AN ABSTRACT OF THE THESIS OF

Steven Andon Kokkinakis for the degree of Master of Science

in Oceanography presented on July 31, 1986.

Title: Utilization of Inorganic and Organic Nitrogen by Phytoplankton off the Washington and Oregon Coasts

Redacted for privacy

Abstract approved:

Patricia A. Wheeler

Uptake of NO_3^-, NH_4^+, and urea was measured using ^{15}N tracers during the July 1985 upwelling period off the Washington and Oregon coasts. Ammonium regeneration was measured in <10 μm and 10-200 μm size fractions of seawater. Surface concentrations of particulate nitrogen (PN), chl-a and NO_3^- decreased in the offshore direction. Surface NO_3^- concentrations were low (<5 μM) at offshore stations and high (>20 μM) at nearshore stations. Nitrogen uptake rates in low NO_3^- waters (i.e. <5 μM) were low (0.020 to .258 μmol N l^{-1} h^{-1}), and were primarily supported by regenerated (NH_4^+ + urea) nitrogen (71% of total uptake). Conversely, high NO_3^- waters (i.e. >20 μM) had high uptake rates (0.281 to 1.480 μmol N l^{-1} h^{-1}) with new (NO_3^-) nitrogen supporting 83% of total uptake. Nanoplankton biomass (< 10 μm) was relatively more abundant in low NO_3^- stations, while netplankton

biomass (10-200 μm) was dominant in high NO_3^- stations. Turnover times for dissolved NH_4^+ and urea pools (ambient concentration + tracer addition) were very short in both low and high NO_3^- waters (0.3 and 1.2 d respectively). Increasing ambient NH_4^+ concentrations, ranging from 0.06 to 1.23 μM, appeared to inhibit urea assimilation.

Ammonium concentrations remained constant throughout most incubations, suggesting that NH_4^+ supply rates are in balance with uptake rates. However, NH_4^+ uptake rates, measured by increases of ^{15}N in particulate material, were consistently lower than regeneration rates, measured using the isotope dilution technique. Significant improvements in regeneration measurements allowed assessment of this apparent imbalance. Mass balance calculations indicate that 10-90% (mean = 43%) of the ^{15}N label originally added was not recovered as dissolved NH_4^+ or labeled particulate nitrogen at the end of incubations. Furthermore, calculations show that a greater amount of ^{15}N-NH_4^+ left the dissolved pool than appeared in the particulate fraction. Release of ^{15}N-labeled dissolved organic nitrogen is a possible cause for the imbalance between measured NH_4^+ uptake and regeneration.

UTILIZATION OF INORGANIC AND ORGANIC NITROGEN BY PHYTOPLANKTON OFF THE WASHINGTON AND OREGON COASTS

by

Steven Andon Kokkinakis

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed July 31, 1986

Commencement June 1987

APPROVED:

Redacted for privacy

Professor of Oceanography in charge of major

Redacted for privacy

Dean of College of Oceanography

Redacted for privacy

Dean of Graduate School

Date thesis is presented July 31, 1986

Typed by Steven A. Kokkinakis for Steven Andon Kokkinakis

Every mast and timber seemed to have a pulse in it that was beating with life and joy, and I felt a wild exulting in my own heart, and felt as if I would be glad to bound along so round the world.

Herman Melville, "Redburn"

The Road goes ever on and on
  Down from the door where it began.
Now far ahead the Road has gone,
  And I must follow, if I can,
Pursuing it with eager feet,
  Until it joins some larger way
Where many paths and errands meet.
  And whither then? I cannot say.

J.R.R. Tolkien, "The Fellowship of the Ring"

It was a clear steel-blue day. The firmaments of air and sea were hardly separable in that all-pervading azure; only, the pensive star was transparently pure and soft, with a woman's look, and the robust and man-like sea heaved with long, strong, lingering swells, as Samson's chest in his sleep.

Herman Melville, "Moby Dick"

Most human history has not afforded men much chance to pursue their curiosity, except as a hobby of the rich or within the refuge of a monastery somewhere. We can count ourselves fortunate to live in a society and at a time when we are actually paid to explore the universe.

--Henry Stommel, on receiving the Bigelow Medal, 1974

ACKNOWLEDGEMENTS

My most sincere appreciation goes to my major professor, Pat Wheeler, for her support, encouragement, and patience throughout all phases of my graduate program. Thanks Pat for providing all the ingredients necessary for an enjoyable and rewarding graduate experience.

I would also like to thank my committee members, Charlie Miller, Peter Bottomley, and Ken Williamson for their interest and helpful suggestions. Extra thanks goes to Charlie Miller for providing valuable suggestions and comments on an earlier draft of this thesis.

Many thanks to Joe Siebenaller and Fred Prahl for providing me with laboratory space on each of their cruises aboard the R/V Wecoma. Thanks also go to Sandy Moore for running the particulate nitrogen samples, Rod Fujita for the nutrient analyses, and John Westall for the printing of this thesis on his LaserJet.

My first exposure to oceanographic research began with my undergraduate participation in the Sea Education Association (SEA). I would like to give special thanks to the people of SEA for providing an invaluable introduction to the ocean world. I am especially grateful to Jude Wilber, chief scientist on SEA-63, for his encouragement and support during the SEA shore and sea components. Thanks Jude for preventing the epidemic spread of "voluntary ignorance" and for your sincere interest in my academic pursuits.

I would also like to thank another early mentor from Colgate University, Ron Hoham, for being an excellent professor and more importantly a friend. Thanks Ron for your teachings on algae and your continued friendship during my graduate years out here in Oregon. I look forward to another spectacular visit to the "Big Sky" country of Montana.

A big thanks goes to Nan "The Teach" Dudek for instructing me in laboratory techniques necessary for maximum efficiency per unit time. I am also grateful to Mark "Dr. Methods" Brzezinski for providing important clues about how one goes about being a graduate student. Thanks Mark for all your helpful suggestions on "designs for success" in the world of methods development.

My past three years at OSU have been especially enjoyable because of the people I have come to know through work and play. Thanks go to: Margaret O'Brien, for being a cheerful cohort on those early morning Sacajawea cruises; Mark "Dr. Methods" Brzezinski, my student mentor; Hal Batchelder, D & D fanatic and provider of neat computer programs; Joe "lets go rafting" Yuska, outdoor enthusiast and all around wild and scenic kind of guy, Annette and Renate, very special friends from Germany; Alton "Gonzo" Chung, for being such an understanding housemate while I was undergoing PDMS (Predefense Manic Syndrome) and for finding humor in almost everything; Julie Ahern, for all those kinky postcards, Jenny "lets go skiing" Brophy, Michel "Beauty eh" Boudrias, Nan "The Teach" Dudek, John Collins, Kevin Krefft, John Jamerlan, Anne-Marie Fagnan, and Anne Poulson.

Of course I have to pay tribute to my east coast college

buddies Gregg "Dr. Bad" Loshin and Jay "The Man" Andretta for always showing me to a good time back in wild NYC during my infrequent visits home. Recent memories include: travels to Jamaica where things were never quite in control; crazy nights out in the Big Apple till 5:00 a.m., Fort Lauderdale Spring Party Week where Uncle Wiggley discovers the depth of my insanity; road trips to Caz for a some predictable fun; and all those detailed and informative long distance communications.

Finally, I want to offer sincere thanks to my parents for their generous support and encouragement during my undergraduate and graduate schooling. I am very proud and fortunate to have parents like you.

Financial support for this work has been provided by grants OCE-8208873 and OCE-8308753 awarded to Dr. Patricia Wheeler from the National Science Foundation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (cont'd)

LIST OF TABLES

UTILIZATION OF INORGANIC AND ORGANIC NITROGEN BY PHYTOPLANKTON OFF THE WASHINGTON AND OREGON COASTS

Chapter I

INTRODUCTION

Primary production in marine waters relies on a supply of dissolved inorganic and organic forms of nitrogen. Dugdale and Goering (1967) initiated the use of ^{15}N-labeled NO_3^- and NH_4^+ for measurement of uptake rates by phytoplankton in marine waters, and proposed a distinction between "regenerated" and "new" sources of nitrogen. Regenerated nitrogen (NH_4^+, urea, and organic N) is produced within the euphotic zone by invertebrate and fish excretion, and bacterial remineralization. Turnover rates for these nutrients can be rapid, minutes to hours (Paasche and Kristiansen 1982a, Kristiansen 1983). Nitrate and molecular nitrogen (N_2) are forms of new nitrogen. Nitrate is introduced into the euphotic zone principally from deep water, while N_2 enters mostly from the atmosphere. Utilization of new nitrogen is necessary for the net transfer of nitrogen to higher trophic levels.

Eppley and Peterson (1979) and Eppley (1981) discuss factors controlling global distribution of new and regenerated production of particulate matter. They argue that new production must approximate

the sum of sinking flux of particulate organic matter to the deep ocean and transfer to higher trophic levels (Eppley and Peterson 1979). Furthermore, they suggest that on a global scale approximately 80% of the nitrogen utilized by phytoplankton is derived from regenerative processes, with the balance coming from vertical mixing and upwelling of deep NO_3^--rich waters.

Past studies have found new production to be quantitatively dominant in regions where inputs of deep nutrient-rich waters are prevalent (Eppley et al. 1979, Harrison et al. 1983, Yoder et al. 1983, Probyn 1985). New production is essential in supporting the expansion of phytoplankton stocks and net transfer to higher trophic levels (e.g. zooplankton and fish) (Dugdale and Goering 1967, Eppley and Peterson 1979, and Eppley 1981). From the perspective of the fish industry, NO_3^- supported production in upwelling and coastal regions sustains 50% of the world's fish catch, while these regions make up only 0.1% of the ocean's area (Ryther 1969). Regenerated production, on the other hand, tends to be quantitatively most important in stratified waters (e.g. ocean gyres, bays and fjords) where all nitrogen nutrients occur in low concentrations in the euphotic zone (Eppley et al. 1973, Eppley et al. 1977, Paasche and Kristiansen 1982a, Harrison et al. 1985, Price et al. 1985). However, there are habitats where NO_3^- concentrations are more than an order of magnitude higher (>20 μM) than NH_4^+ and urea concentrations (<2 μM), yet utilization of regenerated sources

dominates (Olson 1980, Glibert et al. 1982a, Probyn and Painting 1985). Relatively low NO_3^- uptake, in high NO_3^- waters, may be attributed to inhibition of NO_3^- use by ambient NH_4^+ concentrations >0.5 µM (MacIsaac and Dugdale 1972, McCarthy et al. 1977, and Glibert et al. 1982a).

A further interest in phytoplankton productivity is the determination of specific activity within various size classes. Malone (1980) reviewed a number of size-fractionation studies showing that netplankton (~20-200 µm) tend to occur in well-mixed, high NO_3^- waters, while nanoplankton (<20 µm) prevail in stratified, low NO_3^- waters. Furthermore, size-fractionated nitrogen uptake measurements (Glibert et al. 1982b, Probyn 1985) support the conclusion that netplankton production is primarily sustained by new nitrogen, while nanoplankton production is supported by regenerated nitrogen.

Investigation of the regenerated forms of nitrogen has expanded to include measurements of NH_4^+ regeneration rates. Early experiments by Harris (1959) indicated that zooplankton excretion could account for over 70% of the nutrients required for primary production. However, more recent studies suggest that in productive upwelling and coastal areas macrozooplankton supply only 5 to 30% of phytoplankton nitrogen needs (McCarthy et al. 1975, Smith 1978a, b, Whitledge 1978, Caperon et al. 1979). The ^{15}N-NH_4^+ isotope dilution technique (Bremner and Edwards 1965, Bremner and Keeney 1965, 1966) has been adapted and applied to coastal marine waters (Harrison 1978, Caperon et al. 1979). Isotope dilution

studies by Harrison (1978), Caperon et al. (1979), and Glibert (1982) indicate that microzooplankton and bacteria (< 100 μm fraction) are the dominant suppliers of NH_4^+. Furthermore, these studies report that on a daily basis, NH_4^+ regeneration rates were frequently in balance with NH_4^+ uptake rates by phytoplankton. However, studies by Paasche and Kristiansen (1982b) and LaRoche (1983) find that regeneration rates only supply 28% to 36% of NH_4^+ assimilation rates. The imbalance may be due to using only daytime measurements, short incubations, or single depth sampling (Paasche and Kristiansen 1982b).

Although size fraction experiments have demonstrated the dominant role of microheterotrophs (i.e. organisms < 100 μm) in NH_4^+ regeneration, it is uncertain whether microzooplankton (principally protozoans) or bacteria are the major suppliers of NH_4^+ (Johannes 1965, Fenchel and Harrison 1976, Fenchel and Jørgensen 1977, rewiewed by Harrison 1980). It has been assumed that bacteria are responsible for most dissolved organic nitrogen uptake, while phytoplankton primarily utilize inorganic nitrogen (reviewed by Billen 1984). Recent studies, however, support the hypothesis that heterotrophic bacteria also play an important role in the utilization of inorganic nitrogen (i.e. NH_4^+) in the euphotic zone (Eppley et al. 1977, Laws et al. 1985, Wheeler and Kirchman 1986). Thus, NH_4^+ uptake rates include secondary (i.e. heterotrophic) as well as primary (i.e. autotrophic) utilization of NH_4^+.

Study site

The coastal regions of Oregon and Washington provide a dynamic situation for studying nitrogen utilization during the spring and summer upwelling period. Cold, nutrient-rich surface waters are found adjacent to the coast after periods of moderate to strong northern winds. These nearshore cold waters form north-to-south bands of high productivity and biomass. Surface NO_3^- concentrations decrease rapidly as nearshore waters move offshore, due to nutrient utilization by phytoplankton (Small and Menzies 1981). Frequent fluctuations in wind patterns cause largest changes in surface water density within 15 km of the shore and hence a very spatially and temporally variable NO_3^- distribution. This provides an ideal situation for examining relative use of new and regenerated sources of nitrogen as a function of nutrient conditions.

Goals and Objectives

The major goal of this study was to examine the uptake of inorganic and organic nitrogen in coastal waters off Oregon and Washington using ^{15}N tracer techniques. This is the first reported study using ^{15}N tracers to measure uptake or regeneration of nitrogen in these waters. The specific objectives were as follows: (1) To measure uptake rates of NO_3^-, NH_4^+, and urea; (2) To compare the relative utilization of new and regenerated nitrogen;

(3) To compare nitrogen utilization by nano- and netplankton; and (4) To measure NH_4^+ regeneration by <10 μm and 10-200 μm size classes of plankton.

Measurment of uptake rates for NO_3^-, NH_4^+ and urea are presented in chapter II, while NH_4^+ regeneration estimates are presented in chapter III. Chapter IV presents the overall summary and conclusions.

Chapter II

UPTAKE OF INORGANIC AND ORGANIC NITROGEN

METHODS

Sampling

Nitrogen uptake experiments were performed during July 1985, 8 to 115 km off the coast of Oregon and Washington (Fig. II.1). Fifteen stations were sampled from the R/V Wecoma, while one other station (0) was sampled from the R/V Sacajawea. Seawater samples from 15 m were collected aboard R/V Wecoma using 30 l Niskin bottles. Morning collection times were between 0530 and 0830 and midday collection times were between 1015 and 1445. A Wilden (Model MP-2) air driven pump, with a double teflon diaphragm, was used aboard R/V Sacajawea to collect water from 10 m. The sample water was screened through either 200 µm or 10 µm nylon Nitex mesh and pooled into 50 l Nalgene carboys. The prescreened water from the 50 l carboys was then transferred to 2.7 l polycarbonate bottles for experimental incubations. Samples from each station were preserved in 5% gluteraldehyde for determination of relative abundance of phytoplankton genera. Subsamples of 1 or 10 ml were settled and examined using an inverted microscope.

Analyses

Nutrient analyses were performed on seawater after filtration through precombusted, glass fiber filters (Whatman GF/F). Samples for

Figure II.1. Locations for low NO_3^- stations (2-8, 14, and 15) and high NO_3^- stations (0, 1, and 9-13) off the coasts of Oregon and Washington.

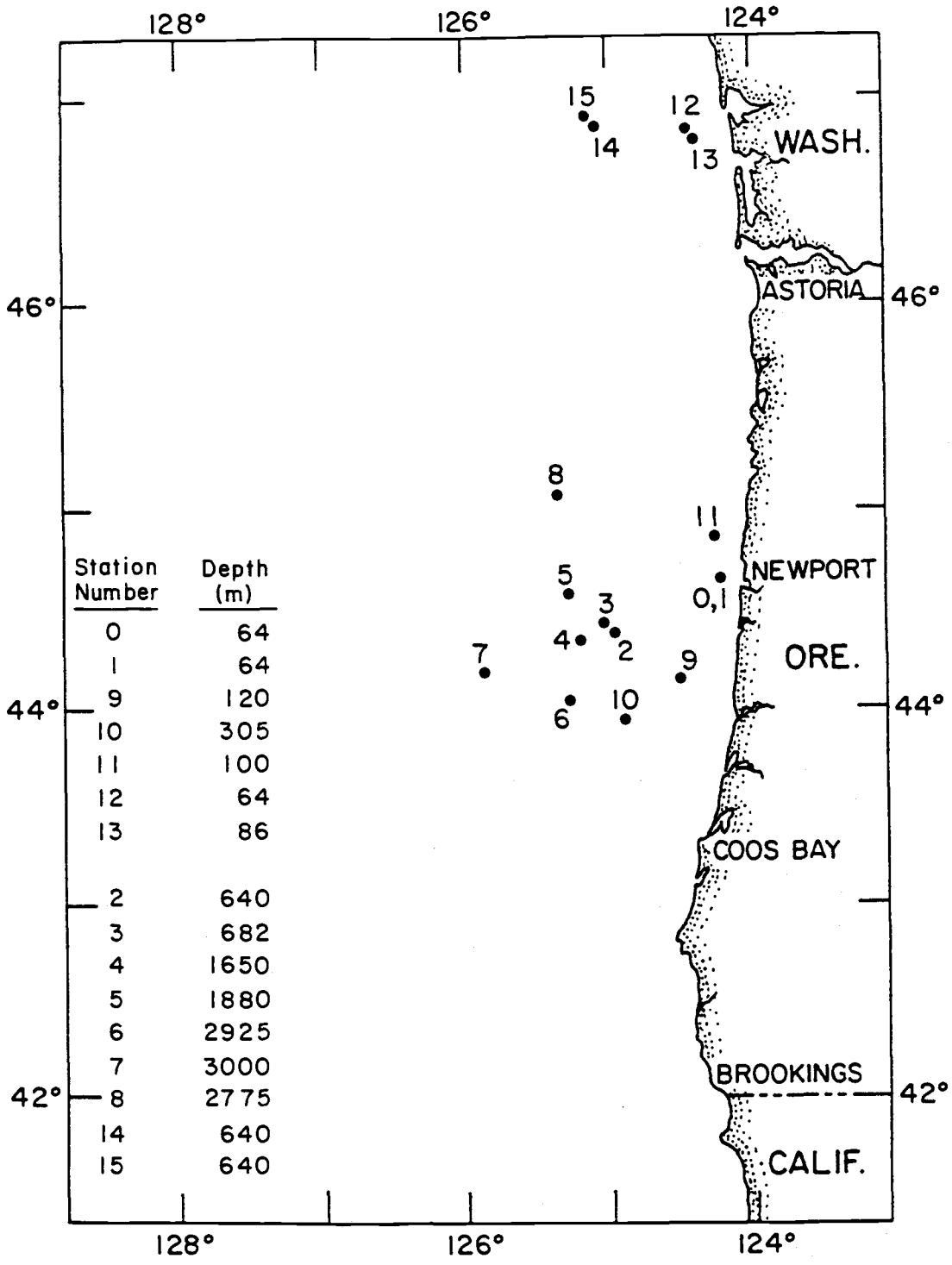
128°
126°
124°
46°
44°
42°
WASH.
ASTORIA
NEWPORT
ORE.
COOS BAY
BROOKINGS
CALIF.
15
14
12
13
8
11
5
3
0,1
4
2
7
9
6
10
Station Number
Depth (m)
0 64
1 64
9 120
10 305
11 100
12 64
13 86
2 640
3 682
4 1650
5 1880
6 2925
7 3000
8 2775
14 640
15 640

Figure II.1

dissolved nitrate, nitrite, and phosphate were frozen (-20° C) in acid washed polyethylene bottles and then measured onshore with a Technicon® autoanalyzer. Dissolved NH_4^+ was measured manually aboard ship using the phenol/hypochlorite reaction as described in Strickland and Parsons (1972), but scaled down to 10 ml volume. The mean precision (SD) of triplicate NH_4^+ analyses was 0.025 µM. Samples for dissolved urea were stored frozen in polyethylene containers for analysis onshore using the urease method (McCarthy 1970). A Sigma® urease (Type IV) preparation was used for the analyses instead of the Worthington urease employed by McCarthy 1970. The Sigma enzyme preparation had lower NH_4^+ contamination and maintained higher activity over a longer storage period than the Worthington preparation. Standard curves for the urea determinations were run using 2000 m filtered seawater which contained undetectable levels of both NH_4^+ and urea. The mean precision (SD) of duplicate urea analyses was 0.021 µM urea-N.

Particulate material for chl-a and particulate nitrogen (PN) analyses was collected on 25 mm, precombusted, glass fiber filters (Whatman GF/F) under vacuum (<180 mm Hg). Chl-a samples were stored frozen under vacuum for 1-2 weeks until analyses could be performed using a Turner Designs fluorometer following procedures described by Strickland and Parsons (1972) (mean coefficient of variation (CV) for duplicates = 10.4%). PN samples were stored frozen and then dried for 24 h at 60° C. Particulate nitrogen and carbon concentrations then were determined using a Perkin Elmer CHN analyzer (mean CV for duplicates = 3.6%). Subsurface light measurements were determined

using a Li-Cor Model Li-185a Quantum/Radiometer/Photometer. Light intensities at 0.5 m, at time of water collection, ranged from 80-600 μEinst m^{-2} s^{-1}.

Nitrogen Uptake

Nitrogen uptake rates were measured using simulated <u>in situ</u> conditions for bottle incubations with ^{15}N tracers for NO_3^-, NH_4^+, and urea (Dugdale and Goering 1967). Uptake rates are presented in units of μmol N l^{-1} h^{-1}. Nitrate uptake rates were measured only on the <200 μm screened water, while NH_4^+ and urea uptake rates were measured on both <10 μm and <200 μm fractioned seawater (only on <200 μm for station 0). The ^{15}N-uptake experiments commenced within 1 to 2.5 h of water collection. Additions of 0.1 μM NH_4^+-N ($(^{15}NH_4)_2SO_4$, 99.7 atom %), 0.2 μM urea-N ($CO(^{15}NH_2)_2$, 95.1 atom %), and 2.9 μM NO_3^- ($Na^{15}NO_3$, 99.2 atom %) were made in separate incubation bottles. Both trace and saturating concentrations (0.2 and 2.0 μM urea-N) of urea were used for midday experiments on <200 μm screened seawater (stations 3, 5, 7, 9, 13, and 15). It was not always possible to use "trace" additions (~10% of ambient concentrations) for NH_4^+ and urea. This was due to analytical limitations, a problem that is common with work in low nutrient waters (e.g. Glibert et al. 1982c). However, the 0.1 μM NH_4^+-N and 0.2 μM urea-N additions of ^{15}N tracers used fell within the range of ambient concentrations. Thus,

enhancement in uptake due to isotope addition should be within limits occurring in the field.

Incubation bottles were covered with one layer of neutral density screening to reduce the sunlight intensity by 50% and placed in Plexiglas deck boxes cooled by circulating surface seawater. Station 0 bottles were maintained at near ambient temperature in a walk-in cold room and exposed to cool-white fluorescent light screened to approximate in situ light intensity. Frequent sampling was employed to assess and avoid potential underestimates of uptake rates caused by substrate depletion or isotope dilution. Ammonium and urea uptake were measured at four time intervals (0, 30, 60 and 120 min) while NO_3^- uptake was measured at two (120 and 240 min). Midday trace and saturating urea uptakes were measured at three time intervals (0, 60, and 180 min). Uptake experiments were terminated by filtration (<180 mm Hg) of 1.2 to 2.7 l of seawater for collection of particulate matter onto 47.5 mm, precombusted, glass fiber filters (Whatman GF/F). The filters were immediately frozen and then dried at 60° C for 24 h after return to shore-based laboratory. Filtrates from the NH_4^+ incubation bottles were saved for determination of the specific activity (atom % ^{15}N) of dissolved NH_4^+ (refer to chapter III for details). Samples were prepared for isotopic analysis by first converting organic and inorganic nitrogen to nitrogen gas using a dry micro-Dumas combustion (Fiedler and Proksch 1975). The atom % ^{15}N for samples was then determined by emission spectrometry (Fiedler and Proksch 1975, Harrison 1983) using a Jasco Model N-150 NIA-1 ^{15}N Analyzer. Details of sample preparation were adapted from

LaRoche (1983) and are described in Dudek et al.(1986). Ammonium uptake measurements were corrected for isotope dilution of the nutrient during the course of an incubation (Harrison 1978, Caperon et al. 1979, Glibert 1982, Paasche and Kristiansen 1982b). Analogous procedures for the determination of urea isotope dilution are not yet available. Therefore, rates of urea uptake may be underestimated for the "trace" addition experiments. Ammonium uptake rates were calculated by dividing the rate of increase of ^{15}N in the particulate material (atom % ^{15}N) by the atom % enrichment of NH_4^+ in the dissolved pool (Glibert et al. 1982c):

$$\text{Uptake} = [d(\text{atom \% } ^{15}\text{N of PN})/dt]/R \times (PN)$$

where R is the exponential mean of atom % enrichment of dissolved NH_4^+ during the incubation period, and PN is the amount of nitrogen in the particulate material (μmol N l^{-1}). Three to four time points were used for each linear regression. Standard errors for the slopes and the calculated estimates of other parameters were determined as described by Bevington (1969).

A relative preference index (RPI) (McCarthy et al. 1977) was used to compare the utilization of NH_4^+ and urea relative to their availability in seawater. For example, for NH_4^+:

$$RPI_{NH_4^+} = \frac{\dfrac{P_{NH_4^+}}{P_{NH_4^+} + P_{urea}}}{\dfrac{[NH_4^+]}{[NH_4^+] + [urea]}}$$

where P_{NH_4}+ and P_{urea} = absolute uptake rates (μmol N l^{-1} h^{-1}) for each nutrient; [NH_4^+] and [urea] = respective concentrations.

Turnover times for NH_4^+ and urea were calculated as follows:

Turnover time = [ambient nutrient conc. + tracer addition]/uptake rate

For NH_4^+, uptake rates were assumed to be continuous over a 24 h period, while for urea dark uptake was assumed to be ~50% of light uptake. These assumptions are supported by recent diel studies by Wheeler (pers. comm.) and Price et al. 1985, but should be considered rough approximations.

Daily nitrogen-specific uptake rates indicate the rate at which particulate nitrogen is being labeled (Dugdale and Goering 1967). The rates were calculated as follows:

$$v = \frac{\text{mass N taken up}}{\text{mass N present} \cdot t} = t^{-1}$$

using a 15 h uptake period for NO_3^- (photoperiod was 15 h), since NO_3^- assimilation is generally restricted to the light period (Syrett 1981), a 24 h period for NH_4^+, and a 19.5 h period for urea.

RESULTS

Physical and chemical parameters

Table II.1 summarizes nutrient concentrations, biomass, and physical parameters for the 16 stations. The data demonstrate a strong correspondence between surface (i.e. 15 m depth) nutrient concentrations and depth of the water column (Fig. II.2). Surface NO_3^- concentrations were high (>20 µM) for shallow stations (<0.4 km) and low (< 5 µM) for deeper stations (Fig. II.2A). Similarly, surface PO_4^{3-} concentrations were relatively high (~2.8 µM) at the shallow stations and low (~0.7 µM) at deeper stations (Fig. II.2B). Ratios of NO_3^-/PO_4^{3-} in the surface water were relatively high (9-13) for shallow stations (<0.4 km) and low (1.5-4) for deeper stations (Fig. II.2C).

For the presentation of the results, stations have been separated into 2 groups according to ambient concentrations of NO_3^-: shallow nearshore stations with NO_3^- concentrations >20 µM (Sta. 0, 1, and 9-13) and deeper offshore stations with NO_3^- concentrations <5 µM (Sta. 2-8, 14 and 15). The high NO_3^- waters result from strong upwelling along the shore. Ambient NO_3^- concentrations were inversely correlated with surface water temperatures (Table II.1). Water temperatures ranged from 10-12.2° C in nearshore, high NO_3^- waters and from 13-18° C in offshore, low NO_3^- waters.

Table II.1. Physical parameters, nutrient concentrations and biomass.

Sampling date	Sta. no.	Station location	Sta. depth (m)	Temp. (°C)	Size fraction (µm)	NH_4^+ (µM)	UREA (µM)	NO_3^- (µM)	NO_2^- (µM)	PO_4^{3-} (µM)	PN (µM)	Chl-a (µg/l)
LOW NO_3^-												
22 July	2	44°22.35'N 124°57.55'W	640	16.5	<10		0.46				2.19	0.63
					<200	0.16	0.34	1.2	nd	0.58	2.71	1.58
22 July	3	44°22.60'N 124°58.30'W	685	*	<200	0.11	0.18	0.8	nd	0.32	4.35	2.54
23 July	4	44°18.75'N 125°11.51'W	1650	17.2	<10		0.16				0.68	0.12
					<200	0.18	0.27	0.7	nd	0.42	0.80	0.16
23 July	5	44°29.39'N 125°15.47'W	1880	18.0	<200	0.26	0.10	0.6	nd	0.44	0.80	0.23
24 July	6	43°59.10'N 125°16.47'W	2925	13.0	<10		nd				2.53	1.19
					<200	0.51	nd	3.3	0.11	1.06	4.45	4.14
24 July	7	44°09.54'N 125°52.54'W	3000	16.0	<200	0.16	0.34	0.8	nd	0.50	1.31	0.32
5 July	8	45°02.21'N 125°15.60'W	2775	16.5	<10		0.07				0.84	0.14
					<200	0.06	nd	1.1	nd	0.38	1.08	0.22
29 July	14	46°50.13'N 125°05.88'W	640	14.0	<10		0.18				1.73	1.20
					<200	1.23	0.03	4.6	0.44	1.28	1.74	1.17
29 July	15	46°50.17'N 125°07.02'W	640	14.3	<10		0.04				2.15	1.64
					<200	0.84	0.04	2.3	0.23	1.16	2.76	1.86
HIGH NO_3^-												
3 July	0	44°40.00'N 124°12.00'W	64	11.5	<200	0.18	0.02	21.5	0.26	1.81	18.46	23.10
21 July	1	44°40.00'N 124°12.00'W	64	11.8	<10		0.33				1.70	1.07
					<200	0.27	0.03	49.1	0.39	4.26	3.25	5.17
25 July	9	44°06.47'N 124°30.88'W	120	10.2	<200	0.06	0.23	22.3	0.34	1.79	8.49	11.29
26 July	10	43°52.47'N 124°54.83'W	305	10.2	<10		nd				2.23	1.42
					<200	0.62	0.10	20.1	0.36	2.06	6.51	11.12
27 July	11	44°50.98'N 124°13.70'W	100	10.0	<10		0.08				2.06	1.32
					<200	0.44	0.13	31.2	0.34	2.61	6.35	9.29
28 July	12	46°49.60'N 124°26.30'W	64	11.7	<10		0.33				1.49	1.59
					<200	0.16	0.35	36.9	0.69	3.12	3.72	5.31
28 July	13	46°49.24'N 124°24.51'W	86	12.2	<200	nd	0.34	48.3	0.48	3.89	1.70	1.95

nd = non-detectable

* no reading available

Figure II.2. Surface nutrient concentrations plotted against station depth. A. NO_3^-, B. PO_4^{3-}, and C. ratio of NO_3^-/PO_4^{3-}.

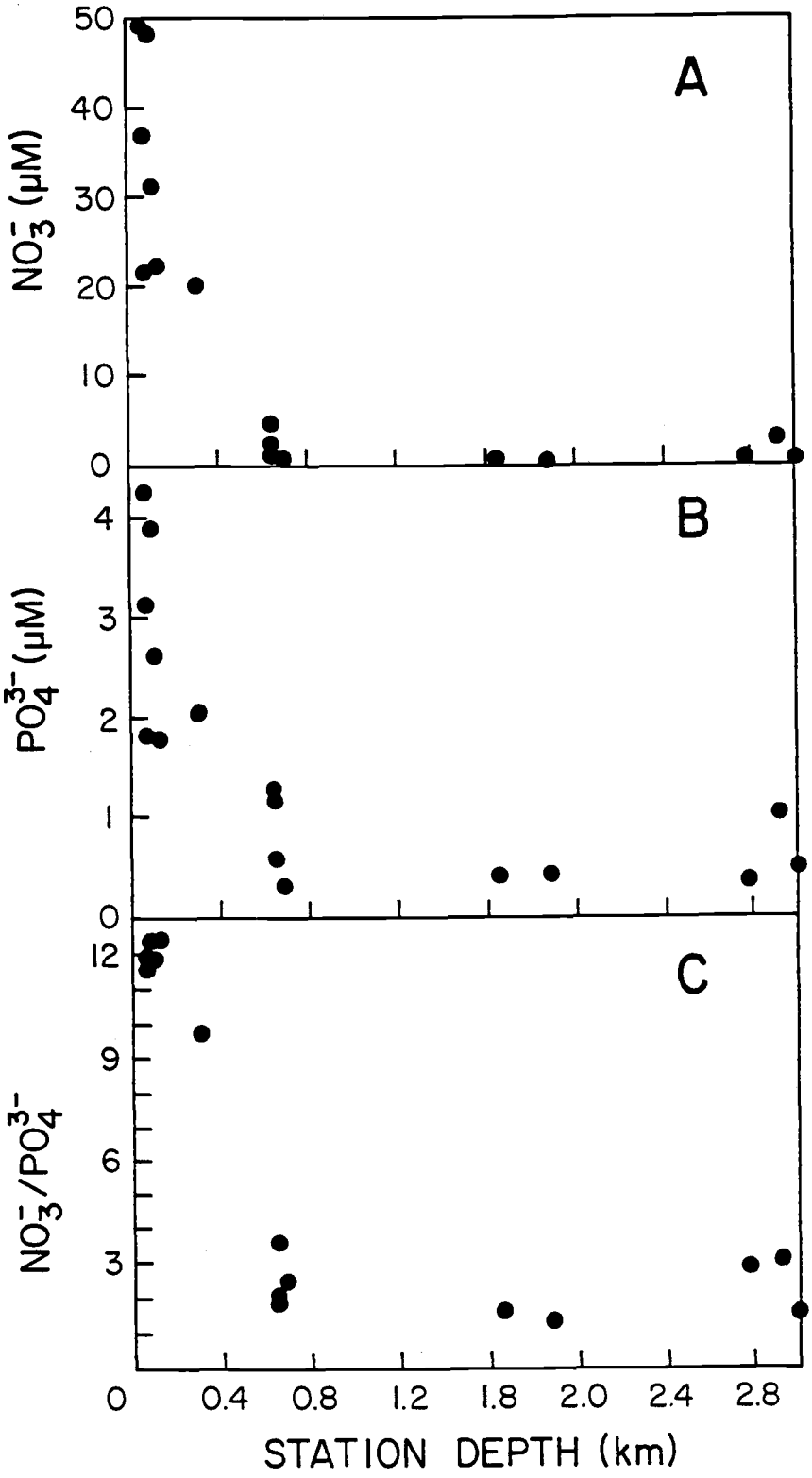
A
B
C
NO_3^- (μM)
PO_4^{3-} (μM)
NO_3^-/PO_4^{3-}
50
40
30
20
10
0
4
3
2
1
0
12
9
6
3
0
0.4
0.8
1.2
1.8
2.0
2.4
2.8
STATION DEPTH (km)

Figure II.2

Concentrations of regenerated forms of nitrogen (NH_4^+ + urea) ranged from 0.20 to 1.26 µM, and were generally much lower than those of NO_3^- (0.6 to 49.1 µM). In general, urea concentrations were high (>0.15 µM) when NH_4^+ was low (<0.2 µM). Conversely, urea concentrations were low (<0.15 µM) when NH_4^+ was high (>0.2 µM, Fig. II.3).

Particulate nitrogen (PN) and chl-a concentrations are useful indicators of phytoplankton biomass. Both PN and chl-a concentrations were low in lowest and highest NO_3^- waters, while highest biomass concentrations were present in waters with intermediate NO_3^- levels (Fig. II.4). Table II.2 lists the dominant phytoplankton genera found in the waters sampled and their relative abundance. Pronounced differences occur between the genera present in low vs. high NO_3^- waters. Low NO_3^- waters were primarily dominated by the diatoms Rhizosolenia and Nitzschia, and an unidentified naked dinoflagellate. High NO_3^- waters were dominated by the diatoms Asterionella, Nitzschia and Thalassiosira. Most of the diatom genera present in high NO_3^- waters were chain-forming types. Nitzschia was dominant at both low and high NO_3^- stations; however, dominance at low NO_3^- stations occurred only at the higher NO_3^- concentrations within that group. The diatom Chaetoceros was common in both high and low NO_3^- waters.

Figure II.3. The relationship between surface concentrations of urea and NH_4^+ for all stations. Urea and NH_4^+ concentrations are from the <200 µm samples in Table II.1.

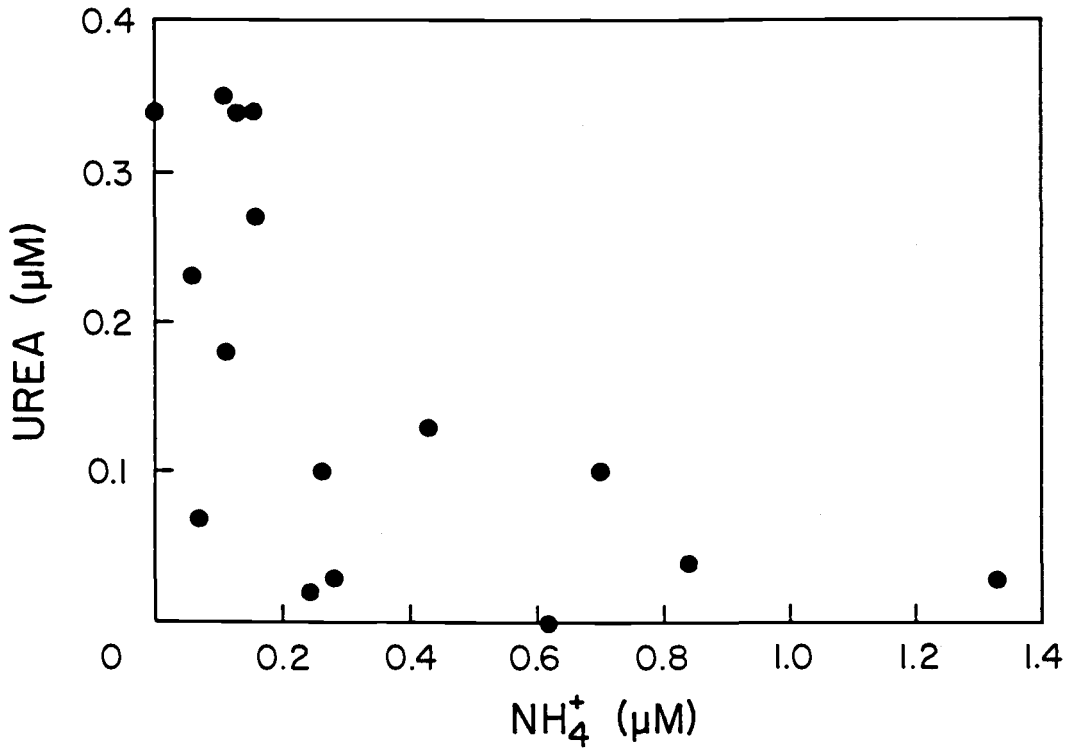

Figure II.3

Figure II.4. Biomass plotted against surface NO_3^- concentration. A. Particulate nitrogen (PN). B. Chl-a.

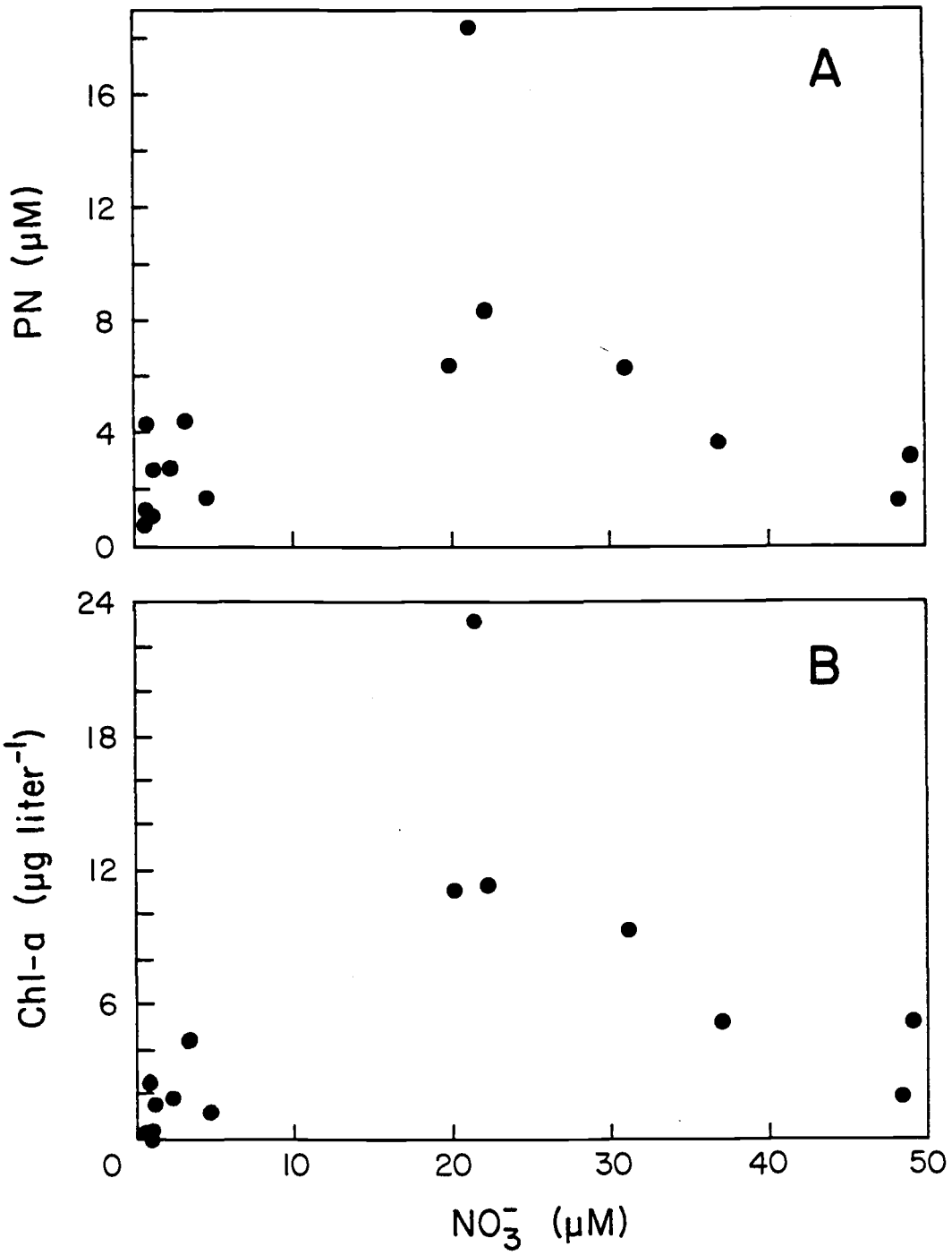

Figure II.4

Table II.2. Relative abundance of phytoplankton genera.

	LOW NO_3^-								HIGH NO_3^-					
Sta.* No.	4	5	7	8	2	15	6	14	9	10	0	11	12	13
GENERA														
DIATOMS														
Asterionella							2		2	1	2	1	4	
Bacteriastrum		4	4	2										
Chaetoceros	2	3	4	4			4			1		3	4	3
Ditylum													4	4
Leptocylindrus					4					4				4
Nitzschia					4	3	1	1	2	4		3	1	1
Rhizosolenia	1	3	1	2	1		2			4				
Skeletonema										4		2	4	4
Thalassionema													4	
Thalassiosira					4		4		1	4	1	3	4	
Thalassiothrix										4				
Uniden. pennates	2	4	4	4									4	
DINOFLAGELLATES														
Ceratium	4	4												
Unidentified forms		1	2	1		1	4	1	4	4			2	4
SILICOFLAGELLATES														
Unidentified forms	3												4	

* No samples collected for sta. 1 and 3.

LEGEND 1=Dominant (>50% of cell numbers)
2=Abundant (25-50%)
3=Common (10-25%)
4=Present (<10%)

Nitrogen uptake rates

The time course of assimilation of $^{15}N\text{-}NH_4^+$ into PN was frequently non-linear over the two hour incubation period (Fig. II.5). The rate of accumulation of ^{15}N often decreased by the 1 and 2 h time points. Only the initial "linear" portion of the time course data was used for calculating uptake rates. Furthermore, all NH_4^+ uptake rates reported were corrected for changes in ^{15}N enrichment over the incubation period (see chapter III).

Nitrate uptake rates were positively correlated with ambient NO_3^- concentrations, ranging from 0.004 to 0.147 μmol N l^{-1} h^{-1} in low NO_3^- waters and from 0.236 to 1.20 μmol N l^{-1} h^{-1} in high NO_3^- waters (Table II.3). Ammonium and urea uptake rates were comparable to NO_3^- uptake in low NO_3^- waters (0.002 to 0.092 μmol N l^{-1} h^{-1}, but were much lower than NO_3^- uptake in high NO_3^- waters (0.008 to 0.223 μmol N l^{-1} h^{-1}) (Table II.3). Figure II.6 presents a comparison of uptake rates for the three nitrogenous nutrients. Ammonium uptake dominated in low NO_3^- waters, ranging from 36 to 64% of total uptake, with an average of 50%. Urea uptake was also important ranging from 7.5 to 37% of total uptake, with an average of 21%, while NO_3^- uptake ranged from 13 to 57%, with an average of 29% (Fig II.6A). However, in high NO_3^- waters, NO_3^- uptake dominated, accounting for an average of 83% of the total nitrogen assimilated by the plankton. Ammonium uptake averaged 13% of the total while urea uptake averaged only 4% of total (Fig. II.6B).

Figure II.5. Time course of assimilation of $^{15}N\text{-}NH_4^+$ into particulate nitrogen over a two hour time period. A. Station 0. B. Station 1. (<200 μm fraction).

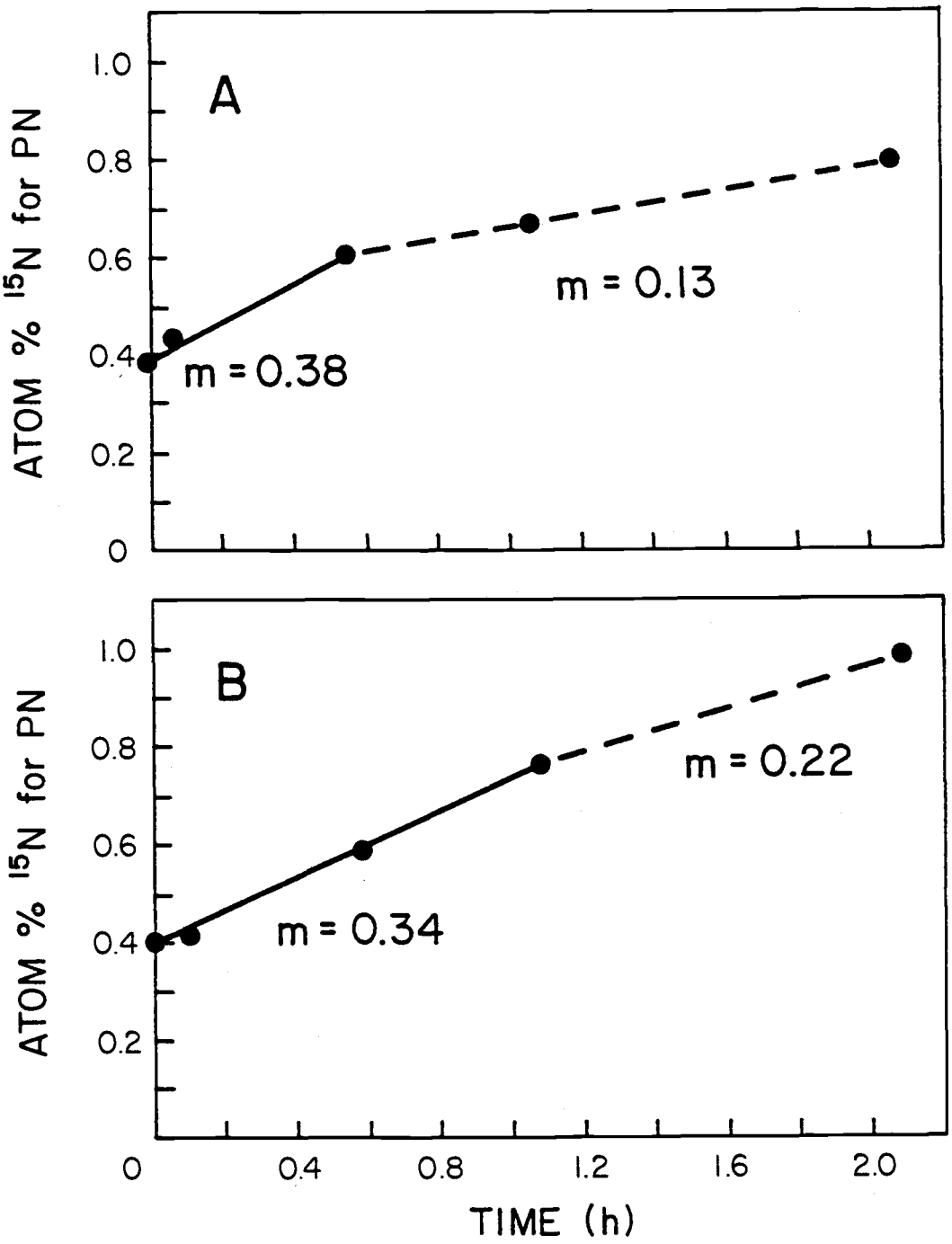
A
B
m = 0.38
m = 0.13
m = 0.34
m = 0.22
ATOM % 15N for PN
ATOM % 15N for PN
TIME (h)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.6
2.0

Figure II.5

Table II.3. Nitrogen uptake rates.

Sta. No.	NO_3^- (μM)	NO_3^- Uptake	NH_4^+ Uptake	UREA Uptake
		(μmol N $liter^{-1}$ h^{-1})		
Low NO_3^-				
4	0.7	0.004	0.018	0.011
8	1.1	0.013	0.031	0.008
2	1.2	0.043	0.072	0.067
6	3.3	0.147	0.092	0.019
14	4.6	0.005	0.013	0.002
High NO_3^-				
10	20.1	0.421	0.081	0.018
0	21.5	1.200	0.223	0.057
11	31.2	0.653	0.118	0.018
12	36.9	0.236	0.025	0.020
1	49.1	0.360	0.047	0.008

Figure II.6. Nitrate, NH_4^+, and urea uptake as percentages of total nitrogen uptake. Labels on the x-axis denote ambient NO_3^- concentrations at each station. A. Low NO_3^- stations. B. High NO_3^- stations.

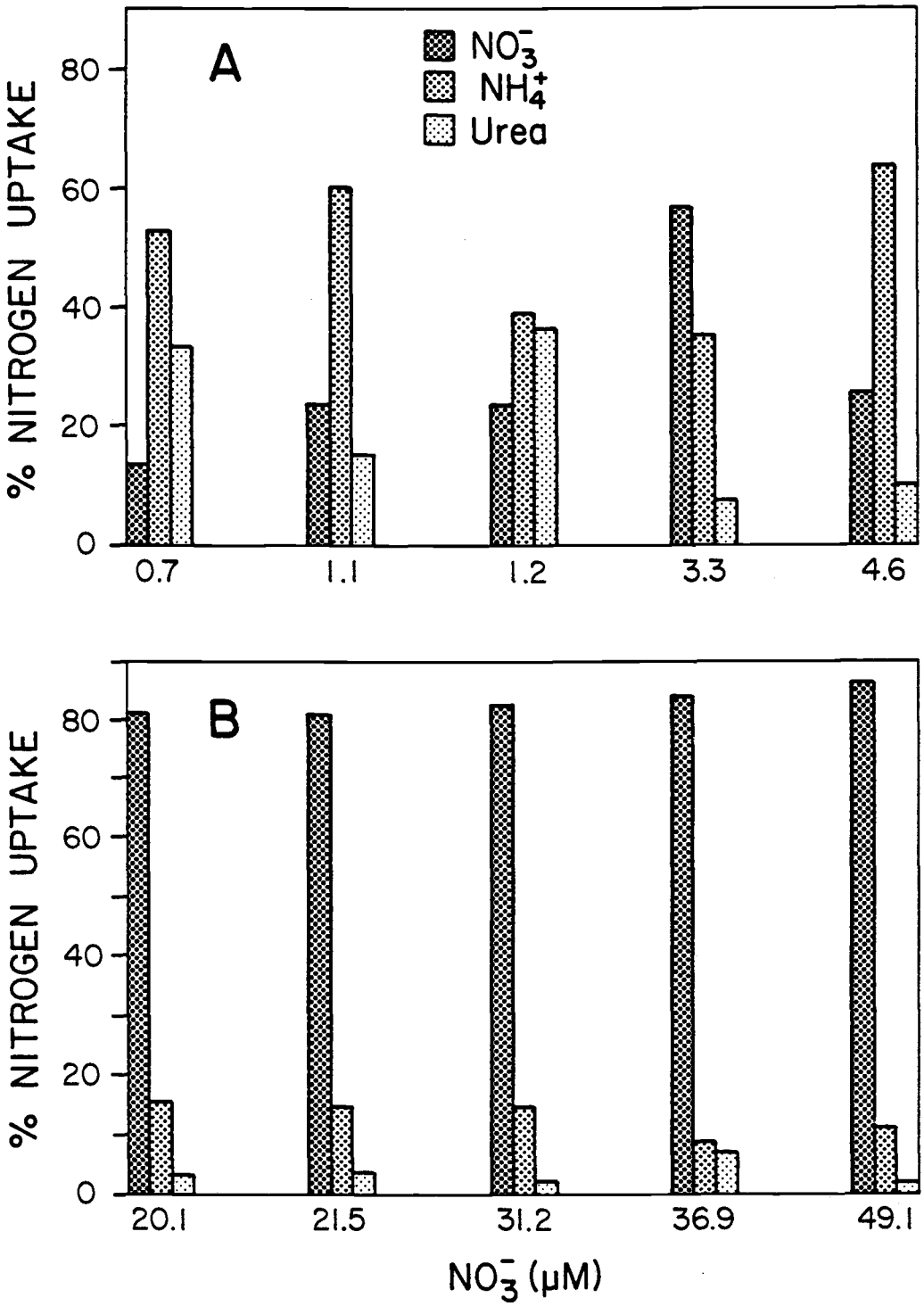
A
NO_3^-
NH_4^+
Urea
% NITROGEN UPTAKE
80
60
40
20
0
0.7
1.1
1.2
3.3
4.6
B
% NITROGEN UPTAKE
80
60
40
20
0
20.1
21.5
31.2
36.9
49.1
NO_3^- (μM)

Figure II.6

Thus, uptake of regenerated nitrogen (NH_4^+ and urea) was dominant (71%) in low NO_3^- waters, while uptake of new nitrogen (NO_3^-) was dominant (83%) in high NO_3^- waters. The percentage of new nitrogen uptake was hyperbolically related to ambient concentration of NO_3^- (Fig. II.7).

Urea uptake measurements were made for both trace and saturating additions (0.2 and 2.0 µM urea-N) at six stations (Table II.4). The ratio of saturating/trace uptake ranges from 0.4 to 1.7, with a mean value of 1.0. Consequently, urea uptake rates were not significantly different for the two additions.

Uptake rates normalized to phytoplankton biomass (chl-a) are plotted against ambient NO_3^- concentrations in Fig. II.8. In general, NO_3^- uptake rates increased linearly with increasing ambient levels of NO_3^- (Fig. II.8A). The very low NO_3^- uptake value for station 14 (<0.01 µmol N/ug chl-a/h) may have been due to an inhibitory effect of the high NH_4^+ concentration (1.23 µM). Ammonium and urea uptake rates decreased dramatically with increasing NO_3^- concentrations between 0-5 µM NO_3^-, and then remained low and relatively constant at higher NO_3^- concentrations (Fig. II.8, B and C).

Turnover times for NH_4^+ and urea (ambient conc. + tracer addition) are presented in Figure II.9. Turnover times represent the amount of time required for the plankton to completely utilize the dissolved pool of a particular nitrogenous nutrient. Except for station 14 (NO_3^- = 4.6 µM), the mean turnover times for NH_4^+ in low and high NO_3^- stations were comparable (0.32 d and 0.27 d, respectively). The same was true for urea turnover times in low and

Figure II.7. The percent new production [$NO_3^-/(NO_3^- + NH_4^+ +$ urea)] X 100% for low and high NO_3^- stations plotted against ambient NO_3^- concentration.

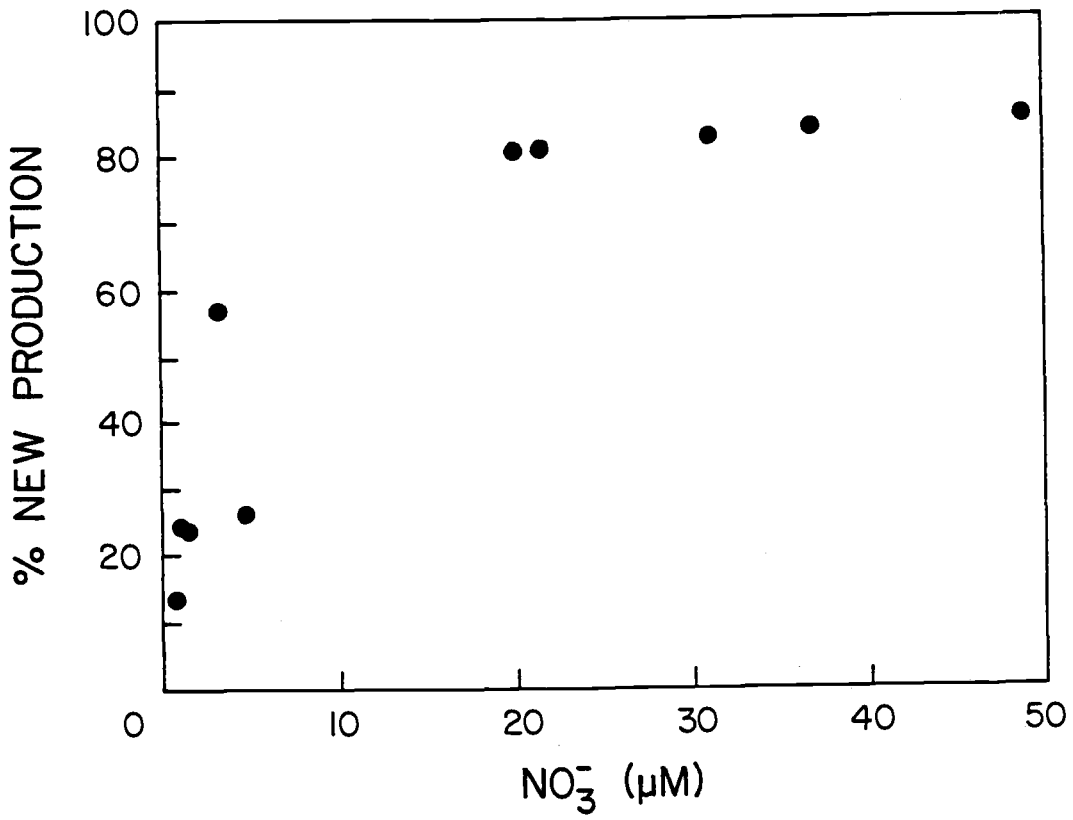

Figure II.7

Table II.4. Urea uptake with trace and saturating urea additions.

Sta. No.	Urea Uptake TRACE	Urea Uptake SATURATING	Uptake Ratio SAT./TR.	NO_3^-	NH_4^+	UREA
	(μmol N l^{-1} h^{-1})			(μM)	(μM)	(μM)
3	0.071	0.029	0.4	0.8	0.11	0.18
5	0.008	0.007	0.9	0.6	0.26	0.10
7	0.019	0.011	0.6	0.8	0.16	0.34
15	0.006	0.010	1.7	2.3	0.84	0.04
9	0.041	0.052	1.3	22.3	0.06	0.23
13	0.009	0.008	0.9	48.3	nd	0.34

nd = non-detectable.

Figure II.8. Uptake rates normalized to chl-a (μmol N/ug chl-a/h) plotted against ambient NO_3^-. A. NO_3^-, B. NH_4^+, and C. urea. The linear regression equation for NO_3^- uptake = .0008x + .0284 (r = 0.85). Points marked with arrows were excluded from the regression analysis. Note that uptake scale in A. is different than in B. and C.

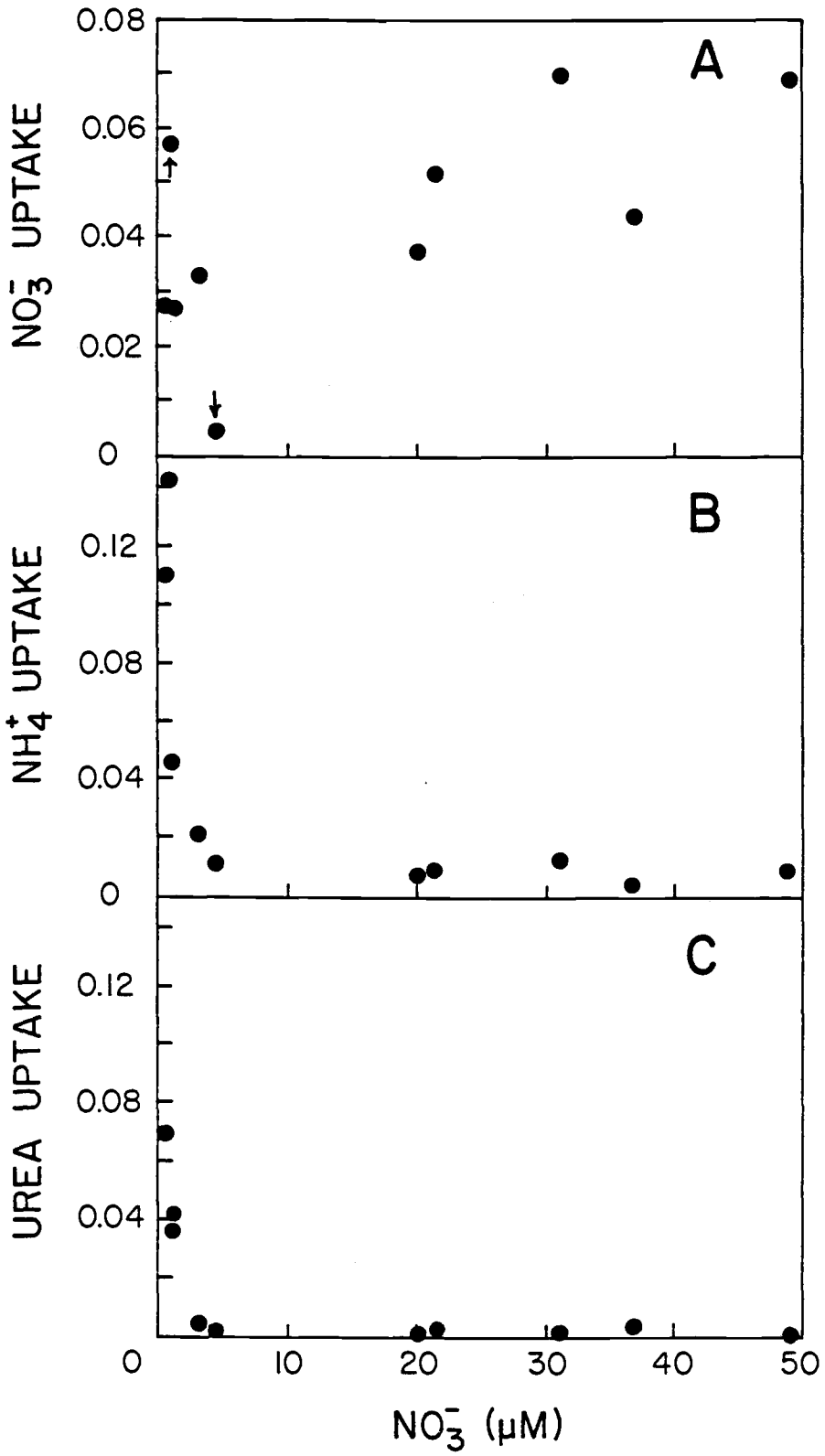
A
B
C
NO_3^- UPTAKE
NH_4^+ UPTAKE
UREA UPTAKE
0.08
0.06
0.04
0.02
0
0.12
0.08
0.04
0
0.12
0.08
0.04
0
10
20
30
40
50
NO_3^- (μM)

Figure II.8

Figure II.9. Turnover times (d) for NH_4^+ and urea (ambient concentration + tracer addition). Labels on the x-axis denote ambient NO_3^- concentrations at each station. A. Low NO_3^- stations. B. High NO_3^- stations.

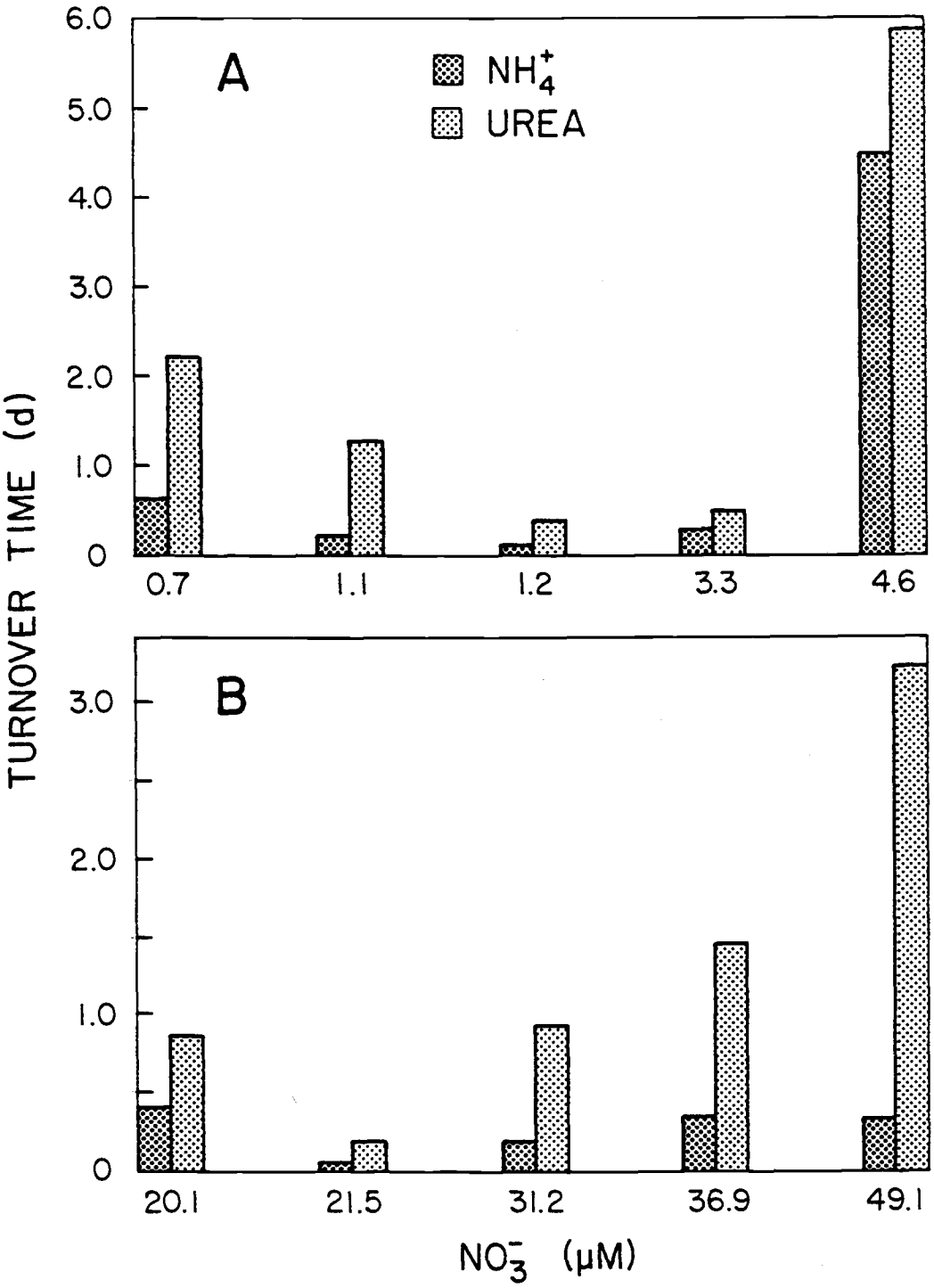
A
NH_4^+
UREA
TURNOVER TIME (d)
6.0
5.0
4.0
3.0
2.0
1.0
0
0.7
1.1
1.2
3.3
4.6
B
3.0
2.0
1.0
0
20.1
21.5
31.2
36.9
49.1
NO_3^- (μM)

Figure II.9

high NO_3^- waters (1.11 d and 1.33 d, respectively). Station 14 had very slow turnover times for both NH_4^+ and urea compared to all the other stations. On the average, turnover of urea was roughly 3.5 times slower than turnover of NH_4^+ in low NO_3^- waters and 5 times slower in high NO_3^- waters.

Nitrogen-specific uptake rates (v) are approximately equal to the relative growth rate of phytoplankton in terms of nitrogen. If NO_3^-, NH_4^+, and urea uptake are ~equal to total N use, then "v" gives a minimum estimate of specific growth rate. The degree of underestimate is proportional to the amount of non-phytoplankton N. Except for station 14, the mean nitrogen-specific uptake rates for low and high NO_3^- stations were comparable (1.1 d^{-1} and 1.6 d^{-1}, respectively, Fig. II.10). Significantly higher nitrogen-specific uptake rates were measured at stations 1 and 11 (avg. 2.05 d^{-1}) compared to the other high NO_3^- stations (avg. 1.29 d^{-1}). Station 14 had an unusually low nitrogen-specific uptake rate of 0.25 d^{-1}).

Uptake of NH_4^+ and Urea in Size Fraction Experiments

Size fractionation of NH_4^+ and urea uptake was carried out for nine stations. Figure II.11 presents PN and chl-a concentrations for each size fraction (<10 μm = nanoplankton and 10-200 μm = netplankton). The average percent PN in the netplankton fraction was markedly higher in high NO_3^- waters than in low (60.2% and 20.0%

Figure II.10. Nitrogen-specific uptake rates (d^{-1}) for NO_3^-, NH_4^+ and urea as nitrogen sources. Labels on the x-axis denote ambient NO_3^- concentrations at each station.

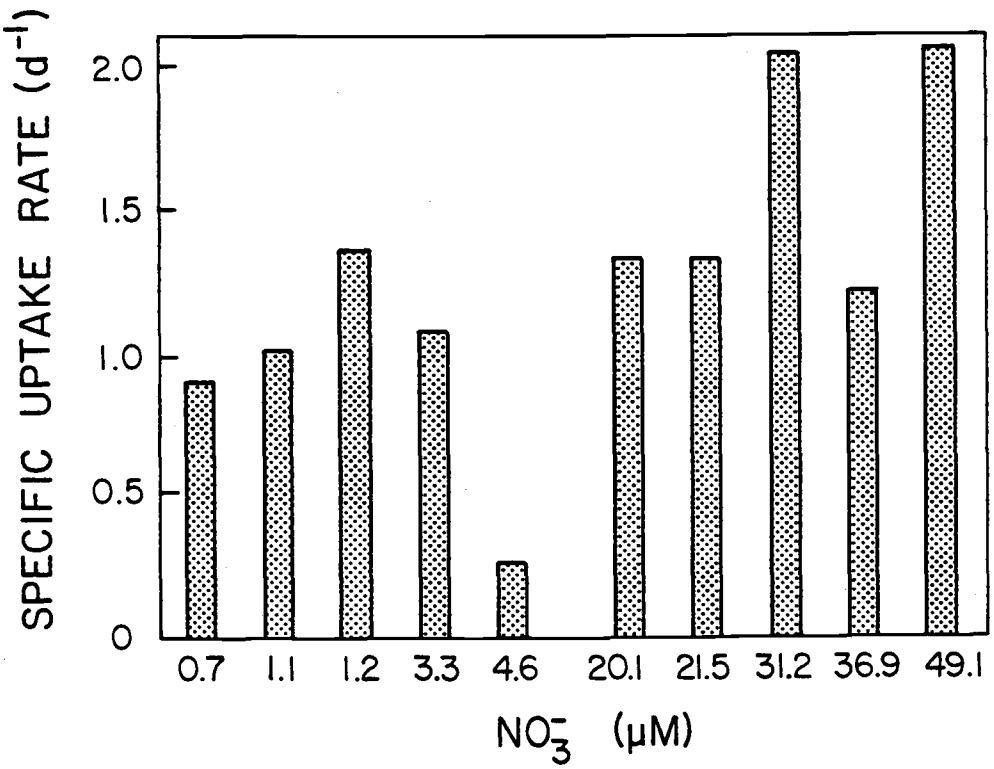
SPECIFIC UPTAKE RATE (d^{-1})
2.0
1.5
1.0
0.5
0
0.7
1.1
1.2
3.3
4.6
20.1
21.5
31.2
36.9
49.1
NO_3^- (μM)

Figure II.10

Figure II.11. A. Particulate nitrogen (PN) concentrations for nano- (<10 μm) and netplankton (10-200 μm) fractions. B. Chl-a concentrations for nano- and netplankton. Labels on the x-axis denote NO_3^- concentrations at each station. (Note that scales are different for the low and high NO_3^- stations).

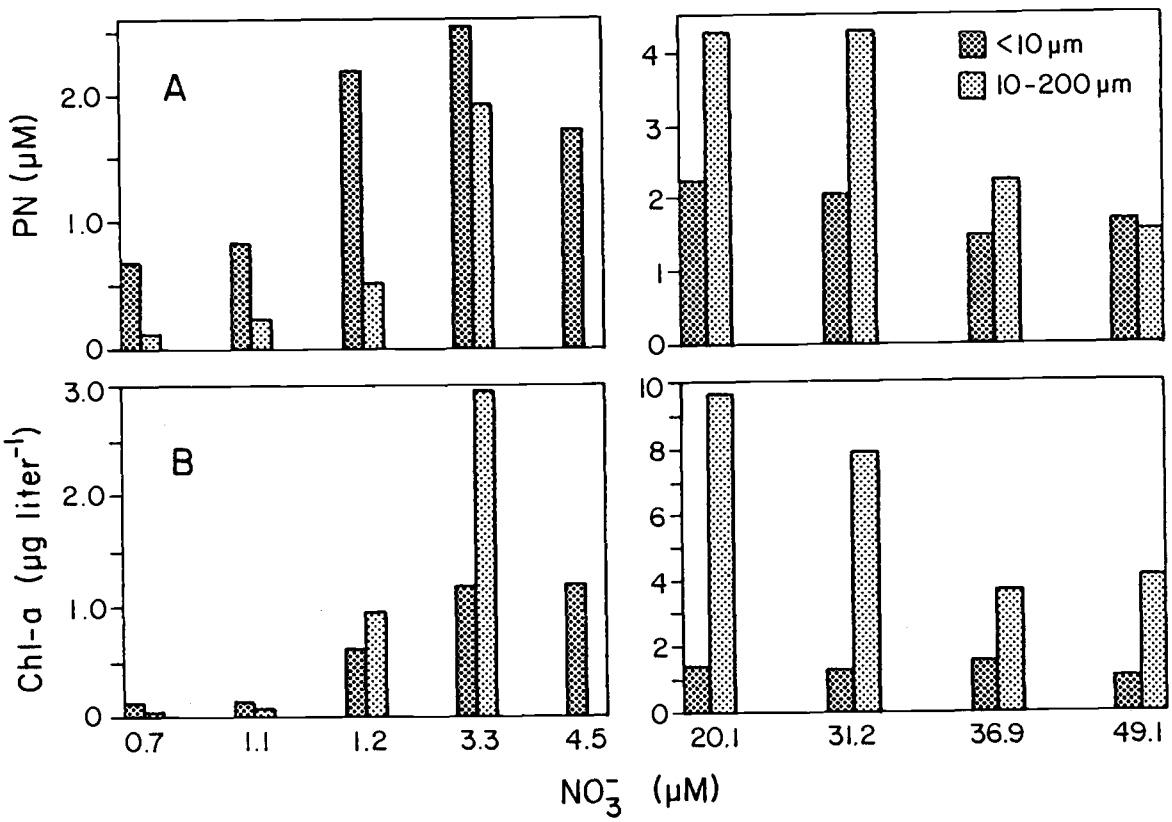
A
B
PN (μM)
Chl-a (μg liter⁻¹)
<10 μm
10-200 μm
0.7
1.1
1.2
3.3
4.5
20.1
31.2
36.9
49.1
NO3- (μM)

Figure II.11

respectively. The same was true for the average percent chl-a in the netplankton fraction (80.6% and 38.6% respectively). The variability in concentrations of PN and chl-a was greater at low than high NO_3^- stations.

Nanoplankton dominated the utilization of regenerated nitrogen in low NO_3^- waters (61.3% of NH_4^+ and 63.5% of urea uptake), while netplankton dominated regenerated nitrogen use in high NO_3^- waters (72.3% of NH_4^+ and 84.0% of urea uptake, Table II.5). Figure II.12 compares percent uptake of NH_4^+ and urea, in each size fraction, to percent PN and chl-a. The average percent uptake of NH_4^+ and urea closely paralleled the percent chl-a in each size fraction. Station 1 results were omitted because the ambient concentrations of NH_4^+ and urea in the <10 μm fraction were greatly elevated (0.83 and 0.33 μM, respectively) above NH_4^+ and urea concentrations present in the <200 μm fraction (0.28 and 0.03 μM, respectively). The 10 μm Nitex screening of the seawater may have enhanced excretion of NH_4^+ and urea by microzooplankton. This problem was not encountered during any other size-fractionation experiment.

In the high NO_3^- waters, the average ratio of (% urea uptake/% PN) was significantly higher in netplankton (1.3) than in nanoplankton (0.4) (Table II.6). The average ratio of (% NH_4^+ or % urea uptake/% chl-a) was not significantly different between size fractions. Ratios for the low NO_3^- stations were more variable than for high NO_3^- stations (Table II.6). Station 6 (NO_3^- = 3.3 μM) closely resembles the pattern seen in the high NO_3^-

Table II.5. Uptake of NH_4^+ and urea by nano- and netplankton.

Sta. No.	NH_4^+ UPTAKE (µmol N l^{-1} h^{-1})		NH_4^+ % Uptake		UREA UPTAKE (µmol N l^{-1} h^{-1})		UREA % Uptake	
	<10 µm	10-200 µm	<10 µm	10-200 µm	<10 µm	10-200 µm	<10 µm	10-200 µm
LOW NO_3^-								
4	0.011	0.007	60.9	39.1	0.005	0.006	47.6	52.4
8	0.015	0.017	46.7	53.3	0.008	0.000	99.5	0.5
2	0.045	0.027	62.9	37.1	0.029	0.036	44.5	55.5
6	0.033	0.059	35.9	64.1	0.005	0.014	26.0	74.0
14	0.015	0.000	100.0	0.0	0.003	0.000	100.0	0.0
HIGH NO_3^-								
10	0.017	0.065	20.3	79.7	0.002	0.016	12.6	87.4
11	0.011	0.108	9.0	91.0	0.002	0.015	13.2	86.8
12	0.013	0.011	53.8	46.2	0.004	0.016	21.0	79.0
1	0.088	0.000	*	*	0.008	0.001	*	*

* Comparison not possible because NH_4^+ and urea concentrations were elevated in <10 µm fraction.

Figure II.12. Comparison of percent NH_4^+ and urea uptake (μmol N l^{-1} h^{-1}), to percent particulate nitrogen and chl-a in nano- and netplankton fractions. Stations 2, 4, 6, 8, and 14 are low NO_3^- stations. Stations 10-12 are high NO_3^- stations.

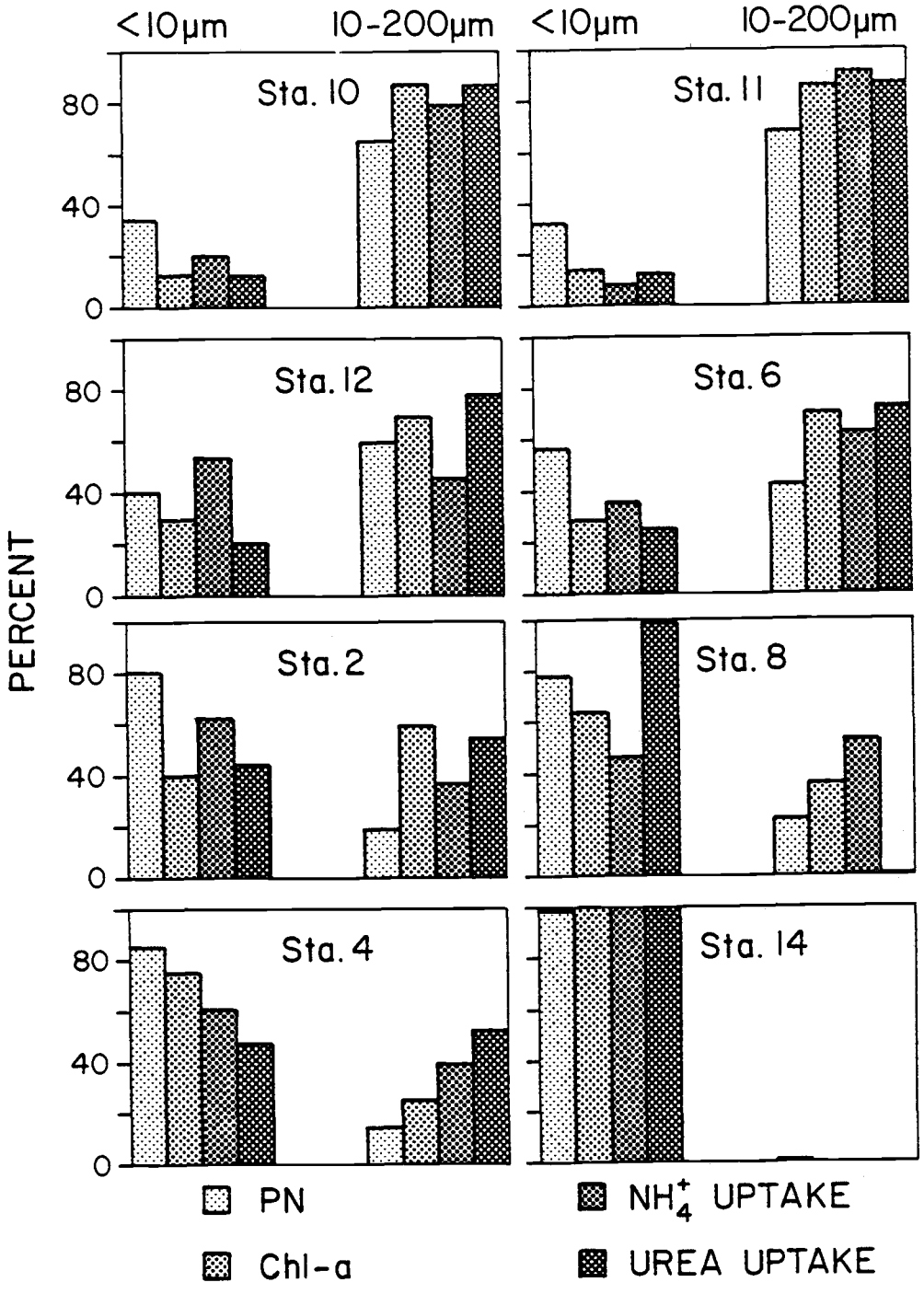
<10μm
10-200μm
<10μm
10-200μm
Sta. 10
Sta. 11
Sta. 12
Sta. 6
Sta. 2
Sta. 8
Sta. 4
Sta. 14
PERCENT
0
40
80
PN
Chl-a
NH_4^+ UPTAKE
UREA UPTAKE

Figure II.12

Table II.6. Ratios of NH_4^+ and urea uptake to PN and chl-a.

Sta. No.	% NH_4^+ Uptake/ % PN		% UREA Uptake/ % PN		% NH_4^+ Uptake/ % Chl-a		% UREA Uptake/ % Chl-a	
	<10 μm	10-200 μm	<10 μm	10-200 μm	<10 μm	10-200 μm	<10 μm	10-200 μm
LOW NO_3^-								
4	0.7	2.6	0.6	3.5	0.8	1.6	0.6	2.1
8	0.6	2.4	1.3	0.0	0.7	1.5	1.6	0.0
2	0.8	1.9	0.6	2.9	1.6	0.6	1.1	0.9
6	0.6	1.5	0.5	1.7	1.2	0.9	0.9	1.0
MEAN =	0.7	2.1	0.8	2.0	1.1	1.2	1.0	1.0
SD =	0.1	0.5	0.4	1.5	0.4	0.5	0.4	0.9
HIGH NO_3^-								
10	0.6	1.2	0.4	1.3	1.6	0.9	1.0	1.0
11	0.3	1.3	0.4	1.3	0.6	1.1	0.9	1.0
12	1.3	0.8	0.5	1.3	1.8	0.7	0.7	1.1
MEAN =	0.7	1.1	0.4	1.3	1.3	0.9	0.9	1.0
SD =	0.5	0.3	0.1	0.0	0.6	0.2	0.2	0.1

Station 1 not included because NH_4^+ and urea were elevated in <10 μm fraction.

Station 14 not included because all uptake and biomass occurred in the <10 μm fraction.

stations (Fig. II.12). In general, the mean ratio of (% NH_4^+ or urea uptake/% PN) was noticeably larger in the netplankton than in the nanoplankton. As with the high NO_3^- stations, the ratio of (% NH_4^+ or urea uptake/% chl-a) was not significantly different between size fractions.

The ratio of urea/(urea + NH_4^+) uptake was inversely related to increasing NH_4^+ concentration (Fig. II.13), with ratios ranging from 0 to 0.6. The relative utilization of urea by nanoplankton was not influenced by ambient concentrations of urea (Fig. II.14A). For netplankton, however, relative urea utilization increased as ambient urea concentrations increased (Fig. II.14B).

Daily, nitrogen-specific uptake rates for regenerated forms of nitrogen (NH_4^+ and urea) are higher in the netplankton fraction in both low and high NO_3^- waters (3.0 and 2.2 times, respectively, Fig. II.15). A relative preference index (RPI) for NH_4^+ and urea was calculated using uptake rates for both size fractions (Table II.7). Both ambient and tracer concentrations of NH_4^+ and urea were used in the calculations, because tracer additions frequently made up the majority of the nutrient present. The RPI values for NH_4^+ were always >1 while those of urea were always less than one. Results indicate a consistent preference for NH_4^+, and discrimination against urea utilization relative to their ambient concentration. These results were uniform for all stations regardless of NO_3^- concentration or size fraction.

No significant isotope dilution occurred during NH_4^+ uptake experiments at stations 0, 4 (<10 μm), 6, 10, and 12. For the

Figure II.13. The ratio of urea/(urea + NH_4^+) uptake as a function of ambient NH_4^+ (μM). A. Nanoplankton. B. Netplankton.

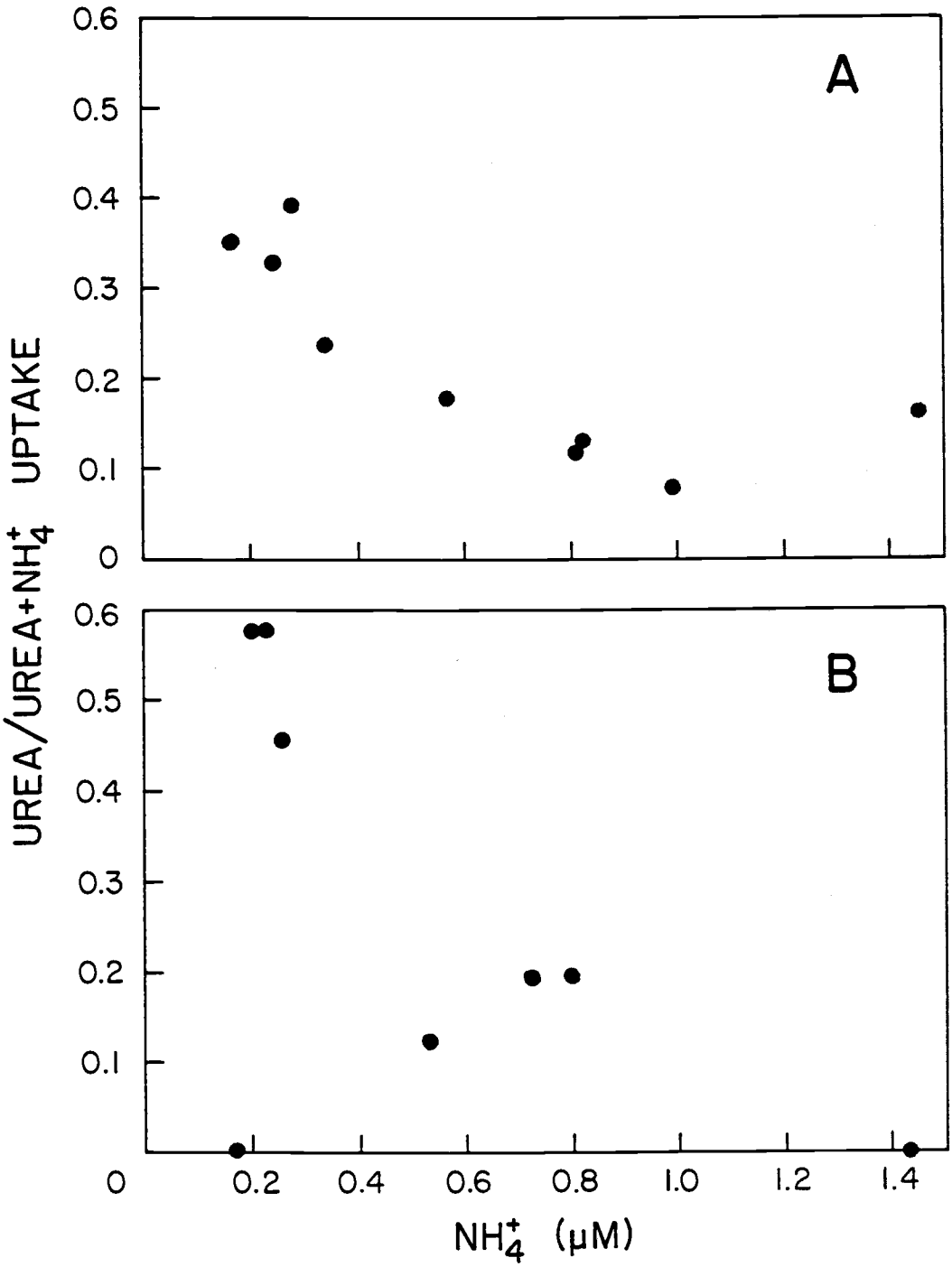
A
B
0.6
0.5
0.4
0.3
0.2
0.1
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
UREA/UREA+NH4+ UPTAKE
NH4+ (μM)

Figure II.13

Figure II.14. The ratio of urea/(urea + NH_4^+) uptake as a function of ambient urea-N (μM). A. Nanoplankton. B. Netplankton.

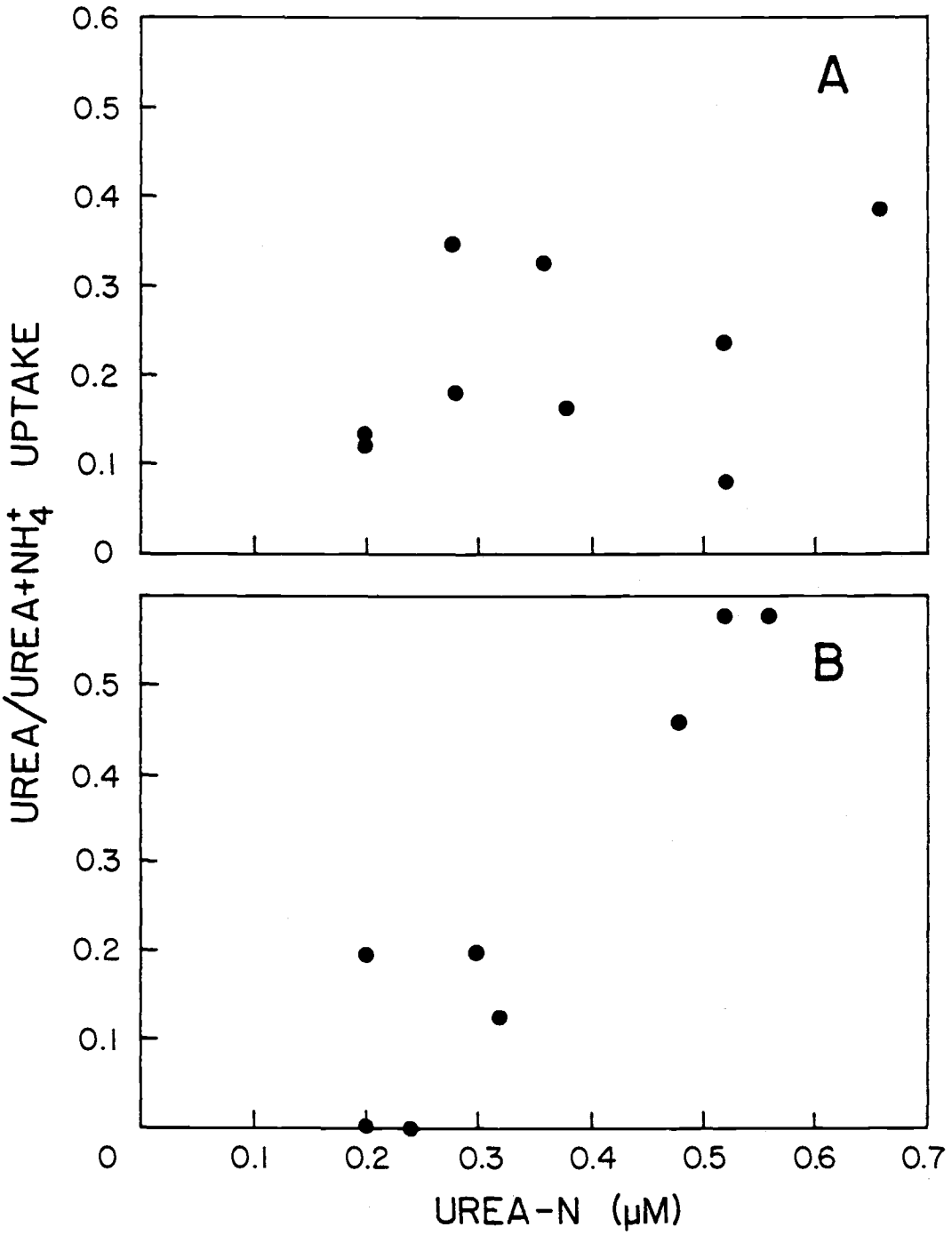
A
B
0.6
0.5
0.4
0.3
0.2
0.1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
UREA/UREA+NH$_4^+$ UPTAKE
UREA-N (μM)

Figure II.14

Figure II.15. Nitrogen-specific uptake rates (d^{-1}) for regenerated nitrogen sources for nano- and netplankton. Labels on the x-axis denote ambient NO_3^- concentrations at each station. A. Low NO_3^- stations. B. High NO_3^- stations.

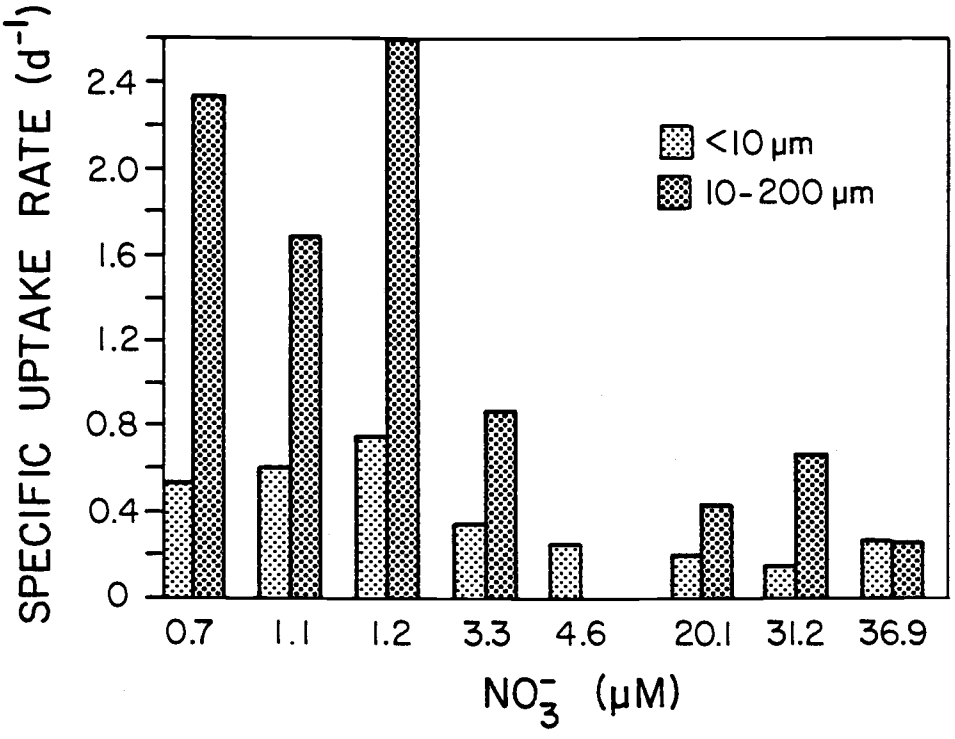
SPECIFIC UPTAKE RATE (d⁻¹)
2.4
2.0
1.6
1.2
0.8
0.4
0
<10 µm
10-200 µm
0.7
1.1
1.2
3.3
4.6
20.1
31.2
36.9
NO3- (µM)

Figure II.15

Table II.7. Relative preference index (RPI) for size-fractionated NH_4^+ and urea uptake.

Sta. No.	RPI NH_4^+ <10 μm	RPI UREA <10 μm	RPI NH_4^+ 10-200 μm	RPI UREA 10-200 μm
LOW NO_3^-				
4	1.7	0.5	1.5	0.7
8	1.8	0.6	2.2	0.0
2	2.1	0.5	1.4	0.8
6	1.1	0.6	1.0	0.9
14	1.1	0.8	*	*
HIGH NO_3^-				
10	1.1	0.6	1.1	0.7
11	1.3	0.5	1.4	0.3
12	2.0	0.4	1.5	0.8
1	1.4	0.2	*	*

* All uptake occurred in the <10 μm fraction.

remaining experiments, significant isotope dilution did occur and the mean atom % ^{15}N of dissolved NH_4^+ is reported in Table II.8. The ratio of corrected/uncorrected uptake rates ranged form 0.87 to 3.57, averaging 1.42.

DISCUSSION

Surface NO_3^- concentrations and water temperatures off the Oregon coast during this study were similiar to surface measurements of NO_3^- and temperature reported by Small and Menzies (1981). Highest surface NO_3^- concentrations and coldest water temperatures occurred in the nearshore region. Conversely, lowest NO_3^- levels and warmest water temperatures were found in surface waters farther offshore (Table II.1, Fig. II.1). Small and Menzies (1981) recorded a surface temperature as low as 8° C, with a corresponding NO_3^- concentration of 25 µM during a strong upwelling period nearshore. Nearshore surface temperatures, in this study, were never below 10° C while surface NO_3^- concentrations were sometimes twice as high (>48 µM).

Surface PO_4^{3-} concentrations in offshore and nearshore waters were high, ranging from 0.32 to 1.28 µM offshore and from 1.79 to 4.26 µM nearshore (Table II.1). An average k_s (half-saturation) value for PO_4^{3-} uptake in the Central North Pacific was 0.20 µM (in review by Nalewajko and Lean 1980). The concentrations of PO_4^{3-} measured in this study appear sufficient to preclude this nutrient as limiting to phytoplankton growth.

Table II.8. Effect of isotope dilution on estimate of NH_4^+ uptake rates.

Sta. No.	Size Frac. (μm)	Mean NH_4^+ Enrichment (atom % ^{15}N)	SD	Corr. Uptake/ Uncorr. Uptake
LOW NO_3^-				
4	<10	*		
	<200	34.7	1.63	1.10
8	<10	57.0	10.72	1.09
	<200	29.8	2.22	1.97
2	<10	28.8	4.58	1.33
	<200	26.0	4.11	1.67
6	<10	*		
	<200	*		
14	<10	8.4	0.04	0.87
	<200	7.8	0.06	0.89
HIGH NO_3^-				
0	<200	*		
10	<10	*		
	<200	*		
11	<10	20.9	1.16	0.90
	<200	15.7	5.31	1.20
12	<10	*		
	<200	*		
1	<10	3.0	0.62	3.57
	<200	24.6	1.26	1.07

* The calculated atom % enrichment was used in determination of NH_4^+ uptake rates. The calculated value was determined by dividing the ^{15}N added to the medium by the total ^{15}N and ^{14}N present (tracer + ambient concentration).

Combined NH_4^+ and urea concentrations (μmol N l^{-1}) at all stations were variable and showed no relationship to NO_3^- concentrations. However, urea concentrations were relatively high at low NH_4^+ and low at high NH_4^+ concentrations. While the variability of urea concentrations in marine waters is well established (Remsen 1971, McCarthy and Kamykowski 1972, Avilova 1983, and Harrison et al. 1985), the relationship between NH_4^+ and urea presented in this study has not been reported previously.

Ammonium is the major nitrogenous excretory product of most marine animals (Parry 1960). Recent studies of NH_4^+ regeneration have shown that microorganisms (<100 μm) are the principal producers of NH_4^+ (Harrison 1978, Caperon et al. 1979, Glibert 1982) and the latest studies suggest that eukaryotes (e.g. protozoans) excrete NH_4^+, while heterotrophic bacteria consume NH_4^+ (Goldman et al. 1985, Laws et al. 1985, Wheeler and Kirchman, 1986). The sources of urea in marine waters are not well known. However, they include excretion by invertebrates and fish (Corner and Newell 1967, McCarthy and Whitledge 1972, Whitledge, 1981) and bacterial degradation of purines, pyrimidines and arginine (Maita et al. 1973, McCarthy 1980). Maita et al. (1973) argue that arginine decomposition by bacteria is the primary source of urea in seawater, and that urea excretion rates from aquatic animals are three to four orders of magnitude lower than bacterial production rates. In the present study, the relationship of ambient NH_4^+ and urea concentrations may result from the relative distribution of NH_4^+ and urea producing organisms.

Biomass distribution

The relationship of total (<200 μm) PN and chl-a to surface NO_3^- concentration followed the normal pattern for upwelled waters (Small and Menzies 1981, MacIsaac et al. 1985). Highest NO_3^- waters had relatively low PN and chl-a concentrations (Sta. 1, 12, and 13), while stations with intermediate concentrations of NO_3^- (Sta. 0, 10, and 11) had highest levels of PN and chl-a. Nitrate was utilized rapidly at high biomass stations. For example, NO_3^- uptake at station 0 was 1.2 μmol N l^{-1} h^{-1}. At this rate, phytoplankton would deplete the 21 μM surface NO_3^- to <5 μM in one day, assuming no new inputs of NO_3^- and assuming no decrease in NO_3^- uptake rates in response to lowered NO_3^- concentrations.

In this study, surface PN, chl-a and NO_3^- concentrations decreased in the offshore direction. This trend has been found often in other coastal studies (Eppley et al. 1978, Small and Menzies 1981, MacIssac et al. 1985, Probyn 1985). Size-fractionated biomass also displayed an onshore-offshore trend. In high NO_3^- waters most of the PN and chl-a were in the 10-200 μm fraction (netplankton), while in low NO_3^- waters most biomass was in the <10 μm fraction (nanoplankton). Microscopic analyses of phytoplankton genera showed unicellular diatoms and dinoflagellates dominating low NO_3^- waters (cell types likely to pass through <10 μm screening). In high NO_3^- waters, large chain-forming diatoms such as Asterionella and Thalassiosira were dominant. However, in recently upwelled water (Sta. 12 and 13) unicellular diatoms were dominant, while

chain-forming diatoms were present in smaller numbers. Recent evidence suggests that phototrophic picoplankton play an important role as primary producers in the pelagic environment (Gieskes et al. 1979, Joint and Pomeroy 1983, Li et al. 1983). In this study, picoplankton were not examined, but were probably an important component of the plankton in the low NO_3^- waters (Herbland et al. 1985, Probyn 1985). Malone (1980) describes two important size classes of phytoplankton: netplankton (~20-200 μm) and nanoplankton (<20 μm). His review of literature on size distribution of phytoplankton shows netplankton biomass being relatively more abundant in continental shelf and coastal upwelling waters, whereas nanoplankton (including picoplankton component) tend to dominate in open ocean waters. Results in this study support the spatial distribution of nanoplankton and netplankton described by Malone (1980).

Nitrogen assimilation

Mass balance calculations indicate that more $^{15}N\text{-}NH_4^+$ left the dissolved pool than was recovered in the particulate fraction. These results imply that the NH_4^+ uptake rates reported here are underestimates. Details of the mass balance calculations and possible mechanisms leading to the underestimates in NH_4^+ uptake rates are discussed fully in chapter III.

Combined nitrogen uptake rates for NO_3^-, NH_4^+ and urea were on average 6.4 times greater in high NO_3^- than in low NO_3^- waters. Nitrate was quantitatively the most important nitrogen source at the high NO_3^- stations (83% on avg.). The average for total nitrogen assimilated in high NO_3^- stations, 0.70 μmol N l^{-1} h^{-1}, is close to averages reported by MacIsaac et al. (1985) (NO_3^- assimilation only) and Probyn (1985) in other upwelling regions (0.45 and 0.35 μmol N l^{-1} h^{-1} respectively).

Diatom blooms are observed commonly in upwelled waters. Eppley et al. (1979) conclude that, "the transport of NO_3^- into the euphotic zone appears to be a major factor regulating the standing stock and production of phytoplankton in southern California". Probyn (1985) reached similiar conclusions in a nitrogen uptake study in the Benguela upwelling system. He found that total nitrogen assimilation in phytoplankton communities increased with the proportion of NO_3^- utilized [$NO_3^-/(NO_3^- + NH_4^+ + \text{urea})$]. It is clear in the present study that >20 μM NO_3^- waters are associated with higher standing stocks of phytoplankton and higher rates of nitrogen utilization.

Nitrogen-specific uptake rates for NH_4^+ and urea were usually higher for the netplankton fraction than for the nanoplankton fraction. This agrees with results presented by Probyn (1985) for the Benguela upwelling area (specific uptake rates for NH_4^+, urea, and NO_3^- combined). Probyn found that picoplankton (<1 μm) and nanoplankton (<10 μm) size classes generally had lower nitrogen specific uptake rates than the whole plankton community (<212 μm).

Work by Furnas (1983) in Narragansett Bay, however, shows that nitrogen-specific uptake rates for nanoplankton were similiar to rates for the total population (<153 μm fraction).

In this study, the percentage of NH_4^+ and urea uptake in each fraction closely paralleled the percentage of chl-a (Fig. II.12). Furthermore, the proportion of chl-a in netplankton was uniformly higher than the proportion of PN. If the chl-a/PN ratio was comparable for all size classes of phytoplankton within a sample, then the results suggest that the nanoplankton fraction may contain a greater portion of non-phytoplankton N than the netplankton fraction. Banse (1977) found a predominance of detrital carbon in the nanoplankton fraction, which led to an underestimate of chl-a/carbon for nanoplankton. The presence of detrital PN or heterotrophic PN (microzooplankton and bacteria) leads to underestimates of nitrogen-specific uptake rates for natural assemblages of phytoplankton (Dugdale and Goering 1967). Thus, in this study, lower nitrogen-specific uptake rates for nanoplankton, relative to netplankton, may be attributed to a larger percentage of non-phytoplankton N in the <10 μm fraction.

Assimilation of new and regenerated nitrogen

Assimilation of regenerated nitrogen usually dominates in open ocean waters (Dugdale and Goering 1967, Eppley et al. 1973, Eppley et al. 1977, Eppley 1981). On the other hand, coastal upwelling regions

and frontal zones display large variability in percentages of new and regenerated nitrogen production (Olson 1980, Price et al. 1985, Probyn 1985). In the present study, regenerated production (NH_4^+ and urea supported) was most important in low NO_3^- waters (avg. 71%), while new production (NO_3^- supported) was consistently dominant in high NO_3^- waters (avg. 83%).

Nitrogen uptake rates normalized to phytoplankton biomass (chl-a) followed the same pattern observed by Price et al. (1985): highest chl-a specific uptake rates of NH_4^+ and urea occurred in stratified waters (low NO_3^-), while highest rates for NO_3^- occurred in frontal waters where concentrations of NO_3^- were much higher. The percent new production in high NO_3^- waters resembles averages reported for similiar regions (Yoder et al. 1983, >50% in SE continental shelf of U.S, Harrison et al. 1983, 67% in Middle Atlantic Bight, Probyn 1985, 71% shelf waters). However, Probyn (1985) found percent new nitrogen production averaging only 48% in higher NO_3^- nearshore waters (8-25 μM) and 71% in lower NO_3^- shelf waters (<7 μM). Ammonium inhibition of NO_3^- uptake may be responsible for the low percentage of new production at two of Probyn's three nearshore stations (NH_4^+ was 0.40 and 0.75 μM). Similar concentrations of NH_4^+ in this study (e.g. sta. 10 and 11) did not inhibit NO_3^- uptake in high NO_3^- waters.

Ammonium and urea uptake are quantitatively most important in low NO_3^- waters in this study. Ammonium is usually the dominant nitrogen source in low nutrient waters (Dugdale and Goering 1967, Eppley et al. 1973, Eppley et al. 1977, Axler et al. 1981). Other

studies have demonstrated that urea assimilation is also significant and at times can be as important as NH_4^+ use (McCarthy 1972, Kristiansen 1983, Harrison et al. 1985). Urea provided an average of 21% of nitrogen assimilation in low NO_3^- waters in this study. This average resembles urea assimilation averages reported by others in similiar low NO_3^- waters (McCarthy 1972, Kristiansen 1983, Harrison et al. 1985: 28, 19, and 32% of total nitrogen assimilation respectively).

Average turnover times for dissolved NH_4^+ and urea (excluding sta. 14) were short regardless of NO_3^- concentration (0.30 d and 1.2 d, respectively). These turnover times are in agreement with findings of McCarthy (1972), Herbland (1976), Paasche and Kristiansen (1982a), and Kristiansen (1983). However, Mitamura and Saijo (1980) and Harrison et al. (1985) found urea turnover times to be an order of magnitude longer. In fact, Mitamura and Saijo (1980) measured extremely long urea turnover times (110 d) in NW Pacific Central and subarctic Pacific waters.

The relative preference index (RPI) has been used to evaluate phytoplankton preference for NO_3^-, NH_4^+ and urea. Previous studies report that NH_4^+ and urea are usually preferred and NO_3^- rejected, relative to their respective ambient concentrations (McCarthy 1977, Glibert et al. 1982a, Probyn 1985, Probyn and Painting 1985). The RPI index was used in this study to compare NH_4^+ and urea preference by nano- and netplankton. Size-fractioned NO_3^- uptake was not measured, and therefore the RPI did not include this nutrient. While NH_4^+ and urea are typically preferred over

NO_3^-, results from this study also show NH_4^+ being "preferred" relative to urea. This preference is reflected in other comparisons of NH_4^+ and urea use as well. For example, NH_4^+ assimilation was greater than that of urea in all experiments, and turnover times for NH_4^+ were consistently shorter. It must be noted, however, that some NH_4^+ uptake rates were corrected for ^{15}N isotope dilution, whereas no procedures are presently available to correct for effects of urea isotope dilution. In general, corrections of NH_4^+ uptake rates were small (Table II.8), and the comparison of NH_4^+ and urea uptake rates is probably reliable.

Ammonium concentrations >0.5 μM often inhibit NO_3^- uptake (MacIsaac and Dugdale 1972, McCarthy et al. 1977, Glibert et al. 1982a, Paasche and Kristiansen 1982a). In this study, urea uptake in both size fractions also appeared to be inhibited by increasing concentrations of NH_4^+. Kristiansen (1983) found a similiar inhibition, but only at higher NH_4^+ concentrations (1-2 μM). Urea uptake by netplankton, but not nanoplankton, increased with increasing urea concentrations. Since additions of saturating urea (2.0 μM urea-N) resulted in little increase in urea uptake relative to trace additions, urea uptake appears to be regulated more by ambient NH_4^+ than ambient urea concentrations.

Conclusions

1. Low NO_3^- waters were characterized by low rates of nitrogen utilization, a high percentage of regenerated production, and an abundance in nanoplankton biomass.

2. High NO_3^- waters were characterized by high rates of nitrogen utilization, a high percentage of new production, and an abundance in netplankton biomass.

3. Ammonium and urea uptake in each size fraction was proportional to chl-a, but not to PN. Detrital PN or heterotrophic PN in each size fraction may have influenced nitrogen-specific uptake rates.

4. Turnover times for ambient NH_4^+ and urea pools were very short in low and high NO_3^- waters. Ammonium turnover times were on average four times shorter than those for urea. Increasing NH_4^+ concentrations appeared to have an inhibitory effect on urea assimilation.

Chapter III

REGENERATION OF AMMONIUM

THEORETICAL BACKGROUND

Classically it was assumed that the most abundant nitrogen form (i.e. NO_3^-) was of greatest importance to phytoplankton growth (Glibert 1981). Justification for this view was based on the positive correlation between productivity and NO_3^- concentration in surface waters. The introduction of ^{15}N-tracer techniques to measure uptake of nitrogenous nutrients by phytoplankton, demonstrated the importance of NH_4^+ to phytoplankton growth (Dugdale and Goering 1967). However, low ambient NH_4^+ concentrations lead to some difficulty in the accurate determination of uptake rates. Dugdale and Goering noted that one potential source of error in NH_4^+ uptake determinations was dilution of the isotope during the incubation. Release of ^{14}N-NH_4^+, by invertebrates or during bacterial remineralization, can decrease the ^{15}N enrichment of the NH_4^+ pool during experimental incubations. Such isotope dilution would result in an underestimate of NH_4^+ uptake rates. Since ^{15}N-NH_4^+ additions were usually high in early studies, isotope dilution errors were assumed to be small.

Blackburn (1979) and Caperon et al. (1979) independently proposed similiar models for calculating rates of NH_4^+ uptake and regeneration using results from isotope dilution measurements. Their method requires simultaneous determination of changes in NH_4^+ concentration and the atom % enrichment of the dissolved NH_4^+ pool. The models cannot be applied to calculations of uptake and

regeneration rates however, when ambient NH_4^+ is at or near the limit of detection or in the absence of significant changes in ambient NH_4^+ (Glibert et al. 1982c). Consequently, Glibert et al. (1982c) suggested alternate equations for calculating rates of regeneration when NH_4^+ concentrations remain constant during experimental incubations.

Ammonium uptake rates can be measured using two different approaches. The Blackburn-Caperon model estimates NH_4^+ uptake from changes in NH_4^+ concentration and ^{15}N enrichment of the dissolved pool, while an earlier model employed by Dugdale and Goering (1967) estimates uptake from increases in ^{15}N in particulate material. The original equations used to calculate uptake rates from accumulation of ^{15}N in the particulate fraction (assuming no isotope dilution) are as follows:

$$(1)\quad V_{NH_4^+} = \frac{\text{atom \% } ^{15}N \text{ of PN}}{(R_o) \cdot \text{ time of incubation}}$$

$$(2)\quad \rho_{NH_4^+} = V_{NH_4^+} \cdot (PN)$$

where (V) is the specific uptake rate of a particular form of nitrogen (in units of reciprocal time), atom % ^{15}N of PN is the enrichment of the particulate fraction, (R_o) is the initial enrichment of ^{15}N in the medium, and p (rho) is the absolute uptake rate of N (μmol N l^{-1} h^{-1}). PN is the concentration of particulate nitrogen (in units μmol N l^{-1}).

Measurement of isotope dilution allows an adjustment of calculated uptake rates for any significant changes in R (Harrison 1978, Glibert 1982, Glibert et al. 1982c). Studies by Glibert (1982) and Harrison and Harris (1986) have shown that isotope dilution can lead to a 2-3 fold underestimate in NH_4^+ uptake rates if a constant enrichment value for dissolved $^{15}N\text{-}NH_4^+$ is assumed. If the isotopic enrichment of NH_4^+ is monitored during the experiment, the equation proposed for calculating uptake rates (Glibert et al. 1982c) is:

$$P_{NH_4^+} = \frac{\text{atom \% } ^{15}\text{N of PN}}{(\overline{R})\bullet \text{ time of incubation}} \bullet (PN)$$

where P (uppercase rho) is used to distinguish the rate corrected for isotope dilution from p (lowercase rho), the uncorrected rate. $\overline{R}$ is the mean atom % enrichment of dissolved NH_4^+ between the initial and final time points assuming an exponential decrease.

Laws (1984) has recently reviewed problems (i.e. "missing $^{15}N\text{-}NH_4^+$") associated with interpretation of NH_4^+ isotope dilution models and estimates of NH_4^+ uptake rates. Results from the present study will be discussed in light of recent views concerning the best estimate for NH_4^+ uptake rates, as well as associated regeneration rates (Laws 1984, Glibert et al. 1985).

METHODS

Ammonium regeneration measurements were made on <10 μm and <200 μm size-fractioned seawater for stations 0-2, 4, 6, 8, 10-12, and 14. Regeneration measurements in station 0 only include the <200 μm fraction. Collection procedures and other details are described in Chapter II.

Immediately prior to $^{15}N\text{-}NH_4^+$ additions, triplicate 10 ml samples were withdrawn from each incubation bottle for analysis of initial NH_4^+ concentrations. Additions of $^{15}N\text{-}NH_4^+$ (0.1 μmol N l^{-1}, 99.7 atom %) were made to a series of four 2.7 l polycarbonate, incubation bottles for each size fraction. Each bottle was filtered at specific time intervals (0, 30, 60 and 120 min) to obtain time course measurements for regeneration of NH_4^+. One liter of filtrate, from each incubation bottle, was transferred to acid-washed 1 liter polyethylene bottles. Triplicate 10 ml subsamples were removed from each filtrate bottle for final NH_4^+ analysis, while the remaining filtrate was stored at $<0^o$ C prior to distillation aboard ship the same day for concentration and recovery of NH_4^+ for isotopic analysis.

The basic procedures for concentration of NH_4^+ from the filtrate were adapted from those described by Glibert et al. (1982c) and Paasche and Kristiansen (1982b). Triplicate standards were run prior to experimental samples. These standards consisted of the same $^{15}N\text{-}NH_4^+$ stock (10.0 atom % ^{15}N) used as a carrier for

experimental samples. A carrier addition (3 μM)) was necessary to satisfy mass requirements for emission spectrometry. Deionized water was used for standards at stations 0 and 1, while filtered seawater was used at all other stations.

Duplicate filtrate samples (425 ml) were poured into flasks, spiked with $^{15}N\text{-}NH_4^+$ (carrier addition, 10 atom % enrichment), and buffered to pH >9.3 using a 1.8 ml addition of 1 M borate buffer. Approximately 50 ml of distillate were collected in 10 ml of 0.01 M HCl. Duplicate distillates for each time point were combined, boiled down to ~ 5 ml, then frozen in acid washed serum bottles. The final concentration step was completed later by boiling to a volume of <0.5 ml, transfer to a precombusted, glass fiber filter (Whatman GF/F), and a brief (<45 min) drying period at 60° C. Recovery of NH_4^+ after distillation and evaporation was approximately 70 to 80%. All dried filter samples were stored under vacuum prior to determination of isotopic enrichment. The relative abundance of ^{15}N was determined by emission spectrometry (Fiedler and Proksch 1975). Procedures for sample preparation were adapted from LaRoche (1983) and are described in detail by Dudek et al. (1986).

In this study, steps were taken to improve NH_4^+ regeneration measurements so that results could be critically assessed for accuracy and precision. Triplicate standards were processed for each experiment, providing daily estimates of analytical accuracy and precision. Experimental samples were taken at four time intervals during each 2 h incubation period. The standard deviation for the calculated rate of regeneration was determined by using statistical formulas for the propagation of error (Bevington 1969).

Calculations

Rates of NH_4^+ regeneration were determined employing either the Blackburn-Caperon "variable NH_4^+" equations (Blackburn 1979, Caperon et al. 1979) or the Glibert et al. (1982c) "constant NH_4^+" equation. Both models utilize data on NH_4^+ concentrations and changes in isotopic enrichment. Occasionally, calculated values for initial ^{15}N-NH_4^+ enrichment (R_o) were substituted for measured values when analytical measurements were poor. The variable NH_4^+ model was employed when a significant change in ambient NH_4^+ occurred over the course of an incubation (Stations 0, 6, and 12). The constant NH_4^+ model was employed when there was no significant change in NH_4^+ concentration. Both isotope dilution models assume an exponential decrease in the atom percent enrichment of the dissolved NH_4^+ pool.

Mass balance was calculated to compare the amount of ^{15}N NH_4^+ removed from the dissolved pool to the amount which appeared in the particulate fraction (Laws 1984). The initial ^{15}N present as dissolved NH_4^+ was known from the standard experimental addition (0.1 μmol N l^{-1}, 99.7% ^{15}N). The final ^{15}N content of the NH_4^+ pool was calculated by multiplying the measured isotopic enrichment (R_f) by the measured NH_4^+ concentration (S_f). Reliable NH_4^+ measurements were sometimes difficult to obtain. Occasionally one replicate was noticably discrepant from the other values. This is attributed to NH_4^+ contamination of sample tubes

or to handling of samples. If the standard deviation (SD) of replicate samples was greater than twice the normal analytical SD, the outlier was excluded from the data set. If only one experimental determination was available, an analytical standard deviation of 0.03 uM was used for calculations. Individual SD's, for measured ^{15}N enrichment (R_f) in the NH_4^+ pool, were derived from standard errors for values predicted from the regressed time course data. Standard deviations for NH_4^+ concentrations (S_f) and ^{15}N enrichment (R_f) were used in calculating the SD for final ^{15}N content ($R_f \cdot S_f$) in the NH_4^+ pool.

The ^{15}N content present in the particulate fraction was determined by multiplying the $^{15}N/(^{15}N + ^{14}N)$ ratio of the PN by the concentration of PN (μmol N l^{-1}). A mean SD for analysis of ^{15}N in PN (0.016 atom %) was obtained empirically by averaging SD's from triplicate, single time-point estimates. Individual SD's for measured concentrations of PN were calculated from duplicate analyses. The SD for final ^{15}N content in the particulate fraction was calculated using statistical equations for the propagation of error.

RESULTS

The atom % enrichment of the dissolved NH_4^+ pool (R_s) was measured at four time points. Frequently, one of the four measurements was noticably discrepant from the regression fit of the three other points (Fig. III.1). When this occurred, the outlier was excluded from the data set. The variable NH_4^+ equations were used to calculate regeneration rates for stations 0, 6, and 12 (<200 μm fraction only), since significant changes in NH_4^+ occurred during the incubation period (Table III.1). The constant NH_4^+ model was used for the remaining rate determinations since NH_4^+ was constant during the incubation period (Table III.1).

Ammonium regeneration rates for <10 μm and 10-200 μm size fractions are presented along with corresponding NH_4^+ uptake rates in Table III.2. Regeneration rates for the 10-200 μm fraction were determined by the difference between the <10 μm rates and the <200 μm rates. For nine experiments, out of a total of nineteen, regeneration rates could be measured (0.00 to 0.201 μmol N l^{-1} h^{-1}), but as a result of cumulative errors, standard deviations were large and rates were not significantly different from zero (0.05 level). Nevertheless, nine other experiments did result in measurement of significant regeneration rates.

Ammonium regeneration rates span a large range of values (0.041 to 1.160 μmol N l^{-1} h^{-1}). The ratio of NH_4^+ regeneration/ uptake rates range from 0.9 to 13.2, with a mean value of 7.9, in the

Figure III.1. Changes in atom % enrichment of the dissolved NH_4^+ pool over a two hour incubation period for stations 1 (A) and 2 (B) (<200 and <10 μm size fraction, respectively). Discrepant values for the time course are indicated by arrows.

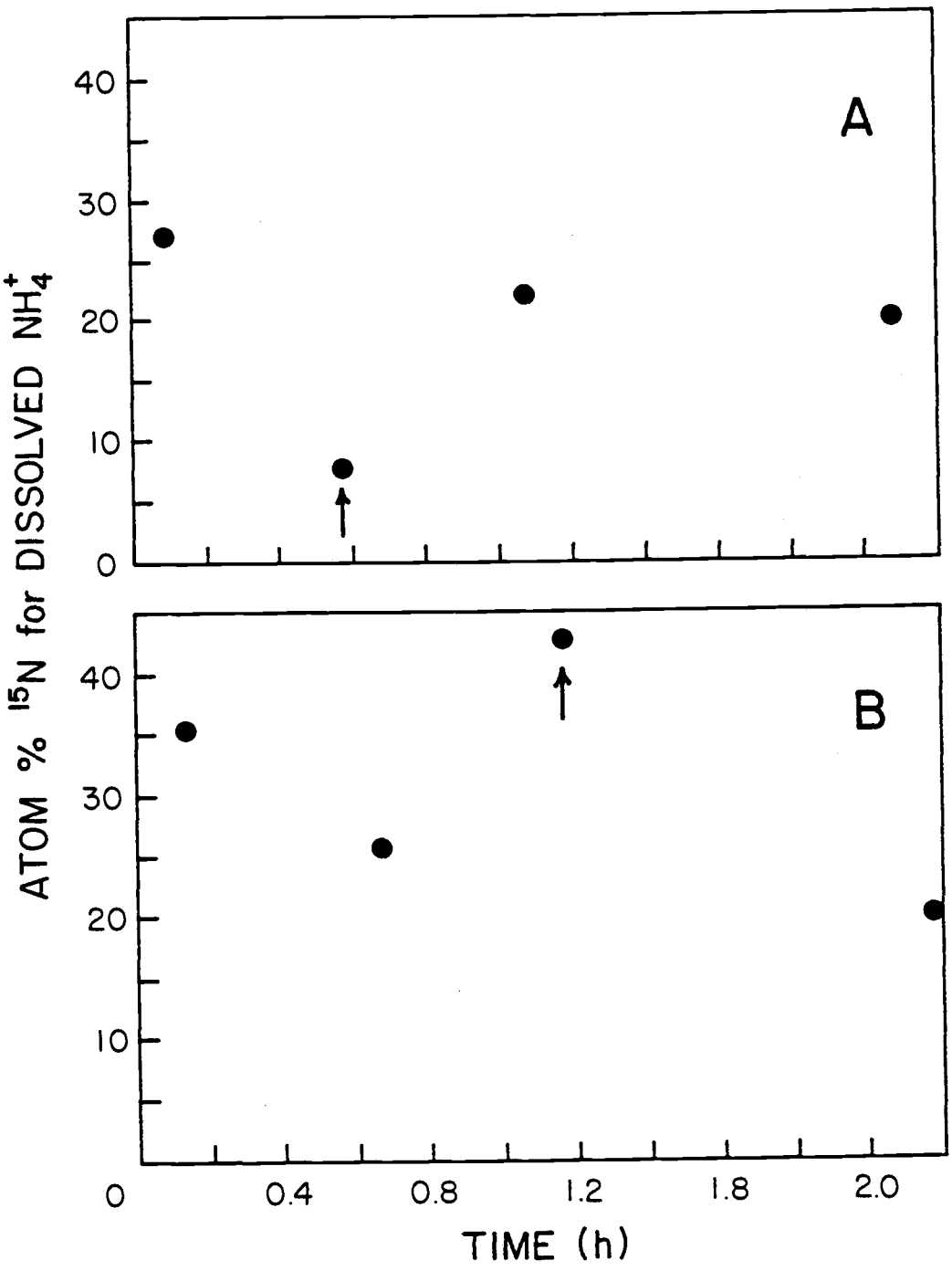

Figure III.1

Table III.1. Concentrations of NH_4^+ over a two hour incubation period for selected "variable" and "constant" NH_4^+ experiments.

	Sta. No.	Size Frac. (µm)	Time (h)	$[NH_4^+]$ (µM)	Slope	SE	$[NH_4^+]$ (µM) (reg. values)
"variable"	0	<200	0.07	0.22			0.19
			0.56	0.14			0.17
			1.07	0.15			0.15
			2.07	0.12	-0.041	0.021	0.11
	12	<200	0.09	0.23			0.22
			0.58	0.19			0.20
			1.07	0.18			0.18
			2.06	0.14	-0.043	0.007	0.14
"constant"	4	<10	0.11	0.22			0.23
			0.58	0.21			0.23
			1.12	0.27			0.22
			2.19	0.18	-0.016	0.027	0.20
	4	<200	0.12	0.20			0.22
			0.60	0.24			0.21
			1.11	0.20			0.21
			2.10	0.19	-0.012	0.016	0.19
	14	<10	0.15	1.38			1.38
			0.62	1.40			1.37
			1.12	1.31			1.36
			2.14	1.37	-0.012	0.031	1.35
	6	<10	0.15	0.78			0.74
			0.64	*			0.75
			1.13	0.68			0.76
			2.14	0.81	0.016	0.067	0.77

* Determination of NH_4^+ concentration not possible because of sample contamination.

Table III.2. Comparison of NH_4^+ uptake and regeneration in size fraction experiments.

Sta. No.	Size Frac. (µm)	NH_4^+ (µM)	NO_3^- (µM)	PN (µM)	Chl-a (µg l^{-1})	P N-Uptake (µmol N l^{-1} h^{-1})	SD	D N-Regeneration (µmol N l^{-1} h^{-1})	SD	REGEN/ UPTAKE
LOW NO_3^-										
4	<10	0.13		0.68	0.12	0.0108	0.003	0.132*	0.13	
	10-200	0.16	0.7	0.12	0.04	0.0069	0.001	0.080	0.01	11.6
8	<10	0.06		0.84	0.14	0.0147	0.003	0.078	0.01	5.3
	10-200	0.07	1.1	0.24	0.08	0.0168	0.004	-0.029*	0.03	
2	<10	0.16		2.19	0.63	0.0450	0.007	0.041	0.02	0.9
	10-200	0.13	1.2	0.52	0.95	0.0266	0.015	0.058	0.03	2.2
6	<10	0.67		2.53	1.19	0.0328	0.005	0.178*	0.22	
	10-200	0.62	3.3	1.92	2.95	0.0587	0.009	0.201*	0.17	
14	<10	1.26		1.73	1.20	0.0153	0.004	0.184	0.01	12.1
	10-200	1.33	4.6	0.01	0.00	0.0000		0.396	0.04	∞
#HIGH NO_3^-										
10	<10	0.66		2.23	1.42	0.0166	0.009	0.0*		
	10-200	0.70	20.1	4.28	9.70	0.0649	0.037	0.0*		
11	<10	0.43		2.06	1.32	0.0106	0.001	0.027*	0.03	
	10-200	0.43	31.2	4.29	7.97	0.1078	0.041	0.437	0.21	4.1
12	<10	0.22		1.49	1.59	0.0133	0.003	0.027*		
	10-200	0.11	36.9	2.23	3.72	0.0114	0.005	0.112*		
1	<10	0.83		1.70	1.07	0.0877	0.020	1.160	0.21	13.2
	<200**	0.28	49.1	3.25	5.17	0.0470	0.003	0.051	0.01	1.1

* not significantly > 0.

** Elevated NH_4^+ concentrations occurred in the <10 µm fraction and therefore the difference between the <200 µm and <10 µm fraction was not valid.

The regeneration rate for station 0 was not determined because of poor analytical results.

<10 μm fraction. In the 10-200 μm fraction, the ratio ranges from 1.1 to ∞, with a mean value of 4.8 (excluding ∞ value). There does not appear to be any relationship between PN or NO_3^- concentrations and rates of NH_4^+ regeneration (Table III.2). Regeneration rates between size fractions display no discernible trends. Rates were highest in the 10-200 μm fraction at stations 4 and 11, in the <10 μm fraction at stations 1 and 8, and were equivalent in the two size fractions at stations 2 and 14. Comparison of rates for size fractions, within stations, was hampered by low precision of the rate estimates in some cases (e.g. Sta. 4, 8, and 11).

Ammonium concentrations decreased over the incubation period for stations 0, 6, and 12 (<200 μm fraction only). Comparison of changes in NH_4^+ concentration to ^{15}N-estimated uptake rates showed that incorporation of NH_4^+ into PN averaged 59.5% (n=3) of net NH_4^+ removal from the dissolved pool (Table III.3).

Mass balance calculations indicate that a substantial amount of ^{15}N-NH_4^+, removed from the dissolved pool, was not recovered in the particulate fraction (Table III.4). The ratio of (^{15}N-NH_4^+ removed from the dissolved pool/^{15}N assimilated into PN) ranges from 1.5 to 20.0, with a mean of 6.8. Calculation of missing ^{15}N NH_4^+ as (missing ^{15}N-NH_4^+/total ^{15}N-NH_4^+ added) showed an average of 60% ^{15}N-NH_4^+ missing in the <10 μm fraction and 36% missing in the <200 μm fraction.

Table III.3. Changes in dissolved NH_4^+ over the time course versus NH_4^+ assimilation into the particulate fraction.

Sta. No.	Decrease in NH_4^+ pool over time course (μmol N l^{-1})	Assimilation of NH_4^+ into the particulate fraction (μmol N l^{-1})
0	0.38	0.220
6	0.14	0.092
12	0.08	0.044

Table III.4. Recovery of $^{15}N\text{-}NH_4^+$ in uptake and regeneration experiments.

Sta. No.	Size Frac. (µm)	Time Interval (min)	^{15}N Removed* (diss. pool) (µmol ^{15}N)	SD	^{15}N Uptake (into PN) (µmol ^{15}N)	SD	Missing ^{15}N (µmol ^{15}N)	SD	^{15}N Removed/ ^{15}N Uptake	Missing ^{15}N/ Total ^{15}N (Percent)
LOW NO_3^-										
4	<200	0-120	0.05	0.008	0.014	0.0002	0.04	0.008	3.6	40.0
8	<10	0-60	0.04	0.016	0.017	0.0006	0.03	0.016	ns	ns
	<200	0-120	0.07	0.008	0.021	0.0011	0.05	0.008	3.3	50.0
2	<10	0-60	0.06	0.010	0.028	0.0004	0.03	0.010	2.1	30.0
	<200	0-120	0.09	0.003	0.045	0.0014	0.04	0.003	2.0	40.0
14	<10	0-120	0.00	0.010	0.003	0.0003	0.00	0.010	ns	ns
	<200	0-120	0.04	0.002	0.002	0.0003	0.04	0.002	20.0	40.0
HIGH NO_3^-										
11	<200	0-60	0.05	0.023	0.035	0.0011	0.01	0.023	ns	ns
12	<10	0-60	0.03	0.019	0.008	0.0003	0.02	0.019	ns	ns
1	<10	0-120	0.09	0.002	0.006	0.0003	0.08	0.002	15.0	90.0
	<200	0-60	0.03	0.004	0.020	0.0018	0.01	0.004	1.5	10.0

ns = not significantly >0.

* Significant ^{15}N removal from the dissolved pool did not occur for stations 4 (<10 µm), 6 (<10 and <200 µm), 10 (<10 and <200), 11 (<10), and 12 (<200).

DISCUSSION

The use of isotope dilution techniques to measure rates of NH_4^+ regeneration has greatly increased our understanding of nitrogen dynamics in marine waters. However, the interpretation of results from recent studies may be compromised by methodological problems which have not been critically assessed. Caperon et al. (1979) and Paasche and Kristiansen (1982b) used saturating concentrations of labeled NH_4^+ (e.g. 40 µM), but the effects of this perturbation of natural conditions are not known. Some studies have used incubation periods as long as 24 h (Harrison 1978, Caperon et al. 1979). Long incubations could lead to ^{15}N isotopic equilibration between the NH_4^+ pool and particulate fraction, violating an assumption required for the calculation of regeneration rates. Finally, regeneration measurements have been determined using only two time points, which precludes empirical evaluation of the precision of the rate determinations (Harrison 1978, Glibert 1982).

Improvements, such as shorter incubation times and "trace" enrichments of NH_4^+ were initiated by Glibert (1982). Although these improvements are important, Glibert's NH_4^+ regeneration rate estimates are based only on initial and final determinations, and errors were estimated from assumed analytical standard deviations, rather than empirically derived values. When ambient NH_4^+ is low (at or near the analytical limit of detection) and significant changes

in ambient NH_4^+ do not occur, Glibert (1982) assumed that the regeneration rate, D, was equal to the corrected uptake rate P. In comparing measured uptake and regeneration values, Glibert et al. (1982c) found that the regeneration rate (D) was 80% larger that the uptake rate (P). They attributed the discrepancy between the estimates to analytical uncertainty in determination of initial NH_4^+ concentrations. Glibert et al. (1982c) conclude that, "the best estimate of D in this case is P...because pool size does not enter into its formulation". Methods employed in the present study followed Glibert's recommendations and also included more extensive time course data and an empirical analysis of errors. Ambient NH_4^+ concentrations were well above the limit of detection, and, therefore, measured regeneration rates are reported for all experiments in this study.

Successful measurement of regeneration rates are usually more difficult than uptake rates. In 50% of the regeneration measurements, standard deviations were so large that rates determined were not significantly greater than 0 (Table III.2). Close scrutiny of the present results suggest that findings in earlier studies using only two time points may be compromised by disregarding errors inherent in the techniques used (Fig. III.1). Mass balance calculations were performed to see if the added $^{15}N\text{-}NH_4^+$ could be completely accounted for at the end of incubations. Results show that a large portion (avg. 42.8%) of the original $^{15}N\text{-}NH_4^+$ added was not recovered in the NH_4^+ pool or in the particulate fraction (Table III.4). Laws (1984) performed similiar calculations on data from

Glibert et al. (1982c) and showed that about 40% of the ^{15}N added at the start of the incubations was not recovered. Wheeler (pers. comm.) also found an average of 13.3% ^{15}N missing at the end of experimental incubations in the subarctic Pacific. Closer examination of Glibert et al. (1982c) results revealed that the $^{15}N\text{-}NH_4^+$ removed from the dissolved pool was greater than the ^{15}N recovered in PN by factors ranging from 1.5 to 5.6 (Laws 1984). Similiarly, in this study, the ratio of [$^{15}N\text{-}NH_4^+$ removed from the dissolved pool/^{15}N recovered in PN] ranges from 1.5 to 20.0, averaging 6.8.

Comparison of NH_4^+ regeneration rates and NH_4^+ uptake rates also illustrates an apparent discrepancy. All regeneration rates significantly >0 in this study were from experimental incubations where NH_4^+ concentrations were constant. Since NH_4^+ concentrations did not change over the course of the incubations, it is justified to conclude that regeneration rates are in balance with uptake rates. However, experimental rate measurements suggest that regeneration rates are significantly greater than uptake rates by a factor of 7.9 in the <10 μm fraction and 11.0 in the <200 μm fraction (Table III.5). Thus, the two types of experimental measurements lead to conflicting conclusions. Additional published data (Table III.5) illustrate the generality of the problem. Although NH_4^+ concentrations are relatively constant, experimental measurements of NH_4^+ regeneration and uptake do not demonstrate a close equality (Table III.5).

These results imply that either NH_4^+ regeneration is overestimated and/or NH_4^+ uptake is underestimated by present

Table III.5. Comparison of NH_4^+ regeneration (D) and uptake (P).

Location	Size Frac. (μm)	Incubation Time (h)	Regeneration/ Uptake RANGE	MEAN	n	Reference
Sargasso Sea						
Pierce Main Sta.	<130	2-4	0.0 - 9.8	5.2	11	Glibert (1982)*
Pierce 7, 16, 27	<130	2-4	3.9 - 45.0	13.8	6	"
(2100 hr)	<35	2-4	6.0 - 29.1	11.4	5	"
	<10	2-4	6.8 - 49.2	25.3	4	"
Scotia Sea	<202	4	0.0 - 59.0	14.8	5	"
Vineyard Sound, Mass.	<202	1	0.0 - 12.0	3.0	4	"
	<35	1	11.6 - 43.2	28.6	5	"
	<10	1	0.0 - 14.0	3.5	6	"
Chesapeake Bay	35-202	1	0.9 - 1.2	1.0	2	"
	10-35	1	10.1	10.1	1	"
	1-10	1	0.3	0.3	1	"
Oregon coast: NH5 (8.3 km offshore)	<200	2-4	0.4 - 18.7	9.3	6	Wheeler et al., unpublished data
Oregon-Wash. coast	<10	1-2	0.9 - 13.2	7.9	4	Present study
	<200	1-2	1.1 - 44.1	11.0	5	"

* Values were estimated from figures in Glibert (1982).

isotopic techniques. Results from station 1 suggest that regeneration was overestimated in the <10 μm fraction. It is not clear why the <10 μm regeneration rate was 20 times larger than the <200 μm rate. Regeneration may be enhanced as a result of increased excretion by microzooplankton due to sample handling, passage through Nitex screening (e.g. 10 μm size), and subsequent bottle confinement (Glibert et al. 1982c). However, enhanced regeneration does not explain the discrepancy between measured uptake and regeneration rates.

Further evidence supports the idea that uptake rates are underestimated. Ammonium concentrations decreased during some experiments in the present study. Incorporation of NH_4^+ into PN averaged 59.5% (n = 3) of net NH_4^+ removal from the dissolved pool. This evidence, in conjunction with findings mentioned previously, clearly demonstrates that accumulation of ^{15}N in PN is likely to underestimate actual NH_4^+ uptake rates.

Laws (1984) suggests that possible mechanisms for loss of ^{15}N during incubation experiments include nitrification, adsorption of NH_4^+ to the walls of the polycarbonate containers or to clay particles, and release of ^{15}N as dissolved organic nitrogen (DON). Release of ^{15}N as DON could be ascribed to methodological artifacts as well as genuine biological processes such as phytoplankton excretion and zooplankton grazing (Laws 1984). Goldman and Dennett (1985) find that certain species of marine phytoplankton are susceptible to cell breakage during filtration. Leakage of metabolites from broken cells is a potential source of nutrient uptake

loss from the particulate fraction in short incubations using polycarbonate filters. This source of error would be largest in pelagic environments where small and fragile chrysophytes, cryptophytes, and other microflagellates are frequently important (Goldman and Dennett 1985). However, in the present study the use of glass fiber filters and the avoidance of a rinsing step should have minimized losses due to cell leakage.

On the other hand, the release of DON may be an important naturally occurring biological process. Larsson and Hagström (1979) claim that as much as 45% of primary production can be channeled into bacterial growth after excretion of organic metabolites by phytoplankton. It is likely that some fraction of this material contains organic nitrogen. Furthermore, Ittekkot (1982) found that concentrations of total dissolved amino acids increased in the early stages of a Chaetoceros bloom. Taken together, these results suggest that a portion of the missing ^{15}N could be attributed to uptake of $^{15}N\text{-}NH_4^+$ into the particulate fraction, followed by biological release of the label as ^{15}N-DON during the incubation period.

Concentrations of labile DON generally remain constant in marine waters, presumably as a result of bacterial utilization. DON production rates are unknown. However, if we assume that bacterial production is supported by and equivalent to release of DON, then recent estimates of bacterial production can be used as a rough approximation of DON production. Bacterial production, in terms of nitrogen, ranges from 0.2-178 nmol N l^{-1} h^{-1} (Wheeler and Kirchman

1986). The rate of loss of ^{15}N in this study range from -4.0 to 1,070 nmol N l^{-1} h^{-1}, with a mean of 253. Excluding extreme values from stations 14 (<200 μm) and 1 (<10 μm) gives a mean value of 92 nmol N l^{-1} h^{-1} (SD= 116). This mean value for rate of decrease in ^{15}N-NH_4^+ from the dissolved pool without recovery in the particulate fraction compares favorably with reported rates of bacterial nitrogen production in coastal waters. Thus, ^{15}N-DON release from the particulate fraction could account for the apparent underestimates of NH_4^+ uptake rates. Other processes may also contribute to the apparent loss of ^{15}N in NH_4^+ uptake experiments. For example, Wheeler and Kirchman (1986) have demonstrated that heterotrophic bacteria may be responsible for 78% of total NH_4^+ uptake in a <1 μm fraction sample. Some bacteria pass through the GF/F filters typically used for uptake measurements (Wheeler, per. comm.), and some missing ^{15}N may result from uncaptured labeled bacteria.

The occurrence of a significant fraction of "missing ^{15}N" in uptake experiments has important implications, whether it results from methodological artifacts or natural processes. If most of the ^{15}N loss reflects naturally occurring release, uptake rates for NH_4^+ may be determined best by loss of ^{15}N from the dissolved NH_4^+ pool. Differences between removal of NH_4^+ from the dissolved pool and accumulation in the particulate fraction could be considered that portion of primary production made available for use by bacteria. Future studies should approach the problem systematically, using controlled laboratory experiments to test possible artifacts

(absorption of ^{15}N to wall containers and clay particles), as well as biological processes resulting in the loss of ^{15}N from the particulate fraction.

In this study, results from regeneration measurements were often less precise than uptake measurements. Recent improvements for NH_4^+ isotope analysis might reduce errors associated with regeneration measurements (Fisher and Morrissey 1985, Dudek et al. 1986). Along with improvements in experimental protocol described in this study, measurement of precision could be obtained by running duplicate or triplicate analyses for atom % ^{15}N at each time point. Developing a more accurate and precise NH_4^+ analysis would also improve estimates of uptake and regeneration especially for open ocean waters.

Conclusions

Ambient NH_4^+ concentrations remained relatively constant throughout experimental incubations in this study suggesting that supply rates are in balance with uptake rates. Isotopic determinations however indicate that rates of regeneration usually exceed rates of uptake. Mass balance calculations indicate that 10-90% (mean= 43%) of the ^{15}N label originally added is not recovered as dissolved NH_4^+ plus labeled particulate nitrogen at the end of the incubation. Further progress in understanding the dynamics of N cycling will be dependent on refinements in

determination of NH_4^+ regeneration rates, as well as determination of whether NH_4^+ uptake or regeneration provides the best estimate of NH_4^+ utilization.

Chapter IV

SUMMARY AND CONCLUSIONS

The main objective of this study was to characterize utilization of NO_3^-, NH_4^+, and urea during upwelling off the Washington and Oregon coasts. Some water samples were separated into <10 um and 10-200 μm size fractions to compare use of NH_4^+ and urea by nano- and netplankton. Ammonium regeneration was also measured for <10 μm and 10-200 μm size fractions.

In general, utilization of new and regenerated nitrogen followed a consistent pattern. In low NO_3^- waters (<5 μM), regenerated nutrients (NH_4^+ and urea) were the main nitrogen sources. Nitrogen uptake rates and biomass were low, and nanoplankton were dominant. Conversely, in regions of upwelled water (NO_3^- >20 μM), new nitrogen (NO_3^-) was the main nitrogen source. Nitrogen uptake rates and biomass were highest in upwelled waters, and phytoplankton communities were dominated by large chain-forming diatoms (netplankton).

High and low NO_3^- stations were similiar in some respects. For instance, NH_4^+ and urea uptake, in each size fraction, was found to be proportional to chl-a concentrations. Also, turnover times for ambient NH_4^+ and urea pools were very short in low, as well as high NO_3^- waters. Ammonium turnover times were on average four times shorter than urea turnover times. The relative preference

index showed NH_4^+ being preferred over urea in all cases. Increasing NH_4^+ concentrations, from 0.06 to 1.23 μM, appeared to inbibit urea uptake.

While NH_4^+ is generally the form of nitrogen preferred by phytoplankton, results from high NO_3^- stations demonstrate the quantitative importance of NO_3^- to phytoplankton growth. Nitrate provided 83% of total nitrogen uptake in high NO_3^- waters. During June-August 1985 Wheeler et al. (unpub.) found that NO_3^- provided only 58% of total nitrogen uptake in similiar, high NO_3^- waters. It is difficult at this time to provide generalizations about nitrogen use in upwelling waters off the coasts of Washington and Oregon. Results from this study demonstrate the importance of NO_3^- in supporting high nitrogen uptake rates in upwelled waters. However, the dominance of new production can change over a small spatial scale.

Ammonium isotope dilution measurements are necessary to correct for changing atom % ^{15}N of the dissolved NH_4^+ pool in tracer uptake experiments. Corrected uptake rates were on average 1.4 times higher than uncorrected rates.

Ammonium concentrations remained relatively constant throughout incubations. This occurrence clearly suggests that NH_4^+ supply rates are in balance with uptake rates by plankton. However, results demonstrate that NH_4^+ uptake rates, measured by increases of ^{15}N in particulate material, were consistently lower than regeneration rates. Mass balance calculations show that a greater amount of ^{15}N NH_4^+ left the dissolved pool than appeared in the particulate

fraction. This implies that some unmeasured process is also occurring. Biological release of ^{15}N-labeled DON is a possible cause for the imbalance between measured NH_4^+ uptake and regeneration.

Future efforts should be focused on identifying the mechanisms responsible for the ^{15}N missing in NH_4^+ uptake experiments. In addition, refinements in measuring low concentrations of NH_4^+ would improve both uptake and regeneration rate determinations. Critical evaluation of estimates of NH_4^+ regeneration will also require greater attention to error analysis than has been evident in the past. Ultimately, progress in understanding the dynamics of N cycling will be dependent on our ability to follow movement of all ^{15}N during uptake and regeneration experiments.

BIBLIOGRAPHY

Avilova, S. D. (1983). Urea in the waters of the northwestern Indian Ocean. Oceanology 23: 439-443.

Axler, R. P., Redfield, G. W., and Goldman, C. R. (1981). The importance of regenerated nitrogen to phytoplankton productivity in a subalpine lake. Ecology 62: 345-354.

Banse, K. (1977). Determining the carbon-to-chlorophyll ratio of natural phytoplankton. Mar. Biol. 41: 199-212.

Bevington, P. R. (1969). Data reduction and error analysis for the physical sciences. McGraw-Hill, Inc., p. 336.

Blackburn, T. H. (1979). Method for measuring rates of NH4 turnover in anoxic marine sediments, using a 15N-NH4 dilution technique. Appl. Environ. Microbiol. 37: 760-765.

Billen, G. (1984). Heterotrophic utilization and regeneration of nitrogen. In: Hobbie, J. E., Williams, P. J. leB. (ed.) Heterotrophic activity in the sea. Plenum Press, p. 313-355.

Bremner, J. M., and Edwards, A. P. (1965). Determination and isotope-ratio analysis of different forms of nitrogen in soils: I. Apparatus and procedure for distillation and determination of ammonium. Soil Sci. Soc. Proc. 29: 504-507.

Bremner, J. M., and Keeney, D. R. (1965). Steam distillation methods for determination of ammonium, nitrate, and nitrite. Anal. Chim. Acta 32: 485-495.

Bremner, J. M., and Keeney, D. R. (1966). Determination and isotope-ratio analysis of different forms of nitrogen in soils: 3. Exchangeable ammonium, nitrate, and nitrite by extraction distillation methods. Soil Sci. Soc. Amer. Proc. 30: 577-582.

Caperon, J., Schell, D., Hirota, J., and Laws, E. (1979). Ammonium excretion rates in Kanoehe Bay, Hawaii, measured by a 15N isotope dilution technique. Mar. Biol. 54: 33-40.

Corner, E. D. S., and Newell, B. S. (1967). On the nutrition and metabolism of zooplankton. IV. The forms of nitrogen excreted by Calanus. J. Mar. Biol. Ass. U. K. 47: 113-120.

Dudek, N., Brzezinski, M. A., and Wheeler, P. A. (1986). Recovery of ammonium nitrogen by solvent extraction for the determination of relative ^{15}N abundance in regeneration experiments. Mar. Chem. 18: 59-69.

Dugdale, R. C., and Goering, J. J. (1967). Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. 12: 196-206.

Eppley, R. W. (1981). Autotrophic production of particulate matter. In: Longhurst, A. R. (ed.) Analysis of marine ecosystems. Academic Press, London, p. 343-361.

Eppley, R. W., and Peterson, B. J. (1979). Particulate organic matter flux and planktonic new production in the deep ocean. Nature, Lond. 279: 210-215.

Eppley, R. W., Renger, E. H., and Harrison, W. G. (1979). Nitrate and phytoplankton production in southern California coastal waters. Limnol. Oceanogr. 24:483-494.

Eppley, R. W., Renger, E. H., Venrick, E. L., and Mullin, M. M. (1973). A study of plankton dynamics and nutrient cycling in the central gyre of the north Pacific Ocean. Limnol. Oceanogr. 18: 534-551.

Eppley, R. W., Sapienza, C., and Renger, E. H. (1978). Gradients in phytoplankton stocks and nutrients off southern California in 1974-76. Estuar. Coast. Mar. Sci. 7: 291-301.

Eppley, R. W., Sharp, J. H., Renger, E. H., Perry, M. J., and Harrison, W. G. (1977). Nitrogen assimilation by phytoplankton and other microorganisms in the surface waters of the central north Pacific Ocean. Mar. Biol. 39: 111-120.

Fenchel, T., and Harrison, P. (1976). The significance of bacterial grazing and mineral cycling for the decomposition of particulate detritus. In: Anderson, J. M., Macfadyen (ed.) The role of terrestrial and aquatic organisms in decomposition processes. Blackwell Scientific Publications, Lond., p. 285-299.

Fenchel, T. M., and Jørgensen, B. B. (1977). Detritus food chains of aquatic ecosystems: the role of bacteria. In: Alexander, M. (ed.) Advances in microbial ecology, Vol. 1, p. 1-58.

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

Fisher, T. R., and Morrissey, K. M. (1985). A new method for the recovery of ammonium from natural waters for measurement of ^{15}N composition in isotope dilution experiments. Mar. Chem. 16: 11-21.

Furnas, M. J. (1983). Nitrogen dynamics in lower-Narragansett Bay, Rhode Island. I. Uptake by size-fractionated phytoplankton populations. J. Plankton Res. 5: 657-675.

Glibert, P. M. (1981). Uptake and remineralization of ammonium by marine plankton. Ph.D. thesis. Harvard University, Cambridge, Mass. p. 216.

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

Glibert, P. M., Biggs, D. C., and McCarthy, J. J. (1982a). Utilization of ammonium and nitrate during austral summer in the Scotia Sea. Deep-Sea Res. 29: 837-850.

Glibert, P. M., Goldman, J. C., and Carpenter, E. J. (1982b). Seasonal variation in the utilization of ammonium and nitrate by phytoplankton in the Vineyard Sound, Massachusetts, USA. Mar. Biol. 70: 237-249.

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

Glibert, P. M., Lipschultz, F., McCarthy, J. J., and Altabet, M. A. (1985). Has the mystery of the vanishing ^{15}N in isotope dilution experiments been resolved? Limnol. Oceanogr. 30: 444-447.

Gieskes, W. G. C., Kraay, G. W., and Baars, M. A. (1979). Current ^{14}C methods for measuring primary production: gross underestimates in oceanic waters. Neth. J. Sea Res. 13: 58-78.

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

Goldman, J. C., and Dennett, M. R. (1985). Susceptibility of some marine phytoplankton species to cell breakage during filtration and post-filtration rinsing. J. Exp. Mar. Biol. Ecol. 86: 47-58.

Harris, E. (1959). The nitrogen cycle in Long Island Sound. Bull. Bingham Oceanogr. 17: 31-65.

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

Harrison, W. G. (1980). Nutrient regeneration and primary production in the sea. In: Falkowski, P. G. (ed.) Primary productivity in the sea. Plenum Press, New York, pl 433-460.

Harrison, W. G. (1983). Use of isotopes. In: Carpenter, E. J., Capone, D. G. (ed.) Nitrogen in the marine environment. Academic Press, New York, p. 763-807.

Harrison, W. G., Douglas, D., Falkowski, P., Rowe, O., and Vidal, J. (1983). Summer nutrient dynamics of the Middle Atlantic Bight: nitrogen uptake and regeneration. J. Plankton Res. 5: 539-556.

Harrison, W. G., and Harris, L. R. (1986). Isotope-dilution and its effects on measurements of nitrogen and phosphorus uptake by oceanic microplankton. Mar. Ecol. Prog. Ser. 27: 253-261.

Harrison, W. G., Head, E. J. H., Conover, R. J., Longhurst, A. R., and Sameoto, D. D. (1985). The distribution and metabolism of urea in the eastern Canadian Arctic. Deep-Sea Res. 32: 23-42.

Herbland, A. (1976). In situ utilization of urea in the euphotic zone of the tropical Atlantic. J. Exp. Mar. Biol. Ecol. 21: 269-277.

Herbland, A., LeBouteiller, A., and Raimbault, P. (1985). Size structure of phytoplankton biomass in the equatorial Atlantic Ocean. Deep-Sea Res. 32: 849-836.

Ittekkot, V. (1982). Variations of dissolved organic matter during a plankton bloom: Qualitative aspects, based on sugar and amino acid analyses. Mar. Chem. 11: 143-158.

Johannes, R. E. (1965). Influence of marine protozoa on regeneration. Limnol. Oceanogr. 10: 434-442.

Joint, I. R., and Pomeroy, A. J. (1983). Production of picoplankton and small nanoplankton in the Celtic Sea. Mar. Biol. 77: 19-27.

Kristiansen, S. (1983). Urea as a nitrogen source for the phytoplankton in the Oslofjord. Mar. Biol. 74: 17-24.

LaRoche, J. (1983). Ammonium regeneration: its contribution to phytoplankton nitrogen requirements in a eutrophic environment. Mar. Biol. 75: 231-240.

Larsson, U., and Hagström, Å. (1979). Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. Mar. Biol. 52: 199-206.

Laws, E. (1984). Isotope dilution models and the mystery of the vanishing ^{15}N. Limnol. Oceanogr. 29: 379-386.

Laws, E. A., Harrison, W. G., and DiTullio, G. R. (1985). A comparison of nitrogen assimilation rates based on ^{15}N uptake and autotrophic protein synthesis. Deep-Sea Res. 32: 85-95.

Li, W. K. W., Subba Rao, D. W., Harrison, W. G., Smith, J. C., Cullen, J. J., Irwin, B., and Platt, T. (1983). Autotrophic picoplankton in the tropical ocean. Science 219: 292-295.

MacIsaac, J. J., and Dugdale, R. C. (1972). Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. Deep-Sea Res. 19: 209-232.

MacIsaac, J. J., Dugdale, R. C., Barber, R. T., Blasco, D., and Packard, T. T. (1985). Primary production cycle in an upwelling center. Deep-Sea Res. 32: 503-529.

Maita, Y., Matsunaga, K., and Nishimura, M. (1973). Estimation of urea production from amino acid in the ocean by marine bacteria. Bull. Fac. Fish. Hokkaido Univ. 23: 185-190.

Malone, T. C. (1980). Algal size. In: Morris, I. (ed.) The physiological ecology of phytoplankton. University of California Press, p. 433-463.

McCarthy, J. J. (1970). A urease method for urea in seawater. Limnol. Oceanogr. 15: 309-313.

McCarthy, J. J. (1980). Nitrogen. In: Morris, I. (ed.) The physiological ecology of phytoplankton. University of California Press, p. 191-233.

McCarthy, J. J., and Kamykowski, D. (1972). Urea and other nitrogenous nutrients in La Jolla Bay during February, March, and April 1970. Fish. Bull. 70: 1261-1274.

McCarthy, J. J., Taylor, W. R., and Taft, J. L. (1975). The dynamics of nitrogen and phosphorus cycling in the open waters of the Chesapeake Bay. In: Church, T. M. (ed.) Marine chemistry in the coastal environment. Am. Chem. Soc., p. 664-681.

McCarthy, J. J., Taylor, W. R., and Taft, J. L. (1977). Nitrogenous nutrition of the plankton in the Chesapeake Bay. I. Nutrient availability and phytoplankton preferences. Limnol. Oceanogr. 22: 996-1011.

McCarthy, J. J., and Whitledge, T. E. (1972). Nitrogen excretion by anchovy (Engraulis mordax and E. ringens) and jack mackeral (Trachurus symmetricus). Fish. Bull. 70: 395-401.

Mitamura, O., and Saijo, Y. (1980). In situ measurement of the urea decomposition rate and its turnover rate in the Pacific Ocean. Mar. Biol. 58: 147-152.

Nalewajko, C., and Lean, D. R. S. (1980). Phosphorus. In: Morris, I. (ed.) The physiological ecology of phytoplankton. University of California Press, p. 235- 258.

Olson, R. J. (1980). Nitrate and ammonium uptake in Antarctic waters. Limnol. Oceanogr. 25: 1064-1074.

Paasche, E., and Kristiansen, S. (1982a). Nitrogen nutrition of the phytoplankton in the Oslofjord. Estuar. Coast. Shelf Sci. 14: 237-249.

Paasche, E., and Kristiansen, S. (1982b). Ammonium regeneration by microzooplankton in the Oslofjord. Mar. Biol. 69: 55-63.

Parry, G. (1960). Excretion. In: Waterman, T. H. (ed.) The physiology of Crustacea. Vol. I, Academic Press, N.Y., p. 341-366.

Price, N. M., Cochlan, W. P., and Harrison, P. J. (1985). Time course of uptake of inorganic and organic nitrogen by phytoplankton in the Strait of Georgia: comparison of frontal and stratified communities. Mar. Ecol. Prog. Ser. 27: 39-53.

Probyn, T. A. (1985). Nitrogen uptake by size-fractionated phytoplankton populations in the southern Benguela upwelling system. Mar. Ecol. Prog. Ser. 22: 249-258.

Probyn, T. A., and Painting, S. J. (1985). Nitrogen uptake by size-fractionated phytoplankton populations in Antarctic surface waters. Limnol. Oceanogr. 30: 1327-1331.

Remsen, C. C. (1971). The distribution of urea in coastal and oceanic waters. Limnol. Oceanogr. 16: 732-740.

Ryther, J. H. (1959). Potential productivity of the sea. Science 130: 602-608.

Small, L. F., and Menzies, D. W. (1981). Patterns of primary productivity and biomass in a coastal upwelling region. Deep-Sea Res. 28A: 123-149.

Smith, S. L. (1978a). Nutrient regeneration by zooplankton during a red tide off Peru, with notes on biomass and species composition of zooplankton. Mar. Biol. 49: 125-132.

Smith, S. L. (1978b). The role of zooplankton in the nitrogen dynamics of a shallow estuary. Estuar. Coast. Mar. Sci. 7: 555-565.

Strickland, J. D. H., and Parsons, T. R. (1972). A practical handbook of seawater analysis. (2nd ed.) Bull. Fish. Res. Bd. Can. 167: 1-311.

Syrett, P. J. (1981). Nitrogen metabolism of microalgae. In: Platt, T. (ed.) Physiological bases of phytoplankton ecology. Can. Bull. Fish. Aquat. Sci. 210: 182-210.

Wheeler, P. A. (unpub. ms.). Nitrogen utilization in the subarctic Pacific during the spring and summer of 1984.

Wheeler, P. A., and Kirchman, D. L. (1986). Utilization of inorganic and organic forms of nitrogen by bacteria in marine systems. Limnol. Oceanogr.

Wheeler, P. A., Kokkinakis, S. A., and O'Brien, M. M. (unpub. ms.). A seasonal study of nitrate uptake and ammonium uptake and regeneration off the coast of Oregon.

Whitledge, T. (1978). Regeneration of nitrogen by zooplankton and fish in the northwest Africa and the Peru upwelling ecosystems. In: Boje, R., Tonezak, M. (ed.) Upwelling ecosystems. Berlin: Springer-Verlag, p. 90-100.

Whitledge, T. E. (1981). Nitrogen recycling and biological populations in upwelling ecosystems. In: Richards, F. A. (ed.) Coastal upwelling. Washington, D. C.: American Geographical Union, p. 257-273.

Yoder, J. A., Atkinson, L. P., Bishop, S. S., Hofmann, E. E., and Lee, T. N. (1983). Effect of upwelling on phytoplankton productivity of the outer southeastern United States continental shelf. Cont. Shelf Res. 1: 385-404.