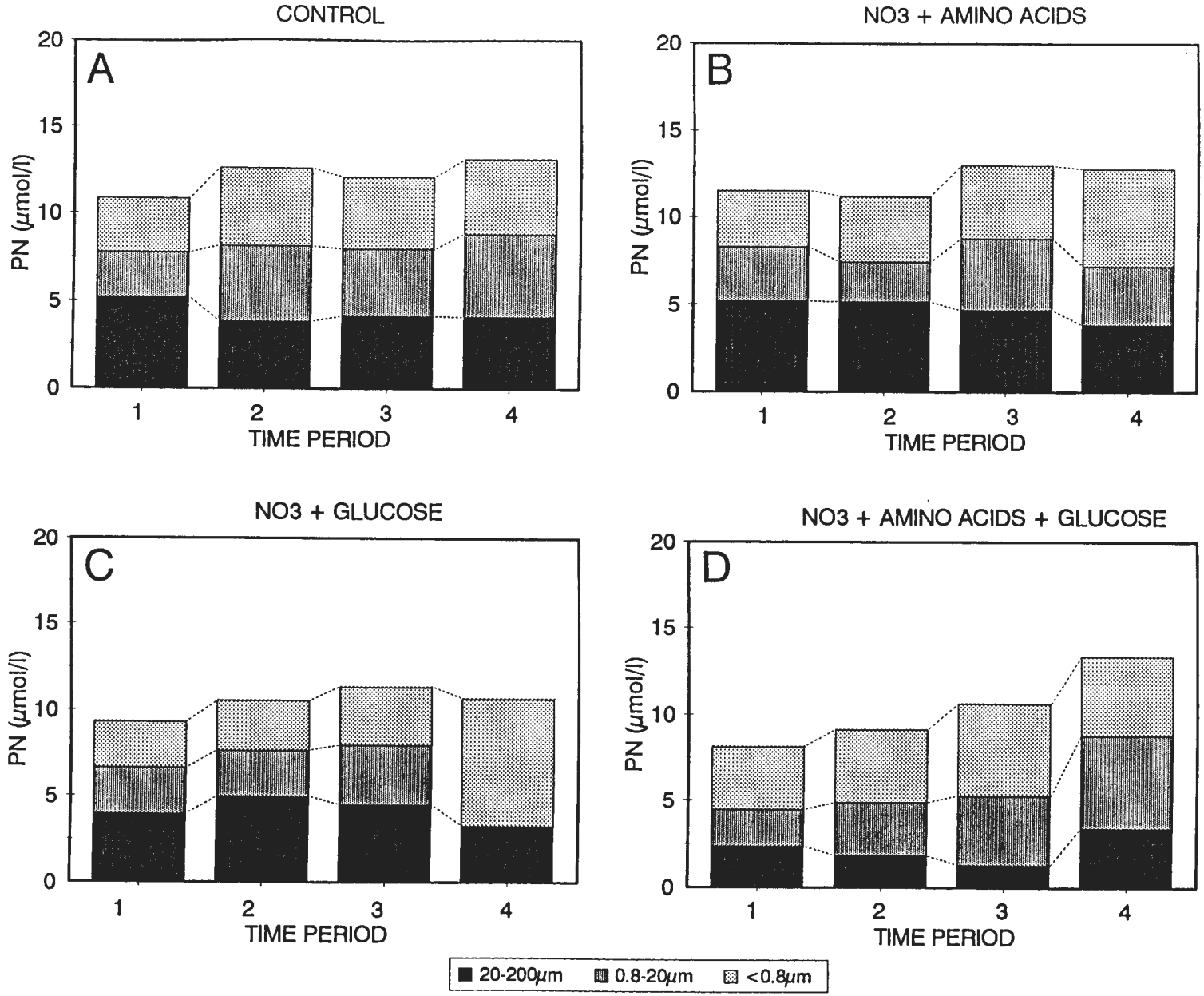

Figure 3: Biomass with time expressed in terms of particulate nitrogen (µmolN/l) for the 15N-NO3 spiked incubations.

less air induced mixing than the rest, resulting in a slower nitrate uptake rate. All bags follow a similar pattern of steady nutrient depletion. NO_3^- is depleted by T_2 (<0.05 $\mu molN.l^{-1}$) in all cases excepting the NO_3+AA+GLU incubation (1.640 $\mu molN.l^{-1}$), where NO_3^- is still present until T_4 when the concentration reaches zero.

Biomass:

Biomass changes with time, expressed in terms of µmoles of particulate nitrogen per litre, are presented in figures 2 and 3. There appears to have been a slight increase of biomass with time throughout the range of treatments and size fractions.

Inorganic nitrogen uptake with time:

Ammonium uptake:

Time course representations of ammonium and nitrate uptake by the three size fractions are given in figures 4-6. The abrupt peak and decline (uptake rates of between about 0.1-0.45 $\mu molN.l^{-1}.h^{-1}$ at T_1 declining to zero by T_2) in ammonium uptake is extremely striking. It has been well documented that phytoplankton are able to exhibit rapid uptake in response to a pulse of ammonium especially under conditions of nutrient limitation (e.g. Glibert & Goldman 1981; Wheeler *et al.* 1982), and this is supported by these results.

Nitrate uptake:

Nitrate uptake rates follow a less striking pattern. In all incubations, with the exception of the NO_3+AA+GLU bag, there is an initial uptake maximum at T_1 throughout the range of size fractions (between 0.109-0.144, 0.037-0.047 and 0.010-0.012 $\mu molN.l^{-1}.h^{-1}$ for the net-, nano-, and bacterio-plankton respectively).

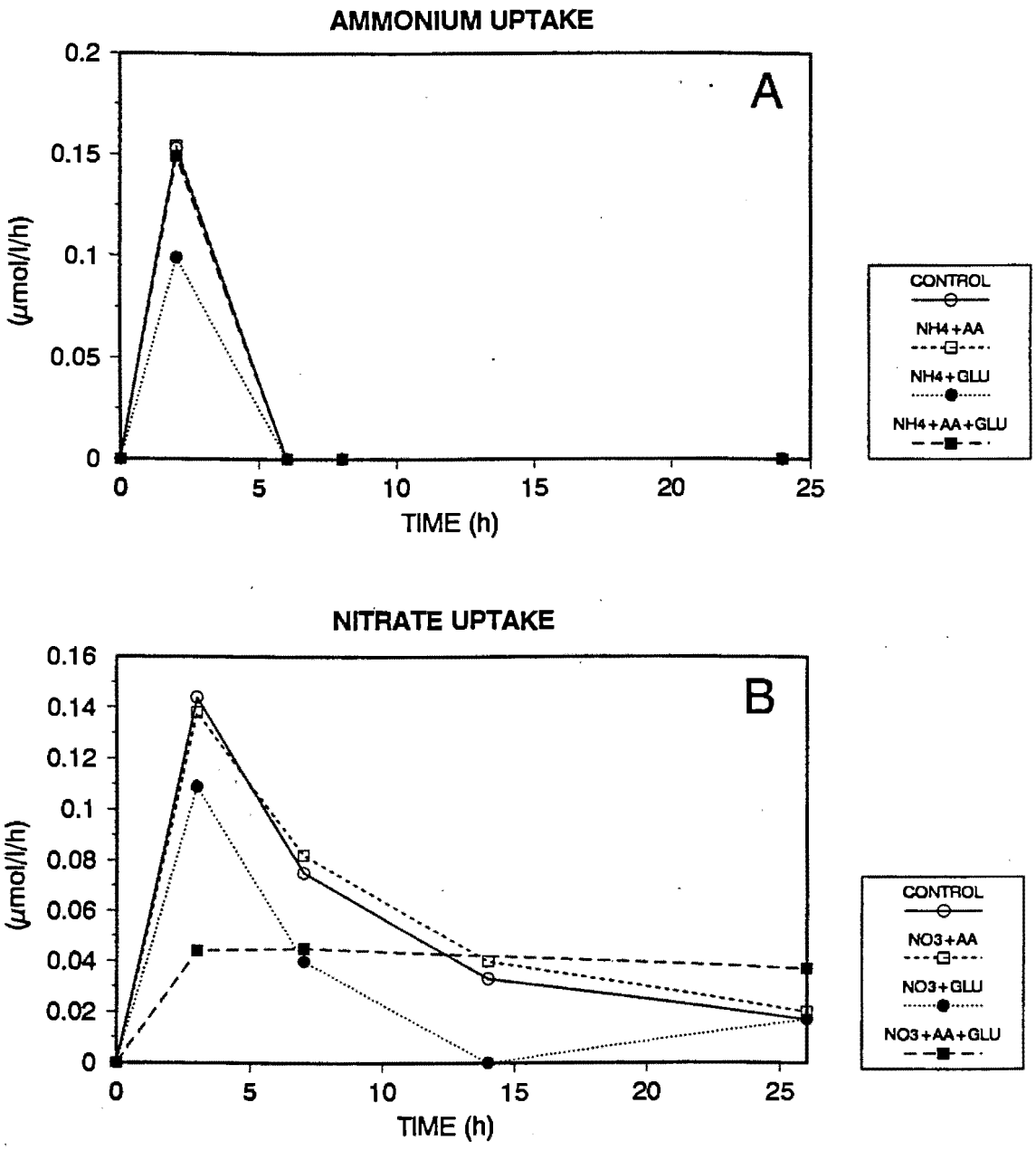

Figure 4: Nitrogen uptake (µmolN/l/h) by the netplankton (20-200µm).

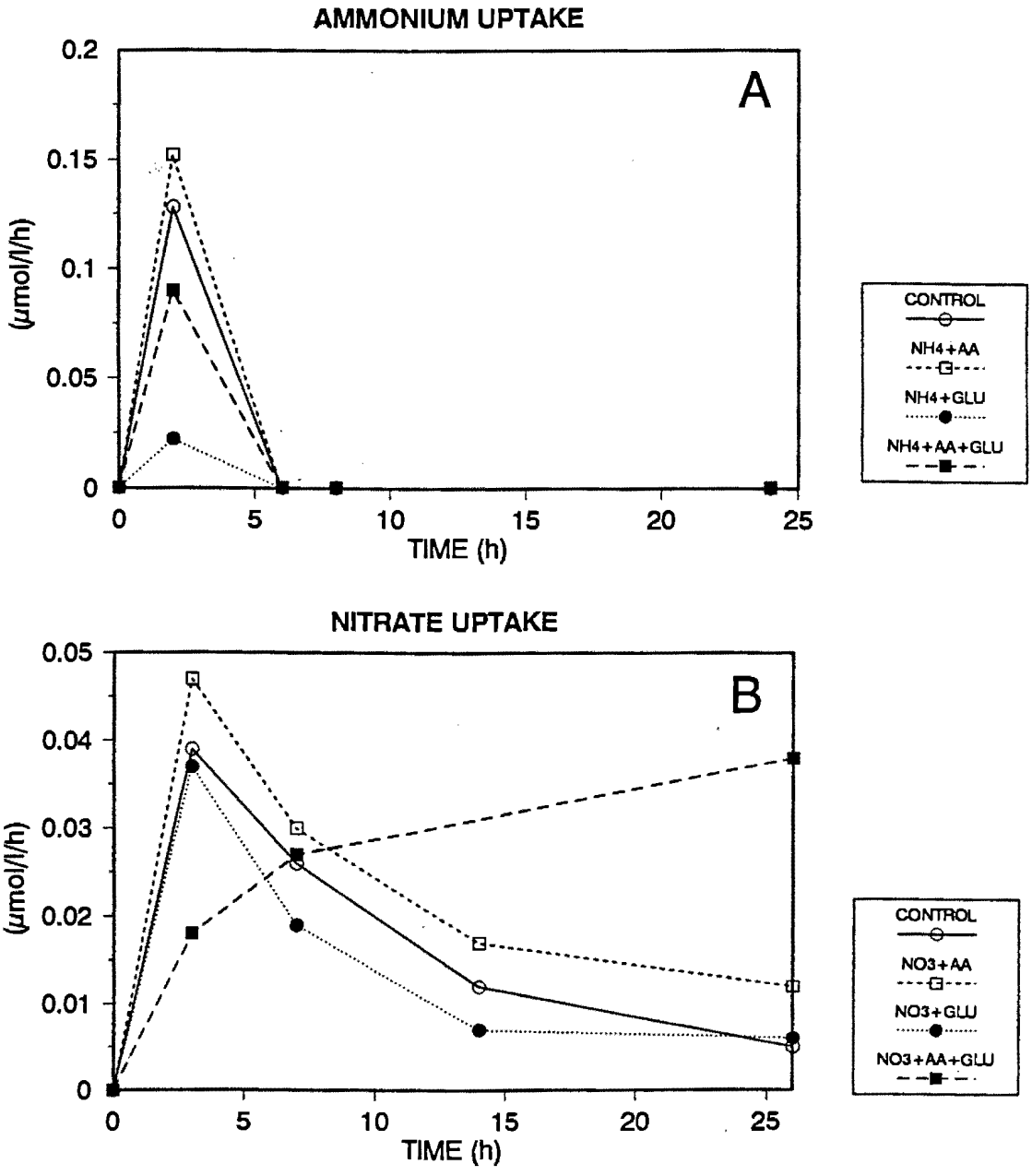

Figure 5: Nitrogen uptake (µmolN/l/h) by the nanoplankton (0.8-20µm).

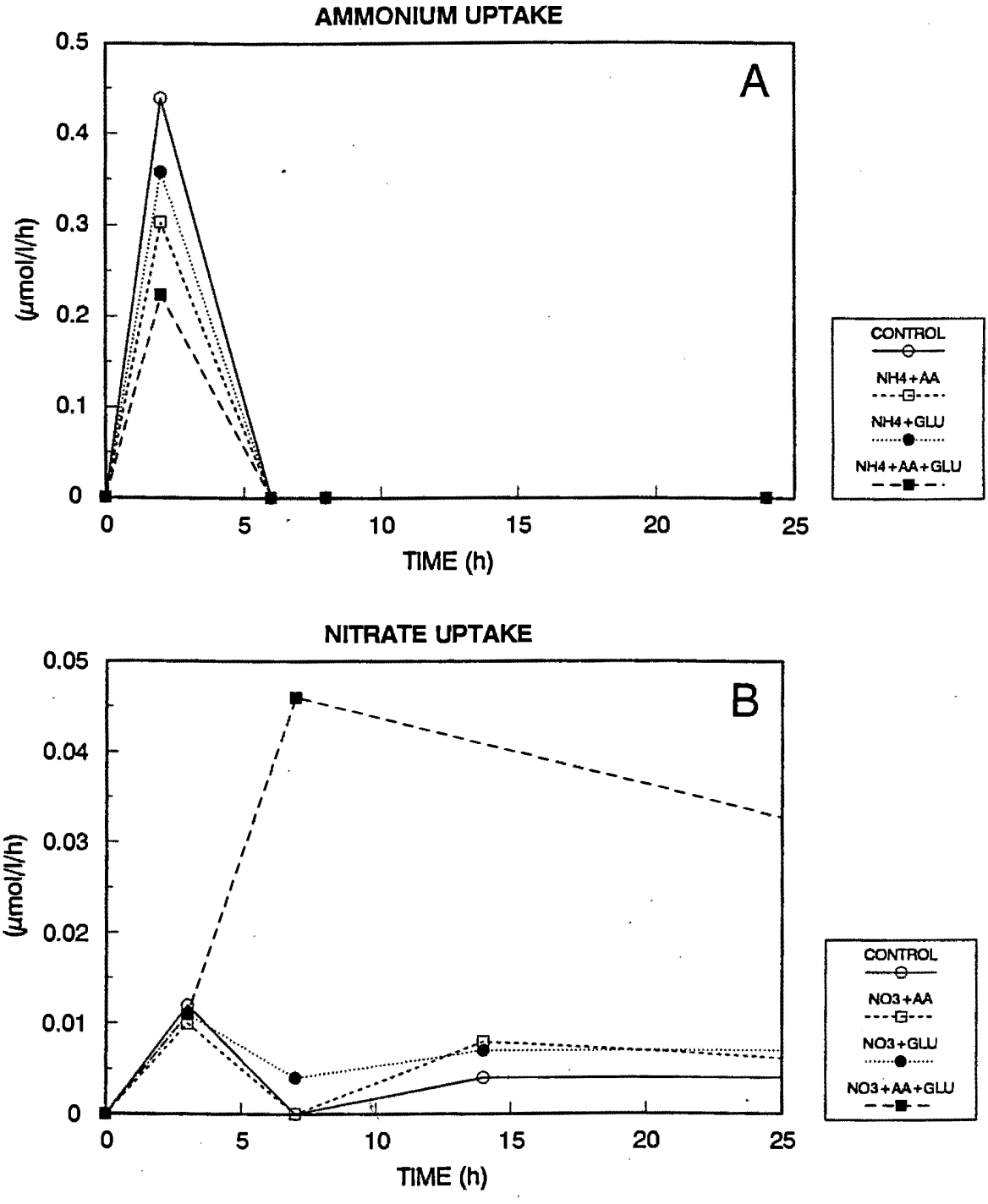

Figure 6: Nitrogen uptake (µmolN/l/h) by the bacterioplankton (<0.8µm).

This was followed by a fairly steady decline till the end of the time course, with uptake by all fractions decreasing to below 0.02 $\mu molN.l^{-1}.h^{-1}$. In the case of the NO_3+AA+GLU incubation there is a slight variation between the three size fractions.

The netplanktonic fraction initially take up a relatively small amount of nitrate (approximately 0.044 $\mu molN.l^{-1}.h^{-1}$ in the NO_3+AA+GLU incubation, compared with 0.109-0.144 $\mu molN.l^{-1}.h^{-1}$ in the cases of the remaining incubations), however, instead of a subsequent decline, the uptake rate remains approximately the same throughout the experiment.

The nanoplanktonic fraction exhibited an uptake rate increasing with time, starting from an initial uptake of 0.018 $\mu molN.l^{-1}.h^{-1}$ in the NO_3+AA+GLU incubation (as opposed to between 0.037-0.047 $\mu molN.l^{-1}.h^{-1}$), and increasing to 0.038 $\mu molN.l^{-1}.h^{-1}$ at T_4.

The bacterioplankton fraction shows an initial uptake rate in the NO_3+AA+GLU incubation which is similar to that of the other incubations (approximately 0.01 $\mu molN.l^{-1}.h^{-1}$), before increasing significantly by T_2 to 0.045 $\mu molN.l^{-1}.h^{-1}$, followed by a decline in the uptake rate (reaching 0.032 $\mu molN.l^{-1}.h^{-1}$ at T_4).

It is suggested that the reason for the disparity seen in the case of this incubation is the comparatively high abundance of available nitrate in this treatment (nitrate concentrations measured in the CONTROL 2, NO_3+AA and NO_3+GLU incubations being between 22-43% of that measured in the NO_3+AA+GLU incubation: see figure 1B), maintaining the substrate concentration above detection limits throughout the course of the experiment.

The netplanktonic fraction was found to take up greater quantities of nitrate than the nanoplankton and bacterioplankton, exhibiting a maximum absolute uptake of 0.144 $\mu mol.l^{-1}.h^{-1}$, compared with a maximum of less than 0.05 $\mu mol.l^{-1}.h^{-1}$ shown by the latter fractions.

Atom % excess with time:

The atom % excess (a%e) provides an indication of ^{15}N in the cellular nitrogen pool. This provides a useful indication of isotopic dynamics, enabling detection of any isotope loss from the particulate fraction, shown by a decreased a%e. The atom % excess values with time for the various treatments are illustrated in figures 7 (^{15}N-NH_4^+ spiked) and 8 (^{15}N-NO_3^- spiked).

Ammonium spiked incubations:

Given the exhaustion of substrate ammonium and the subsequent decline in ammonium uptake, it would be expected that the atom % excess would remain constant (if uptake ceased and no isotope loss occurred), or decline (if isotope loss occurred at a greater rate than uptake, which would cease in the absence of available substrate) after T_1. The observed decline of atom % excess for these incubations (figure 7) is thus consistent with this expectation, indicating a loss of isotope from the particulate fraction. This indicates the possibility of isotope leakage into the aqueous phase after T_1, and/or predation of the measured particulate fraction. Given the measured depletion of ammonium substrate in the incubations this would suggest that the observed loss of isotope was probably not due to leakage and indicates predation as the most likely cause. This is supported by the later reappearance of ^{15}N-NH_4^+ in the aqueous phase, which is compatible with a time lag between predation and excretion.

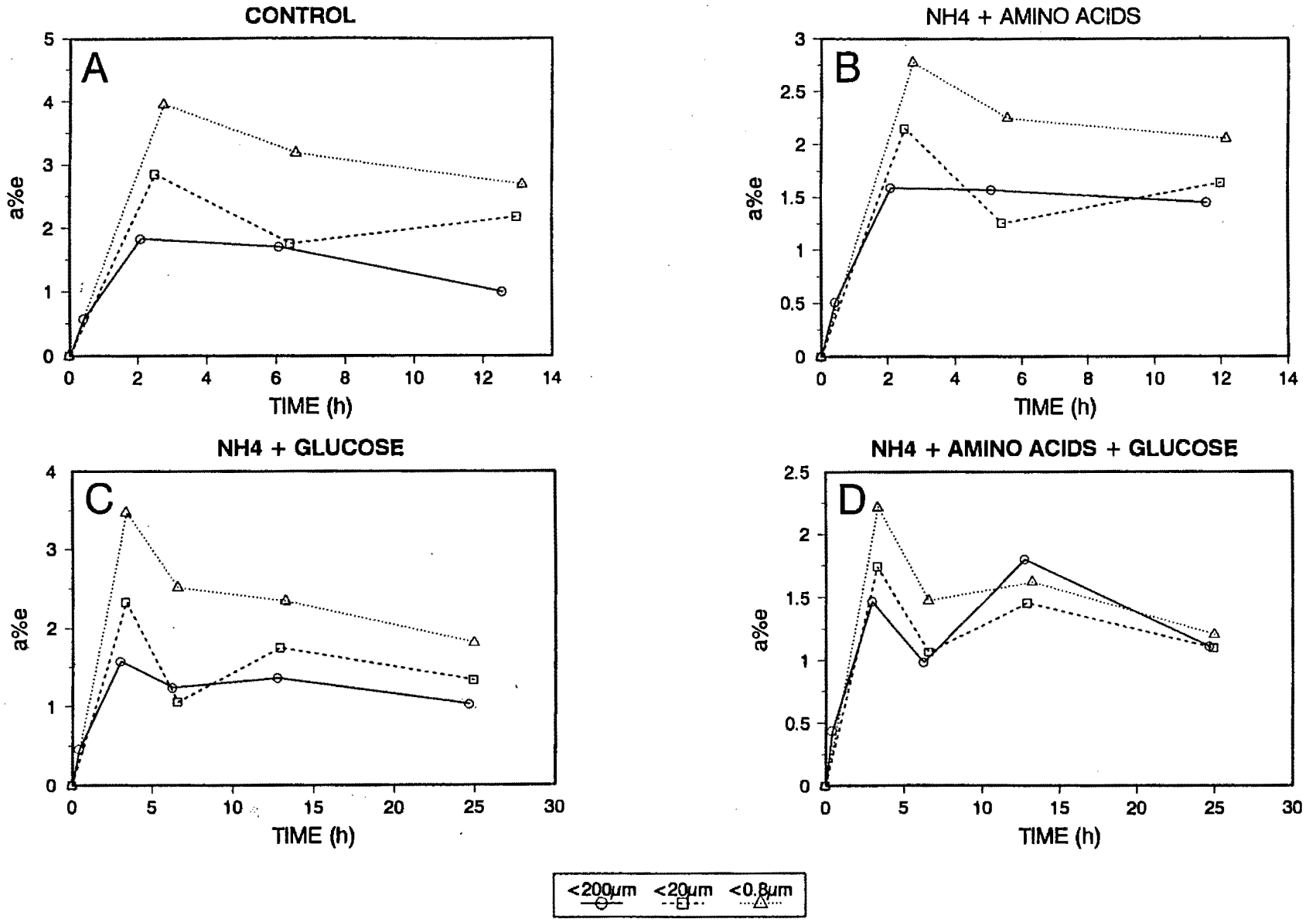

Figure 7: Atom % excess with time for the incubations spiked with 15N-NH4 for the size ranges <200µm, <20µm and <0.8µm.

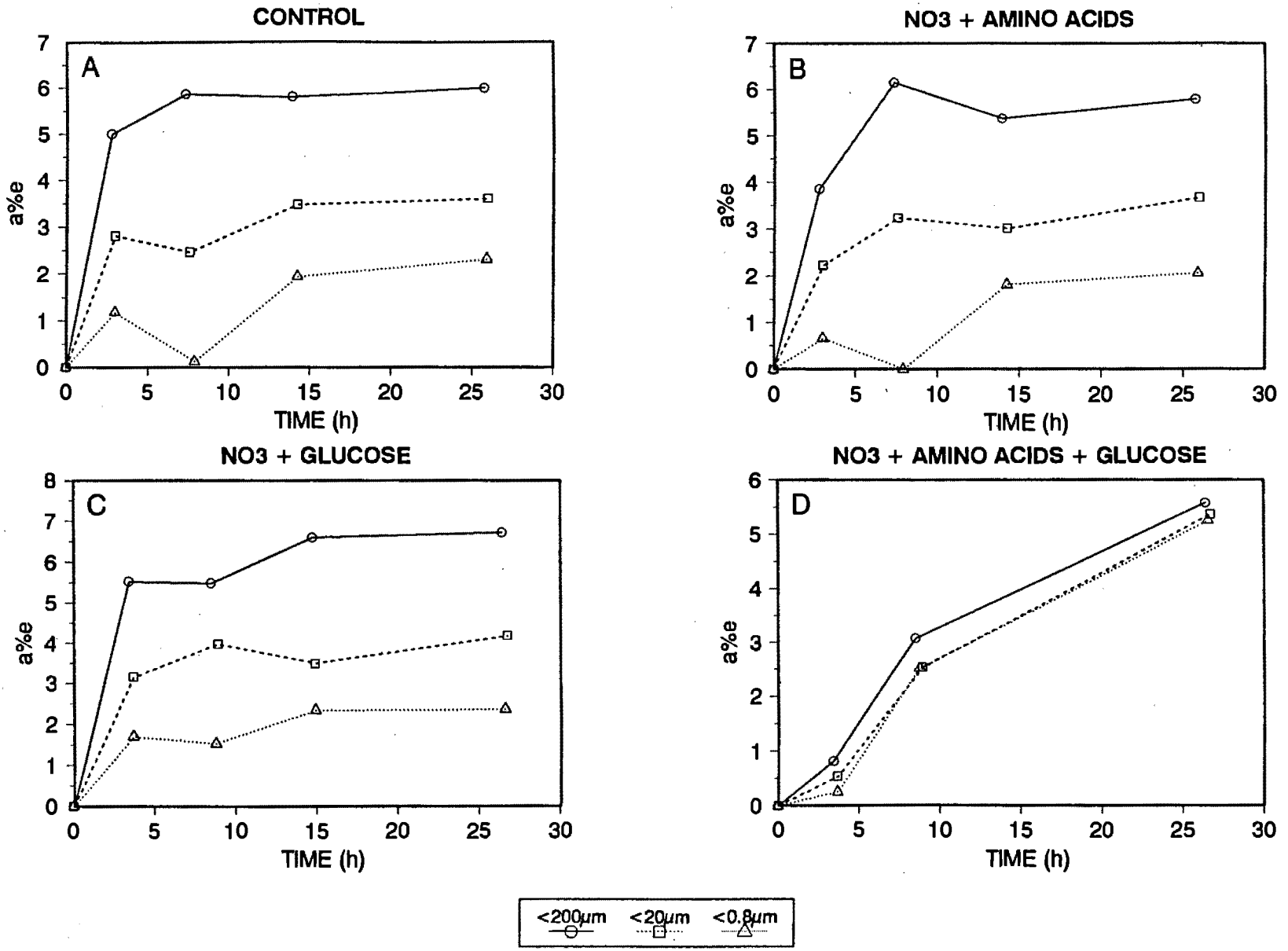

Figure 8: Atom % excess with time for the incubations spiked with 15N-NO3 for the size ranges <200µm, <20µm and <0.8µm

Loss of ^{15}N isotope from the particulate fraction would in any case lead to underestimation of uptake rates. Given this, the observed ammonium substrate exhaustion, and the resultant decline of ammonium uptake after T_1, all further comparisons of ammonium and nitrate uptake data are limited to the first incubation phase (T_0-T_1).

Nitrate spiked incubations:

Atom % excess values for the ^{15}N-NO_3^- spiked incubations (figure 8) show an increasing trend which is fairly constant. As is to be expected given the size related differential uptake of inorganic nitrogen, there is a positive relation between increase in cell size and a%e, indicating the increased uptake of nitrate per unit nitrogen biomass by larger cells. While this trend holds for all treatments, there is little variation between the a%e values for the three size ranges in the incubation with NO_3+AA+GLU. This relation is negative in the case of ammonium spiked incubations, where the size range containing only bacterioplankton demonstrates the highest a%e values and that containing the intact community the lowest, indicating the relatively high uptake of this isotope by the bacterial fraction.

Nitrogen uptake at T_1:

Absolute uptake rates at T_1 of NH_4^+ and NO_3^- ($\mu molN.l^{-1}.h^{-1}$) by the different size fractions are given in figure 9, while figure 10 shows uptake of total nitrogen (NO_3^- + NH_4^+) by these size fractions.

Netplanktonic uptake:

The netplankton (figure 9A) are seen to take up the most NO_3^-, 0.144 $\mu molN.l^{-1}.h^{-1}$ in the CONTROL 2 incubation at T_1 as compared with 0.039 and 0.012 $\mu molN.l^{-1}.h^{-1}$

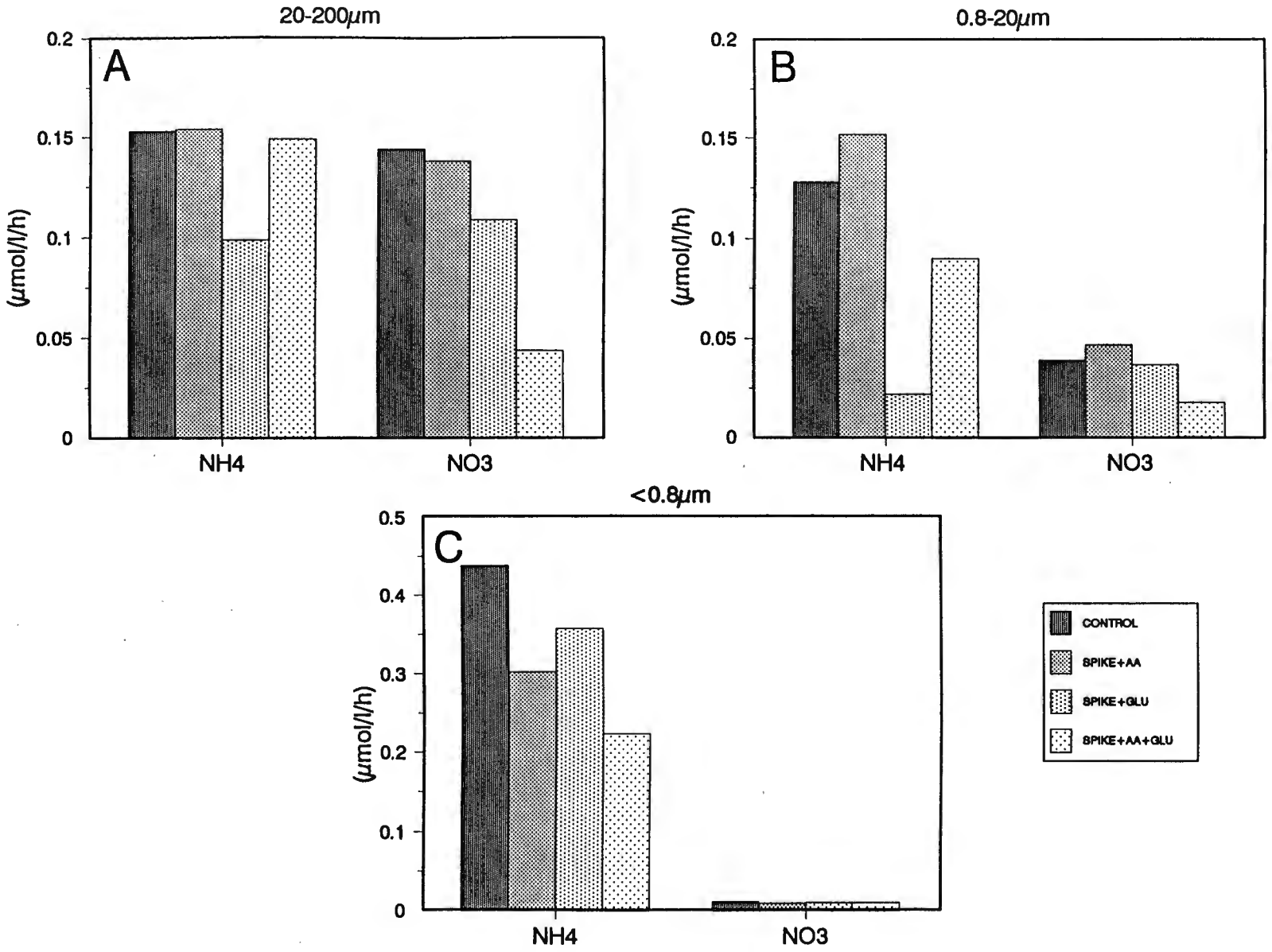

Figure 9: Absolute uptake rates (μmolN/l/h) of ammonium and nitrate at T1 by the three size fractions

taken up by the nanoplankton and bacterioplankton respectively. Nitrate uptake by the netplankton from the $^{15}N\text{-}NO_3^-$ spiked incubation (0.144 $\mu molN.l^{-1}.h^{-1}$) is similar to measured ammonium uptake from the $^{15}N\text{-}NH_4^+$ spiked control incubation (0.153 $\mu molN.l^{-1}.h^{-1}$). It is interesting to note that NH_4^+ uptake by this fraction does not differ significantly for the different treatments (approximately 0.15 $\mu mol.l^{-1}.h^{-1}$), except in the case of the NH_4+GLU incubation where NH_4^+ uptake was decreased by approximately 0.05 $\mu molN.l^{-1}.h^{-1}$ from 0.153 $\mu molN.l^{-1}.h^{-1}$ to 0.099 $\mu mol.l^{-1}.h^{-1}$. NO_3^- uptake by the netplanktonic fraction was greatest in the CONTROL 2 and NO_3+AA bags which showed similar uptake rates (0.144 $\mu molN.l^{-1}.h^{-1}$ and 0.138 $\mu molN.l^{-1}.h^{-1}$ respectively). Uptake was decreased in the NO_3+GLU bag (0.109 $\mu molN.l^{-1}.h^{-1}$) and even more so in the NO_3+AA+GLU bag (0.044 $\mu molN.l^{-1}.h^{-1}$).

Nanoplanktonic uptake:

The nanoplankton selected NH_4^+ in preference to NO_3^- (figure 9B). Here again it is noted that NH_4^+ uptake is decreased in the NH_4+GLU bag, with 0.022 $\mu molN.l^{-1}.h^{-1}$ taken up as opposed to 0.128 and 0.152 $\mu molN.l^{-1}.h^{-1}$ in the CONTROL 1 and NH_4+AA bags. The NH_4+AA+GLU incubation also showed decreased NH_4^+ uptake (0.090 $\mu molN.l^{-1}.h^{-1}$), though not so marked as that of the NH_4+GLU incubation. Nitrate uptake by this fraction did not appear to differ greatly between the treatments with the exception of the NO_3+AA+GLU incubation, where an uptake rate of 0.018 $\mu molN.l^{-1}.h^{-1}$ was measured compared with between 0.037 and 0.047 $\mu molN.l^{-1}.h^{-1}$ for the CONTROL 2, NO_3+AA and NO_3+GLU incubations.

Bacterioplanktonic uptake:

An elevated NH_4^+ uptake relative to NO_3^- uptake by the bacterioplankton was observed (figure 9C). It is also clear that this fraction is responsible for the bulk of

NH_4^+ uptake, taking up between 1.5 and 3.6 times the ammonium taken up by the netplanktonic fraction at T_1, and between 2 and 16 times that due to the nanoplankton. When comparing uptake in the control incubation, the bacterial fraction is seen to utilise 0.439 $\mu molN.l^{-1}.h^{-1}$ of ammonium as opposed to 0.128 and 0.153 $\mu molN.l^{-1}.h^{-1}$ by the nanoplanktonic and netplanktonic fractions respectively. Differences between the control and the three treatments with respect to ammonium uptake rates vary between the highest uptake rate of 0.439 $\mu molN.l^{-1}.h^{-1}$ in the CONTROL 1 incubation, and the lowest uptake rate of 0.224 $\mu molN.l^{-1}.h^{-1}$ occurring in the NH_4+AA+GLU incubation. Uptake in the NH_4+AA incubation is 0.303 $\mu molN.l^{-1}.h^{-1}$ and in the NH_4+GLU incubation, 0.358 $\mu molN.l^{-1}.h^{-1}$. Nitrate uptake by this size fraction is uniformly low (between 0.010 $\mu molN.l^{-1}.h^{-1}$ and 0.012 $\mu molN.l^{-1}.h^{-1}$), contributing only between 5 and 15% of overall nitrate uptake by the intact community

Total inorganic nitrogen uptake:

Figure 10 illustrates total inorganic nitrogen uptake which is a summation of ammonium and nitrate taken up from the corresponding treatments. This representation gives a useful indication of relative uptake rates of the two inorganic nitrogen species.

When comparing total inorganic nitrogen uptake by the different size fractions (figure 10) it can be seen that the bacterial size fraction dominates nitrogen uptake, with about 50% of intact community uptake due to this fraction.

Uptake by the net- and nanoplanktonic size fractions is diminished in the presence of added glucose (approximately 68 and 46% of nitrogen taken up in the absence of added

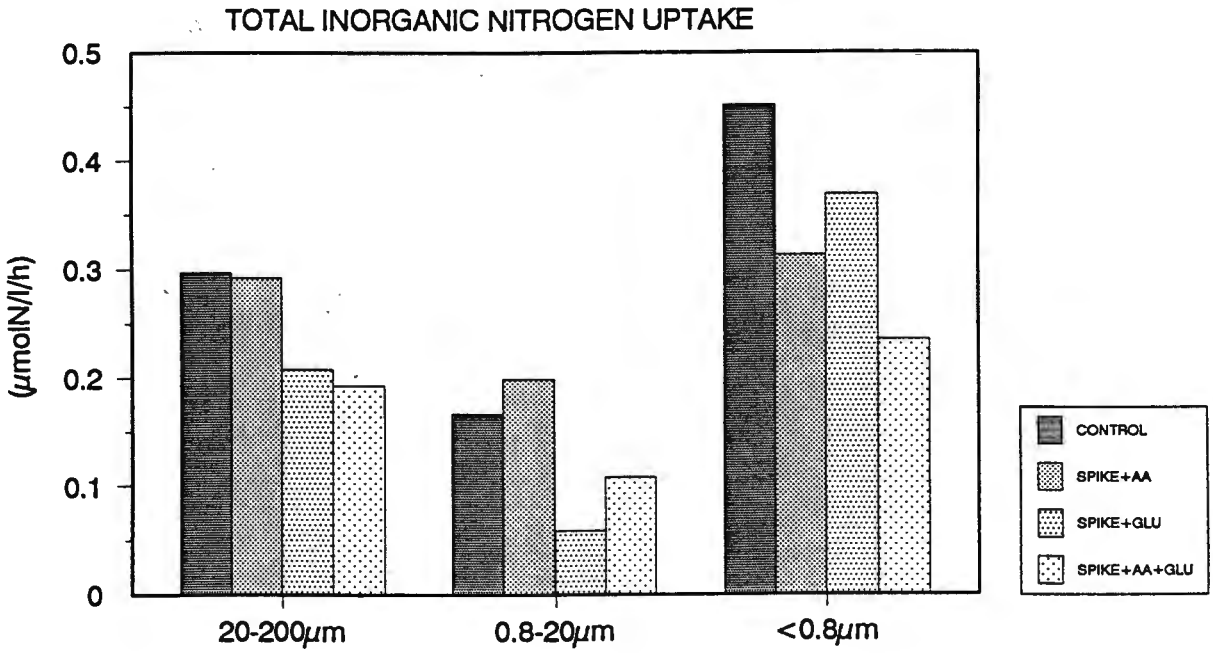

Figure 10: Summed values of ammonium and nitrate absolute uptake rates (µmolN/l/h) by the three size fractions at T1.

glucose by the netplankton and nanoplankton respectively). This may be explained with reference to regeneration rates and will be discussed later.

Netplankton: There appears to be no real difference between the controls and treatments with amino acid enrichment (^{15}N+AA), where uptake of inorganic nitrogen was measured to be 0.297 and 0.292 $\mu molN.l^{-1}.h^{-1}$ respectively; nor between the glucose enriched (^{15}N+GLU: 0.208 $\mu molN.l^{-1}.h^{-1}$), and the glucose plus amino acids enriched (^{15}N+AA+GLU: 0.193 $\mu molN.l^{-1}.h^{-1}$) treatments. Thus it is seen that the presence of added glucose depressed uptake rates in this size fraction by approximately 68% and that amino acids had no noticeable effect.

Nanoplankton: This was also the case in the nanoplanktonic fraction, although not as marked (approximately 46%). It would appear that, in the case of this fraction, while added glucose resulted in reduced uptake of inorganic nitrogen (0.059 $\mu molN.l^{-1}.h^{-1}$ compared with 0.167 $\mu molN.l^{-1}.h^{-1}$ for the control incubations), the addition of amino acids gave rise to a slight increase in uptake of inorganic nitrogen (0.199 $\mu molN.l^{-1}.h^{-1}$ compared with 0.167 $\mu molN.l^{-1}.h^{-1}$ taken up from the controls). The net effect of enrichment with both compounds is a depression of nitrogen uptake of lesser magnitude than that due to added glucose alone (0.108 $\mu molN.l^{-1}.h^{-1}$ as compared with 0.059 $\mu molN.l^{-1}.h^{-1}$ taken up from the glucose enriched incubations and 0.167 $\mu molN.l^{-1}.h^{-1}$ from the controls).

Bacterioplankton: In the case of the bacterial size fraction the trend is rather different. The addition of amino acids gave rise to decreased inorganic nitrogen utilisation, the treatments with added amino acids showing uptake rates lower than those not amended with amino acids (0.313 and 0.235 $\mu molN.l^{-1}.h^{-1}$ as compared with 0.451 and 0.369

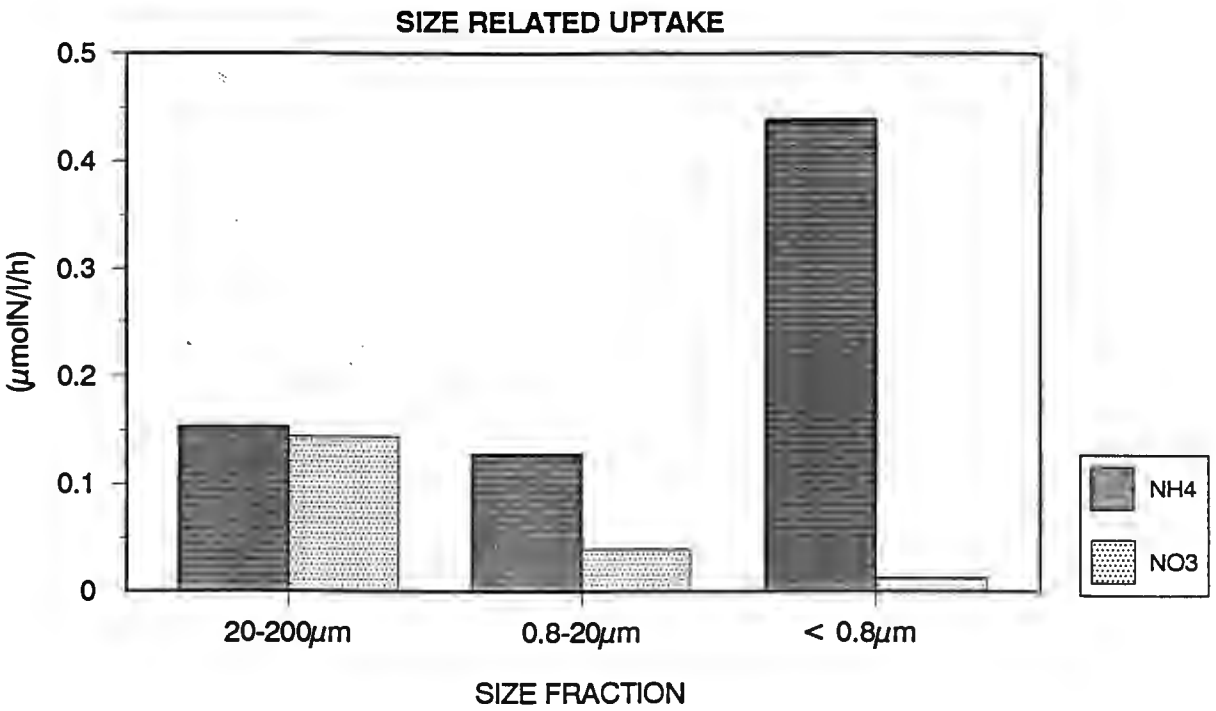

Figure 11: Absolute uptake rates (µmolN/l/h) of ammonium and nitrate in the control incubations at T1 related to plankton size.

$\mu molN.l^{-1}.h^{-1}$). It is also apparent that uptake of inorganic nitrogen by this fraction is depressed in the presence of added glucose. The treatments with added glucose yielded a lower uptake rate than the controls (0.369 $\mu molN.l^{-1}.h^{-1}$ as compared with 0.451 $\mu molN.l^{-1}.h^{-1}$), while the treatment with both amino acids and glucose resulted in the most marked retardation of inorganic nitrogen uptake (0.235 $\mu molN.l^{-1}.h^{-1}$ compared with 0.451 $\mu molN.l^{-1}.h^{-1}$ calculated for the control incubations).

Size related uptake:

Figure 11 shows size related uptake of ammonium and nitrate at T1 in the absence of any organic additions. A definite relationship between size and nitrogen species taken up is evident. Uptake of nitrate and ammonium by the netplanktonic fraction is similar (0.153 $\mu molN.l^{-1}.h^{-1}$ of ammonium, and 0.144 $\mu molN.l^{-1}.h^{-1}$ of nitrate; 51.5 and 48.5% of total inorganic nitrogen taken up), while both the nanoplanktonic and bacterioplanktonic fractions exhibit selection for ammonium over nitrate. This selectivity is most marked in the bacterial fraction where ammonium uptake (0.439 $\mu molN.l^{-1}.h^{-1}$) comprises 97.3% of total inorganic nitrogen uptake, and nitrate (0.012 $\mu molN.l^{-1}.h^{-1}$) only 2.7%. Ammonium uptake (0.128 $\mu molN.l^{-1}.h^{-1}$) comprises 76.6%, and nitrate (0.039 $\mu molN.l^{-1}.h^{-1}$) 23.4% of nanoplanktonic uptake. It is also apparent that the bacterioplankton were responsible for the bulk of ammonium uptake (61% of ammonium taken up by the intact community) while netplankton were responsible for the major portion of nitrate taken up (74% of nitrate taken up by the intact community). Ammonium uptake comprised 79% of total inorganic nitrogen taken up by the intact assemblage.

Figure 12 shows uptake of total inorganic nitrogen, ammonium and nitrate due to the different size fractions throughout the range of treatments expressed as a percentage of

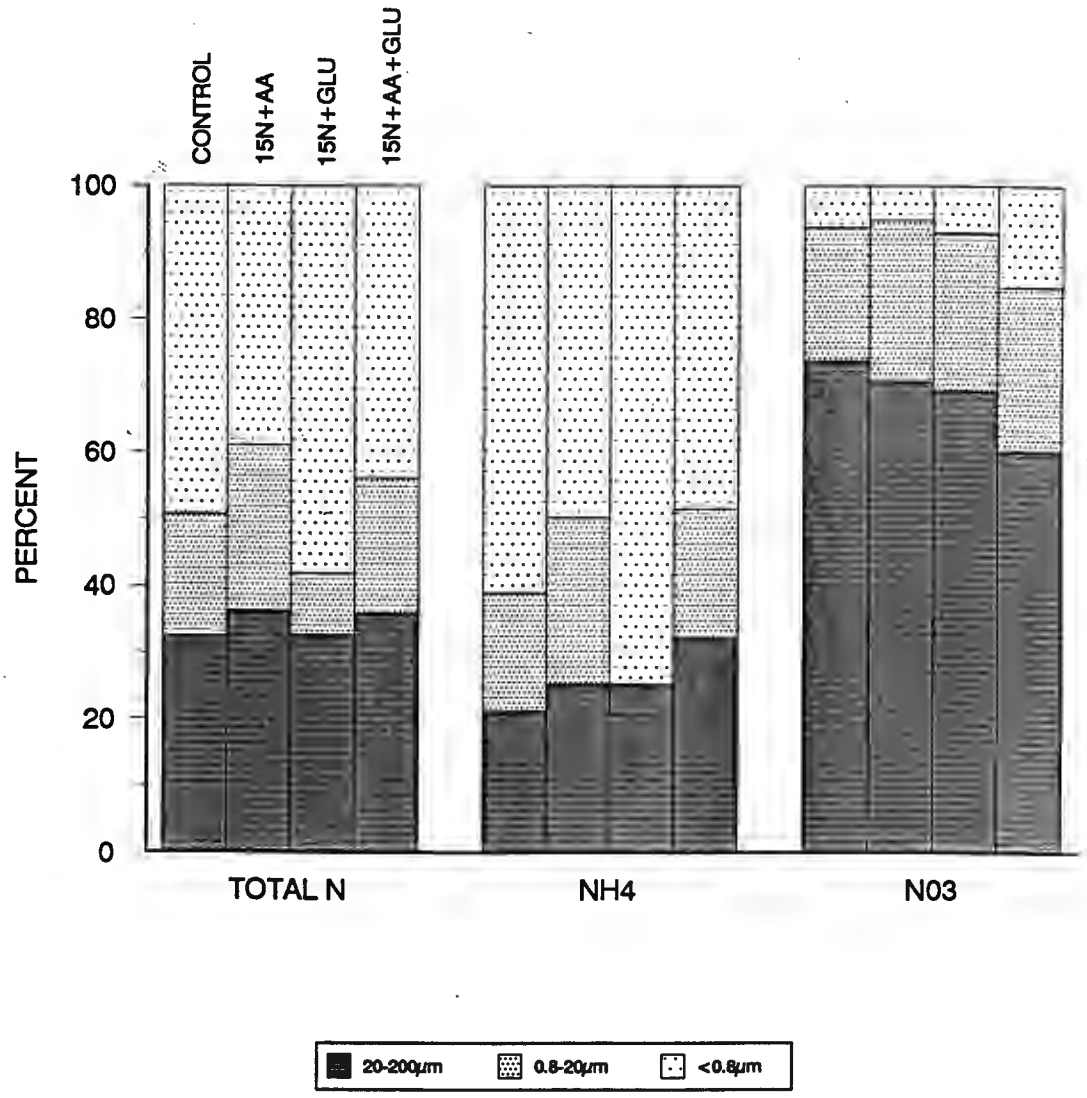

Figure 12: Percentage of intact community uptake of total inorganic nitrogen (NH4 + NO3), ammonium and nitrate due to the different size fractions.

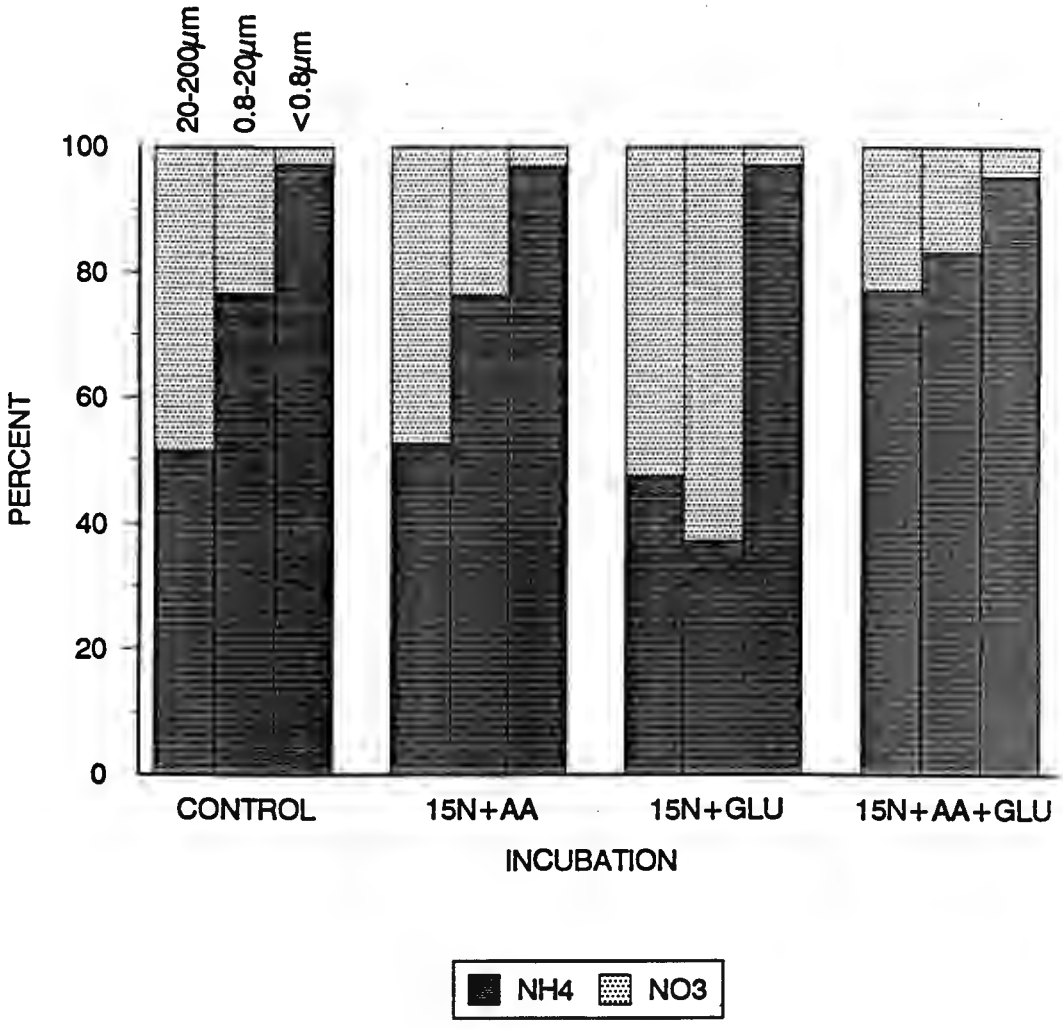

Figure 13: Percent of ammonium versus nitrate taken up by the three size fractions at T1

total community uptake. It is evident that netplankton is responsible for the bulk of nitrate uptake (between 60 and 74%), and bacterioplankton for the bulk of ammonium uptake (between 48 and 75%). When examining total inorganic nitrogen uptake, nanoplankton appear to be responsible for the least amount of uptake (9-25% of intact community uptake) with netplankton taking up 32-36% and bacterioplankton 43-60% of intact community uptake.

Figure 13 illustrates the uptake of ammonium and nitrate due to the different size fractions, expressed as a percentage of total inorganic nitrogen taken up by each fraction. Preferential uptake of ammonium occurs throughout the range of size fractions, with bacterioplankton consistently exhibiting the most strongly preferential ammonium uptake (between 95 and 98% of total inorganic nitrogen uptake by this fraction, compared with 37-83% and 48-77% by the nano- and net- plankton respectively), and netplankton the strongest preferential uptake of nitrate (between 23 and 50 of total inorganic nitrogen taken up compared with 16-23% (with the exception of the incubation with added glucose where nitrate comprised 63% of total inorganic nitrogen uptake) and 2-5% by the nano- and bacterio- plankton respectively.).

Relative preference indices:

A significant constraint is placed on the validity of relative preference indices by the observed depletion of nitrate prior to the start of the experiment. A basic assumption for the calculation of relative uptake of different nutrients from separate incubations is that the nutrient availability to the assemblages in these incubations is similar. This was evidently not the case here where the added $^{15}N\text{-}NO_3^-$ represented a significant portion of the nitrate available to the assemblage. As a result the RPI values calculated

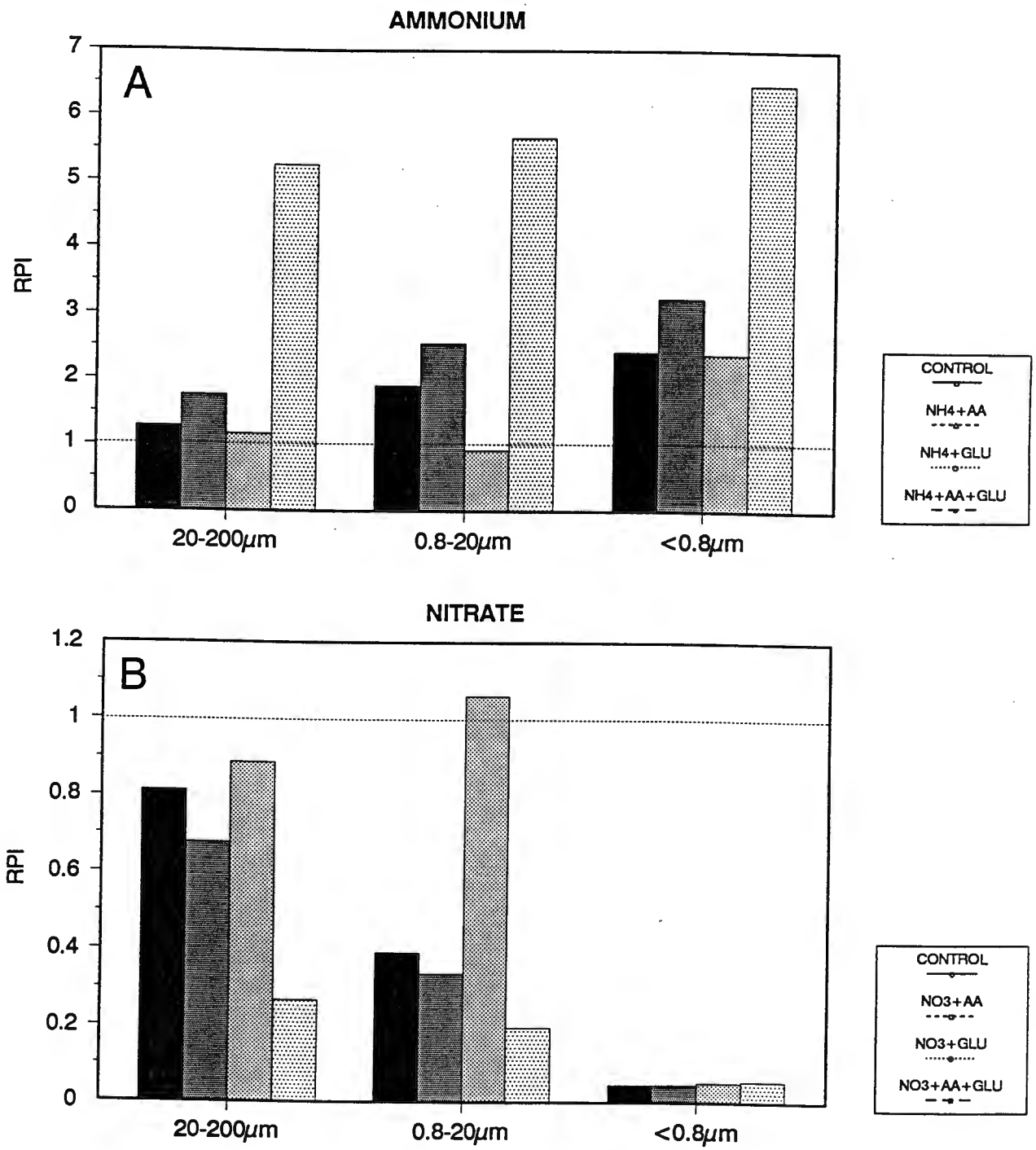

Figure 14: Relative preference indices calculated for the different incubations and size fractions at T1. The dotted line passes through a value of unity.

here cannot be regarded as definitive. They do, nevertheless, provide a useful indication of preferential uptake, particularly with respect to size related differences.

Relative preferential uptake of ammonium:

When examining the ammonium relative preference indices (RPI: figure 14A), it is evident that ammonium was the favoured nitrogen source. RPI_{NH4} values were consistently greater than unity, with the exception of that for nanoplanktonic fraction in the ^{15}N+GLU incubation which was 0.914. Ammonium preference was exhibited by all size fractions, although there is indication of increasing selection for ammonium with decreasing organism size. The RPI values are markedly increased in the treatment containing both added amino acids and glucose (between 5 and 6.5 as compared with values between 0.9 and 3.5 for the other incubations). It must however be born in mind that, while these values do reflect a very strong selection for ammonium over nitrate, the greatly elevated nitrate concentrations in the NO_3+AA+GLU treatment disproportionately increase these values, due to the nature of the equation used to generate them.

Relative preferential uptake of nitrate:

Nitrate RPI values, on the other hand, are consistently less than unity (figure 14B), indicating selection against this nitrogen compound, with the exception of the nanoplanktonic fraction in the ^{15}N+GLU (RPI_{NO3} = 1.059). A very strong size influence is evident. The bacterial fraction exhibits the strongest selection for ammonium over nitrate (RPI_{NO3} between 0.045 and 0.055 as compared with between 0.196 and 1.059 calculated for the net- and nanoplankton). The netplanktonic fraction generally showed the weakest discrimination against nitrate (with RPI_{NO3} values from

0.196-0.885, and an average RPI_{NO3} of 0.660 compared with an average of 0.496 and 0.049 exhibited by the nano- and bacterio- plankton respectively).

There appears to be a relatively greater preference for nitrate in the presence of added glucose among the net- and nano-plankton (RPI_{NO3} values of 0.885 and 1.059 respectively). This effect is particularly pronounced in the nanoplanktonic fraction giving the only RPI_{NO3} value greater than unity. The elevated RPI_{NO3} values obtained here may be explained by the low ammonium substrate concentration measured in the incubation amended with glucose. On the other hand, the addition of both amino acids and glucose appear to depress the nitrate RPI values calculated for the net- and nanoplanktonic fractions compared with the remainder of treatments (RPI_{NO3} values were 0.267 and 0.196 for the net- and nano- plankton respectively in these incubations, as compared with 0.337 to 1.059 in the remainder of incubations). Few distinctions can be drawn between the different treatments for the bacterial fraction as the nitrate RPI values here were similar and low. However it would appear that in the presence of glucose there is less discrimination against nitrate (RPI_{NO3} = 0.051 and 0.055 as opposed to between 0.045 and 0.046 for the treatments without added glucose).

f-ratios:

The f-ratio values are depicted in figure 15. A general trend is displayed of decreasing f-ratio with decreasing size. This is to be expected, given that netplankton were observed to utilise the greater amount of nitrate, and bacterioplankton the greater amount of ammonium. There are, however, two marked deviations from this trend. In the presence of added amino acids and glucose, the netplankton exhibit an f-ratio of less than half that of the other incubations which exhibit similar f-ratios (0.228 opposed

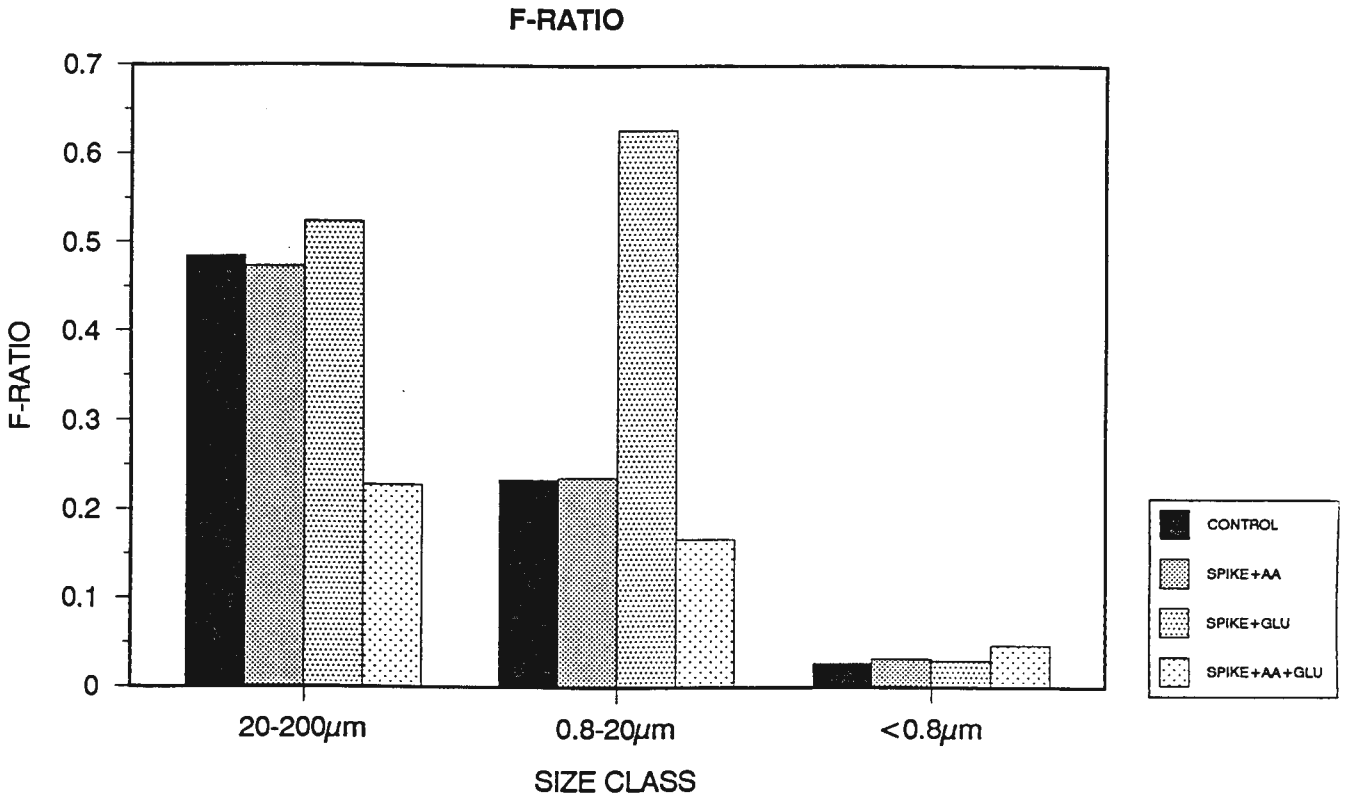

Figure 15: f-ratios calculated for the different treatments and size fractions.

to between 0.473 and 0.524). On the other hand, in the presence of added glucose, the nanoplankton give rise to an f-ratio about three times that of the other incubations (0.627 as opposed to between 0.167 and 0.236). These patterns logically follow similar trends to those shown by RPI values.

Ammonium regeneration:

Ammonium regeneration rates are depicted in figure 16. It is evident that the presence of added glucose has an inhibitory effect on the regeneration of ammonium. Both NH_4+GLU and NH_4+AA+GLU incubations exhibited regeneration of limited duration, with regeneration declining to zero within about three hours. On the other hand, the presence of added amino acids appears to enhance ammonium regeneration relative to the control incubation. Both decline to zero regeneration within about 10 hours, with the control exhibiting a peak regeneration rate of approximately 0.24 $\mu molN.l^{-1}.h^{-1}$ as compared to 0.27 $\mu molN.l^{-1}.h^{-1}$ exhibited by the incubation with added amino acids only.

Amino acid concentration with time:

Total measured amino acid concentrations with time are shown in figure 17. These represent a summation of the individual amino acid concentrations measured. Amino acid concentrations show much fluctuation over short (order of hours) time periods, indicating potentially complex uptake and regeneration dynamics. It is noted that, with the exception of the NH_4+AA incubation, those incubations amended with amino acids do not exhibit an elevated initial amino acid concentration as would be expected. Given this fact and the fact that equipment failure resulted in a delay in sample analysis, it is suggested that these data may be corrupted to some degree by process error. Due to this uncertainty, definite conclusions can not be drawn from these data.

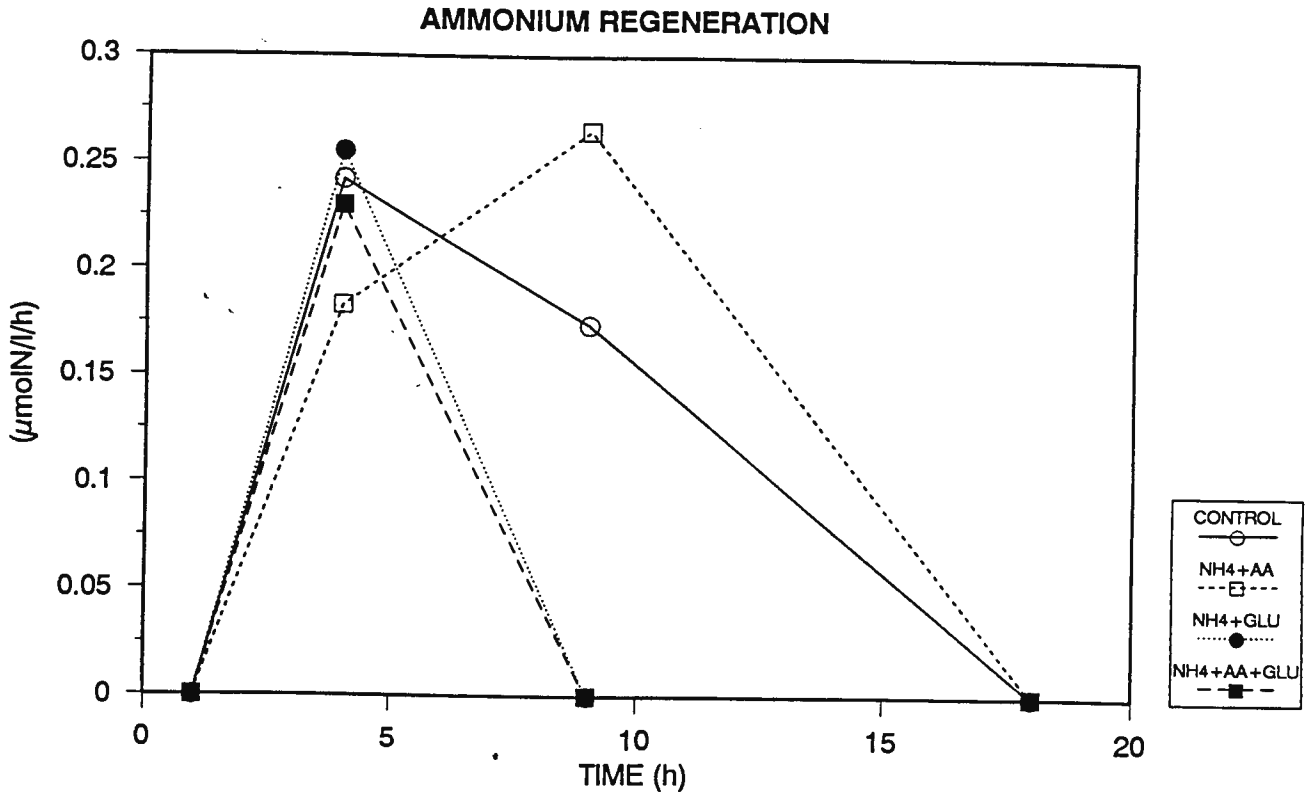

Figure 16: Ammonium regeneration (µmolN/l/h) with time for the different treatments.

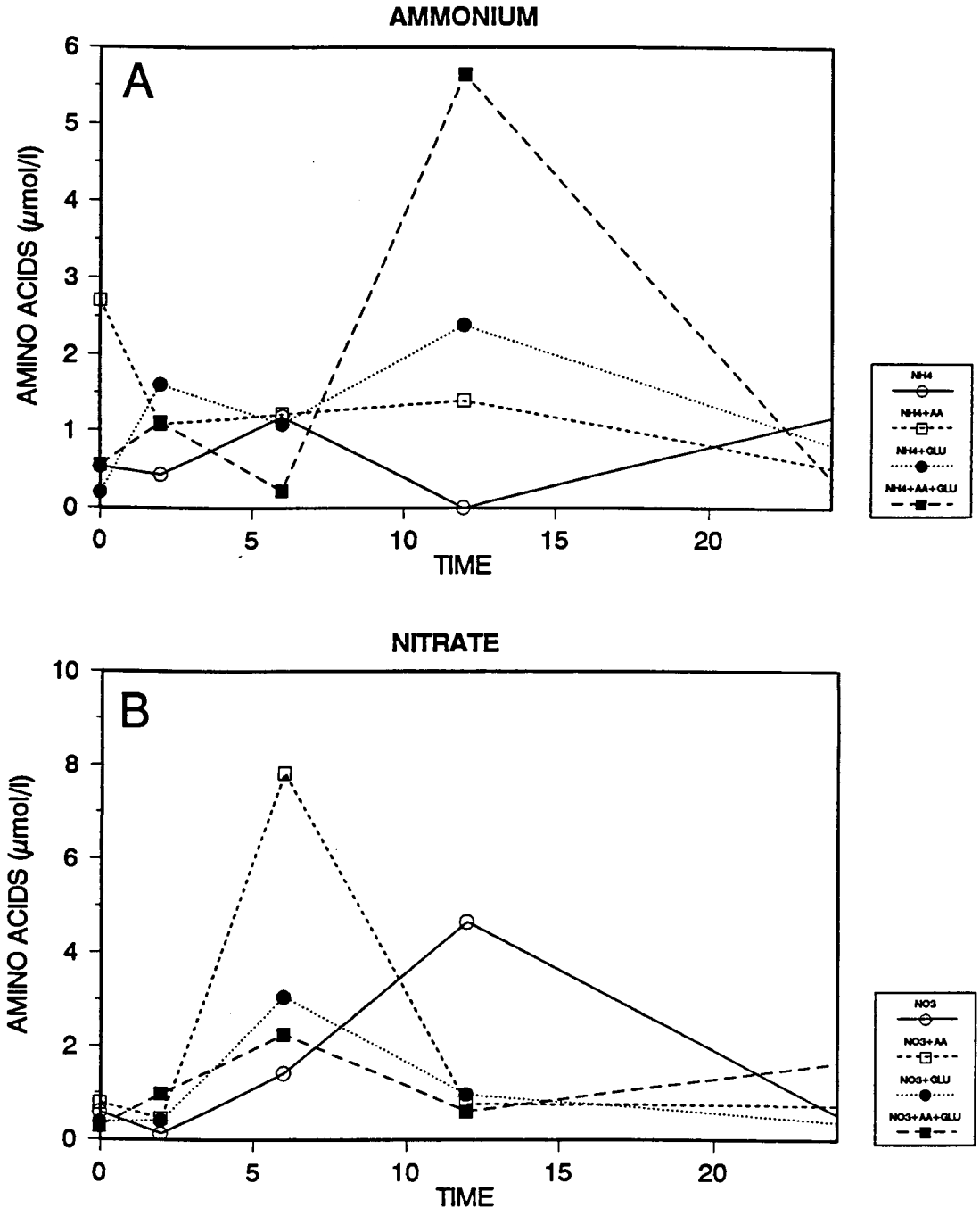

Figure 17: Summed amino acid concentrations (µmol/l) with time (h) for the ammonium and nitrate spiked incubations.

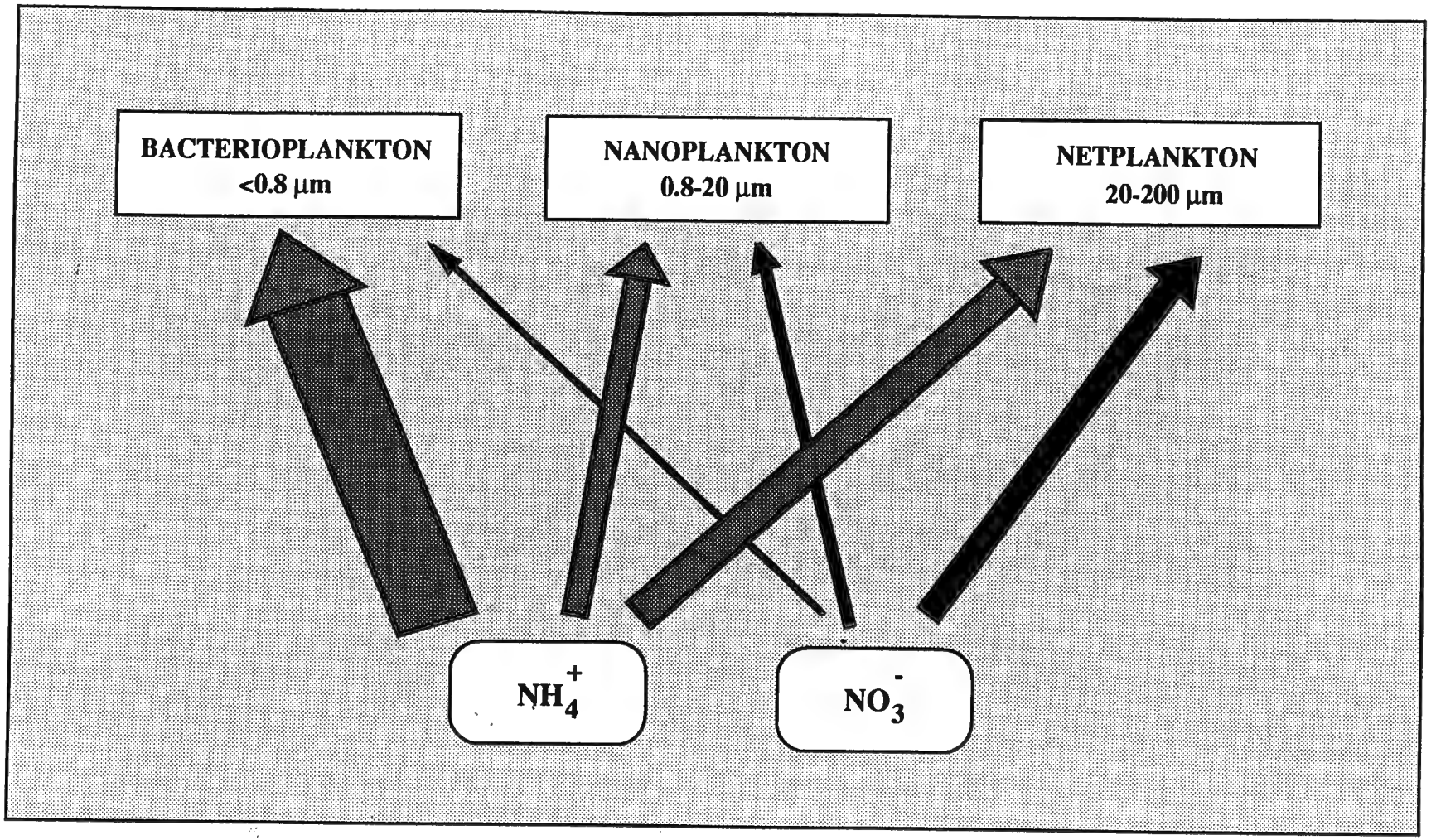

Figure 18: Simplified, semi-quantitative model of ammonium and nitrate uptake by the different size fractions from the control incubations.

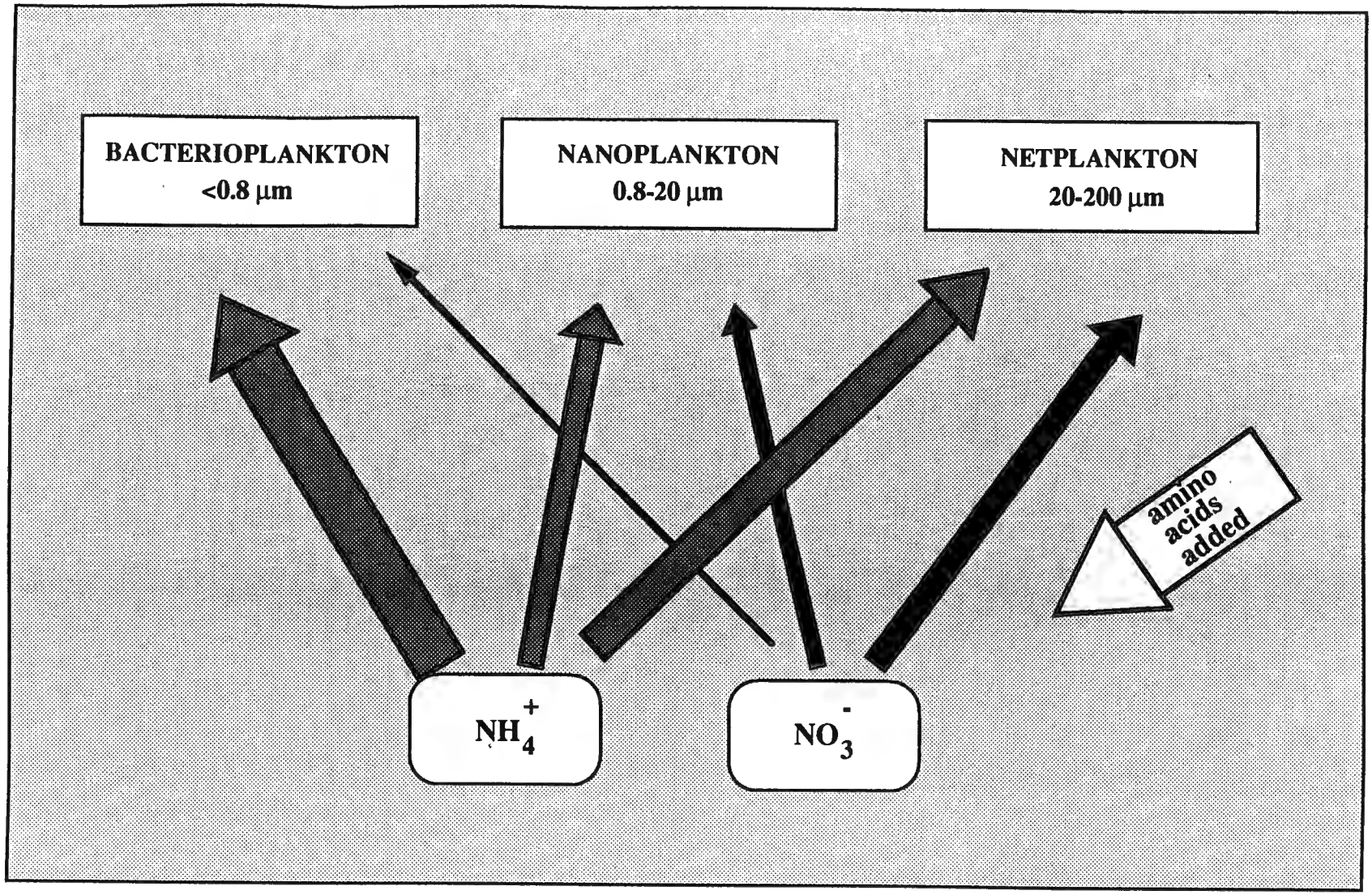

Figure 19: Simplified, semi-quantitative model of ammonium and nitrate uptake by the different size fractions from the incubations with added amino acids.

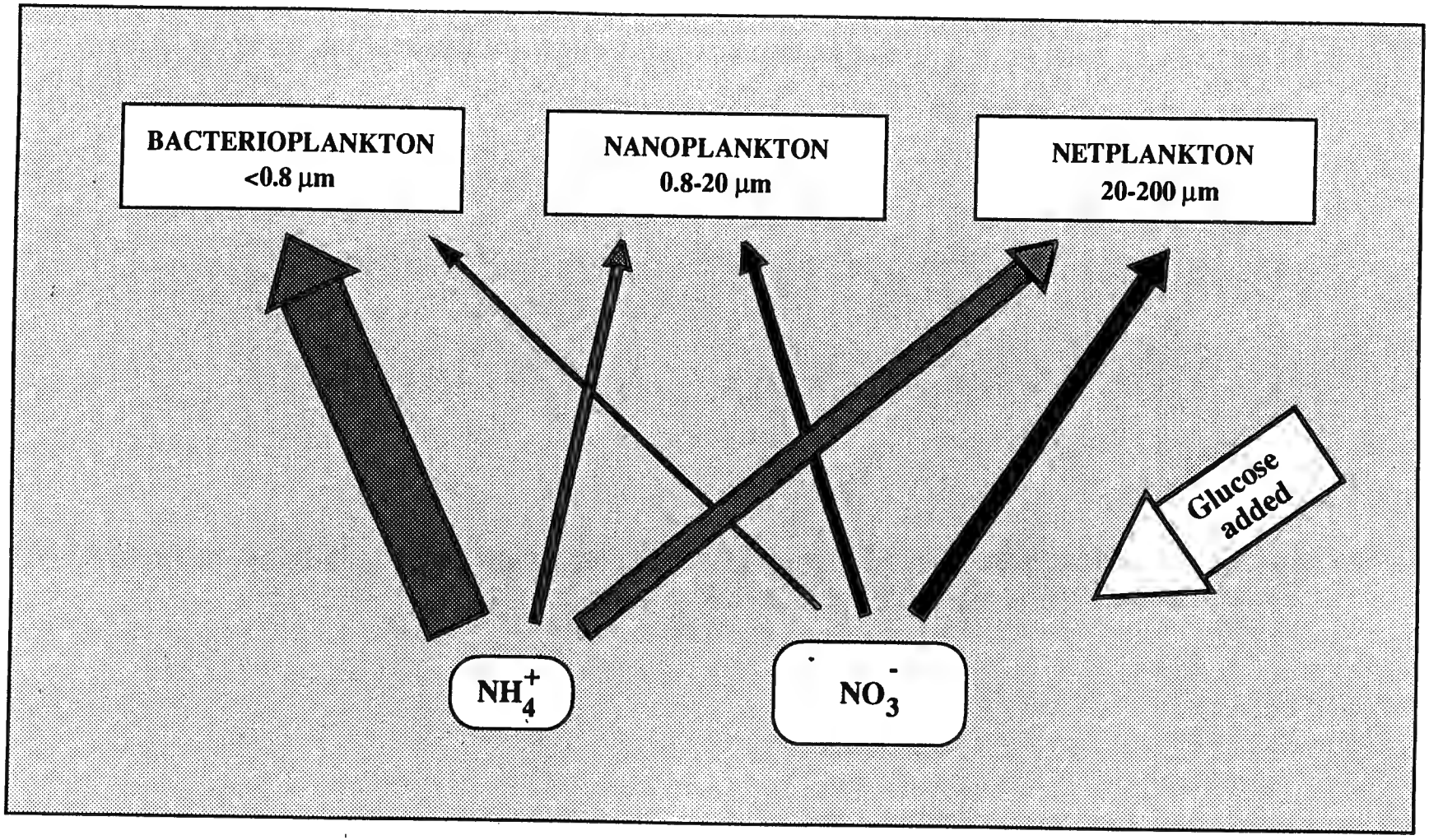

Figure 20: Simplified, semi-quantitative model of ammonium and nitrate uptake by the different size fractions from the incubations with added glucose.

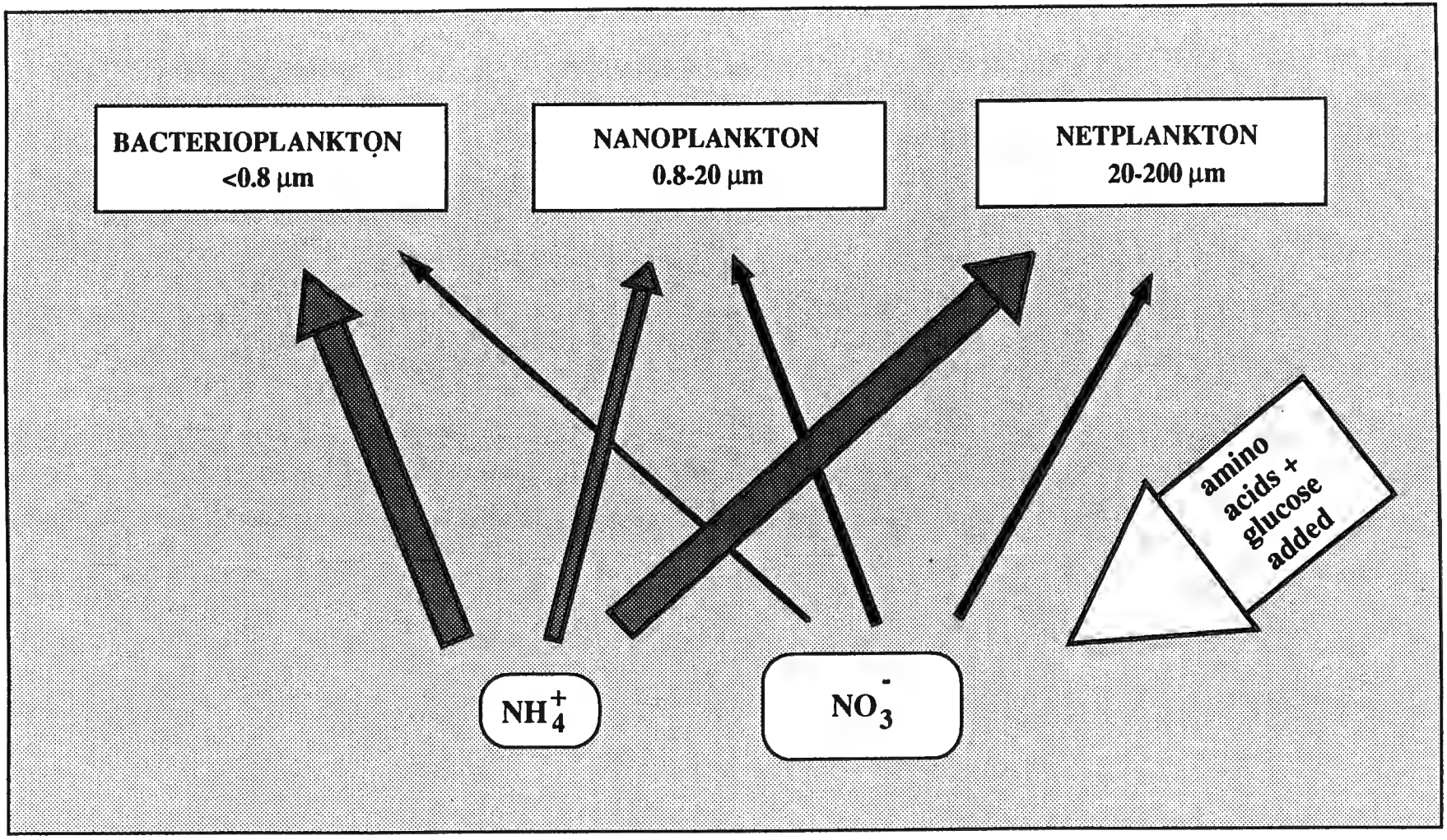

Figure 21: Simplified, semi-quantitative model of ammonium and nitrate uptake by the different size fractions from the incubations with added amino acids and glucose.

Modelling measured nutrient fluxes:

Simplified semi-quantitative models of inorganic nitrogen fluxes in the presence of the different organic additions are presented in figures 18-21.

DISCUSSION

Nitrogen in the ocean:

Nitrogen dynamics have been shown to be of supreme importance to the nutrition of planktonic systems in the ocean, with nitrogen frequently featuring as the rate limiting nutrient (e.g. Dugdale & Goering 1967; Fuhrman *et al.* 1988). Much work in recent years has consequently focussed on the relationships between autotrophic organisms and their nutritional environment. The species of nitrogen utilized as the predominant nitrogen source for growth has important implications for the carbon flux of the system as a whole. The amount of production based on new nitrogen sets limits on the productivity of the system. The rate at which organic nitrogen may be exported from the system is dependant on the rate of new nitrogen imported into it (Dugdale & Goering 1967). Production based on new nitrogen can thus be seen as that which determines production throughput to higher trophic levels of the system (Eppley & Peterson 1979). Goldman and Caron (1985) suggested that a regeneration based system implied a longer more complex food chain leading to the mesozooplankton than one based on new nitrogen. This study examines the effects of competition between bacterioplanktonic and phytoplanktonic organisms for nitrogen, and the effects of nutrient enrichments on this competition.

Bacterial utilization of ammonium as a nitrogen source:

Work done in recent years has pointed to the importance of bacteria in planktonic biomass and productivity. Historically bacteria were considered to utilize DON to satisfy their nitrogen growth requirements (Wright & Hobbie 1965; Dugdale & Goering 1967). More recently, however, it has been shown that bacterial nitrogen uptake is not limited to dissolved organic nitrogen (DON), but that heterotrophic bacteria may in fact be responsible for a significant proportion of dissolved inorganic nitrogen (DIN) taken up (e.g. Horstmann & Hoppe 1981; Laws *et al.* 1985; Wheeler & Kirchman 1986).

The results of this study support these findings, indicating that a large proportion of ammonium uptake is due to the bacterioplankton. Comparing ammonium uptake by the different size fractions (figure 12), 48-75% of that taken up by the intact assemblage was due to uptake by the bacterial fraction. Similarly it was found that between 39 and 58% of inorganic nitrogen (ammonium and nitrate) uptake was due to this fraction. This is comparable to the results of Harrison and Wood (1988) who found the bacterioplankton to be responsible for 30-72% of intact community uptake, and Laws *et al.* (1985) who calculated that >50% of ammonium uptake was due to bacterioplankton. However, Probyn (1985) found bacterial (<1μm) uptake of ammonium to comprise only 27% of that taken up by the intact assemblage in shelf waters and as little as 10% in coastal waters. The proportion of ammonium uptake due to bacteria may increase with decreasing ammonium concentrations given their efficient nutrient transport systems and high substrate affinity (Billen 1984), coupled with low cell volume:surface area ratios and k_s values (half-saturation constants: Morita 1984), which allow them to compete successfully with phytoplankton for this substrate. Nitrate was not found to

comprise a significant portion of nitrogen taken up by the bacterioplankton, and was estimated to supply as little as 2-5% of nitrogen taken up.

Uptake in response to a pulse of ammonium:

It has been noted that nitrogen is frequently growth limiting in the marine environment. The ability of phytoplankton to exploit ammonium occurring in nutrient micropatches has been well documented (e.g. Glibert *et al.* 1982a). According to Glibert and Goldman (1981), and Glibert *et al.* (1982a), phytoplankton in nitrogen poor waters may obtain nitrogen by rapid uptake of ammonium, and uptake of this substrate may occur more rapidly than assimilation and subsequent metabolism (Wheeler and McCarthy 1982). Wheeler *et al.* (1982) noted that, where ammonium concentrations were low, a spike of just 0.05μM gave rise to rapid initial uptake of ammonium which then declined after 10-15 minutes. A similar pattern of rapid uptake followed by a steep decline in the ammonium uptake rate was observed in this study. This pattern was consistent throughout the range of treatments and size fractions, where uptake was observed to peak over the first two hours and decline to zero by T_2 due to the exhaustion of available ammonium.

Nitrogen resource partitioning by the size fractions:

Preferential uptake of ammonium over nitrate:

The preference exhibited by all phytoplankton for ammonium as the nitrogen source for growth has been well documented. It has been shown that even in regions where nitrate comprises as much as 90% of available inorganic nitrogen, ammonium may be taken up selectively (McCarthy *et al.* 1977; Glibert *et al.* 1982b; Probyn & Painting 1985; Koike *et al.* 1986; Probyn 1988). Relative preference indices calculated for the community under study here clearly indicate this preference for ammonium as the

primary nitrogen source. RPI values for ammonium were typically greater than unity, ranging from 0.914 to 6.485, and generally between 1 and 3. It has also been noted that size correlated differential uptake of ammonium and nitrate occurs. Larger phytoplanktonic organisms (netplankton) appear typically to utilize a high proportion of nitrate, while the smaller phytoplankton (nanoplankton) appear to take up a greater proportion of ammonium (e.g. Malone 1980; Nalewajko & Garside 1983; Probyn 1985; Probyn & Painting 1985). RPI_{NH4} values were found to increase with decreasing size, those obtained for netplankton ranging from 1.278 to 5.252 (average RPI_{NH4} = 2.364), nanoplankton from 0.914 to 5.667 (average RPI_{NH4} = 2.757), and bacterioplankton from 2.377 to 6.485 (average RPI_{NH4} = 3.626).

Nitrate RPI values logically displayed the reverse trend, with the netplankton exhibiting values of between 0.267 and 0.885 (average RPI_{NO3} = 0.66); the nanoplankton between 0.196 and 1.059 (average = 0.496); and the bacterioplankton less than 0.06 (average 0.049). Although bacteria have been found to be capable of nitrate uptake, this was completely suppressed in the presence of amino acids and ammonium (Brown *et al.* 1972), and they are seldom found to utilize nitrate as a significant nitrogen source (Wheeler & Kirchman 1986). The RPI values calculated for this fraction here demonstrate this clearly.

Netplanktonic uptake of nitrate

Netplankton generally exhibit rapid growth rates in comparison with smaller phytoplanktonic organisms (Furnas 1990). Typical rates of regenerated nitrogen production are insufficient to support these rates of growth and alternative forms of nitrogen are required to supplement nitrogen growth requirements. Malone (1980) suggested that netplankton use primarily nitrate as a nitrogen source, proliferating in

nitrate rich waters. Probyn and Painting (1985) note that evidence suggests the likelihood of the dominant size classes within the phytoplankton community being correlated with the proportion of new production; and Probyn (1985) noted a correlation between netplanktonic abundance and nitrate availability, with netplankton abounding in newly upwelled nitrate rich waters. Results of this study indicate that the proportion of ammonium taken up increased with decreasing size, while nitrate uptake followed the opposite trend. Comparing uptake of ammonium and nitrate by the different size fractions in the control incubations at T1, it was found that nitrate comprised 48% of nitrogen taken up by the netplankton, 23% of that taken up by the nanoplankton and only 2.5% of that taken up by the bacterioplankton. This trend was consistent throughout the range of treatments (figure 13).

Given the preference for ammonium demonstrated by all phytoplankton, it would appear that the nanoplankton are able to outcompete the netplankton with their relatively large cell volume:surface area ratios, thus necessitating a greater uptake of nitrate by this fraction in order to satisfy their nitrogen growth requirements. In the presence of bacterial uptake of ammonium, ammonium availability would be further reduced and this effect would become even more pronounced. It has been suggested in the literature, that bacteria utilising ammonium as a nitrogen source are able to compete successfully with phytoplankton for this nutrient (e.g. Eppley *et al.* 1977; Horstmann & Hoppe 1981). Whereas Koike *et al.* (1986) found nanoplankton to be more efficient than netplankton at nitrogen uptake; Kirchman *et al.* (1990) suggested that large phytoplankton using nitrate may outcompete smaller phytoplankton using ammonium in the presence of bacterial competition for this nitrogen source. This would imply that, while netplankton may switch to nitrate uptake to supplement their nitrogen requirements, small phytoplankton are not able to do so to the same level of efficiency.

The intact assemblage (<200μm) in this study was observed, in the absence of any organic additions, to exhibit consistently higher a%e (nitrate) than that of the fraction excluding the netplankton (<20μm: see figure 8). This indicates increased nitrate uptake per unit netplanktonic biomass relative to the <20μm community, which would suggest that netplankton have an enhanced ability to utilize nitrate. If the above suppositions are correct, it would appear that nanoplankton are potentially outcompeted for ammonium nitrogen by the bacterioplankton on the one hand, and for nitrate nitrogen by netplankton on the other.

Enrichment with organic nutrients:

It is evident that competition effects are complex, as are their impacts on the productivity of the system. The effects of competition on the proportion of primary production deriving from new nitrogen (predominantly nitrate) versus regenerated nitrogen (urea and ammonium) may have far-reaching consequences for the carbon flux of the system. This study was carried out in an attempt to examine these competition effects by means of organic additions. It was hypothesized that the addition of certain key nutrients would elucidate the effects of competition, either through enhancing or mediating them.

Dissolved free amino acids:

The utilization of dissolved free amino acids by bacterioplankton as a source of the nitrogen and energetic requirements for growth has been widely documented (Billen 1984; Fuhrman *et al.* 1988; Kirchman *et al.* 1989). Bacteria are able to utilize these compounds directly for protein synthesis in preference to *de novo* synthesis of amino acids (Kirchman *et al.* 1985). The utilization of amino acids therefore saves bacteria the energetic expense incurred by the utilization of ammonium or nitrate, and these

compounds represent the preferred bacterial nitrogen source for growth (Kirchman *et al.* 1989). It has been suggested however that concentrations of DFAA in the ocean are frequently limiting and that bacteria will switch to DIN to supplement their nitrogen growth requirements. Thus the availibility, or otherwise, of sufficient amino acid nitrogen may have important implications for phytoplankton productivity due to the effects of competition for ammonium. In this study it was hypothesized that enrichment of the dissolved nitrogen pool with amino acids would decrease bacterial ammonium demand. This would liberate this substrate for phytoplanktonic uptake; and, given the well-documented phytoplanktonic preference for ammonium as a nitrogen source, would tend to suppress nitrate uptake by the phytoplanktonic community.

Dissolved inorganic carbon:

While dissolved inorganic carbon (phytoplanktonic carbon source) is plentiful in the oceans, the availability of dissolved organic carbon (heterotrophic bacterial carbon source), if limiting, may inhibit ammonium assimilation by bacteria (Kirchman *et al.* 1990). When dissolved organic carbon concentrations are insufficient to supply the energetic requirements for growth, ammonium uptake is limited stoichiometrically by carbon uptake in a ratio of at least 4:1 (carbon:ammonium: Kirchman *et al.* 1990). Thus addition of an organic carbon source (glucose) would be expected to enhance bacterial ammonium uptake, and thereby any potential competition for ammonium.

The results of this study indicate that organic nutrient enrichment is influential with respect to nitrogen uptake dynamics. The bacterial fraction responded differently from the net- and nano- planktonic fractions to these additions, indicating the differing uptake physiologies of the bacterioplankton and phytoplankton.

Enrichment with glucose:

Upon examination of total inorganic nitrogen taken up at T_1, it appeared that, for both the net- and nano-planktonic fractions, glucose addition suppressed ammonium uptake rates. Uptake rates in the presence of added glucose were about 65% (netplankton) and 18% (nanoplankton) of the average uptake rates in the absence of glucose. Kirchman *et al.* (1990) found that addition of glucose stimulated bacterial ammonium uptake, and Sherr *et al.* (1986) found glucose addition to increase ammonium depletion. On examination of ammonium substrate concentrations (figure 1), it is evident that, while initial substrate concentrations are similar for all treatments, the treatments enriched with glucose show greatly depleted ammonium concentrations by the end of the first incubation stage (2 hours). It therefore appears that the observed inhibition of nitrogen uptake was due to ammonium substrate depletion.

Examination of ammonium regeneration data (figure 16) indicates inhibition of regeneration in these incubations (with regeneration declining to zero by T_2) relative to those unamended with glucose. Although ammonium concentrations within the incubations decreased, the bacterial fraction did not exhibit a concommitant increase in uptake, thus eliminating competition by this fraction as the cause of decreased uptake rates by these fractions. It appeared, rather, that glucose enrichment gave rise to decreased ammonium regeneration.

It is proposed that protozoans may be indirectly responsible for this effect. Microscopic examination revealed the presence of protozoans such as ciliates and flagellates in the incubations. It is generally assumed that heterotrophic protozoans are incapable of competing for relatively low molecular weight compounds, such as

glucose, at the typically low *in situ* concentrations occurring in aquatic systems (Sherr 1988). She demonstrates that these flagellates are, however, able to take up higher molecular weight compounds, which are often found to occur at comparatively high concentrations. The flagellates under study here were typically of the order of 10μm. However, Rasmussen and Orias (1975) found *Tetrahymena* cells to be capable of axenic uptake of glucose. Protozoans are recognized to frequently be the primary agents of ammonium regeneration in the pelagial (e.g. Johannes 1965; Kiefer & Atkinson 1984; Probyn 1987; Caron *et al.* 1988). It may be that, where carbon is in short supply, excess nitrogen taken up by heterotrophic protozoans would not be assimilated but excreted in the form of ammonium. The corollary of this argument is, that, under non-limiting conditions, this nitrogen is retained and assimilated leading to cessation or a decrease of ammonium regenerated. It is suggested that under non-glucose enriched conditions, excess nitrogen taken up by the protozoans observed in the incubations was regenerated as ammonium. Upon enrichment with added glucose however, these organisms are theoretically capable of taking up glucose directly from the environment leading to retention of nitrogen. The effect of nitrogen retention would logically be a decrease in ammonium regeneration rates as observed, resulting in the measured decline in ammonium concentration. A simple model illustrating this proposal is presented in figure 22.

Enrichment with amino acids:

Enrichment with amino acids appeared to have no impact on ammonium uptake by the netplankton. This can be explained in the light of competition between net- and nano-plankton for ammonium, and the relatively successful uptake of this nutrient by the nanoplanktonic fraction. The increased net availability of ammonium may have little impact on the amount of ammonium available to the netplankton, any additional

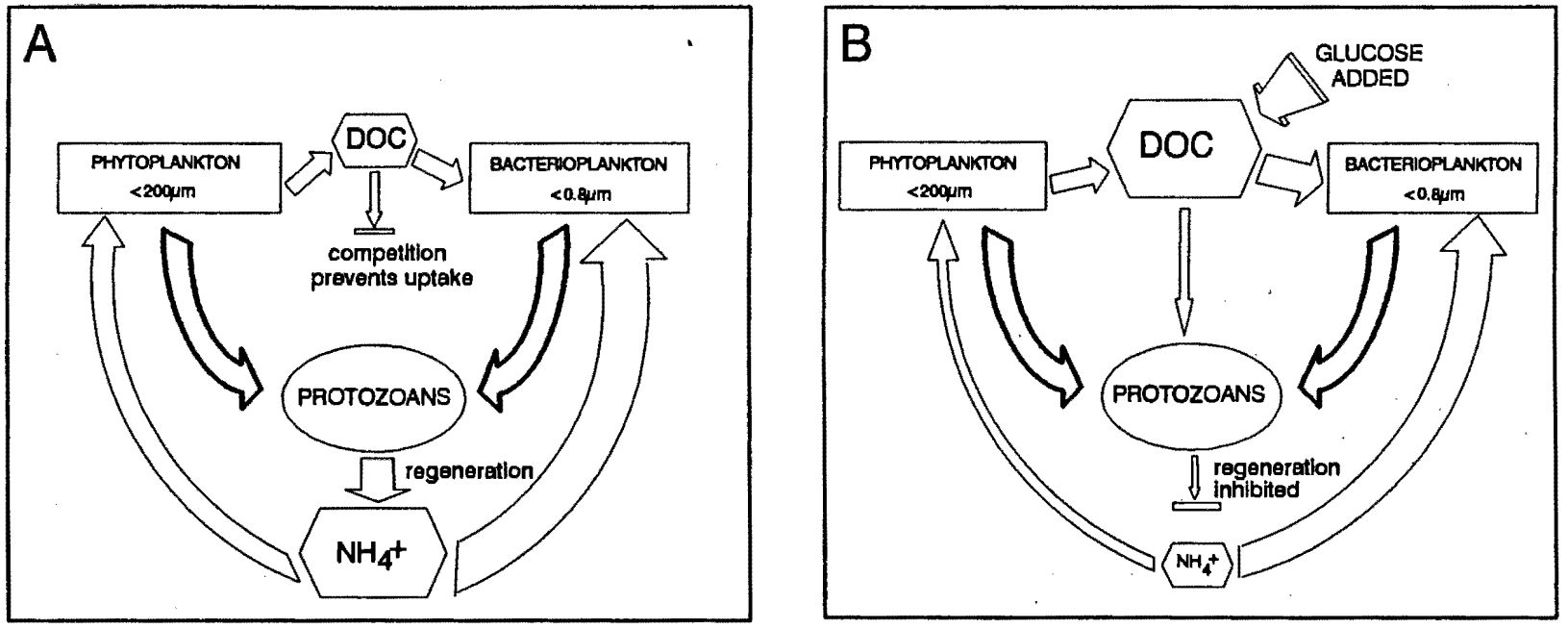

Figure 22: A simplified model illustrating the postulated impact of protozoan uptake and regeneration of organic substrates on nutrient fluxes through the phytoplankton and bacteria. Fluxes of particulate matter are distinguished from nutrient fluxes by bold arrows.

ammonium available to the phytoplankton being taken up by the nanoplankton. The nanoplankton demonstrated enhancement of ammonium uptake in the presence of added amino acids. It is suggested that bacterial ammonium demand was minimized by enrichment of their DON supply freeing a greater proportion of ammonium for nanoplanktonic uptake. This is supported by the effect of amino acid enrichment on the bacterial fraction (figure 9C), which was found to take up less ammonium in the presence of added amino acids. As a corollary to this, nitrate uptake by both the netplanktonic and nanoplanktonic fractions appeared to be depressed in the incubations amended with amino acids. If bacterial competition for ammonium is abated by an increased supply of dissolved organic nitrogen, then it is to be expected that phytoplankton will utilize a greater proportion of their preferred nitrogen source, resulting in lower nitrate uptake rates.

Enrichment with both amino acids and glucose:

Little effect on netplanktonic ammonium uptake was observed in the treatment enriched with both amino acids and glucose. This is inconsistent with the observed depletion of ammonium in this incubation and the argument expounded above, and is difficult to explain. Given the observed ammonium depletion, and the lack of observable effect within the incubation enriched with amino acids only, this result cannot be attributed to a mediation of the effects of bacterial competition on ammonium availability by amino acid enrichment. While process error is a possible cause of this anomaly, I am hesitant to disregard this result, as the theory discussed above concerning the effects of protozoans remains to be tested.

While nitrate uptake by the bacterial fraction was insignificant for all treatments, ammonium uptake appeared to be inhibited in the presence of amino acids and to a

lesser extent by glucose. The compound effect of a combination of both amino acid and glucose enrichment resulted in the greatest inhibition of uptake. It is suggested that bacteria were able to utilise the added amino acids as a nitrogen source, thus decreasing the amount of ammonium required to supplement their nitrogen requirements for growth. Kirchman *et al.* (1990) found no consistent effect of glucose addition on bacterial uptake of primary amines, and free amino acid uptake appears to be dependent only on amino acid concentrations (Kirchman pers. comm.). It has been noted, however by Keil and Kirchman (Mar. Ecol. Prog. Ser., in press) that glucose apparently can increase uptake of DON compounds other than amino acids thus diminishing DIN (and consequently ammonium) demand. It is also possible, however, that ammonium depletion may be responsible for decreased uptake.

Enrichment with these compounds mediated to an extent the observed ammonium uptake inhibition by glucose enrichment within the nanoplanktonic fraction. This indicates that bacterial demand for ammonium was in fact lessened in the presence of these compounds, resulting in a greater proportion of ammonium available to the nanoplankton.

Competition and its effect on the f-ratio:

Nutrient enrichment demonstrated an observable effect of nutrient availability (in terms of both quality and quantity) on competition for nitrogen and its partitioning between the different community components. The f-ratio (new versus total production) serves as an indication of the proportion of primary production becoming available as carbon to the system, and provides a useful indication of how competition impacts on system productivity. A trend of decreasing f-values with decreasing cell size was observed, indicating size correlated roles played by the different fractions. The netplankton were

found to be responsible for the bulk of new production, and hence the greater energy throughput to the higher trophic levels of the system. The bacterial fraction were observed to take up only insignificant amounts of nitrate and this is reflected by extremely low f-values. As expected, bacterial production was largely based on regenerated nitrogen, while netplankton were responsible for the bulk of new production. Variations of the f-value for the bacterial fraction, while observable, are insignificant given their small magnitude.

While the f-ratio values obtained demonstrated a clear size correlated trend, no strong trends were observable with respect to nutrient additions. The f-value for netplanktonic uptake was depressed in the incubation amended with both glucose and amino acids, but not that with only amino acids. This was seen to be the case to a lesser extent for the nanoplankton. Particularly striking is the greatly elevated f-ratio exhibited by this fraction in the presence of added glucose, shown to a lesser extent by the netplankton. The elevated f-values obtained for the nano- and net- plankton are correlated with the low ammonium uptake values obtained for that treatment (see figures 12 and 9B), and are thus most likely a function of the depressed ammonium concentrations and regeneration rates measured in this incubation. The lower values obtained for these fractions in the treatment amended with both amino acids and glucose relate to the low nitrate uptake rates measured in this incubation. It has been suggested that this is due to the observed reduction in bacterial ammonium demand, which is believed to have resulted in increased ammonium availability to the phytoplankton. It is interesting to note that the bacterial f-value for this incubation is elevated with respect to the rest. This would indicate that ammonium uptake was depressed to a greater extent than nitrate uptake in the presence of added amino acids. Why this should be

the case is not certain, however the magnitude of the f-ratios for this fraction approach zero and any variations thus approach insignificance.

CONCLUSIONS

1. The bacterioplankton were found to be responsible for a significant portion of community uptake, 48-75% of the ammonium taken up by the intact community being due to this fraction.

2. Of the measured inorganic nitrogen taken up by the bacterioplankton, ammonium comprised 95-98% and nitrate as little as 2-5%, indicating the importance of reduced nitrogen to bacterial production.

3. Phytoplankton were observed to exert strong selection for ammonium over nitrate, evidenced by ammonium RPI values significantly greater than unity, and nitrate RPI values consistently below unity.

4. The netplanktonic fraction was responsible for a major portion of nitrate taken up (approximately 50% of intact community uptake) indicating that this fraction is responsible for the bulk of primary production becoming available to higher trophic levels.

5. Interactive competition between the fractions examined here were complicated by the presence of larger organisms such as ciliates and heteroflagellates. It is proposed that the observed inhibition of ammonium uptake in the presence of glucose may have been

due to ammonium depletion facilitated by this component of the assemblage. This theory remains to be tested, and may be done by means of selective screening of the assemblage coupled with nutrient enrichment.

6. Bacterial uptake of ammonium was moderated in the presence of added amino acids, indicating their preference for DON as a nitrogen source for growth. Phytoplanktonic uptake of ammonium increased in response to this mediation of competition pressure.

7. While the different roles of the fractions were clearly demonstrated, the effects of organic additions on system productivity could not be conclusively defined. Future experiments focussing on this facet of competition should be carried out in an attempt to clarify these effects further.

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Appendix 1: Ammonium and nitrate substrate concentrations with time in the different incubations.

TREATMENT	TIME INTERVAL	CONCENTRATION (μmolN.l^{-1}) NH_4^+-N	NO_3^--N
CONTROL	T_0	0.667	0.990
	T1	0.564	0.140
	T2	0.150	0.000
	T3	0.051	0.000
	T4	0.190	0.000
^{15}N+AA	T0	0.549	1.280
	T1	0.528	0.570
	T2	0.190	0.050
	T3	0.051	0.000
	T4	0.118	0.000
^{15}N+GLU	T0	0.579	0.840
	T1	0.000	0.180
	T2	0.154	0.000
	T3	0.067	0.000
	T4	0.667	0.000
^{15}N+AA+GLU	T0	0.651	3.790
	T1	0.051	3.000
	T2	0.103	1.640
	T3	0.067	0.520
	T4	0.221	0.000

Appendix 2: Particulate nitrogen (μmolN.l-1) with time for the different treatments and size fractions.

TREATMENTS	TIME INTERVAL	PARTICULATE NITROGEN (μmolN.l-1) 20-200μm	0.8-20μm	<0.8μm
CONTROL 1 (NH_4)	T1	2.829	1.656	2.643
	T2	2.553	1.768	3.682
	T3	4.289	0.000	4.012
	T4	1.761	4.252	5.745
TREATMENT 1 (NH_4+AA)	T1	2.325	2.008	2.588
	T2	2.621	4.067	3.810
	T3	2.471	1.474	4.568
	T4	6.513	3.976	6.118
TREATMENT 2 (NH_4+GLU)	T1	3.161	1.682	3.010
	T2	2.127	4.011	3.984
	T3	2.651	3.173	3.956
	T4	3.507	4.364	5.409
TREATMENT 3 (NH_4+AA+GLU)	T1	2.981	2.207	2.941
	T2	3.928	4.077	3.904
	T3	3.035	3.233	3.583
	T4	3.111	4.902	5.073
CONTROL 2 (NO_3)	T1	5.239	2.556	3.056
	T2	3.838	4.355	4.460
	T3	4.148	3.857	4.126
	T4	4.103	4.738	4.364
TREATMENT 4 (NO_3+AA)	T1	5.185	3.103	3.208
	T2	5.165	2.270	3.730
	T3	4.677	4.115	4.189
	T4	3.805	3.391	5.584
TREATMENT 5 (NO_3+GLU)	T1	3.946	2.679	2.700
	T2	4.949	2.682	2.946
	T3	4.471	3.475	3.426
	T4	3.282	0.000	7.431
TREATMENT 6 (NO_3+AA+GLU)	T1	2.367	2.115	3.622
	T2	1.847	3.035	4.223
	T3	1.294	3.980	5.364
	T4	3.410	5.368	4.599

Appendix 3: Absolute uptake of nitrogen (μmolN.l^{-1}.h^{-1}) with time for the different treatments and size fractions.

TREATMENTS	TIME INTERVAL	ABSOLUTE UPTAKE (μmolN.l^{-1}.h^{-1}) 20-200μm	0.8-20μm	<0.8μm
CONTROL 1 (NH_4)	T0	0.000	0.000	0.000
	T1	0.153	0.128	0.439
	T2	0.000	0.000	0.000
	T3	0.000	0.000	0.000
	T4	0.000	0.000	0.000
TREATMENT 1 (NH_4+AA)	T0	0.000	0.000	0.000
	T1	0.154	0.152	0.303
	T2	0.000	0.000	0.000
	T3	0.000	0.000	0.000
	T4	0.000	0.000	0.000
TREATMENT 2 (NH_4+GLU)	T0	0.000	0.000	0.000
	T1	0.099	0.022	0.358
	T2	0.000	0.000	0.000
	T3	0.000	0.000	0.000
	T4	0.000	0.000	0.000
TREATMENT 3 (NH_4+AA+GLU)	T0	0.000	0.000	0.000
	T1	0.149	0.090	0.224
	T2	0.000	0.000	0.000
	T3	0.000	0.000	0.000
	T4	0.000	0.000	0.000
CONTROL 2 (NO_3)	T0	0.000	0.000	0.000
	T1	0.144	0.039	0.012
	T2	0.075	0.026	0.000
	T3	0.033	0.012	0.004
	T4	0.017	0.005	0.004
TREATMENT 4 (NO_3+AA)	T0	0.000	0.000	0.000
	T1	0.138	0.047	0.010
	T2	0.082	0.030	0.000
	T3	0.040	0.017	0.008
	T4	0.020	0.012	0.006
TREATMENT 5 (NO_3+GLU)	T0	0.000	0.000	0.000
	T1	0.109	0.037	0.011
	T2	0.040	0.019	0.004
	T3	0.000	0.007	0.007
	T4	0.017	0.006	0.007
TREATMENT 6 (NO_3+AA+GLU)	T0	0.000	0.000	0.000
	T1	0.044	0.018	0.011
	T2	0.045	0.027	0.046
	T3	---	---	---
	T4	0.037	0.038	0.032

missing data points are represented by --- .

Appendix 4: Atom % excess with time for the different treatments and size fractions.

TREATMENT	SIZE	TIME	$^{15}NH_4^+$	$^{15}NO_3^-$
CONTROL	TOTAL	T_0	0.000	0.00
		T_i	0.577*	
		T_1	1.829	5.002
		T_2	1.706	5.865
		T_3	0.995	5.810
		T_4	---	6.000
	<20μm	T_0	0.000	0.000
		T_1	2.850	2.811
		T_2	1.757	2.470
		T_3	2.183	3.483
		T_4	---	3.612
	<0.8μm	T_0	0.000	0.000
		T_1	3.952	1.182
		T_2	3.189	0.125
		T_3	2.691	1.946
		T_4	---	2.320
^{15}N+AA	TOTAL	T_0	0.000	0.000
		T_i	0.507*	
		T_1	1.589	3.855
		T2	1.571	6.154
		T3	1.452	5.377
		T4	---	5.800
	<20μm	T_0	0.000	0.000
		T_1	2.147	2.232
		T_2	1.255	3.234
		T3	1.635	3.017
		T4	---	3.669
	<0.8μm	T0	0.000	0.000
		T1	2.775	0.661
		T2	2.245	0.000
		T3	2.052	1.816
		T4	---	2.064
^{15}N+GLU	TOTAL	T0	0.000	0.000
		Ti	0.463*	
		T1	1.579	5.523
		T2	1.241	5.485
		T3	1.365	6.596
		T4	1.030	6.723

Appendix 4 continued:

TREATMENT	SIZE	TIME	$^{15}NH_4^+$	$^{15}NO_3^-$
	<20μm	T0	0.000	0.000
		T1	2.334	3.157
		T2	1.060	3.974
		T3	1.753	3.494
		T4	1.339	4.186
	<0.8μm	T0	0.000	0.000
		T1	3.471	1.698
		T2	2.520	1.531
		T3	2.349	2.339
		T4	1.820	2.363
^{15}N+AA+GLU	TOTAL	T0	0.000	0.000
		Ti	0.428*	
		T1	1.468	0.817
		T2	0.983	3.079
		T3	1.800	---
		T4	1.109	5.579
	<20μm	T0	0.000	0.000
		T1	1.745	0.537
		T2	1.066	2.532
		T3	1.455	---
		T4	1.098	5.370
	<0.8μm	T0	0.000	0.000
		T1	2.216	0.251
		T2	1.475	2.516
		T3	1.621	---
		T4	1.204	5.251

`T_i' represents an interim (unfractionated) sample taken from the incubations spiked with ammonium 20 minutes after the start of the incubation. These data points are marked with an asterisk (*).

` --- ' represents missing data points.

Appendix 5: Particulate nitrogen and absolute uptake of nitrogen for the different treatments and size fractions at T1.

TREATMENT	PARTICULATE N (μmolN.l^{-1})		ABSOLUTE UPTAKE (μmolN.l^{-1}.h^{-1})		
	$^{15}NH_4^{+*}$	$^{15}NO_3^{-*}$	NH_4^+-N	NO_3^--N	TOTAL N
20-200μm					
CONTROL	2.829	5.239	0.153	0.144	0.297
^{15}N+AA	2.325	5.185	0.154	0.138	0.292
^{15}N+GLU	3.161	3.946	0.099	0.109	0.208
^{15}N+AA+GLU	2.981	2.367	0.149	0.044	0.193
0.8-20μm					
CONTROL	1.656	2.556	0.128	0.039	0.167
^{15}N+AA	2.008	3.103	0.152	0.047	0.199
^{15}N+GLU	1.682	2.679	0.022	0.037	0.059
^{15}N+AA+GLU	2.207	2.115	0.090	0.018	0.108
<0.8μm					
CONTROL	2.643	3.056	0.439	0.012	0.451
^{15}N+AA	2.588	3.208	0.303	0.010	0.313
^{15}N+GLU	3.010	2.700	0.358	0.011	0.369
^{15}N+AA+GLU	2.941	3.622	0.224	0.011	0.235

$^{15}NH_4^{+*}$ and $^{15}NO_3^{-*}$ distinguishes the sets of incubations spiked with either ^{15}N-NO_3^- or ^{15}N-NH_4^+.

Appendix 6: Relative preference indices calculated for the different treatments and size fractions.

TREATMENT	SIZE FRACTION 20-200μm	0.8-20μm	<0.8μm
NITRATE			
CONTROL	0.812	0.392	0.045
$^{15}NO_3$+AA	0.676	0.337	0.046
$^{15}NO_3$+GLU	0.885	1.059	0.051
$^{15}NO_3$+AA+GLU	0.267	0.196	0.055
AMMONIUM			
CONTROL	1.278	1.901	2.414
$^{15}NH_4$+AA	1.757	2.547	3.227
$^{15}NH_4$+GLU	1.167	0.914	2.377
$^{15}NH_4$+AA+GLU	5.252	5.667	6.483

Appendix 7: F-ratio values calculated for the different treatments and size fractions.

TREATMENT	SIZE FRACTION 20-200μm	0.8-20μm	<0.8μm
CONTROL	0.485	0.234	0.027
^{15}N+AA	0.473	0.236	0.032
^{15}N+GLU	0.524	0.627	0.030
^{15}N+AA+GLU	0.228	0.167	0.047

Appendix 8: Ammonium regeneration rates (μmolN.l^{-1}.h^{-1}) with time for the different treatments.

TREATMENT	TIME INTERVAL	REGENERATION (μmolN.l^{-1}.h^{-1})
CONTROL 1 (NH_4)	T0	0.000
	T0-T1	0.242
	T1-T2	0.174
	T2-T3	0.000
	T3-T4	0.000
TREATMENT 1 (NH_4+AA)	T0	0.000
	T0-T1	0.183
	T1-T2	0.265
	T2-T3	0.000
	T3-T4	0.000
TREATMENT 2 (NH_4+GLU)	T0	0.000
	T0-T1	0.255
	T1-T2	0.000
	T2-T3	0.000
	T3-T4	0.000
TREATMENT 3 (NH_4+AA+GLU)	T0	0.000
	T0-T1	0.230
	T1-T2	0.000
	T2-T3	0.000
	T3-T4	0.000

Appendix 9: Total amino acid concentrations ($\mu mol.l^{-1}$) with time for the different treatments and size fractions.

TREATMENT	TIME INTERVAL	CONCENTRATION ($\mu mol.l^{-1}$) $^{15}NH_4^+$ SPIKED	$^{15}NO_3^-$ SPIKED
CONTROL	T0	0.533	0.611
	T1	0.430	0.136
	T2	1.185	1.432
	T3	---	4.650
	T4	1.177	0.536
^{15}N+AA	T0	2.705	0.801
	T1	1.089	0.467
	T2	1.213	7.823
	T3	1.396	0.777
	T4	0.512	0.732
^{15}N+GLU	T0	0.198	0.398
	T1	1.603	0.425
	T2	1.082	3.053
	T3	2.384	0.980
	T4	0.816	0.387
^{15}N+AA+GLU	T0	0.552	0.299
	T1	1.110	0.997
	T2	0.215	2.257
	T3	5.639	0.619
	T4	0.373	1.643