NITROGEN UPTAKE BY MARINE PHYTOPLANKTON: THE EFFECTS OF IRRADIANCE, NITROGEN SUPPLY AND DIEL PERIODICITY

by

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ABSTRACT

Diel patterns of nitrogen (NO_3^-, NH_4^+, urea) uptake were investigated in natural assemblages of phytoplankton from neritic and oceanic environments off the coast of British Columbia. This is the first study to report nitrogen uptake rates and extensive measurements of ambient NH_4^+ and urea concentrations in these waters. Calculated rates of N uptake, based on ^{15}N incorporation into particulate matter during time course experiments, were maximal during the day and minimal at night. Besides the obvious effects of irradiance, the amplitude of the periodicity in uptake rate was influenced by phytoplankton community composition, ambient nitrogen concentration, forms of nitrogen available, and depth of sampling. Uptake of nitrogen during the night and in artificial darkness were measurable proportions of daytime and light uptake rates, with the importance of dark uptake generally increasing with increasing N limitation. This is the first study of diel urea uptake by marine phytoplankton in the field. The ratios of dark to light urea uptake over a diel cycle were more similar to those of NO_3^- than those of the other reduced N form, NH_4^+.

Rates of NO_3^- and urea uptake by phytoplankton in the shallow and deep chlorophyll layers of the Strait of Georgia were measured over a gradient of irradiances and results of these experiments could be fitted with a hyperbolic function similar to the Michaelis-Menten equation. Half-saturation constants (K_{LT}) for light-dependent uptake of urea and NO_3^-

ranged from 0 to 14% of the surface irradiance and dark uptake was a variable, but often substantial (> 50%) portion of the total (light + dark) uptake.

The uptake response of nitrate-replete and -starved populations of the picoflagellate, *Micromonas pusilla* (Butch.) Manton et Parke, to urea, NH_4^+ and NO_3^- perturbations was determined by both ^{15}N accumulation and nutrient disappearance from the culture medium. Maximum specific uptake rates (V_{max}) of NH_4^+ were 0.13 h^{-1}, more than 2 times the V_{max} of NO_3^- or urea (ca. 0.05 h^{-1}). The half-saturation constants (K_s) for urea, NH_4^+ and NO_3^- were within ± 0.1 μg-at $N \cdot L^{-1}$ of each other; the average value of 0.41 μg-at $N \cdot L^{-1}$ is within the range reported for small, oceanic diatoms. NO_3^- uptake was completely inhibited following NH_4^+ addition (1-10 μg-at $N \cdot L^{-1}$), whereas urea addition resulted in only a 28% reduction in NO_3^- uptake. Starved cultures of *M. pusilla* exhibited variable uptake of NH_4^+ and urea as a function of time, with an initial "surge" uptake response. This is the first laboratory study of N uptake by an eucaroyotic picoplankter and demonstrates that many of the transient uptake responses reported for diatoms, with which it competes in the field, are common to this picoplankter.

Diel periodicity of nitrogen uptake and assimilation were measured in N-replete batch cultures of *M. pusilla* and also in N-limited cyclostat cultures (14L:10D) at three growth rates corresponding to ca. 75, 50 and 25% of it's maximal growth rate. Nitrate uptake was continuous and independent of the

L:D cycle in the cyclostat cultures at the lowest dilution rate, but NO_3^- uptake rates exhibited pronounced periodicity in the batch and higher dilution rate cultures, a response similar to that seen in previous studies of cyclostat cultures of some diatoms. Diel patterns in cell division, mean cell volume, potential uptake rates and internal pools of NO_3^- were also observed and are discussed with respect to the nutritional status of the cells. The effect of irradiance on the uptake of NH_4^+ and NO_3^- by *M. pusilla* was also described by Michaelis-Menten kinetics; with increasing N limitation the importance of light for nitrogen uptake decreased and dark uptake increased from 5-20% to 21-39% of NO_3^- and NH_4^+ uptake rates, respectively, at saturating irradiance.

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INTRODUCTION

Overview

The growth of marine phytoplankton is directly dependent on their ability to assimilate nutrients and photosynthesize in an environment in which nutrients and/or light are not necessarily optimal for growth. Of the three major inorganic nutrients utilized by marine phytoplankton (nitrogen, phosphorus, silicon) nitrogen is the nutrient whose supply is most likely to limit phytoplankton growth in both coastal (e.g., Ryther and Dunstan, 1971) and oceanic waters (e.g., Thomas, 1966, 1969; Goldman et al., 1979). Phosphorus, although often found in low concentrations, is recycled rapidly and therefore it is less likely to limit marine productivity (Perry and Eppley, 1981; Smith, 1984). Silicon is generally recycled slowly (e.g., Nelson and Goering, 1977), however, it is not required by all marine phytoplankton. When it is limiting, the growth of diatoms is reduced and therefore the major effect is on species composition (Walsh, 1981).

Of the nitrogen (N) sources in the sea that are well-characterized (i.e. not including dissolved organic nitrogen, DON), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and urea ($CO(NH_2)_2$) are the most abundant and, consequently, most relevant research has concentrated on their utilization by phytoplankton. In 1967, Dugdale and Goering introduced a conceptual model that distinguished the relative importance of the various sources of dissolved inorganic nitrogen for phytoplankton growth. Primary production supported by

allochthonous N sources, principally nitrate mixed into surface waters from deep ocean reserves, and secondarily, N_2-fixation, riverine inflow and precipitation, was termed "new production"; production resulting from autochthonous N sources, such as urea and ammonium from animal excretion and microbial remineralization, was termed "regenerated" production. Numerous field and laboratory studies have documented the preference of phytoplankton for the more reduced forms of N (ammonium and urea) relative to the more oxidized forms (nitrate and nitrite) (e.g., Dugdale and Goering, 1967; Eppley et al., 1973; McCarthy et al., 1977, 1982) presumably because reduced forms require less energy to assimilate. Nevertheless the oxidized forms, particularly NO_3^-, may be quantitatively important N sources for phytoplankton, (e.g., Carpenter and Dunham, 1985; Probyn, 1985; Cochlan, 1986; Platt et al., 1989).

In the surface waters of central oceanic gyres, nitrogen concentrations are consistently near or below current limits of detection (generally NO_3^-: 50 ng-at $N \cdot L^{-1}$; NH_4^+: 30 ng-at $N \cdot L^{-1}$; urea: 50 ng-at $N \cdot L^{-1}$) (McCarthy, 1980; Raymont, 1980; Garside, 1985; Price and Harrison, 1987) and show no apparent seasonal pattern (McCarthy, 1980; Sharp, 1983). Concentrations are invariably higher at depth, particularly for NO_3^-. Nitrate, the most abundant form of nitrogen (except for N_2)in coastal regions (Sharp, 1983), exhibits distinctive seasonal trends and is the most important N source in highly productive areas of the world's ocean (e.g., Eppley and

Peterson, 1979; Eppley, 1981; Harrison et al., 1987). Surface nitrate concentrations of coastal waters are usually elevated during the winter (> 20 μg-at $N \cdot L^{-1}$) and, following stratification during the spring and subsequent utilization by phytoplankton, are gradually decreased to detection limits. At this point phytoplankton growth is supported primarily by regenerated N, as in oligotrophic, oceanic gyres,(e.g., McCarthy et al., 1977; Glibert et al., 1982b; Cochlan, 1986). Nitrogen uptake and regeneration are envisaged to be tightly coupled (Goldman, 1984) and the oxidized N forms are of little or no importance. Forms of regenerated N in coastal regions vary in concentration but generally do not exceed 5 μg-at $N \cdot L^{-1}$ and are frequently below 0.5 μg-at $N \cdot L^{-1}$ (McCarthy, 1980; Sharp, 1983; Antia et al., in press).

The utilization of nitrogen by phytoplankton can be divided into two steps; the first step, termed "uptake", describes the actual transport of the particular form of N across the cell's plasmalemma, and the second step, termed "assimilation", refers to the sequence of metabolic events within the cell in which the inorganic N ions are reduced to NH_4^+ (in the case of NO_3^- and NO_2^-) and incorporated primarily into amino acids and proteins (Wheeler, 1983; Lobban et al., 1985). The utilization of nitrogenous nutrients by marine phytoplankton is influenced by a number of factors, including the ambient N concentration, the relative abundance of different N forms, the physiological status of the phytoplankton, the availability of light and temperature.

Uptake interactions between inorganic N forms have been the subject of many culture and field studies (reviews by McCarthy, 1981; Syrett, 1981; Ullrich, 1987) which reveal a range of responses which vary with the phytoplankton species and its nutritional state. Nitrate uptake has been reported to be inhibited to different degrees by NH_4^+ ranging from total suppression (e.g., Syrett and Morris, 1963; McCarthy and Eppley, 1972) to simultaneous and comparable rates of NO_3^- and NH_4^+ uptake (e.g., Conway, 1977; Maestrini et al., 1982, 1986). Interactions between urea and other N forms have attracted less attention partly because only recently has the significance of urea as a source of N for the growth of natural phytoplankton assemblages been generally acknowledged (e.g., McCarthy, 1972,; Kaufman et al., 1983; Kristiansen, 1983; Harrison et al., 1985; Turley, 1985, 1986). Generally, urea suppresses the uptake of NO_3^-, but at a lower level than NH_4^+ (e.g., Grant et al., 1967; Molloy and Syrett, 1988b).

The effect of cellular physiological state on N uptake by phytoplankton was first demonstrated by Syrett (1953) and Harvey (1953) who showed that NH_4^+ and NO_3^- uptake by batch cultures of "N-starved" cells was much more rapid than by "normal" cells that were N-replete. More recent studies have also shown that N-starved or N-deficient phytoplankton have the ability to rapidly take up NH_4^+ upon exposure to an elevated concentration (e.g., Conway et al., 1976; Conway and Harrison, 1977; Glibert and Goldman, 1982). In contrast, after a NO_3^- or urea addition to N-deplete cells enhanced

uptake may or may not occur (e.g., review by Collos, 1983; Price and Harrison, 1988b).

The idea that the rate of steady-state N uptake by marine phytoplankton can be described as a hyperbolic function of the concentration of the limiting nutrient, similar in form to the Michaelis-Menten equation for enzyme kinetics (Dugdale 1967, Eppley and Coatsworth, 1968) has led to numerous studies which have determined kinetic parameters for both laboratory and natural assemblages of phytoplankton (e.g., reviews by McCarthy, 1981; Goldman and Glibert, 1983). It appears that the half-saturation concentrations for uptake are in the range of 10^{-6} to 10^{-7} M for marine phytoplankton and that species (Eppley et al., 1969; MacIsaac and Dugdale, 1969) and clones (Carpenter and Guillard, 1971) which are commonly found in eutrophic regions show consistently higher half-saturation constants than species or clones isolated from oligotrophic waters. Under N-limiting conditions, species with low uptake (half-saturation) constants hold a competitive advantage over species having higher constants. This has been used to explain species distribution in relation to N availability (Eppley et al., 1969; MacIsaac and Dugdale, 1969).

Light intensity and quality exhibit a wide range of temporal and spatial variation. Seasonal, latitudinal and particularly diel (day-night) variations in overall intensity are very pronounced. Light attenuates exponentially with depth, limiting phytoplankton growth to the surface waters (euphotic zone) of the ocean. Many studies have shown that N

uptake is related to light intensity in a hyperbolic fashion, saturation occurring at high light intensities (e.g., Hattori, 1962; Grant, 1967; Eppley and Rogers, 1970). In natural phytoplankton communities the dependence of N uptake upon light intensity has been described by a rectangular hyperbola of the Michaelis-Menten formulation (e.g., MacIsaac and Dugdale, 1972; Fisher et al., 1982). Since neither membrane transport nor reduction of oxidized N forms requires light per se, the dependence of N uptake on light is likely an indirect one (Syrett, 1981). Production of cofactors for NO_3^- and NO_2^- reduction and ATP for membrane transport (Falkowski, 1975a) and the assimilation of NH_4^+ and urea depend on photosynthesis (Syrett, 1981) which may account for the observations of light dependence of N uptake.

In most environments, light intensity exhibits extreme variation, in a periodic fashion every day. Diel physiological rhythms, coupled to fluctuations in light intensity, have been detected in cultures and natural phytoplankton assemblages and include numerous processes (review by Sournia, 1974), most notably photosynthesis (e.g., Doty and Oguri, 1957; MacCaull and Platt, 1977). The term "diel", used almost exclusively by oceanographers, will be used throughout this dissertation to describe any rhythm, whose period is about 24 h, observed in natural conditions. Circadian has the same meaning as diel, but is used to describe such rhythms which persist under constant environmental conditions (i.e. endogenous control). The term

"diurnal", being the opposite of "nocturnal" cannot apply to a 24 h cycle, but only to an event which occurs between sunrise and sunset (Sournia, 1974) and will be used to describe rhythms during the daylight hours. Evidence of the periodicity of N uptake has been reported in cyclostat cultures (e.g., Eppley et al., 1971b; Malone et al., 1975) and natural phytoplankton communities (e.g., Eppley et al., 1970 , 1971a, MacIsaac, 1978; Fisher et al., 1982). Maximum uptake occurs during the day and minimum uptake at night. In an early study, Goering et al., (1964) observed uptake periodicity of NO_3^- and NH_4^+ by Sargasso Sea phytoplankton incubated under constant illumination which suggests that the rhythm was circadian. A dampened amplitude in the diel periodicity of nitrate uptake has been observed in culture and field studies with increased N limitation (e.g., Malone et al., 1975) or N starvation (e.g., Harrison, 1976; Dortch and Maske, 1982).

Objectives

The main objectives of this thesis were as follows:

(1) To determine the nitrogen dynamics (NO_3^-, NH_4^+, and urea) of natural phytoplankton assemblages from coastal and oceanic waters off the coast of British Columbia.

(2) To determine in laboratory studies the nitrogen (NO_3^-, NH_4^+, and urea) uptake kinetics of an ecologically important eucaryotic picoplankter *Micromonas pusilla*, isolated from British Columbian coastal waters.

(3) To determine interactions between nitrogen uptake and

light through diel periodicity and irradiance experiments in the field and the laboratory.

Thesis outline

The underlying premise of this study is that nitrogen uptake and assimilation are a function of both the external environment and the physiological state of the phytoplankter as a result of previous interaction with the environment. The first half of this dissertation examines nitrogen uptake by natural phytoplankton assemblages as a function of light in neritic and oceanic environments. In Chapter 1, time course experiments of nitrogen uptake are described for a variety of phytoplankton communities. These experiments were designed to discern the effect(s) of ambient nitrogen concentrations on nitrogen uptake over day/night cycles. These experiments were conducted in association with phytoplankton nitrogenous nutrition studies and confirm the existence of diel rhythms and the necessity of time course experiments to accurately estimate daily rates of uptake from hourly incubations or vice versa. In Chapter 2 the effects of irradiance, during the daytime, on the uptake of nitrogen by phytoplankton from nitrate-replete frontal and nitrate-deplete stratified, coastal waters are described. These results provide unique information regarding uptake of oxidized and reduced N forms as a function of irradiance in two highly contrasting environments.

Recent work showing the ubiquity of picoplankton (0.2 - < 2.0 μm, Sieburth et al., 1978) (e.g., Gieskes et al.,

1979; Waterbury et al., 1979; Johnson and Sieburth, 1982; Li et al., 1983; Platt et al., 1983) and their importance as photoautotrophs, particularly in oligotrophic, oceanic regions (e.g., Li et al., 1983; Takahashi and Bienfang, 1983; reviews by Joint, 1986; Stockner and Antia, 1986) prompted my choice of a picoplankter to examine the effects of light on nitrogen utilization under controlled laboratory conditions.

In Chapter 3, experiments were conducted with batch cultures of the eucaryotic, picoplankter *Micromonas pusilla*, and designed to examine nitrogen uptake kinetics, the effects of urea and NH_4^+ on NO_3^- uptake, and the transient uptake response(s) to NO_3^- starvation. Requisite information was obtained for subsequent diel studies. This is the first study of picoplankton nitrogenous nutrition besides those employing ^{15}N tracers during size-fractionation of natural communities (e.g., Probyn, 1985; Harrison and Wood, 1988)

Continuous cultures of *M. pusilla* grown on a 14:10 light:dark cycle (i.e. cyclostat) were used in the experiments described in Chapter 4. These experiments were designed to examine the effect of NO_3^- limitation on diel periodicity of N utilization. In situ NO_3^- uptake rates, potential rates of oxidized and reduced N forms during the day/night cycle, and N uptake as a function of irradiance were determined.

Experimental organism

Micromonas pusilla (Butcher) Manton et Parke (1960) (basionym: *Chromulina pusilla*, Butcher, 1952) is a minute (length 1 to 2 μm, width 0.75 to 1 μm) naked, unicellular,

photosynthetic flagellate. *Micromonas pusilla* is usually considered to be an anomalous member of the Prasinophyceae (Manton, 1959; Manton and Parke, 1960) and lacks a cell wall. *Micromonas pusilla* is ubiquitous, occurring in coastal and oceanic samples of tropical, temperate and arctic waters and often achieving numerical dominance (e.g., Throndsen, 1976; Johnson and Sieburth, 1982; Taylor and Waters, 1982; Hallegraeff, 1983; Estep et al., 1984; Hallegraeff and Jeffrey, 1984). It has been reported deeper in the water column than flagellates in general, often well below the euphotic zone (e.g., Manton and Parke, 1960; Throndsen, 1976). *Micromonas pusilla* is frequently encountered in British Columbian coastal waters (e.g. $> 2.5 \cdot 10^7$ cells$\cdot L^{-1}$ in Jervis Inlet, July 1977 Taylor and Waters, 1982; $0.1 - 2.2 \cdot 10^7$ cells$\cdot L^{-1}$ in Fraser River plume, July 1987, Clifford et al., 1989); similar concentrations are often reported in Norwegian waters (e.g., Throndson, 1976 and references therein). Its tolerance of a wide range of temperature and salinity (Throndsen, 1976) or the ability to form strains adapted to different environmental regimes, may contribute to the success of this picoplankter in the world's oceans. The ubiquitous nature and numerical importance of *M. pusilla* enhances the potential for ecologically relevant extrapolation of my laboratory data to natural field populations.

The culture used herein (NEPCC 29-1, Northeast Pacific Culture Collection, Dept. of Oceanography, University of British Columbia) was isolated from English Bay, B.C. by R.

Waters in January, 1971 and subsequently maintained in enriched, natural seawater at 16°C on a 14:10 L:D cycle.

CHAPTER ONE

EFFECTS OF DIEL PERIODICITY ON NITROGEN UPTAKE BY NATURAL ASSEMBLAGES OF PHYTOPLANKTON

INTRODUCTION

Day-night (diel) cycles of biological and related parameters in the ocean are often the manifestation of the effects of sunlight on biological processes. Periodicity in both photosynthetic capacity and in situ photosynthesis is the most obvious diel cycle (e.g., Sournia, 1974) and both diel (and diurnal) rhythms have been reported for many years (e.g., Doty and Oguri, 1957; Verduin, 1957). Diel fluctuations in dissolved inorganic nutrient concentration have been reported in natural populations (e.g., Lorenzen, 1963; Beers and Kelly, 1965) with a concentration decline generally ascribed to the assimilatory activities of phytoplankton (and bacteria) and regenerative increases from zooplankton and heterotrophic remineralization. Goering et al. (1964) first demonstrated marked diel cycles in the potential uptake of NO_3^- and NH_4^+ by phytoplankton in the N-depleted waters of the Sargasso Sea. Since then, numerous accounts of nitrogen uptake periodicity in in situ (e.g., MacIsaac, 1978), shipboard (e.g., Eppley et al., 1971a; Collos and Slawyk, 1976) and N-limited cyclostat cultures (e.g., Eppley et al., 1971b; Malone et al., 1975) of marine phytoplankton have shown maximal uptake during the daylight hours and minimal uptake during the night. Dark uptake of nitrogen has generally been thought to be a response to N limitation (e.g., Syrett, 1981), dampening diel periodicity by a relative enhancement of dark uptake capacity.

Culture studies have demonstrated that the preconditioning N substrate affects the uptake response of phytoplankton to enrichments of different N substrates (e.g., Horrigan and McCarthy, 1981, 1982 ; Dortch and Conway, 1984). Additionally, in N-starved phytoplankton the ability to take up NO_3^- may be lost and must often be induced (Dortch et al., 1982; review by Collos, 1983; Parslow et al., 1984b). Initial NH_4^+ uptake rates are, however, often enhanced upon exposure to an elevated NH_4^+ concentration in culture (e.g., Conway et al., 1976; Conway and Harrison, 1977; Goldman and Glibert, 1982; Parslow et al., 1984a,b) and natural phytoplankton communities (e.g., Glibert and Goldman, 1981; Wheeler et al., 1982; Priscu and Priscu, 1984). In nitrogen-deplete waters, most of the nitrogen demands of phytoplankton are supplied by ammonium and urea from in situ regenerative processes, whereas in N-replete areas, N compounds appear to be utilized at rates proportional to their availability (e.g., Dugdale and Goering, 1967; McCarthy et al., 1977). These observations suggest that phytoplankton communities from N-replete and N-deplete waters may differ in their response to perturbations of nitrogen by their preference for, and uptake rates of, different nitrogen substrates and that these differences may be reflected in diel patterns of N uptake.

Experiments in the present study were designed to examine periodicity of nitrogen uptake in three contrasting environments of relative biomass and nitrogen concentrations: the oceanic subarctic Pacific with low biomass and high NO_3^-

concentrations; a coastal upwelling plume of moderate biomass and varying NO_3^- concentrations; and coastal inshore frontal and stratified waters with elevated and diminished NO_3^- and phytoplankton concentrations, respectively. Time course experiments of 24 h or greater in duration, utilizing either contained samples or repeated sampling of a drogue-tracked water parcel, were conducted with ^{15}N-labelled substrates to relate patterns of uptake to concomitant changes in irradiance and nutrient concentration.

Prior to this study our knowledge of nitrogen uptake by phytoplankton on the west coast of Canada was limited to NO_3^- uptake rates determined in three fjords on the mainland coast of British Columbia (Cochlan et al.,1986). The rates of NO_3^-, NH_4^+, and urea described in this chapter are the first estimates of the uptake capability of natural communities of phytoplankton on the west coast of Canada. The present study was the first to employ ^{15}N methodology (Dugdale and Goering, 1967) and to report ambient concentrations of urea and freshly determined NH_4^+ concentrations on the west coast of Canada and adjacent offshore waters.

MATERIALS AND METHODS

General

Time course experiments of nitrogen uptake were conducted during three cruises aboard the research vessels C.S.S. Vector and C.S.S. Parizeau. The first cruise (OE 84-02), part of Project SUPER (Subarctic Pacific Ecosystem Research) in the Northeast Pacific Ocean was carried out from 7 May to 25 May, 1984, and one time course experiment is reported (TC.1). During the second cruise from July to August, 1984 in the Strait of Georgia, B.C. a coastal basin on the west coast of Canada between the mainland and Vancouver Island, time course studies were conducted in frontal (TC.2) and stratified waters (TC.3). Diel studies on the third cruise (O.E. 86-04) from 18 August to 28 August, 1986 were conducted on the continental shelf off the southwest coast of Vancouver Island (TC.4) and offshore of the shelf (TC.5). Station locations for the experiments are presented in Table 1.1 and shown in Figure 1.1.

Sample collection

Discrete samples were collected from depths, selected to correspond to 50, 30, and 1% of the surface irradiance (I_o), using either 2 or 5 L PVC Niskin bottles (mounted on a rosette or wire) and then transferred into darkened 10 or 20 L Nalgene^R carboys. Vertical profiles of temperature and salinity were obtained from continuous profiles, run prior to bottle sampling, using either a InterOcean model 514A CSTD (cruise 2) or a Guildline model 8701 digital CTD (cruises 1 &

Figure 1.1. Station locations for time course experiments of nitrogen uptake. (A) TC.1 at stn F; TC.4 at stn 24; TC.5 at stn 85. (B) TC.2 at stn A5; TC.3 at stn T4. Panel B is an enlargement of the area delimited by dashed lines in panel A.

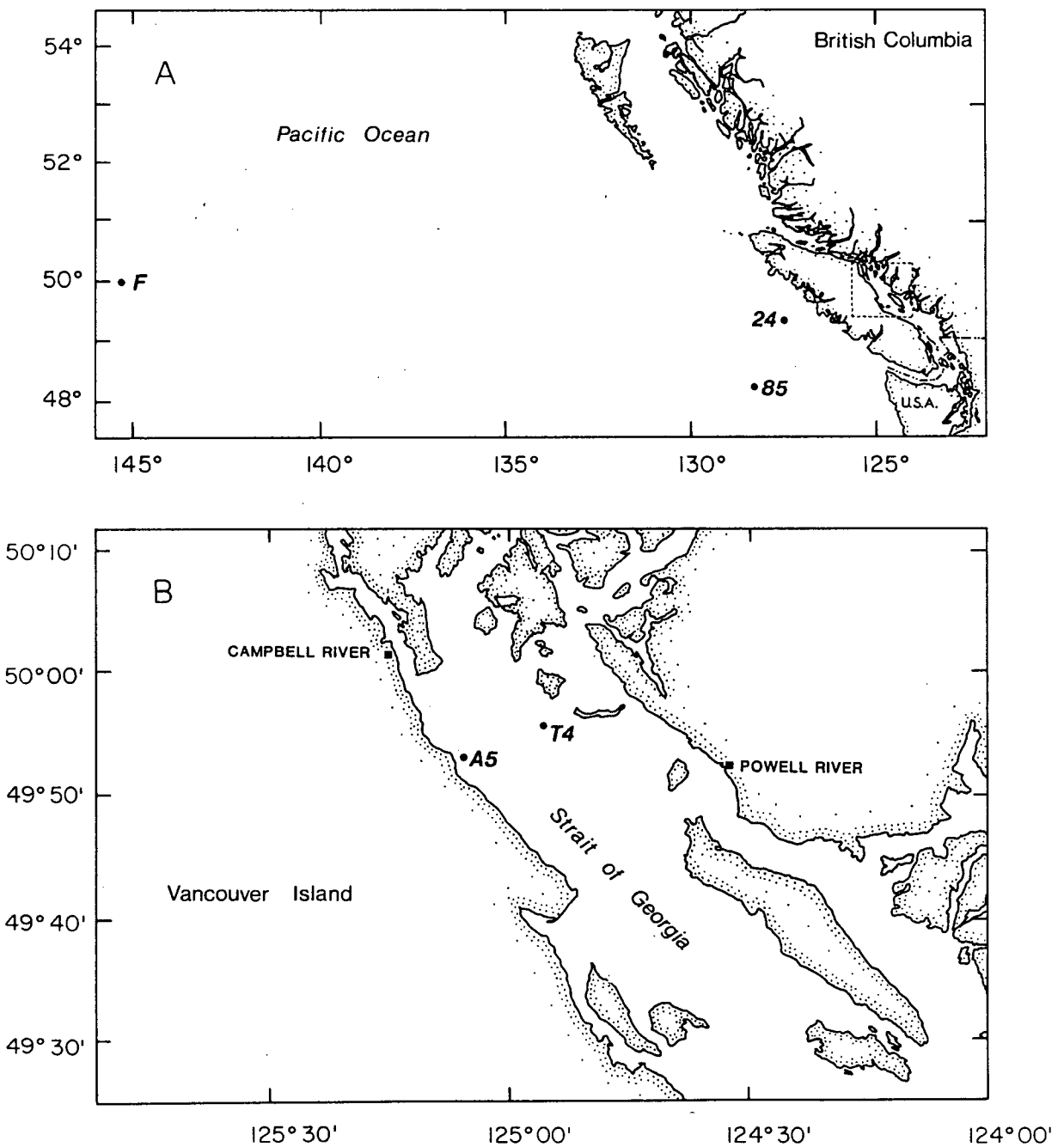


Table 1.1 Initial environmental conditions of seawater collected for time course experiments of nitrogen uptake by natural phytoplankton assemblages. Stations are F: Northeast Pacific Ocean; A5: Strait of Georgia - frontal; T4: Strait of Georgia - stratified; 24: upwelling plume off southwest coast of Vancouver Island; 85: offshore of western Canadian continental shelf, (see Fig. 1.1).

Station and Location	Time Course Number	Date	Starting time of incubation (PDT)	Sample depth (m)	Nitrogen conc. NO_3^-	Urea	NH_4^+	Chl *a*	PON	POC
					($\mu g\text{-at } N \cdot L^{-1}$)			($\mu g \cdot L^{-1}$)	($\mu g\text{-at } N \cdot L^{-1}$)	($\mu g\text{-at } C \cdot L^{-1}$)
F 49°59.5'N 145°14.6'W	TC.1	16 May 1984	0245	8	11.99	0.32*	0.15*	0.59	1.87	19.5
A5 49°53.0'N 125°05.8'W	TC.2	28 July 1984	1000	0	0.27	4.55	0.60	2.12	7.28	47.3
T4 49°55.5'N 124°55.5'W	TC.3	29 July 1984	0800	0	0.19	<.05	0.33	0.39	3.57	31.4
24 49°25.0'N 127°32.1'W	TC.4	20 Aug. 1986	1100	2	12.94	2.38	1.67	10.35	7.75	46.8
				5	11.74	0.82	1.78	11.74	7.35	44.5
				14	11.58	1.02	2.63	7.21	6.15	37.5
85 48°16.7'N 128°18.9'W	TC.5	25 Aug. 1986	1140	1	0.09	1.95	<.05	1.09	2.11	17.3
				28	7.14	0.63	1.25	2.24	2.84	12.6

*Collected from separate bottle casts at similar stations.

3). Simultaneous measurements of chlorophyll fluorescence were determined with a Variosens III in situ fluorometer (cruises 1 & 3) or obtained from pumped samples and measured with a Turner model 111 fluorometer, equipped with a flow-through cell.

Incident solar irradiance (P.A.R.) was monitored continuously with a Lambda Instruments LiCor LI-185 light meter equipped with a LI-190SB Surface Quantum Sensor and recorded with a printing integrator (model LI-550D) or a chart recorder. Subsurface irradiances were determined with a LiCor LI-192S Underwater Quantum Sensor (2π, cruise 2) or a LI-193SB Spherical Quantum Sensor (4π, cruises 1 & 3).

Analytical methods

Subsamples for nutrient analyses were filtered through prewashed, precombusted (460°C for 4 h) Whatman GF/F filters, using an acid-washed syringe and 25 mm Millipore Swinnex^R filter holder, into acid-washed, polypropylene bottles. Ammonium (NH_4^+) concentrations were always determined immediately on board ship with a Technicon Autoanalyzer^R II following the method of Slawyk and MacIsaac (1972). Samples for phosphate (PO_4^{-3}) and silicate (SiO_4^{-4}) were also analyzed fresh following the automated procedures of Hager et al. (1968) and Armstrong et al. (1967), respectively. Samples for nitrate ($NO_3^- + NO_2^-$) and urea were either stored frozen (-20°C), kept dark and cool (< 12 h) or analyzed immediately following the automated procedures of Wood et al. (1967) and Price and Harrison (1987), respectively.

Duplicate samples for chlorophyll *a* (Chl *a*) were filtered (< 125 mm Hg filter pressure differential) onto Whatman GF/F filters with *ca*. 0.5 ml 1% $MgCO_3$ suspension added prior to completion of filtration and either analyzed immediately (< 1 h, cruise 3) or stored frozen in a desiccator (cruises 1 & 2) until analysis ashore. Chlorophyll was extracted in 90% aqueous acetone and analyzed by *in vitro* fluorometry (Parsons et al., 1984) using a Turner Designs model 10 fluorometer, calibrated with crystalline Chl *a* (Sigma Chemical Co.). Duplicate samples for particulate organic carbon and nitrogen (POC & PON) were collected on combusted Whatman GF/F filters, stored similarly, and analyzed by the dry combustion method of Sharp (1974) with either a Perkin-Elmer model 240 or a Carlo Erba model 1106 elemental analyzer. Both instruments were calibrated with acetanilide standards.

Samples for phytoplankton species analysis were preserved in acid Lugol's solution (Parsons et al., 1984) and stored in the dark until analysis. Ten ml subsamples were settled (24 h) and counted on an inverted microscope. During cruise 1 to the northeast Pacific, paired samples were also preserved in alkaline Lugol's solution (Throndsen, 1978) for enumeration of coccolithophorids and the results reported are combined.

Samples for ^{15}N analysis were collected on precombusted Whatman GF/F filters, folded, placed into acid-washed petri-dishes, and immediately frozen for later isotopic analyses. Nitrogen in the particulate samples was converted to dinitrogen gas (N_2) by the micro-Dumas dry combustion

technique as outlined by Cochlan (1982) and LaRoche (1983) and subsequently analyzed for ^{15}N enrichment with a JASCO model N-150 emission spectrometer (Fiedler and Proksch, 1975). Generally each sample was scanned six times (minimum of 3 times) and the average $^{15}N/^{14}N$ peak height ratio was used in the calculation of the percentage ^{15}N (specific activity) in the particulate material. Automatic selection of peak heights during scans and isotopic ratio calculations were performed utilizing in-house software (Jones, unpubl. doc.), with an IBM compatible PC, interfaced with the spectrometer. The emission spectrometer was routinely calibrated with a series of pure N_2 gas standards supplied by JASCO of known ^{15}N enrichment to prepare calibration curves. The precision of the analytical techniques used are presented in Appendix 6.

Tracer experiments

All nitrogen uptake experiments were initiated within 1 h of collection; water was transferred into 500 ml Wheaton glass bottles (clear: light bottles, or darkened with black tape: dark bottles) with teflon-lined caps, and enriched with either $^{15}NH_4Cl$, $Na^{15}NO_3$, or $CO(^{15}NH_2)_2$ (all 99 atom % ^{15}N; Kor Isotopes). Samples were incubated on deck in clear Plexiglas^R incubators cooled with continuously-flowing near surface (3 m) seawater and covered with neutral density screening to simulate the <u>in situ</u> light regime at each sample depth. At selected time intervals, randomly selected bottles were filtered (< 125 mm Hg), for collection of particulate matter for isotopic analysis of ^{15}N atom % excess, and dissolved N

concentrations. Light and dark bottle uptake rates of each nitrogen substrate were measured over the time course and the contents of the sample bottles mixed hourly.

Nitrogen uptake rates were calculated according to the equations of Dugdale and Wilkerson (1986) which are presented in Appendix 1. Specific uptake rates, V (normalized to PON) were estimated using a constant specific uptake model (V_c, equation 6 of Dugdale and Wilkerson). Differences between initial and final ^{15}N atom % excess ($^{15}N_{xs}$) in successsive samples were divided by the length of time interval to obtain average uptake rates during each incubation period. Absolute (transport) rates (were calculated as the product of non-constant specific uptake rates V_i, V_f and the concentrations of PON_i, PON_f, respectively for samples collected at the beginning (i) and end (f) of incubation period. Disappearance uptake rates (V^d) have been calculated from the change in concentration of dissolved nitrogen per unit time and, like the nitrogen-specific and absolute ^{15}N rates, are reported for the time intervals over which they have been calculated.

Experimental procedures

Over the course of this study, subtle and major changes in experimental design were employed, due to both environmental and logistical factors, in the five diel time course experiments reported. In TC.1, conducted in the Northeast Pacific Ocean, water was collected from the 50% I_o light penetration depth (ca. 8 m) and prefiltered through 102 μm Nitex^R nylon netting to remove larger zooplankton before

tracer experiments were initiated. Samples were inoculated with $Na^{15}NO_3$ at a tracer level (usually defined as ≤ 10% of the ambient concentration) to bring final tracer concentration to 1.0 μg-at $N \cdot L^{-1}$ prior to incubation. Triplicate samples were removed and particulates filtered at 3 h intervals for 24 h. Nine hours after isotope enrichment (T_o = 0245 h PDT) triplicate light bottles were darkened for 3 and 5 h during mid-day to estimate dark uptake rates, dark uptake rates were also determined for triplicate samples collected and incubated 24 h in darkness.

During the second cruise, in the Strait of Georgia, samples were collected in the morning (0700-0900 h) and 6.0 μg-at $N \cdot L^{-1}$ of $^{15}NH_4^+$, $^{15}NO_3^-$ or $CO(^{15}NH_2)_2$ were added in TC.2 and 3. Time-zero samples for dissolved nitrogen were withdrawn immediately and analyzed for NH_4^+, NO_3^-, and urea concentrations in all bottles. At 3 h intervals particulate matter, from duplicate samples, was collected by filtration for ^{15}N analysis. Samples for dissolved nitrogen concentrations were taken concurrently and those for Chl *a* and POC and PON every 6 h.

During the third cruise, drogued drifter buoys (Loran-C drifters; described in Mackas et al., 1989) were used to guide repeated sampling from a given water parcel for 48 h (TC.4) and 24 h (TC.5). The drogues were centred at 15 m depth and their positions were reported by radio every half hour; drifter tracks are reported in Forbes et al. (1987). During TC.4, an upwelling plume on the western coast of Vancouver

Island, was sampled at 3 h intervals for physical measurements and at 6 h intervals for N uptake experiments and the concentrations of POC, PON, Chl *a* and dissolved nutrients. Samples from 100, 30, and 1% I_o were collected as described previously and duplicate samples from each depth inoculated with 10 μg-at $N \cdot L^{-1}$ of $Na^{15}NO_3^-$ or $^{15}NH_4Cl$ for 4 h simulated *in situ* incubations. During TC.5, in the waters offshore of the continental shelf, a water parcel was repeatedly sampled at 2 h intervals for biological and physical measurements for 29 h. At each sampling period, duplicate samples from just below the surface and the 1% I_o were enriched with 10 μg-at $N \cdot L^{-1}$ of $Na^{15}NO_3$ and incubated for 4 h in simulated *in situ* conditions. Samples for enumeration of phytoplankton species were collected at the beginning and end of both experiments to determine community composition over the sampling period.

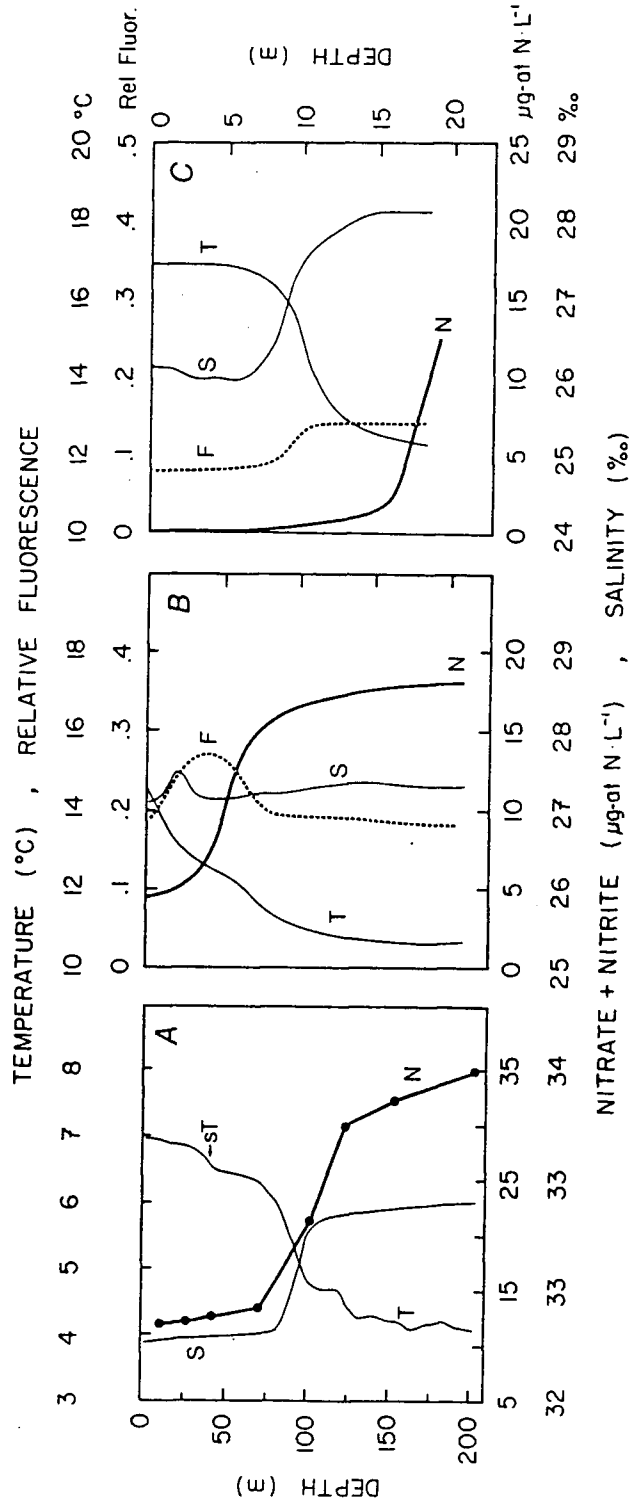
RESULTS

Physical observations

The vertical profiles of temperature, salinity and nitrate concentration for TC. 1 (cruise OE 8402, stn F) in the northeast Pacific Ocean are presented in Figure 1.2 A. The temperature of the upper water column was 6.5-7.0°C and thoroughly mixed until ca. 30 m. A shallow thermocline step (sT, temperature step < 0.5) at 30-40 m effectively divided the euphotic zone (1% I_o = 70 m) into two layers with different turbulence characteristics and dynamics (Denman and Gargett, 1988) above the main pycnocline/seasonal thermocline at 80-100 m. Denman and Gargett (1988) demonstrated that the shallow thermocline step presents a significant barrier to the vertical exchange of phytoplankton and presented two seperate indicators of the physiological state of the phytoplankton which confirm that this physical barrier was sufficient to cause differing degrees of photoadaptation in each of the two layers comprising the euphotic zone. Nitrate (plus nitrite) concentrations were high (ca. 11 μg-at $N \cdot L^{-1}$) in the surface "mixed water" and increased to 20-35 μg-at $N \cdot L^{-1}$ below the pycnocline.

Vertical profiles of temperature, relative in vivo fluorescence and NO_3^- concentrations for the frontal (TC.2, stn A5) and stratified (TC.3, stn T4) waters of the Strait of Georgia are presented in Figure 1.2 B & C. The diagnostic features of the frontal water were the shallow thermocline and high fluorescence at the depth of the nitracline (3 to 7 m).

Figure 1.2. Depth profiles of temperature (T), salinity (S), in vivo fluorescence (F), and nitrate plus nitrite concentration (N) for three stations sampled for containment time course experiments. (A) Oceanic station F, TC.1. (B) Frontal station A5, TC.2. (C) Stratified station T4, TC.3. The shallow thermocline step is indicated by the arrow labelled 'sT' in panel A.



Time course 3 was conducted in warm (17°C), stratified water and the depth profile demonstrated a subsurface fluorescence maximum (ca. 10m) which was overlain by nitrate-depleted mixed water (Fig. 1.2 C).

Time course 4 was conducted by repeated sampling of an upwelled plume off Vancouver Island (stn 24-49) and the depths of sampling (2, 6-7,and 14 m) were all within the mixed surface layer of nitrate-replete (ca. 10 μg-at $N \cdot L^{-1}$), warm (11°C) surface water above the thermocline/halocline (ca. 15 m). The vertical profiles of salinity and temperature (Fig. 1.3 A & B) for the first (stn 24) and last (stn 49) period of sampling (51 h later) demonstrate a slight deepening of the surface mixed layer by a weakening of the shallower, first thermocline over the sampling period. Vertical profiles of NO_3^- and NH_4^+ at 6 h intervals (Fig. 1.4) show relatively little change over time, although elevated NH_4^+ concentrations were initially observed in the surface waters at stn 24; these decreased during the next 6 h to low (< 0.5 μg-at $N \cdot L^{-1}$) but variable concentrations in the surface waters sampled for the remainder of the diel N uptake experiments. Vertical profiles of SiO_4^{-4} and PO_4^{-3} ambient concentration demonstrated little change over the course of the time course experiment (Fig. 1.5).

Time course 5 utilized both surface (0-2 m) nitrate-depleted water from the shallow (ca. 7 m) mixed layer and deep (28 m, 1% I_o) nitrate-replete (> 5 μg-at $N \cdot L^{-1}$) water from the thermocline depth. Profiles of dissolved inorganic nutrients

Figure 1.3. Depth profiles of temperature (T) and salinity (S) for the two stations repeatedly sampled during drogue-type time course experiments. A: stn 24 (beginning of TC.4). B: stn 49 (end of TC.4). C: stn 84 (beginning of TC.5). D: stn 98 (end of TC.5).

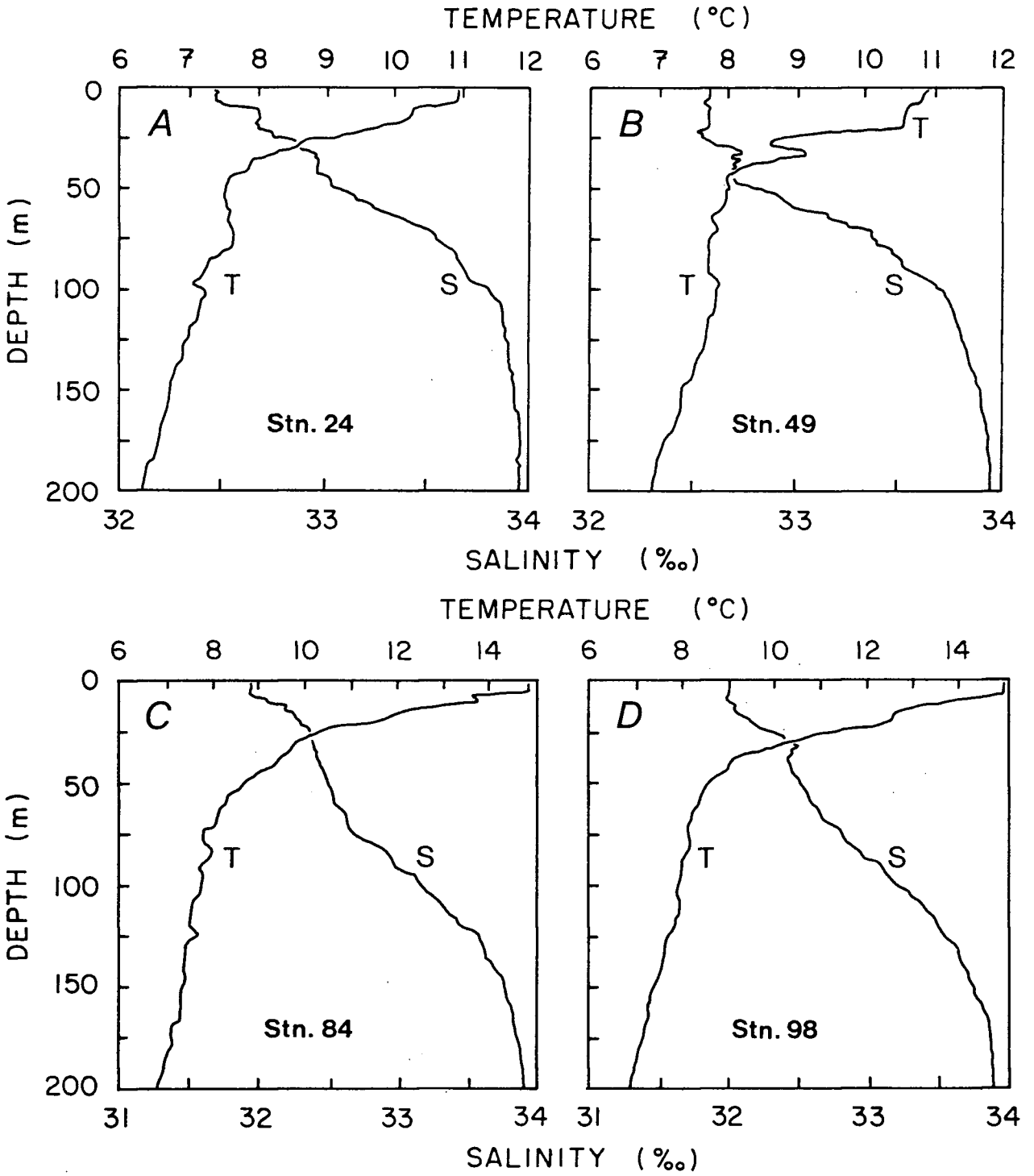


Figure 1.4 Depth profiles of NO_3^- and NH_4^+ at 6 h intervals during time course 4.

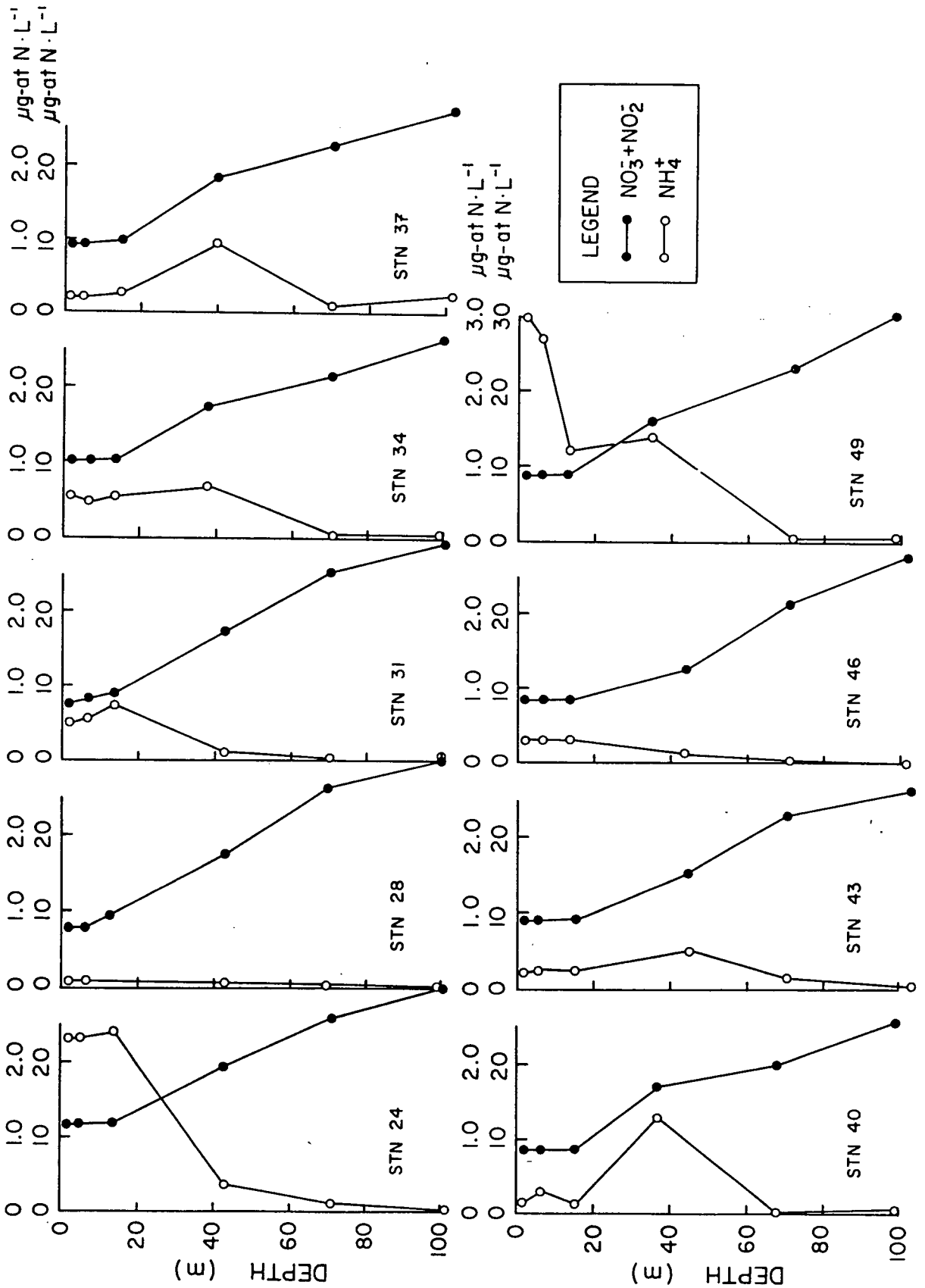


Figure 1.5. Depth profiles of SiO_4^{-4} and PO_4^{-3} at 6 h intervals during time course 4.

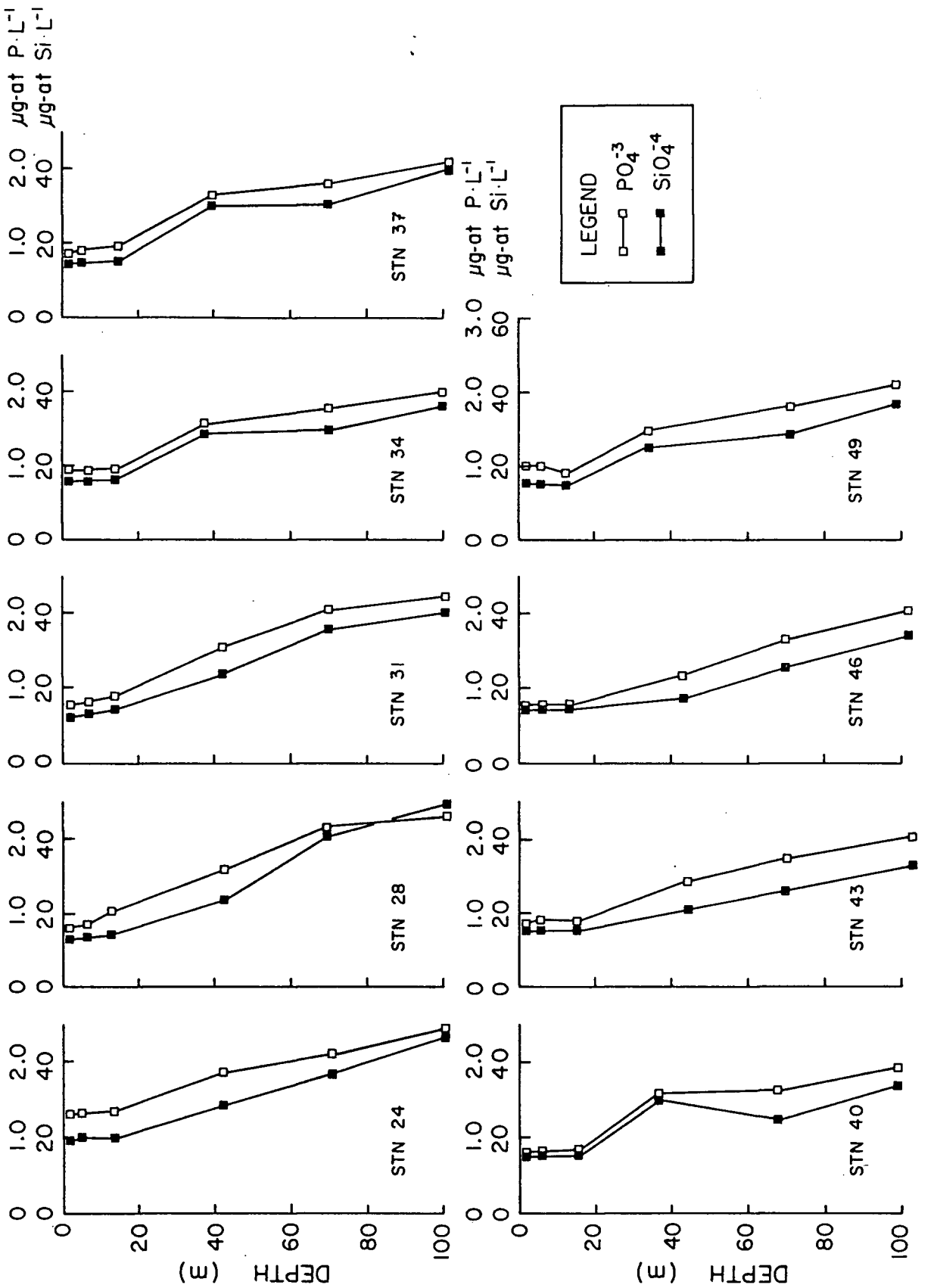
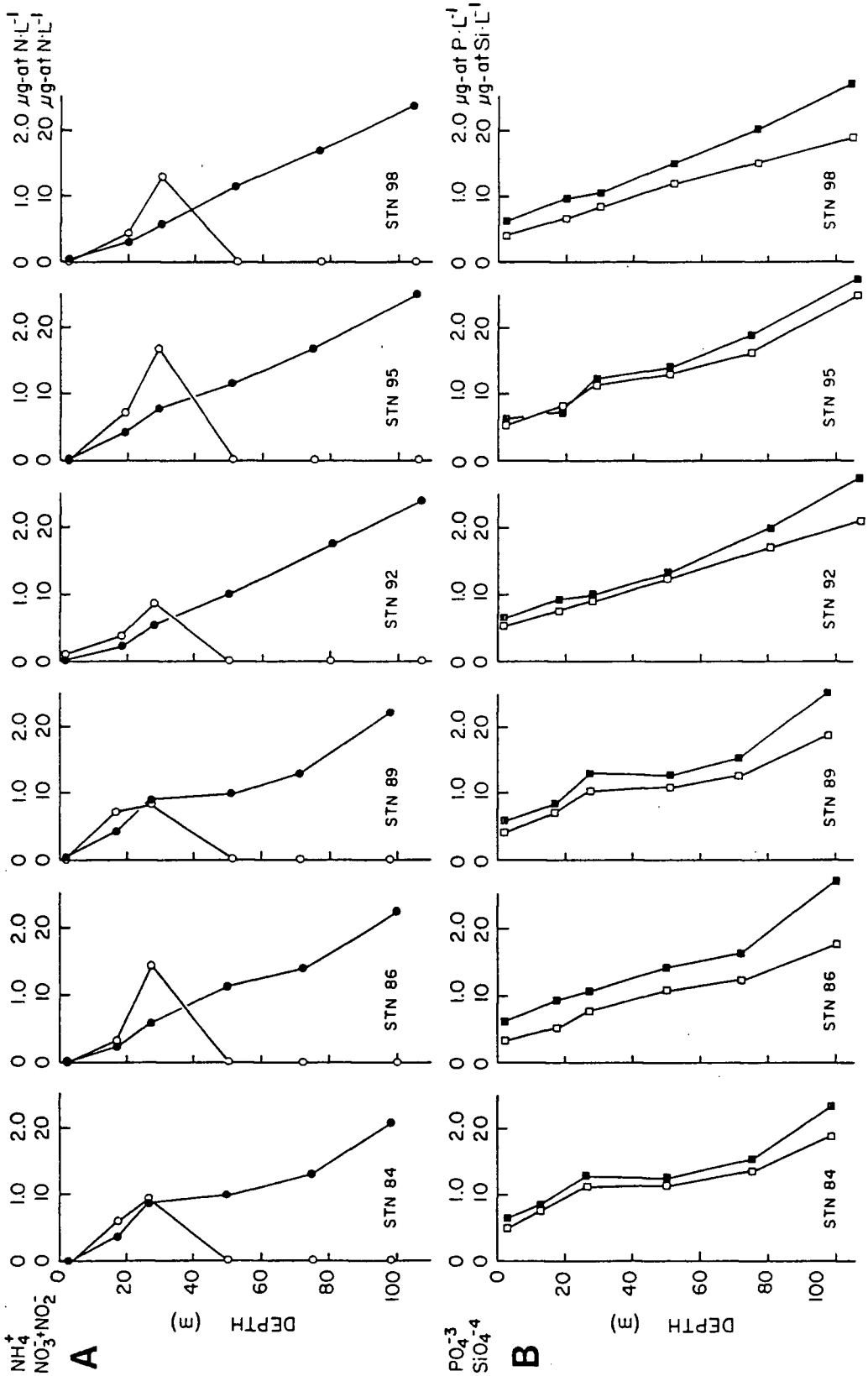


Figure 1.6 A: Depth profiles of NO_3^- (●) and NH_4^+ (○) at 2 h intervals during time course 5. B: Depth profiles of SiO_4^{-4} (■) and PO_4^{-3} (□) at 2 h intervals during time course 5.



(NO_3^-, NH_4^+, SiO_4^{-4}, PO_4^{-3}) are presented in Figure 1.6 and demonstrated little variation in concentration over the 29 h sampling period.

Biological observations

Phytoplankton species were identified and counted using visible light microscopy and thus may be biased by the exclusion of cells < 2 μm (picoplankton), which are difficult to distinguish from inorganic particles (e.g., Booth, 1988). In the water used for TC.1 approximately 83% of the phytoplankton enumerated were small haptophytes belonging to the following genera, *Imantonia, Phaeocystis* or *Chrysochromulina* (4-10 μm). Chrysophytes of the genus *Ochromonas* were 7% of the total cell concentration and pennate diatoms, primarily *Nitzschia cylindrus* and *N. closterium* v. *striatula*, composed an additional 8%; the Raphidophycean flagellate *Heterosigma akashiwo* was ca 1%. Occasional dinoflagellates, (*Gymnodinium* spp. and *Prorocentrum baltica*), centric diatoms (*Chaetoceros peruvianum* and *Thalassiosira* spp.) and prasinophytes (*Nephroselmis* spp.) were observed, but totalled < 1% of the total cell concentration of 4.8×10^6 cells$\cdot L^{-1}$. Samples were not enumerated for picoplankton, although Booth (1988) observed that in samples collected during the same time in the northeast Pacific, 16% of the plant biomass was attributed to cells < 2 μm and 90% of this was composed of the blue-green, coccoid cyanobacterium, *Synechococcus* spp. The majority of the zooplankton was probably removed by screening the water samples through Nitex^R

netting (in order to minimize macrozooplankton predation during incubations) although screened samples were not enumerated; unfiltered zooplankton species and abundance data are reported in Forbes et al. (1988). The species composition of the phytoplankton community in the frontal (TC.2) and stratified (TC.3) water of the Strait of Georgia was very different (Table 1.2.). In the frontal water large, chain-forming diatoms of the genus *Chaetoceros* formed aggregates (≤ 1 mm) which contained some pennate diatoms belonging to *Navicula* and *Nitzschia* spp. The size of the diatom flocs prevented screening prior to ^{15}N experimentation and therefore to remain consistent, none of the future water samples were screened. Small flagellates (< 5 μm) were the most common phytoplankton in the stratified water. *Chaetoceros* spp., *Ch. socialis* and *Skeletonema costatum* were the most abundant diatoms, whereas dinoflagellates were almost exclusively *Gymnodinium* spp. Water samples were not originally taken for zooplankton species enumeration. However the abundance of these animals, as seen in the phytoplankton samples, suggested they could have been important grazers and N remineralizers. As a first approximation the concentration of general categories of these zooplankters are presented (Table 1.2).

The large, centric diatoms, *Skeletonema costatum*, *Thalassiosira nordenskioldii*, and *Chaetoceros* spp. (particularly *Ch. compressum* > *Ch. radicans* > *Ch. ceratosporum*) dominated in terms of relative numbers (70%) in the samples collected at all 3 depths used in TC.4. The

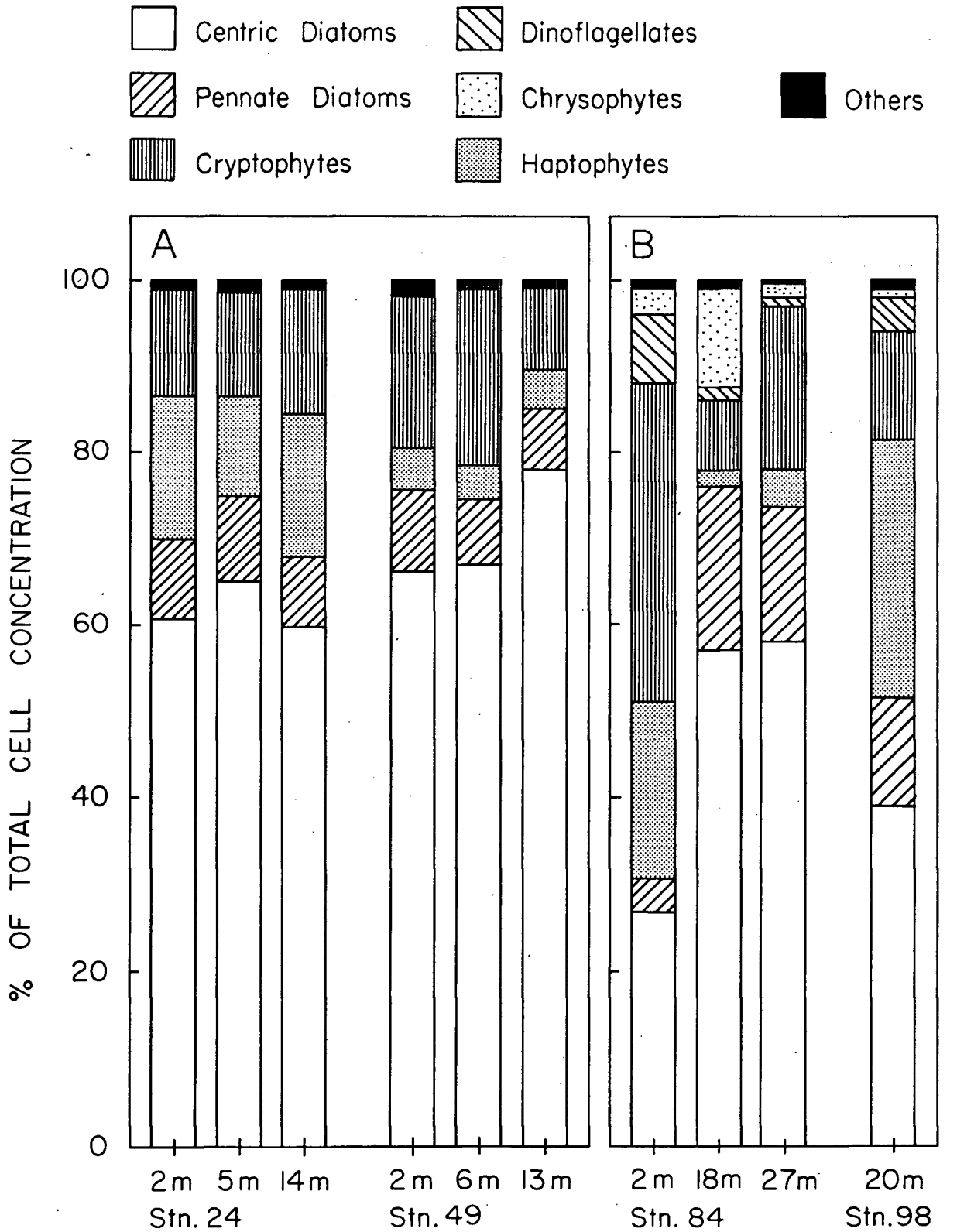
Table 1.2 Plankton community composition in frontal and stratified water of Strait of Georgia, B.C., (see Fig. 1.1 B).

Station	Phytoplankton (10^6 cells·L^{-1})			Zooplankton (animals·L^{-1})			
	Diatoms	Dinoflagellates	Flagellates	Tintinnids	Calanoid Copepods	Ciliates excl. tintinnids	Others
Frontal A5 (TC.2)	2.3	0.023	1.6	470	50	730	280
Stratified T4 (TC.3)	0.43	0.049	1.6	180	60	140	300

remainder of the phytoplankton community consisted primarily of pennate diatoms (*Nitzschia* cf. *subpacifica* > *N. delicatissima* > *N. americana* ≥ *N. longissima*), haptophytes (*Imantonia* and *Phaeocystis* spp.) and unidentified cryptomonads. There were no obvious differences in the species composition and total cell concentration at any of the three depths between the beginning and end of the drogue sampling (Fig. 1.7 A); surface population decreased from 3.0 x 10^6 cells·L^{-1} to 1.3 x 10^6 cells·L^{-1}, the mid-depth (30% I_o) population from 2.8 x 10^6 to 2.3 x 10^6 cells·L^{-1}, and an increase in the population at 1% I_o from 3.6 x 10^6 to 5.0 x 10^6 cells·L^{-1}.

The species composition in the samples collected from the surface (2 m) and depth (27 m) for TC.5 were very different; a dominance of diatoms (74%) both centric (58%) and pennate (15%) in the deeper waters. The most common centric diatoms were *Skeletonema costatum*, *Thalassiosira conferta*, *Chaetoceros* spp. (primarily *Ch. compressum* and *Ch. debilis*) and the pennates were mostly *Thalassionema nitzschioides* and *Nitzschia americana*, *N. delicatissima* and *N. longissima*. In the surface samples only about one-third of the community was composed of centric diatoms, the remainder haptophytes (21%) (primarily *Imantonia* and *Phaeocystis* spp.) unidentified cryptomonads (37%) and dinoflagellates (8%), including genera of *Gymnodinium* and *Protogonyaulax*. Species samples were only collected at mid-depth (20 m) at the end of the time series but this sample showed no major differences in species

Figure 1.7 Composition of the phytoplankton community, A: at the beginning (stn 24) and end (stn 49) of time course 4 B: beginning (stn 84) and end (stn 98) of time course 5.



composition over time, except for an increasing abundance of haptophytes and a decrease in the abundance of diatoms (Fig. 1.7 B).

Nitrogen uptake rates

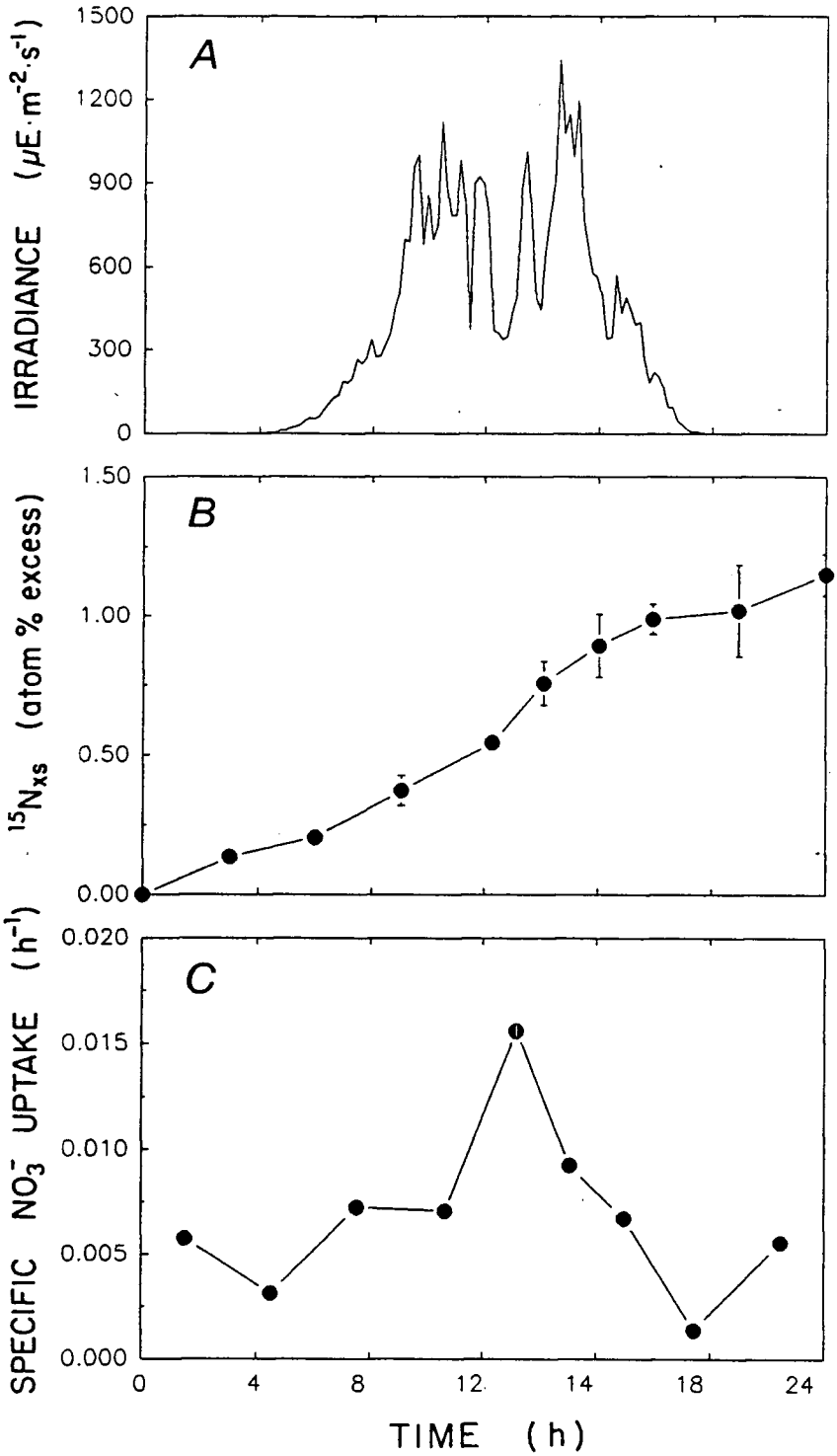
Subarctic Pacific Ocean

During TC.1, in the nitrate-rich subarctic Pacific, the incorporation of ^{15}N-labelled nitrate into particulate matter was followed for 24 h, beginning during the middle of the night (Fig. 1.8 B). A relatively clear diel trend was apparent for specific NO_3^- uptake rates calculated over 3 h intervals (Fig. 1.8 C); maximal uptake rate during the daylight period (mean = 0.0085, S.D. = 0.0045 h^{-1}) and mean rate at night was lower (0.0047 ± 0.0013 h^{-1}). The mean nitrate uptake at night was ca. 55% of the mean daytime value. Samples incubated for 24 h in the dark (0.00063 ± 0.00011 h^{-1}) had significantly lower NO_3^- uptake rates (paired *t*-test, $P \leq 0.01$) than those incubated in the natural light-dark cycle (0.0068 ± 0.00046 h^{-1}). The artificial darkening of triplicate samples for 3 and 5 h during mid-day had no effect during the first 3 h; mean dark uptake was 0.0072 h^{-1} and equivalent to the mean light uptake (0.0071 h^{-1}) during this time, however, during the subsequent 2 h of darkness, uptake declined 92% to 0.00058 h^{-1}, whereas the light uptake rate of NO_3^- attained its maximal value (0.0156 h^{-1}).

Strait of Georgia

During the time course experiments conducted in the Strait of Georgia, the changes in the ambient concentration of

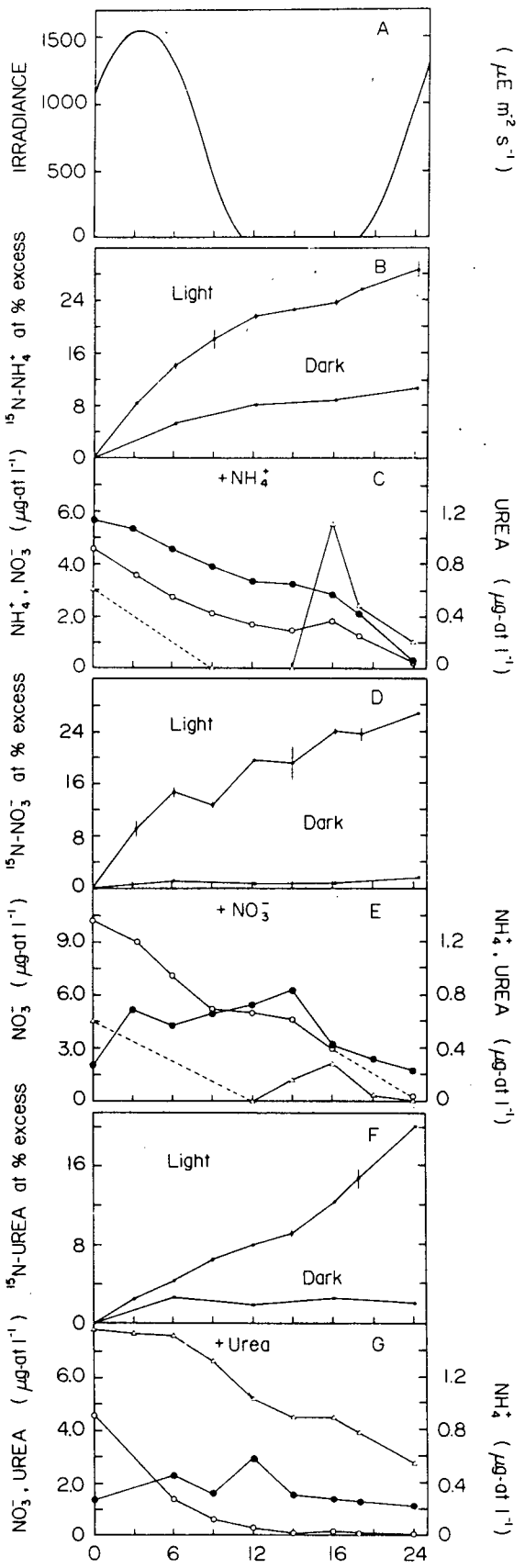
Figure 1.8. Time course measurements at oceanic station F., Time Course 1. (A) Daily incident surface irradiance during experiment. (B) ^{15}N atom % excess in particulate matter for light bottle incubations (error bars represent ± 1 S.D. of triplicates) plotted against elapsed time measured after addition of 1.0 μg-at $N\text{-}NO_3 \cdot L^{-1}$. (C) Nitrogen specific uptake rates of $^{15}NO_3^-$ calculated for 3 h intervals; each point indicates a rate calculated over the time interval between it and the previous point on the curve and plotted against average incubation time between sampling.

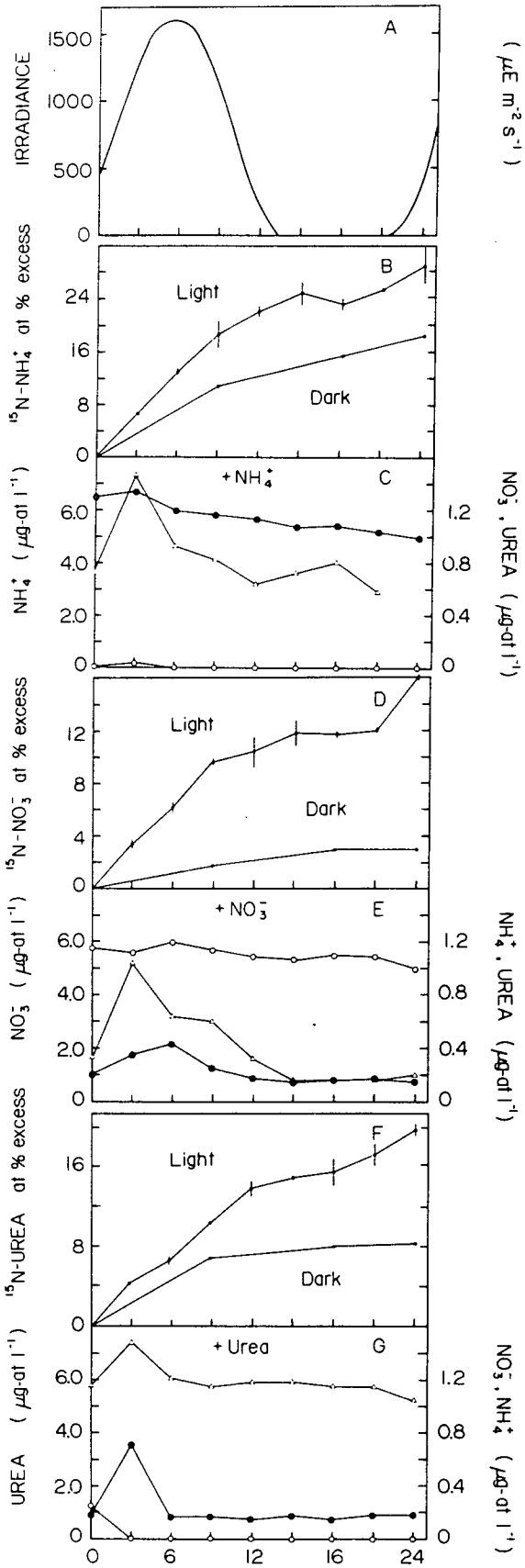


dissolved NH_4^+, NO_3^- and urea and the incorporation of those ^{15}N-labelled substrates into particulate matter were measured for 24 h. Both approaches yield different information concerning nitrogen utilization by the phytoplankton. Changes in dissolved nitrogen concentration represent net community flux of that nutrient and encompass regenerative and uptake processes. By contrast, ^{15} N isotope accumulation is an estimate of gross uptake by the phytoplankton providing there is no recycling of ^{15}N, and ^{15}N enrichment in the dissolved phase remains constant. Results from TC.2 (frontal water) and TC.3 (stratified water) experiments are shown in Fig. 1.9 and Fig. 1.10, respectively. Data from TC.2 demonstrate multiple N substrate uptake by phytoplankton, specifically for NH_4^+, NO_3^- and urea (Fig. 1.9 C, E) and NO_3^- and urea (Fig. 1.9 G). The elevated ambient NO_3^- concentration in the frontal waters allowed the uptake rates of NO_3^- in the NH_4^+- and urea-enriched samples to be determined by N disappearance from the seawater samples. Uptake rates determined by the disappearance of nitrate were similar in the presence (V^d_{0-6h}=0.521 μg-at $N \cdot L^{-1} \cdot h^{-1}$) and absence ($V^d_{0-9h}$=0.567 μg-at $N \cdot L^{-1} \cdot h^{-1}$) of urea, but were reduced in the NH_4^+ enriched samples (V^d_{0-9h}=0.267 μg-at $N \cdot L^{-1} \cdot h^{-1}$). The ^{15}N-urea atom % accumulation rate was constant over the first 15 h, but prior to the end of the dark period it increased and remained linear until the end of the incubation (Fig. 1.9 F). The increase in the urea uptake rate coincided with the depletion of external NO_3^-, moreover the change in urea concentration was minimal

Figure 1.9. Time course measurements at frontal station (A5), Time Course 2. (A) Daily incident irradiance during experiment (B, D, F) ^{15}N atom % excess in particulate matter for light and dark bottle incubations following addition of 6 μg-at $N \cdot L^{-1}$ of (B) NH_4^+, (D) NO_3^- and (F) urea (error bars represent the range of duplicates). (C, E, G) Corresponding measurements of dissolved NH_4^+ (●), NO_3^- (○) and urea (△) in (C) NH_4^+, (E) NO_3^-, and (G) urea-spiked samples. Dashed line indicates no measurements of dissolved urea at 3 and 6 h; (left side of page).

Figure 1.10. As Figure 1.9 except at stratified station (T4), Time Course 3; (right side of page).


A
IRRADIANCE
(μE m⁻² s⁻¹)
1500
1000
500
0
B
Light
Dark
¹⁵N-NH₄⁺ at % excess
24
16
8
0
+NH₄⁺
C
NH₄⁺, NO₃⁻ (μg-at l⁻¹)
UREA (μg-at l⁻¹)
6.0
4.0
2.0
1.2
0.8
0.4
D
¹⁵N-NO₃⁻ at % excess
+NO₃⁻
E
NO₃⁻ (μg-at l⁻¹)
NH₄⁺, UREA (μg-at l⁻¹)
9.0
6.0
3.0
F
¹⁵N-UREA at % excess
+Urea
G
NO₃⁻, UREA (μg-at l⁻¹)
NH₄⁺ (μg-at l⁻¹)
0
6
12
16
24
INCUBATION PERIOD (h)

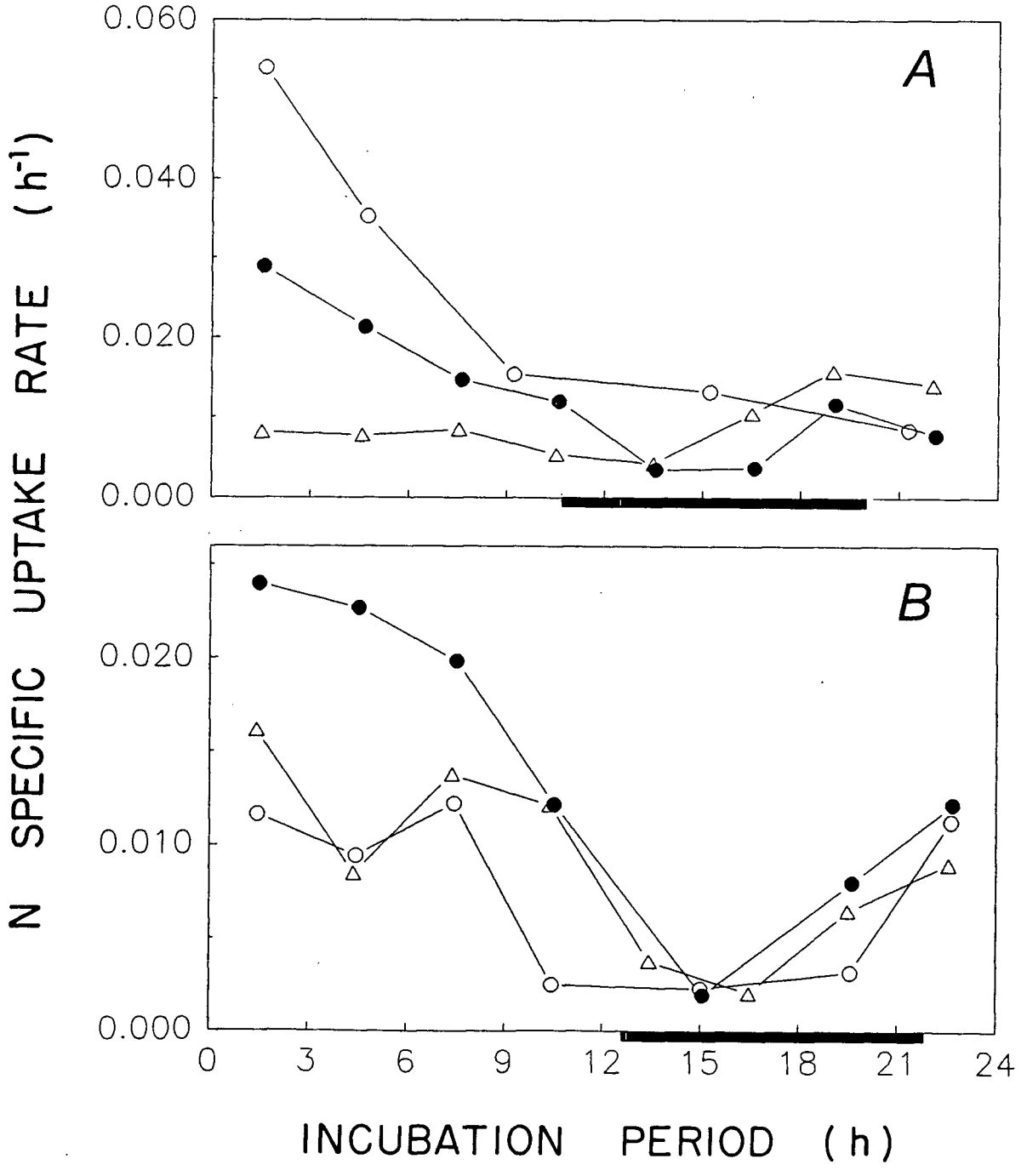

A
IRRADIANCE
(μE m⁻² s⁻¹)
1500
1000
500
0
B
Light
Dark
¹⁵N-NH₄⁺ at % excess
24
16
8
0
+NH₄⁺
C
NH₄⁺ (μg-at l⁻¹)
NO₃⁻, UREA (μg-at l⁻¹)
6.0
4.0
2.0
1.2
0.8
0.4
D
¹⁵N-NO₃⁻ at % excess
12
8
4
+NO₃⁻
E
NO₃⁻ (μg-at l⁻¹)
NH₄⁺, UREA (μg-at l⁻¹)
F
¹⁵N-UREA at % excess
16
+Urea
G
UREA (μg-at l⁻¹)
NO₃⁻, NH₄⁺ (μg-at l⁻¹)
0
6
12
16
24
INCUBATION PERIOD (h)

(Fig. 1.9 G) over the first 6 h, when NO_3^- concentrations were high (4.55 to 1.4 μg-at $N \cdot L^{-1}$) and NO_3^- was being taken up. The incorporation of ^{15}N-NO_3^- and ^{15}N-NH_4^+ was non-linear with time, generally reduced during the nighttime and substrate exhaustion occurred during the 21 to 24 h time interval.

The pattern of ^{15}N uptake by the phytoplankton in the stratified water was similar in the NH_4^+, NO_3^- and urea-enriched samples (Fig. 1.10 B, D, F,). Uptake was constant over the first 9 to 12 h, then decreased during the night and increased again in the early morning. Substrate depletion did not occur in these experiments and the total utilization after 24 h of nitrogen isotopes was minimal in the NH_4^+, NO_3^- and urea-enriched samples (23, 18 and 10%, respectively).

Clear indications of urea regeneration, and to a lesser extent NH_4^+ regeneration, were evident from increases in substrate concentrations in TC.2 and TC.3 and are discussed in detail by Price et al. (1985). The pattern of ^{15}N-labelled NH_4^+, NO_3^- and urea uptake rates suggests the existence of diel periodicity in nitrogen uptake in both frontal and stratified water (Fig. 1.11). The decrease in uptake of NH_4^+ and NO_3^- from 21 to 24 h in TC.2 was due to substrate exhaustion (see Fig. 1.9 C,E). In the frontal community, uptake rates of NO_3^- were greatest throughout the time course, in contrast to the stratified community where NH_4^+ uptake rates were highest and NO_3^- and urea uptake rates similar but lower than NH_4^+ uptake rates. In both experiments nitrogen uptake rates increased prior to the onset of the light period

Figure 1.11. Nitrogen-specific uptake rates of NH_4^+ (●), NO_3^- (O) and urea (Δ) in (A) frontal and (B) stratified water. Rates determined for 3 or 6 h intervals; each point indicates a rate calculated over the time interval between it and the previous point on the curve. Shaded area on the abscissa delimits the dark period.



and this was most marked in the urea-enriched samples.

The ratio of dark to light ^{15}N uptake rate (V_D:V_L) for NH_4^+, NO_3^- and urea is given in Table 1.3. In both frontal and stratified communities, dark NH_4^+ uptakes were a major portion of the light uptake rates throughout the entire time courses. The V_D:V_L for NH_4^+ in the frontal water was constant (38%) and less then the ratio in stratified water (52 to 102%). Initial dark rates of urea uptake were 60 to 66% of the light rates in both TCs but dark uptake declined to a negligible portion of light uptake during the remainder of the TC.2 and 6-24% of V_L in TC.3. The light dependence of NO_3^- uptake was more similar to that of urea than ammonium in both stratified and frontal water.

Uptake rates normalized per unit Chl *a* demonstrated that NH_4^+ and urea uptake rates were on average 2 and 2.4 times greater in the stratified water than in frontal water, whereas NO_3^- uptake rates were on average 1.6 times higher in the frontal water (Table 1.4). Chl *a* specific uptake rates for each substrate, when compared between stations, were most similar over the dark period (12 to 18 h) and the greatest disparity was found initially (0 to 6 h).

Offshore waters

Time course 4 was conducted from samples collected at 6 h intervals from 3 depths in an upwelled plume of water on the continental shelf off Vancouver Island. The initial environmental conditions of the water sampled during TC.4 are presented in Table 1.5. The specific rates of NO_3^- uptake for

Table 1.3. Ratio of dark to light uptake rates ($V_D:V_L$) of NH_4^+, NO_3^- and urea for frontal and stratified water of the Strait of Georgia, B.C., (see Fig. 1.1 B).

Station	Time interval (h)	NH_4^+ ($V_D:V_L$)	NO_3^- ($V_D:V_L$)	Urea ($V_D:V_L$)
Frontal A5	0 - 6	0.37	0.08	0.60
(TC.2)	6 - 12	0.39	<.01	<.01
	12 - 18	0.37	<.01	<.01
	18 - 24	0.39	<.01	<.01
Stratified T4	0 - 9	0.58	0.18	0.66
(TC.3)	9 - 18	1.02	0.60	0.24
	18 - 24	0.52	<.01	0.06

Table 1.4. Chlorophyll *a* specific uptake rates of NH_4^+, NO_3^- and urea in frontal (A5) and stratified (T4) water of the Strait of Georgia, B.C., (see Fig. 1.1 B). The dark period occurs during the 12 to 18 h time interval.

Nitrogen substrate	Time interval (h)	Chl *a* specific N-uptake rate [μg at N (μg Chl *a*)$^{-1}$ h^{-1}] Frontal Stn	Stratified Stn
NH_4^+	0 - 6	0.091	0.261
	6 - 12	0.060	0.133
	12 - 18	0.025	0.030
	18 - 24	0.028	0.047
NO_3^-	0 - 6	0.162	0.098
	6 - 12	0.075	0.082
	12 - 18	0.042	0.019
	18 - 24	0.068	0.039
Urea	0 - 6	0.040	0.127
	6 - 12	0.028	0.125
	12 - 18	0.026	0.019
	18 - 24	0.050	0.053

samples collected from the near-surface (1-2 m) and 30% I_o depth (6-7 m) were not significantly different (paired *t*-test, $P \geq 0.01$) and demonstrated pronounced diel periodicity (Fig. 1.12 B). Maximum rates of uptake were observed during the daylight hours, reduced rates in the early evening (1900-2300 h) and minimal rates during the night. The mean nighttime uptake rate was 15-16% of the daytime rate. The NO_3^- uptake rates of samples collected and incubated at the 1% I_o depth (simulated) were variable and did not show a clear diel pattern, although the greatest values were observed during daylight and the mean nighttime rate was ca. 70% of average daytime value. The specific rates of NH_4^+ uptake demonstrated a similar pattern of diel periodicity as the NO_3^- uptake rates. The potential specific uptake rates of NH_4^+ for the 2 shallow depths were again not significantly different (paired *t*-test, $P \geq 0.01$), were minimal during the night, maximal during daytime and reduced in the early evening. The mean nighttime rate was 30-36% of the daytime value. No diel trend was observed in the uptake rates of the deeper samples and average nighttime rates were 120% of daytime rates. Absolute (transport) rates of NO_3^- and NH_4^+ reflect the patterns discussed for specific rates (Fig. 1.13). Depth profiles of the dissolved nutrients NO_3^-, NH_4^+, SiO_4^{3-} and PO_4^{3-} are presented in Figures 1.4 and 1.5. and show little change in ambient concentration over the sampling period.

Time course 5 was conducted by sampling, at 2 h intervals, the phytoplankton community from the NO_3^--deplete

Table 1.5 Initial environmental conditions of seawater collected for nitrogen uptake experiments during time course 4.

Station and location	Date	Starting time of incubation (PDT)	Sample depth (m)	Nitrogen conc. NO_3^- (μg-at N·L^{-1})	Urea (μg-at N·L^{-1})	NH_4^+ (μg-at N·L^{-1})	Chl *a* (μg·L^{-1})	PON (μg-at N·L^{-1})	POC (μg-at C·L^{-1})
24	49°25.0'N	20 August 1986	1100	1.7	12.90	2.38	1.67	10.35	7.75	46.8
	127°32.1'W			5.4	11.80	0.82	1.78	11.74	7.35	44.5
				14.4	11.60	1.02	2.63	7.21	6.15	37.5
28	49°21.2'N	20 August 1986	1900	2.0	7.98	0.31	0.12	10.25	11.21	72.8
	127°28.9'W			5.8	7.98	0.26	0.12	12.89	11.50	74.5
				14.4	8.96	0.85	0.14	9.16	8.58	60.6
31	49°20.3'N	21 August 1986	0134	2.0	8.78	-	0.82	19.49	8.18	51.6
	127°27.6'W			5.8	8.59	0.71	0.60	19.58	8.27	51.6
				14.4	9.07	0.62	0.92	18.54	8.01	52.9
34	49°18.5'N	21 August 1986	0736	2.0	9.84	0.75	0.67	21.90	8.31	53.2
	127°27.7'W			7.3	9.89	2.27	0.62	22.73	7.36	45.8
				13.7	10.10	0.72	0.75	20.85	7.81	62.6
37	49°17.1'N	21 August 1986	1327	2.0	9.35	0.30	0.20	14.70	9.34	55.3
	127°26.9'W			7.1	9.16	0.41	0.17	15.04	10.06	58.7
				14.1	9.42	0.36	0.19	16.12	9.40	56.1
40	49°16.1'N	21 August 1986	1934	2.0	8.50	0.61	0.11	14.66	9.80	46.7
	127°26.3'W			5.0	8.58	1.26	0.12	15.21	8.57	55.9
				14.8	8.58	0.26	0.10	14.83	8.25	57.6
43	49°16.3'N	22 August 1986	0128	2.0	9.09	0.41	0.17	13.03	7.81	46.6
	127°23.2'W			5.0	9.07	0.29	0.17	14.33	7.39	46.1
				13.4	9.14	0.32	0.18	14.90	6.96	48.7

Table 1.5 continued

Station and location		Date	Starting time of incubation (PDT)	Sample depth (m)	Nitrogen conc. NO_3^- (μg-at N·L^{-1})	Urea (μg-at N·L^{-1})	NH_4^+ (μg-at N·L^{-1})	Chl *a* (μg·L^{-1})	PON (μg-at N·L^{-1})	POC (μg-at C·L^{-1})
46	49°16.0'N 127°21.4'W	22 August 1986	0730	2.0	8.49	0.33	0.37	15.80	7.20	40.8
				7.0	8.49	0.32	0.33	15.50	7.22	41.4
				14.8	8.50	0.42	0.34	15.38	7.17	50.6
49	49°17.4'N 127°21.3'W	22 August 1986	1347	1.5	6.34	0.33	0.08	16.10	9.26	58.7
				7.2	7.63	0.48	0.26	14.00	8.73	53.7
				13.9	8.57	0.61	0.41	13.41	7.09	50.0

Figure 1.12. Time course measurements at upwelled plume stations 24-49, time course 4. (A) Daily incident surface irradiance during experiment. (B) Nitrate and (C) ammonium specific uptake rates at 100% I_o (○), 30% I_o (●) and 1% I_o (△) calculated over 4 h incubation periods and plotted against average incubation period.

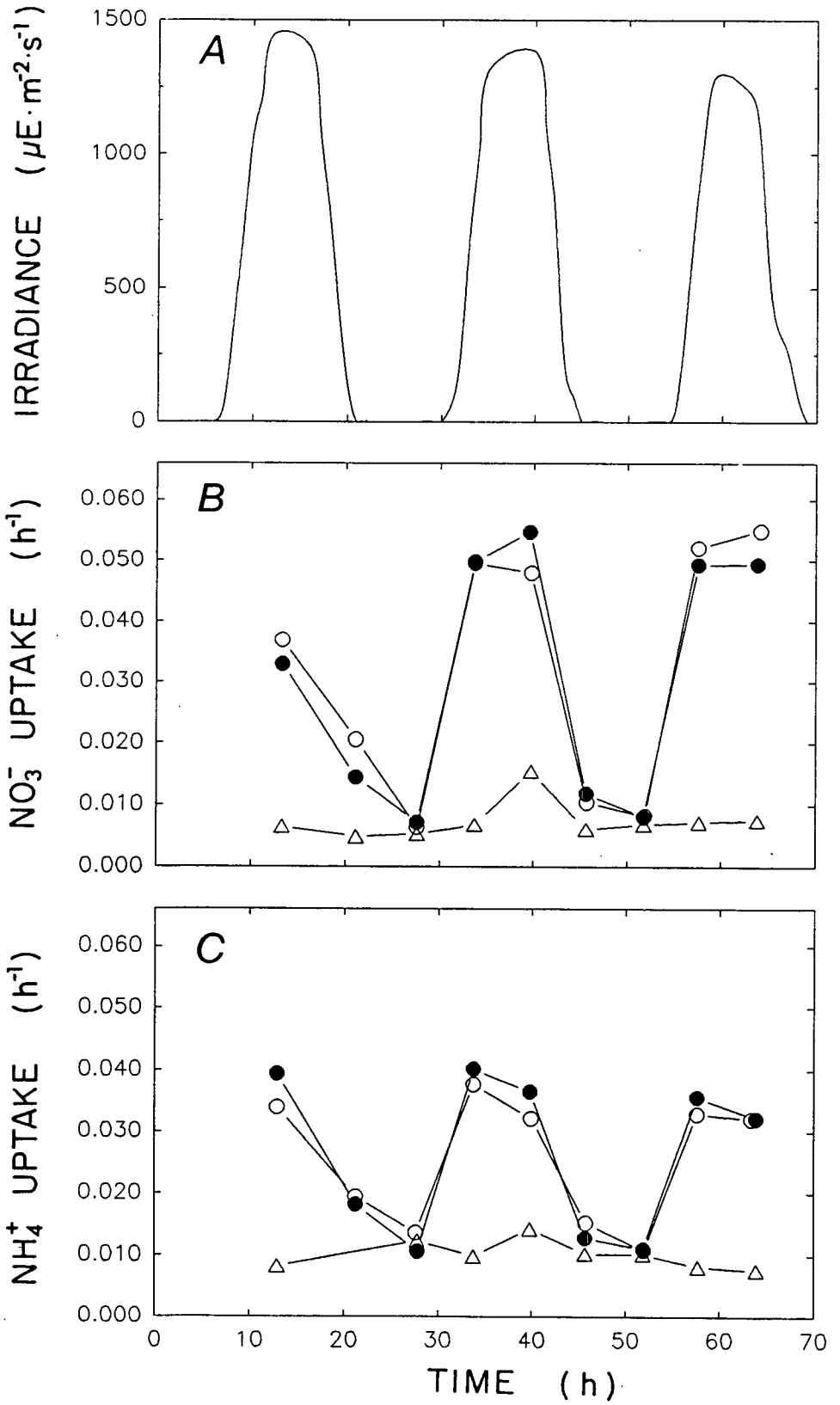
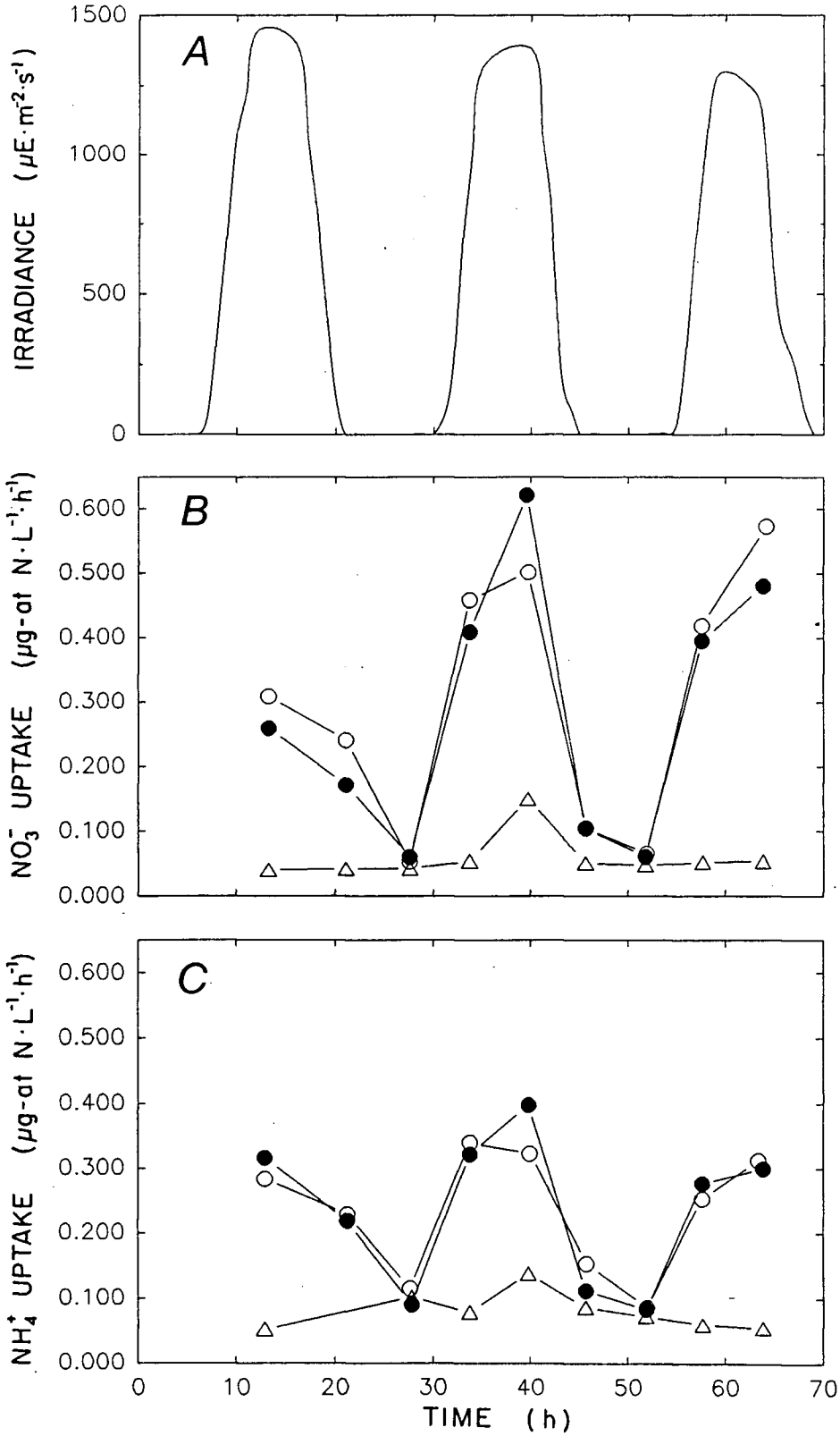


Figure 1.13. Time course measurements at upwelled plume stations 24-49, time course 4. (A) Daily incident surface irradiance during experiment. (B) Nitrate and (C) ammonium absolute uptake rates at 100% I_o (○), 30% I_o (●) and 1% I_o (△) calculated over 4 h incubation periods and plotted against average incubation period.



(0.07-0.35 μg-at $N \cdot L^{-1} \cdot h^{-1}$) surface water and the NO_3^--rich (5.08-8.82 μg-at $N \cdot L^{-1} \cdot h^{-1}$) water from the 1% I_o (28 m) depth (Table 1.6). Specific uptake rates, determined from the incorporation of $^{15}N\text{-}NO_3^-$ during 4 h incubation periods are plotted against average incubation in Fig. 1.14 B. The pattern of NO_3^- specific uptake rates suggests the existence of diel periodicity in the surface samples, similar to that in TC.4, with minimal values at night and maximal values during the daytime. The mean nighttime uptake was 37-47% of the daytime rate.

NO_3^- specific uptake rates of the samples collected at depth were variable during the day/night cycle but suggestive of a diel pattern although displaced (ca. 5 h) later in time. The absolute uptake rates of the surface and deep communities reflect the patterns observed for specific uptake rates and are presented in Fig. 1.14 C. Depth profiles of dissolved nutrients NO_3^-, NH_4^+, SiO_4^{-3} and PO_4^{-3} are presented in Fig. 1.6 and show little change over time.

Table 1.6. Initial environmental conditions during time course 5 conducted off the west coast of Vancouver Island on August 25-26, 1986.

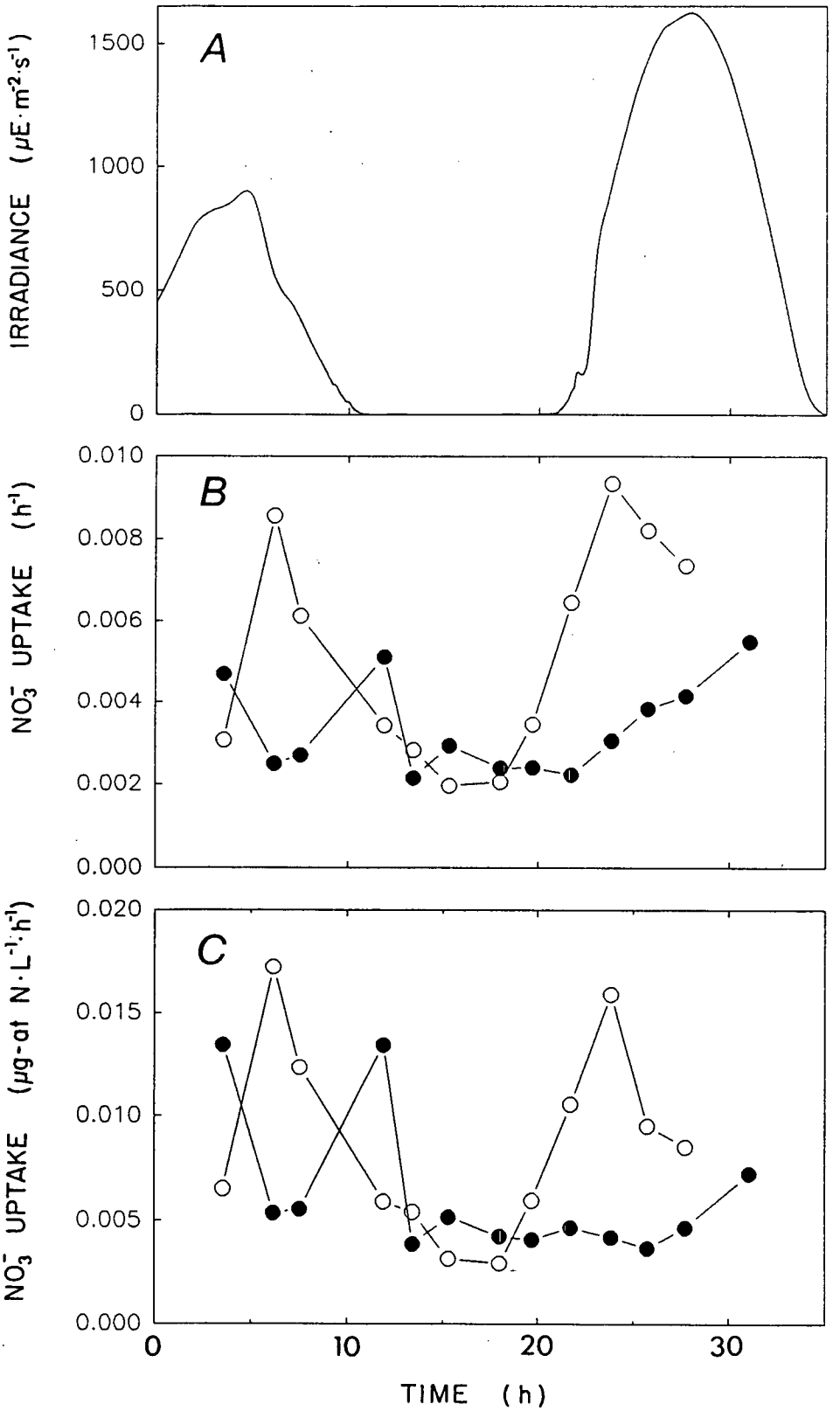
	Station and location	Starting time of incubation (PDT)	Sample depth (m)	Nitrogen conc. NO_3^-	Urea	NH_4^+	Chl *a* ($\mu g \cdot L^{-1}$)	PON (μg-at $N \cdot L^{-1}$)	POC (μg-at $C \cdot L^{-1}$)
				(μg-at $N \cdot L^{-1}$)					
84	48°17.5'N	0912*	2.4	0.13	-	<0.03	0.92	-	-
	128°19.3'W		26.6	8.44	0.93	1.44	-	-	-
85	48°16.7'N	1140	1.3	0.09	1.95	<0.03	1.09	2.11	17.3
	128°18.9 W		28.4	7.14	0.63	1.25	2.24	2.84	12.6
86	48°16.0'N	1422	1.5	0.09	0.77	<0.03	0.96	1.98	18.3
	128°18.9'W		26.3	6.36	1.11	1.79	3.68	2.13	13.8
87	48°15.8'N	1532	1.7	0.02	0.41	<0.03	1.00	2.00	25.2
	128°18.9'W		28.4	6.38	-	1.59	3.74	2.03	16.7
88	48°15.3'N	1736	1.1	0.09	0.39	<0.03	0.73	1.52	14.8
	128°19.2'W		27.2	5.99	0.57	1.19	3.60	1.79	15.8
89	48°16.3'N	1957	1.1	0.31	0.30	<0.03	1.02	1.71	18.6
	128°17.0'W		27.0	9.39	0.50	0.29	3.55	1.80	15.8
90	48°16.1'N	2128	2.0	0.11	-	<0.03	0.94	1.90	15.4
	128°17.3'W		28.0	5.19	-	0.95	2.88	1.81	15.7
91	48°15.4'N	2322	1.9	0.33	-	<0.03	2.04	1.61	11.2
	128°16.4'W		28.4	7.66	-	1.25	2.06	1.75	13.0
92	48°14.9'N	0205	1.9	0.13	0.41	0.11	1.21	1.44	13.3
	128°16.8'W		26.8	5.08	0.98	1.01	2.50	1.77	15.9

Table 1.6 continued

	Station and location	Starting time of incubation (PDT)	Sample depth (m)	Nitrogen conc. NO_3^-	Urea	NH_4^+ ($\mu g\text{-at } N \cdot L^{-1}$)	Chl *a* ($\mu g \cdot L^{-1}$)	PON ($\mu g\text{-at } N \cdot L^{-1}$)	POC ($\mu g\text{-at } C \cdot L^{-1}$)
93	48° 14 5'W	0340	1.4	0.25	-	<0.03	0.70	1.72	13.3
	128° 16.5'W		27.4	6.48	-	1.50	2.85	1.69	15.7
94	48° 14.7'N	0545	1.7	0.35	-	<0.03	0.84	1.62	13.5
	128° 17.7'W		25.8	5.73	-	1.45	3.10	2.10	16.0
95	48° 14.6'N	0747	1.3	0.33	0.37	0.05	0.83	1.67	13.1
	128° 17.0'W		28.9	8.50	2.93	1.19	2.70	1.36	12.9
96	48° 14.6'N	0944	2.2	0.08	-	<0.03	-	1.14	9.09
	128° 16.2'W		27.7	8.24	-	0.78	1.30	0.96	9.77
97	48° 13.9'N	1154	0.5	0.17	-	<0.03	0.56	1.14	11.3
	128° 16.5'W		30.1	7.75	-	1.94	1.72	1.12	11.0
98	48° 13.5'N	1457	1.9	0.21	2.27	<0.03	0.74	1.75	15.3
	128° 17.6'W		29.1	5.67	1.30	1.25	3.99	1.30	9.86

* designates cast time; no N uptake experiments at stn 84.

Figure 1.14. Time course measurements at stations 85-98, time course 5. (A) Daily incident surface irradiance during experiment. (B) Nitrogen specific uptake rates of nitrate at 100% I_o (○) and 1% (●) calculated over 4 h incubation periods and plotted against average incubation period. (C) Absolute uptake rates of nitrate.



DISCUSSION

Experimental considerations

Previous diel studies of N uptake by natural phytoplankton assemblages have taken three basic approaches: (1) samples are collected at one location, transferred to incubation containers, and then subsampled over time (e.g., Eppley et al., 1971b; Collos and Slawyk, 1976; Kristiansen and Lund, 1989); (2) sampling occurs at one geographic location over time (e.g., MacIsaac, 1978; Tobiesen, 1987; Fisher et al., 1988); or (3) an attempt is made to follow a water parcel which is sampled over time (e.g., MacIsaac, 1978). Each approach has its advantages and disadvantages. Containment has the advantage of knowing that the same water is being subsampled over time and eliminates potential complicating factors such as advection, diel migration of phytoplankton (e.g., Blasco, 1978; Cullen and Horrigan, 1981) and diel variability in ambient N concentration (e.g., Lorenzen, 1965; Beers and Kelly, 1965). The major disadvantage is the problem associated with bottle incubation techniques in general; these include changes in plankton species composition (Venrick et al., 1977), bottle size effects (Gieskes et al., 1979), and toxicity due to trace metals present as bottle contaminants (Carpenter and Lively, 1980). These "bottle-effects" may substantially alter the contained water; large, "clean" containers will minimize these effects.

Sampling at one geographic location has the advantage of dealing with natural samples, but does not take into account

the problem of advection which may result in different communities being sampled at different times. Advection is potentially a more pronounced problem in tidally influenced regions such as the Strait of Georgia where a pattern caused by tides could be mistaken for a diel rhythm in phytoplankton.

Sampling a particular parcel of water over time has the distinct advantage that the same planktonic community is likely sampled each time. However such an approach requires knowledge of the physics of the water parcel and a means of following it, a logistically more difficult and expensive experimental approach. Both of the latter methods assume that biological activity such as diel migratory behaviour (e.g., Cullen and Horrigan, 1981; Frempong, 1984) and phytoplankton sinking (e.g., Bienfang et al., 1982) are minimal in order to achieve constancy in phytoplankton community composition.

In the current study both the containment approach and following of a water parcel over time were utilized. Attempts were made to minimize zooplankton predation in the contained samples during the incubation period by screening out macrozooplankton with netting prior to ^{15}N inoculation. In all time course experiments saturating additions of ^{15}N-labelled substrates were used so that the effects associated with isotope dilution of the isotope enrichment factor by unlabeled regenerated N would be minimal (e.g. Glibert et al., 1982c; Price et al., 1985) and substrate exhaustion would not occur during the incubation period (e.g., Goldman et al., 1981; Fisher et al., 1981). Uptake rates reported are

therefore not necessarily in situ values but indicative of potential rates of N uptake that can be realized by the phytoplankton community when provided with saturating concentrations of N, a condition often achieved for NO_3^- uptake in the surface, and especially deeper communities, but seldom observed for the uptake of regenerated N in natural situations.

The incubation (sampling) intervals in the time course experiments were long (3-6 h) relative to the rapid uptake response of phytoplankton seen in the laboratory (e.g., Conway et al., 1976; Parslow et al., 1984a, b) and the field (e.g., Glibert and Goldman, 1981; Priscu and Priscu, 1984) and thus I was unable to detect short term variations in uptake rate. Enhanced uptake of NH_4^+ and urea by NO_3^--sufficient phytoplankton have been previously reported (Horrigan and McCarthy, 1981, 1982; Parslow et al., 1984b). In light of the slower, long term rates of regenerated N uptake relative to NO_3^- uptake in the frontal station of Strait of Georgia (TC.2) and the upwelled plume (TC.4) off Vancouver Island it is unlikely that such enhanced uptake processes occurred on time scales shorter than the sampling intervals employed in the present study. Bottle containment effects have been shown to lead to serious underestimates of rate processes (Venrick et al., 1977). One would expect the consequences of containment to be most severe in higher biomass communities but the constant rates of Chl a and POC and PON synthesis in TC.2 and TC.3 indicate no such artifacts in these experiments.

Simultaneous uptake of nitrogen compounds

Simultaneous utilization of NH_4^+ and NO_3^- is well documented in laboratory (e.g., Eppley and Renger, 1974; Bienfang, 1975; Caperon and Ziemann, 1976; DeManche et al., 1979; Dortch and Conway, 1984) and natural phytoplankton assemblages (e.g., Collos and Lewin, 1974; Conover, 1975; Conway, 1977; McCarthy et al., 1977; Maestrini et al., 1982, 1986, Quéguiner et al., 1986; Collos et al., 1989). The results of TC.2 and TC.3, conducted in the frontal and stratified areas of the Strait of Georgia, respectively not only demonstrate dual nitrogen substrate utilization but that NH_4^+, NO_3^-, and urea may be taken up concurrently. As first pointed out by Collos (1987), multiple nitrogen substrate utilization will result in a reduction of the nitrogen-specific uptake rate of the ^{15}N-labelled compound compared to the N-specific uptake rate determined when only the ^{15}N-labelled compound is being taken up. In all the experiments a saturating addition of the ^{15}N-labelled compound of interest was used and the effects of isotopic dilution, due to unlabelled nitrogen being taken up, are potentially only a problem in areas of high ambient nitrogen levels (i.e. NH_4^+ uptake in TC.4), In TC.2 and TC.3 the absolute uptake rates were calculated using the final PON determined at the end of an incubation; which gives an accurate measure of uptake rate of the ^{15}N-labelled nutrient into the phytoplankton and avoids potential artifacts caused by incorporation of non-^{15}N-labelled nitrogen forms.

Maestrini et al. (1982) demonstrated that microalgae of oyster ponds took up NH_4^+ and NO_3^- at the same rate once the NH_4^+ concentration had decreased to ca. 7 μg-at $N \cdot L^{-1}$. The results from the frontal community (TC.2) demonstrated the similarity of NH_4^+ and NO_3^- uptake rates in the NH_4^+ enriched samples. However, the NO_3^- uptake rate was reduced by 50% in the NH_4^+ spiked samples as compared to the NO_3^- spiked samples. Similar NH_4^+ suppression of NO_3^- uptake has been reported for both laboratory (e.g., Grant et al., 1967; Conway, 1977; Cresswell and Syrett, 1979) and natural phytoplankton assemblages (e.g., McCarthy et al., 1977, Blasco and Conway, 1982). The effects of NH_4^+ addition on urea uptake has been less studied than interactions between NO_3^- and NH_4^+. Nevertheless it appears from the few available studies that NH_4^+ suppresses urea uptake and the inhibitory response is either instantaneous (Williams and Hodson, 1977; Lund, 1987) or manifested later (Horrigan and McCarthy, 1982; Molloy and Syrett, 1988a;) In TC.2 and TC.3 the effect of NH_4^+ on urea disappearance uptake rate is difficult to discern due to the low ambient concentrations of urea and evidence of urea regeneration during the incubation period. In TC.2 the NO_3^- disappearance uptake rate was unaffected or slightly enhanced in the presence of urea in agreement with the laboratory study of Lund (1987) who found no suppression of NO_3^- uptake in the marine diatom, *Skeletonema costatum*.

Molloy and Syrett (1988b) found simultaneous uptake of NO_3^- and urea, but urea inhibition (24-26%) of NO_3^- uptake in

cultures of *Chlorella emersonii* and *Phaeodactylum tricornutum* after prolonged (1-2 d) N deprivation. Partial inhibition of urea uptake by NO_3^- has been reported in natural seawater samples (McCarthy and Eppley, 1972) and laboratory cultures of *Skeletonema costatum* (Lund, 1987) and *P. tricornutum* and *C. emersonii* (Molloy and Syrett, 1988b). Ambient concentrations of urea in the frontal and stratified stations (TC.2, TC.3) were low. The low concentrations observed and the possibility of urea regeneration during the incubation period makes it difficult to discern any inhibitory effect of NO_3^- on urea uptake. In TC.4, and TC.5 relatively high ambient concentrations of urea (0.75 - 2.38 μg-at $N \cdot L^{-1}$) were occasionally observed in the upper waters sampled (2-7 m) which may have, either biologically or through isotopic dilution of ^{15}N in particulate material, reduced the potential rates of specific NO_3^- uptakes as reported.

Effects of light/dark regime on nitrogen uptake

Diel periodicity in the uptake rates of NO_3^-, NH_4^+ and urea were evident in the time course experiments of the various natural phytoplankton assemblages. In TC.1, conducted in the NO_3^--replete waters of the northeast Pacific Ocean, nighttime NO_3^- uptake rates were about half those reported during the daytime, with a pattern of maximal rates during mid-day and lower rates in the morning and afternoon. Similar strong, diel patterns in NO_3^- uptake have been observed in the NO_3^--rich Antarctic waters by Koike et al. (1986) where the uptake of NO_3^- and NH_4^+ during the nighttime amounted to ca.

10-30 and 50%, respectively of the daytime values. Olson (1980) found in 2 time course experiments that NO_3^- uptake ceased during the nighttime and NH_4^+ uptake was either 25 or 85% of the daytime rate, whereas Glibert et al. (1982a) found anomalous results; they found no difference in NO_3^- uptake between samples incubated in the dark and those incubated over a normal light-dark regime for samples collected from the NO_3^--rich Scotia Sea. During TC.1 when samples which had normally been exposed to a natural L:D cycle were suddenly darkened during mid-day, a lag of > 3 h occurred before dark uptake rates declined to the rate observed during 24 h of darkness. The initial "dark" NO_3^- uptake rate may have been the result of previous light stimulation and only after some elapsed time were the reductants, cofactors and enzymes that are necessary for NO_3^- assimilation, and produced (or activated) during the light period, used up (or deactivated) during the artificially imposed darkness.

In the coastal waters of the Strait of Georgia similar patterns of diel uptake of all 3 N substrates were observed for the NO_3^--replete and NO_3^--deplete stratified waters. Nitrate and NH_4^+ nighttime uptake rates were about one-third of the average daytime rates in the frontal waters and declined to about half this value in the stratified waters. Nighttime uptake rates of urea were ca. 75 and 15% of the daytime rates for frontal and stratified waters, respectively. Fisher et al. (1982) measured similar diel variations in NH_4^+ uptake in an estuarine phytoplankton community while diel

variation in NO_3^- uptake rates have been recorded for freshwater reservoir plankton (Toetz, 1976) and *Ceratophyllum*-periphyton communities (Toetz, 1971). Price and Harrison (1988a) observed diel periodicity for saturated urea uptake rates of samples collected from NO_3^--deplete areas of the Sargasso Sea, but no definitive light/dark trends in NO_3^--replete areas.

The constancy of V_D:V_L (dark incubation bottle: clear incubation bottle) for NH_4^+ in the frontal water, when NH_4^+ uptake rates of phytoplankton exposed to the natural light/dark cycle were periodic, suggests that NH_4^+ uptake is circadian; in absence of the light/dark cycle the rhythm is free running (see Chisholm, 1981). This conclusion is supported by Goering et al. (1964) who found rhythmic variation in both potential NH_4^+ and NO_3^- uptake by surface phytoplankton communities of the Sargasso Sea under continuous illumination. However, Kristiansen and Lund (1989) found no diel variability in potential uptake rates of NO_3^-, NH_4^+ or urea in samples collected from the N-depleted Barents Sea and incubated under constant light, results which do not support the endogenous theory of uptake control. In the Strait of Georgia the results of V_D:V_L for urea and NO_3^- were similar to each other and demonstrate that their dependency on light was comparable. The light dependence of uptake of these nutrients is discussed in detail in Chapter 2.

Laboratory studies have shown that N-deprived phytoplankton have higher dark uptake rates of nitrogen than

N-replete phytoplankton (e.g., Syrett, 1962; Eppley and Coatsworth, 1968; Harrison, 1976; Rees and Syrett, 1979). In the Strait of Georgia dark N uptake rates normalized to Chl *a* were highest in the N-depleted stratified water in agreement with these observations; also relative to the frontal community, dark uptake rates were a greater proportion of the light rates for NH_4^+, NO_3^- and urea in stratified water. The higher Chl *a* specific uptake rates of NH_4^+ and urea in stratified water and of NO_3^- in frontal water are consistent with the way nitrogen is envisaged to support these areas. Specifically, regenerated N (NH_4^+ and urea) has been shown to supply most of the phytoplankton nitrogen demand in N-depleted waters and as the concentration of ambient NO_3^- increases so does the relative importance (not preference) of NO_3^- for phytoplankton nitrogen ration (e.g., McCarthy et al., 1977; Harrison, 1980; Glibert et al., 1982b; Cochlan 1986). It is important to remember that the species composition of the two phytoplankton communities contrasted markedly and likely contributed to the observed variability in the light response and precludes an explanation of diel periodicity of N uptake based merely on the phytoplankton communities' nitrogen status.

The rhythmic pattern of NO_3^- uptake rates observed in the upper waters (1-7 m) of the upwelled NO_3^--rich plume are indicative of *in situ* uptake diel periodicity where nighttime values were 15-16% of daytime rates; the pattern of potential rates of NH_4^+ uptake was similar but nighttime rates were a

greater proportion (ca. 30-36% of the daytime values). Similar diel periodicity of NO_3^- and NH_4^+ uptake rates of natural phytoplankton communities in upwelled regions have been observed by others. Eppley et al. (1970) found that Peru Current phytoplankton had nighttime rates ca. 25 and 62% of daytime values for NO_3^- and NH_4^+, respectively. Collos and Slawyk (1976) also observed diel variation of NO_3^- uptake in shipboard cultures of surface communities collected in the upwelling area off Northwest Africa; nighttime values were ca. 20% of daytime rates of uptake. In eutrophic Lake Biwa, Japan, Mitamura and Saijo (1986) found nighttime uptake rates of NO_3^- to be only 10% of daytime rates and although the phase for urea and NH_4^+ uptake periodicity corresponded to that of NO_3^- the amplitude was lower with nighttime values 80 and 95% of daytime rates. The absence of an apparent diel rhythm in NO_3^- and NH_4^+ uptake of the deeper (1% I_o) community of the upwelled plume was also observed by MacIsaac (1978) in the NO_3^--replete 1% I_o samples of a phytoplankton community dominated by the dinoflagellate *Gonyaulax polyedra* off Baja, Mexico. Diel periodicity in both potential NH_4^+ and NO_3^- uptake rates was, however, observed down to the 10% I_o depth. The amplitude of diel periodicity of potential NO_3^- uptake rates observed in the NO_3^--deplete surface waters used in TC.5 was greater than that observed for in situ NO_3^- uptake of the NO_3^--replete surface waters of TC.4; nighttime rates were ca. 40% of daytime values compared to 15-16% in TC.4. These rates, however, are potential rates of uptake which represent

rates that may be realized under conditions of concentrations saturating to uptake. Salhsten (1987) could discern no diel pattern for *in situ* uptake rates of NH_4^+ or NO_3^- in the oligotrophic, central North Pacific Gyre. Similarly absence of diel periodicity in *in situ* uptake rates of NO_3^- and NH_4^+ have been observed for natural assemblages collected from N-deplete surface waters of the eastern Canadian Arctic (Harrison, 1983a), the continental shelf off Nova Scotia (Cochlan, 1982, 1986) and the ultraoligotrophic Toolik Lake, Alaska (Whalen and Alexander, 1984a). Although potential rates of NH_4^+ did not show diel periodicity (Cochlan, 1982; Whalen and Alexander, 1984a) diel periodicity of potential NO_3^- uptake rates of the freshwater community was observed. The deeper phytoplankton community in TC.5 was not NO_3^--deplete and more similar in species composition to the upwelled communities of TC.4. Although it did not demonstrate a definitive diel pattern of uptake, lower rates of NO_3^- uptake were generally observed at night with increased uptake rates in the day peaking later than those of the surface community. Miyazaki et al. (1987) observed a similar time delay of uptake maxima for NO_3^- and NH_4^+ during dark bottle incubations of the phytoplankton of Lake Nakanuma, Japan. They attributed this delay to the cumulative increase in stored energy and intermediate carbon compounds produced during photosynthesis and necessary for the uptake and assimilation of nitrogen.

In summary it appears that diel periodicity of nitrogen

uptake is influenced by several confounding factors including: (1) the amount of phytoplankton biomass and its species composition; (2) variation in concentration of ambient nitrogenous compounds as the substrate for N uptake; (3) variation in light intensity (irradiance): and (4) the depth from which plankton is collected, hence preconditioned light history of phytoplankton. In addition, the inhibitory effects of other N forms (e.g., NH_4^+, Tobiesen, 1987), the effect of irradiance and ambient water temperature on periodicity of enzyme activities (e.g., NO_3^- and NO_2^- reductase, Eppley et al., 1970, 1971b) may also be reflected in the observed periodicity of nitrogen uptake by natural phytoplankton assemblages. Eppley et al. (1971a) suggested that the structure and diversity of the phytoplankton community might be affected by diel periodicity of cell division occurring at different times of the day. By the same line of reasoning diel rhythms in N uptake may also play a significant role in the regulation of spatial and temporal distribution of phytoplankton. The results of the present study demonstrate the need for controlled laboratory experiments utilizing unialgal populations in order to isolate and determine the effects of N limitation on the periodicity of N uptake by phytoplankton.

CHAPTER TWO

EFFECTS OF IRRADIANCE ON NITROGEN UPTAKE BY PHYTOPLANKTON: COMPARISON OF FRONTAL AND STRATIFIED COMMUNITIES

INTRODUCTION

In most marine and freshwater systems, the uptake of nitrogenous nutrients by phytoplankton is related to the availability of the nutrients (e.g. MacIsaac and Dugdale, 1969; Probyn, 1985) and photosynthetic photon flux density (PPFD) (e.g., MacIsaac and Dugdale, 1972; Priscu, 1984). The dependence of nitrogen uptake upon PPFD has been described by a rectangular hyperbola similar to the Michaelis-Menten formulation in many marine (e.g., MacIsaac and Dugdale, 1972; Fisher et al., 1982) and freshwater (e.g., Priscu, 1984; Whalen and Alexander, 1984b) communities. Although nitrogen uptake and assimilation by phytoplankton are dependent upon PPFD as an energy source, either directly or indirectly through photosynthesis, the exact biochemical mechanism(s) by which light regulates nitrogen metabolism remains unresolved (e.g. see review by Syrett, 1981). The presence of NO_3^--activated ATPase, apparently located within the cell membranes of a number of marine phytoplankters (Falkowski 1975a,b), provides a physiological basis for the coupling between light and NO_3^- uptake and probably specific ATPases exist for the uptake of NH_4^+ and urea as well. The energy (ATP) generated by photophosphorylation is required for the functioning of these uptake enzymes (permeases) and may also drive the reactions of NH_4^+ (GS/GOGAT) and urea (UAL-ase) assimilation.

In addition, the photogeneration of reductants NAD(P)H and reduced ferredoxin will drive the reduction of NO_3^-, NO_2^- and the GOGAT reaction of NH_4^+ assimilation. Other possible interactions of light with inorganic nitrogen metabolism of phytoplankton are discussed in detail by Syrett (1981).

Numerous culture studies have demonstrated that nitrogen-deprived phytoplankton have greater dark uptake rates of N than N-replete phytoplankton (e.g., Syrett, 1962; Eppley and Coatsworth, 1968; Thacker and Syrett, 1972b; Rees and Syrett, 1979) suggesting a lesser light dependence on N uptake, during N stress. This together with field studies which show that deep-living phytoplankton sustain substantial N uptake velocities with little or no light (e.g., Conway and Whitledge, 1979; Nelson and Conway, 1979; Priscu, 1984) suggests that both light exposure and nutritional history of phytoplankton may be important in determining their ability to sequester nitrogen, and that these controlling factors may differ for the various forms of nitrogen.

Shallow sea fronts, located at the boundary between stratified and vertically mixed regimes (see reviews by Denman and Powell, 1984; LeFèvre, 1986) are generally areas of high primary productivity (e.g., Pingree et al., 1975; Parsons et al., 1981, 1983; Holligan et al., 1984). These regions are characterized by having high phytoplankton biomass in the surface water with measurable concentrations of nitrate, and a shallow pycnocline which extends to the surface at the frontal boundary (e.g., Simpson and Pingree 1978).

A surface transect normal to a frontal boundary progresses from high concentrations of dissolved NO_3^- on the well-mixed side to N-deplete stratified waters and thus represents a gradient of both nitrogen and light availability and consequently phytoplankton physiological states. Moreover, the nitrogenous nutrition of the phytoplankton would likely differ along such a transect. In the N-impoverished waters, the N demands of phytoplankton are supplied by reduced N forms such as NH_4^+ and urea from regenerative processes whereas, in N-rich areas, nitrogen compounds are generally utilized at rates proportional to their availability (e.g., Dugdale and Goering 1967; McCarthy et al., 1977).

The experiments presented in this study were conducted in the Strait of Georgia, a partially enclosed coastal basin on the west coast of Canada (see reviews by LeBlond, 1983; Harrison et al., 1983), where several tidally-induced frontal regions have been previously described (Parsons et al., 1981; Price et al., 1985). The influence of PPFD on the uptake of NO_3^- and urea by phytoplankton from nitrate-replete frontal water and nitrate-deplete stratified water was examined and the dependence of N uptake on PPFD by the phytoplankton from the subsurface chlorophyll maximum of these two distinct areas compared. This study is the first to measure both urea and NO_3^- uptake by natural assemblages of phytoplankton as a function of PPFD. Simulated <u>in situ</u> experimental conditions were attempted in order to obtain a better understanding of the true NO_3^- uptake response to PPFD in these physically and

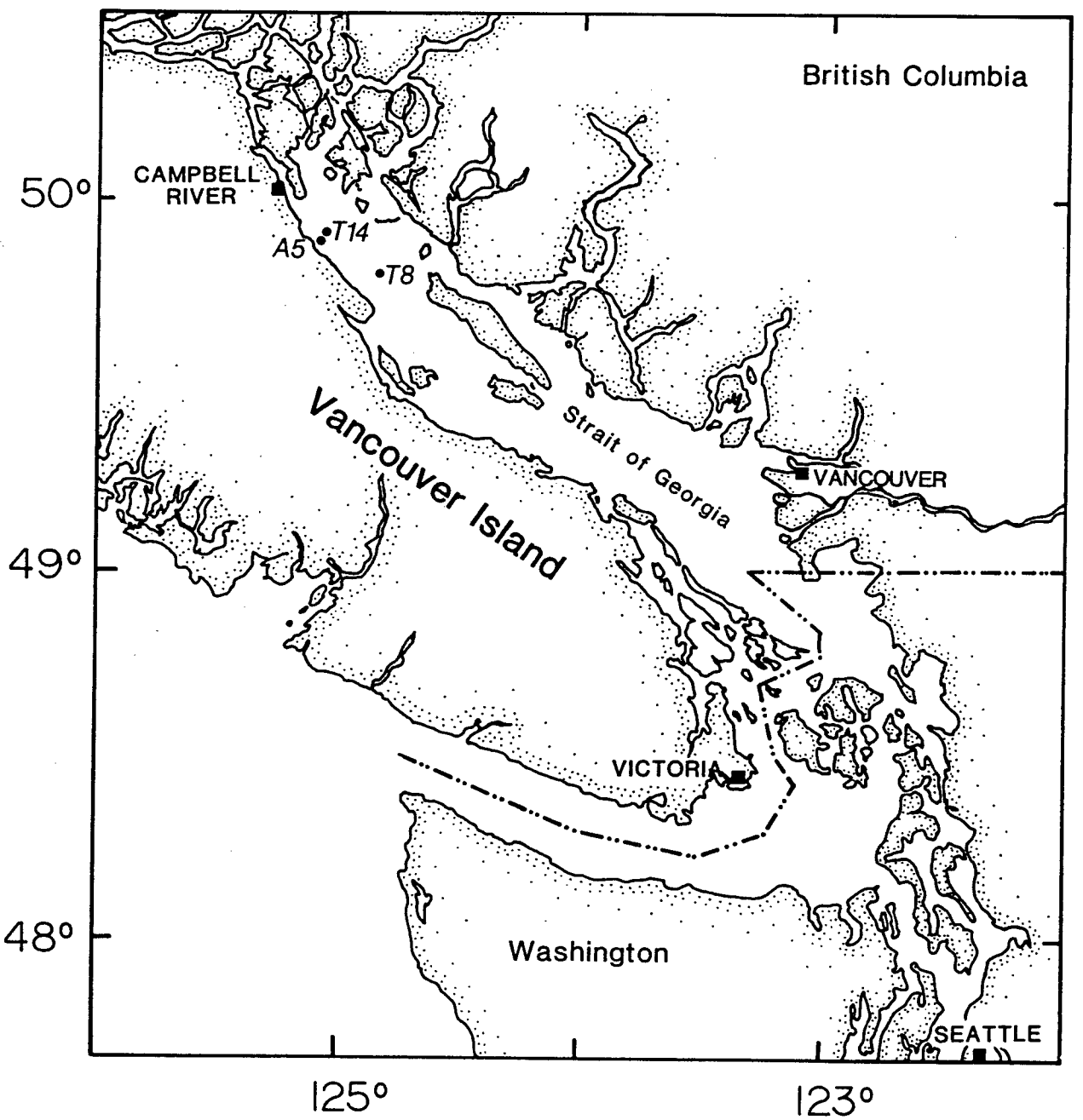
chemically distinct environments. Previous studies of the effect(s) of PPFD on N uptake by phytoplankton have employed saturating enrichments of isotopically labelled N forms (e.g., MacIsaac and Dugdale, 1972; Priscu 1984; Mitamura 1986) and reported uptake rates may reflect the effects of both PPFD and N concentration.

MATERIALS AND METHODS

General

Nitrogen uptake experiments were conducted in the Strait of Georgia, B.C., Canada aboard the C.S.S. Vector during July-August, 1984; station locations are shown in Fig. 2.1. Between 1400 and 1500 h PDT water samples were collected, using 5 L PVC Niskin bottles, from just below the sea surface (0-1 m) and from depths corresponding to the deep chlorophyll maximum (DCM). Samples were shielded from direct sunlight during transfer to 10 L Nalgene^{R} carboys and taken into the ship's laboratory. Subsamples for nutrient analyses were removed with an acid-washed syringe and gently filtered through combusted (460°C for 4 h) Whatman GF/F filters (mounted in 25 mm Millipore Swinex^{R} filter holders) into acid-washed polyethylene bottles. Nitrate plus nitrite (NO_3^- + NO_2^-) and ammonium (NH_4^+) were measured immediately with a Technicon AutoAnalyzer^{R} II, following the procedures outlined in Wood et al. (1967) and Slawyk and MacIsaac (1972), respectively. Urea was determined by the diacetyl monoxime thiosemicarbizide technique described by Price and Harrison (1987). Samples for chlorophyll *a* (Chl *a*) were collected on Whatman GF/F filters and stored frozen in a desiccator. Chl *a* was extracted in 90% acetone overnight and analyzed by *in vitro* fluorometry (Strickland and Parsons, 1972) using a Turner Designs model 10 fluorometer. Particulate organic carbon (POC) and nitrogen (PON), collected on combusted Whatman GF/F filters, were stored similarly and analyzed later after drying (24 h at

Figure 2.1. Station locations for nitrogen uptake experiments. Frontal (T14), shallow stratified (A5) and deeply stratified (T8) stations in the Strait of Georgia, B.C.



<60°C) with a Perkin Elmer model 240 elemental analyzer, using the dry combustion method described by Sharp (1974). The precision of these techniques is given in Appendix 6.

At each station continuous vertical profiles (0-20 m) of temperature, salinity, fluorescence and NO_3^- + NO_2^- were run prior to the bottle casts. Temperature and salinity were determined with an InterOcean 514A CSTD system and *in vivo* fluorescence and NO_3^- + NO_2^- concentrations were obtained from pumped samples (mRoy FR162-144 diaphragm pump, flow rate *ca*. 1 L min^{-1}) and measured with a Turner model 111 fluorometer (equipped with a flow-through cell) and a Technicon AutoAnalyzer^R II, respectively. These data were logged onto a personal computer and plotted in real-time using a custom software programme which compensates for time lags in pumping and machine analyses (Jones, pers. comm.). Incident solar irradiance (PAR, 400-700 nm) was monitored continuously with a Lambda Instruments LI-185 light meter, equipped with a LI-190SB Surface Quantum Sensor, and connected to a chart recorder. Subsurface irradiances were measured with a LI-185B light meter, equipped with a LI-192S Underwater Quantum Sensor.

Phytoplankton samples (250 ml) were preserved in Lugol's solution (Parsons et al., 1984) and stored in the dark until counting. Ten ml subsamples were settled (24 h) and counted on a Wild inverted microscope following Utermöhl (1958).

Experimental

Within 1 h of collection, water samples from each depth

were transferred under reduced light conditions to 500 ml Wheaton glass bottles with teflon-lined caps. Nitrate and urea uptake rates were measured using the stable isotope ^{15}N (Kor Isotopes), as a tracer (Dugdale and Goering, 1967). For the urea experiments, $CO(^{15}NH_2)_2$ (99 atom %) was added to bring the final ^{15}N concentration to 2-4 μg-at $N \cdot L^{-1}$. In the nitrate experiments, $Na^{15}NO_3$ (99 atom %) was added in concentrations of either 0.05 μg-at $N \cdot L^{-1}$ or less than 10% of the ambient NO_3^- + NO_2^- concentration. These enrichments were not always true tracer additions (usually defined as ≤ 10% of ambient), but the term "tracer" will be used here to distinguish the low $^{15}NO_3^-$ enrichments from the saturating enrichments associated with the urea uptake experiments. Following enrichment, bottles were immediately mixed and placed within neutral density screening to simulate the following PPFDs (95, 55, 31, 10, 3.4, 1.1 and 0 $\%I_o$). The screen material used in the incubators was calibrated with a Biospherical Instruments QSL-100 4π sensor placed within an adapted incubation bottle. The 0% PPFD was achieved by wrapping the bottle with black tape. Incubations were conducted at <u>in situ</u> temperature (± 1.5°C) under natural light in clear Plexiglas^R deck incubators. Samples from the surface waters were cooled with flowing surface seawater, while deeper samples were incubated in a separate temperature controlled incubator. Incubations were terminated after 2-4 h by filtration (pressure differential < 125 mm Hg) onto combusted Whatman GF/F filters, placed into plastic petri dishes, and

stored frozen in a desiccator. Based on the ambient nitrogen concentration, the particulate nitrogen concentration and the initial ^{15}N atom % in the particulate fraction, it was calculated that an average (± SD) of 24.1 ± 15.3% and 8.5 ± 5.2% of the NO_3^- and urea, respectively, in solution was incorporated into particulate material during the incubation. At the highest uptake rates achieved, never more than 70% of the NO_3^- and 20% of urea isotope, were incorporated into the PON. Therefore substrate exhaustion was not a problem in the experiments of this study.

Nitrogen in the particulate samples was converted to dinitrogen gas (N_2) by the micro-Dumas dry combustion technique (LaRoche ,1983) and then analyzed for ^{15}N enrichment with a JASCO model N-150 emission spectrometer (Fielder and Proksch, 1975). Nitrogen uptake rates were calculated using equation 7 of Dugdale and Wilkerson (1986) (equivalent to equation 5 of Collos, 1987) which corrects for changes in PON during the incubation period (see Appendix 1, equation 5). Corrections were not made for isotopic dilution from remineralization of ^{14}N-urea during the incubation (Hansell and Goering, 1989) as this correction would probably be negligible given the large amount of ^{15}N-labelled urea added to the bottles. Specific rates of nitrogen transport were calculated by division of the volumetric rates by the phaeophytin-corrected chlorophyll a concentration at the beginning of the experiments. Although chlorophyll a per cell may vary with depth due to PPFD differences, it was chosen as

the normalization parameter because it absorbs the light necessary to fuel cellular transport mechanisms. Chl a specific uptake rates also facilitates the comparison with previously published Chl a normalized nitrogen and carbon uptake versus irradiance studies.

Kinetic parameters of uptake

The kinetic constants for NO_3^- and urea uptake with respect to irradiance were obtained by a direct fit of the data to a modified Michaelis-Menten hyperbola using a computerized, iterative, non-linear least-squares technique (Labtec Notebook Curvefit^R, Laboratories Technclogies Corp.). The Michaelis-Menten equation, modified to account for dark uptake, describes uptake over the hyperbolic light part of the curve (MacIsaac and Dugdale, 1972) and is as follows:

$$V = V_D + V'_{max}\left[\frac{I}{K_{LT} + I}\right]$$

where V is the total uptake of N per unit of chlorophyll, V_D is the dark value of V, I is the integrated average PPFD during the incubation period, V'_{max} is the maximum N uptake per unit chlorophyll at saturating PPFD and K_{LT}, the half-saturation constant for light, is the PPFD at 0.5 V'_{max}. The assumption is made that dark uptake is a constant at all light levels. Only data showing no photoinhibition were used in this analysis.

RESULTS AND DISCUSSION

General description of stations

The vertical profiles of temperature, salinity, relative in vivo Chl a fluorescence and $NO_3^- + NO_2^-$ concentration for the three stations at which N uptake versus PPFD experiments were conducted are presented in Fig. 2.2. The diagnostic features of the frontal water (T14) included both a weak thermocline and halocline which extended from the surface to ca. 9 m, a subsurface fluorescence maximum layer (ca. 5-8 m), a nitracline which extended to the surface and relatively high $NO_3^- + NO_2^-$ concentrations throughout the water column. In the deeply stratified station (T8), fluorescence increased slightly with depth; the nitracline occurred at ca.12 m, and the upper 10 m was devoid of measurable $NO_3^- + NO_2^-$. A strong thermocline and halocline at 5-15 m separated the deep NO_3^--replete water from the NO_3^--depleted mixed surface water. Similar conditions were observed at the shallow-stratified station (A5) but the halocline, thermocline, and nitracline all developed within the upper 5 m of the water column. The initial biomass data and environmental conditions for each station are given in Table 2.1.

The species composition of the phytoplankton community in the frontal and stratified waters varied considerably (Table 2.2). In the frontal waters, large, chain-forming diatoms were the most common phytoplankton at both the surface and the chlorophyll maximum layer (DCM). *Chaetoceros socialis* was the dominant species followed in abundance by *Skeletonema costatum*

Figure 2.2. Depth profiles of temperature (T), salinity (S), <u>in vivo</u> fluorescence (F) and nitrate plus nitrite concentration (N) for the three stations sampled (T14: frontal; A5: shallow stratified; and T8: deeply stratified).

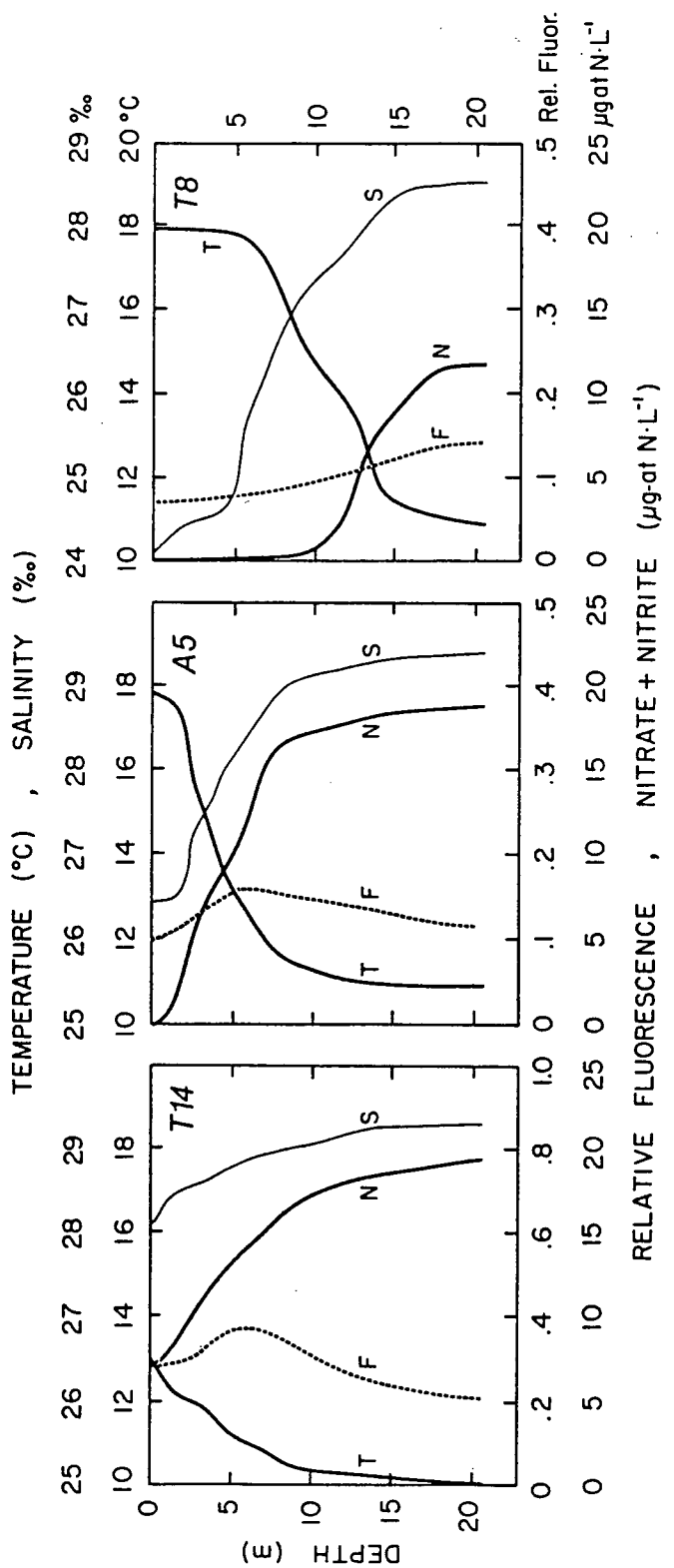


Table 2.1 Initial environmental conditions of seawater collected for N-uptake versus irradiance experiments.

Station and location	Description	Date	Starting time of incubation (PDT)	Sample depth (m)	Nitrogen conc. (μg-at N·L^{-1}) NO_3^-	Urea	NH_4^+*	Chl *a* (μg·L^{-1})	PON (μg-at N·L^{-1})	POC (μg-at N·L^{-1})
T14 49°53'24"N	Frontal	27 Jul 1984	1530	0	6.02	-	0.23	1.29	5.28	43.1
125°05'06"W				8	15.05	-	0.21	2.28	6.96	40.6
A5 49°53'02"N	Shallow	30 Jul 1984	1430	0	<.05	0.63	0.16	0.33	2.57	22.9
125°05'48"W	Stratified			15	20.89	0.72	0.32	0.67	2.01	14.5
T8 49°48'36"N	Deep	1 Aug 1984	1500	0	<.05	0.82	0.17	0.35	2.90	24.1
124°50'39"W	Stratified			15	7.54	0.17	0.40	0.99	3.83	24.1

*NH_4^+ concentrations from separate bottle casts

Table 2.2 Phytoplankton community composition in frontal and stratified water in the Strait of Georgia, B.C.

Station	Depth (m)	Phytoplankton ($\cdot 10^6$ cells$\cdot L^{-1}$) Diatoms	Flagellates*
Frontal T14	0	2.3	0.96
	8	2.2	0.76
Shallow Stratified A5	0	0.23	1.9
	15	0.73	0.79
Deeply Stratified T8	0	0.026	1.5
	15	0.15	1.7

* <5% of flagellates were dinoflagellates

and other diatoms of the genus, *Chaetoceros*, including *C. debilis*. Small pigmented flagellates (<5 μm) were the most abundant phytoplankton in the surface waters of both stratified stations; dominant diatoms were still *Skeletonema costatum* and *Chaetoceros* spp., although *Thalassiosira* spp. and pennate diatoms belonging to *Navicula* and *Nitzschia* genera appeared in small numbers. Dinoflagellates were always a small numerical fraction (<5%) of the total flagellates present and were almost exclusively *Gymnodinium* or *Amphidinium* spp. The deep chlorophyll maximum communities of the two stratified stations differed in the relative abundance of flagellates and diatoms, but the species composition was similar.

Effect of light on nitrogen uptake rates

MacIsaac and Dugdale (1972) first showed that the uptake of nitrate and ammonium by natural phytoplankton assemblages could be related to PPFD by a rectangular hyperbola; PPFD may be treated as a substrate, following Michaelis-Menten kinetics under conditions of no nutrient stress. Such a model assumes that there is no N uptake at zero PPFD (i.e. the PPFD response curve passes through the origin). They suggested that the consequences of not subtracting dark uptake from the light uptake, when uptake in the dark is greater than ca. 15% of uptake at saturating PPFD, can be significant; linear transformations of such kinetic data are distorted beyond usefulness and thus the values of derived parameters questionable. For situations in which dark uptake is a

substantial portion (>10-15% of PPFD - saturated uptake), they proposed a slightly modified equation, employed in the present study, which takes into account a constant dark uptake rate and describes N uptake over the hyperbolic portion of the PPFD response curve, but not photoinhibition. Photoinhibition problems can be overcome by using an equation developed by Parker (1974) or a modification of the equation of Platt et al. (1980) originally developed for the light response of photosynthesis (Lewis and Levine, 1984; Priscu, 1989). Numerous studies in both marine (MacIsaac and Dugdale, 1972; MacIsaac et al., 1974; Nelson and Conway, 1979; Slawyk, 1979) and freshwater natural communities (Priscu 1984; Whalen and Alexander 1984b; Mitamura, 1986) have demonstrated that the uptake response of NO_3^+ and NH_4^- can be successfully described by the Michaelis-Menten formulation.

In the present study, nitrate and urea uptake were dependent on PPFD at both depths sampled in stratified and frontal waters of the Strait of Georgia. Experiments in which the natural phytoplankton communities from the surface and the DCM layers were exposed to a gradient in PPFD yielded data which could be adequately described by the Michaelis-Menten formulation up to inhibiting PPFD levels (Fig. 2.3 and 2.4). Photoinhibition occurred between 55 and 95% of surface PPFD (I_o) and was only observed for samples collected from depth. Photoinhibition of N uptake cannot be adequately discussed in this study due to the paucity of data at high PPFD, but suffice to say, it is not likely a problem for the surface

Figure 2.3. Nitrate uptake of the surface (O) and DCM (●) phytoplankton communities of the Strait of Georgia. The curved plots are fitted directly to the Michaelis-Menten equation; the linear (dashed line) PPFD-inhibited portions were not included in the calculations. Stations are T14 (frontal), A5 (shallow stratified) and T8 (deeply stratified).

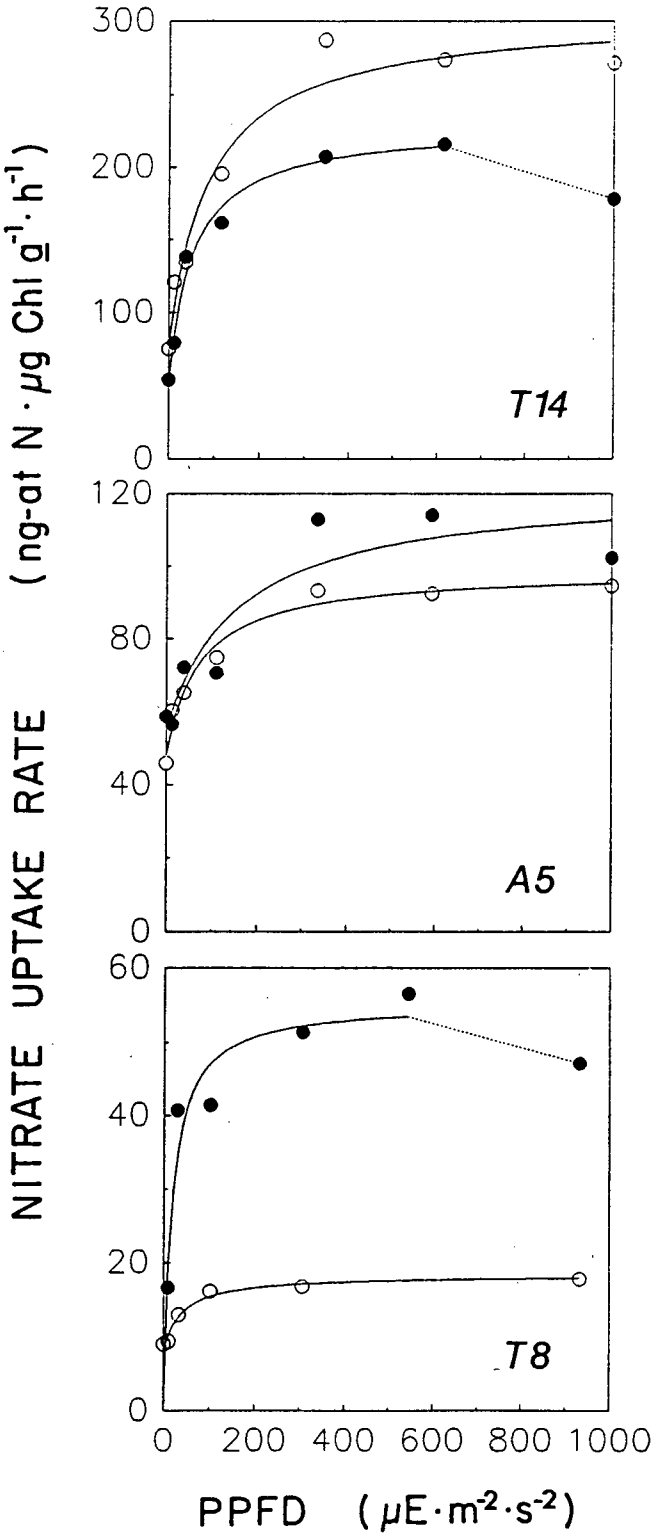
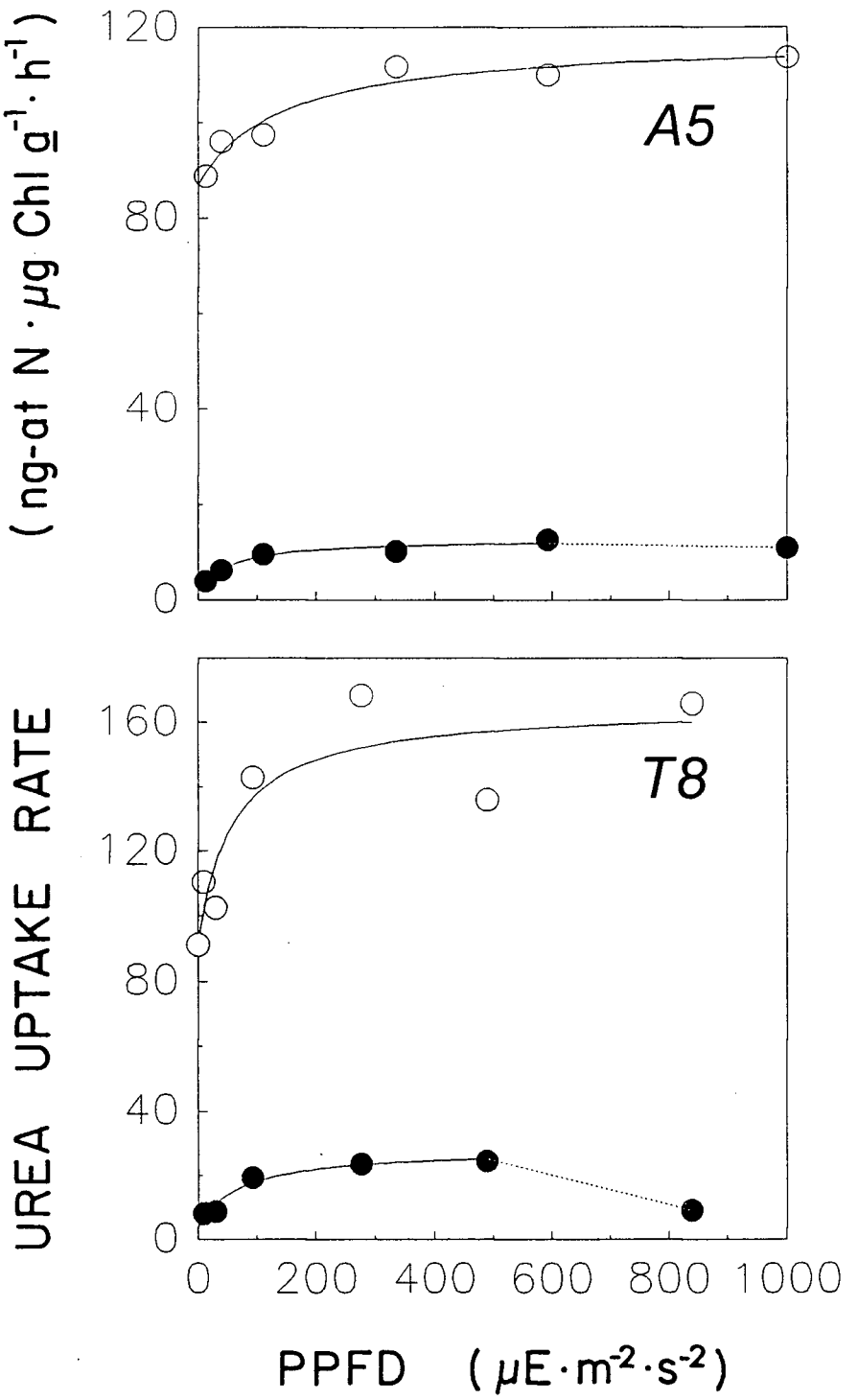


Figure 2.4. Urea uptake of the surface (o) and DCM (●) phytoplankton communities of the Strait of Georgia. The curved plots are fitted directly to the Michaelis-Menten equation; the linear (dashed line) PPFD-inhibited portions were not included in the calculations. Stations are A5 (shallow stratified) and T8 (deeply stratified).



samples, which are naturally exposed to high PPFD; phytoplankton collected near the bottom of the euphotic zone, are effectively excluded from the high PPFD in the mixed surface waters by the pycnocline and are not likely to encounter such high PPFDs naturally.

Kinetic parameters of nitrogen uptake

Dark uptake, the half-saturation constant (K_{LT}), and maximum nitrogen uptake velocity (V'_{max}) for light dependent urea and nitrate nitrogen uptake are summarized in Table 2.3. The K_{LT} values in the present study are those representing the PPFD at which 0.5 V'_{max} occurs. However, it is important to remember that these Michaelis-Menten parameters only represent uptake data from the hyperbolic (or light) portion of the PPFD response curve and do not include the substantial dark N uptake observed. Some investigators (e.g., Priscu, 1984) have ignored dark uptake in the linear transformation of their kinetic data and forced their PPFD response curves to pass through the origin even though dark uptake was substantial (ca. 50% of total N-uptake). Half-saturation constants derived in this manner are not an accurate measure of PPFD at which $V = V_{max}/2$ and should be interpreted with caution, particularly as an indicator of the phytoplankton communities' abilities to assimilate specific N substrates at low PPFD. A better estimate of the PPFD at which one-half the total maximal N uptake of the phytoplankton community is achieved (K_{LT}') can be calculated by a simple rearrangement of the Michaelis-Menten equation employed in the present study:

Table 2.3 Parameters describing the characteristics of nitrogen uptake, as a function of PPFD, for phytoplankton assemblages in the Strait of Georgia, B.C. Stations are T14: frontal; A5: shallow stratified; and T8: deeply stratified. Definitions are given in the text, estimated standard errors of parameters in parentheses.

Station	Nitrogen substrate	Depth (m)	V_D (ng-at N (μg Chl$\underline{a}$)$^{-1}$ h^{-1})	V_{max} (ng-at N (μg Chl$\underline{a}$)$^{-1}$ h^{-1})	K_{LT} ($\mu E \cdot m^{-2} \cdot s^{-1}$)	K_{LT} (%I_o)
T14	NO_3^-	0	80 (16.8)	225 (22.3)	91 (40.1)	8.2 (3.6)
		8	53 (9.4)	174 (12.5)	53 (15.7)	4.8 (1.4)
A5	NO_3^-	0	48 (3.2)	50 (4.0)	74 (25.9)	6.7 (2.3)
		15	55 (8.8)	67 (15.3)	156 (151)	14 (14)
	Urea	0	87 (4.2)	31 (4.3)	140 (103)	13 (9.3)
		15	1.8 (2.3)	11 (1.9)	54 (40.7)	4.9 (3.7)
T8	NO_3^-	0	8.5 (0.79)	9.8 (1.02)	45 (18.7)	4.6 (1.9)
		15	0	55 (4.2)	18 (6.6)	1.8 (0.7)
	Urea	0	92 (14.1)	73 (17.6)	59 (63.4)	6.7 (7.2)
		15	4.0 (3.9)	24 (3.6)	72 (56.2)	8.2 (6.4)

$$K_{LT}' = (V - V_D) \cdot K_{LT}/(V'_{max} - V + V_D)$$

where $V = (V'_{max} + V_D)/2$, and V'_{max} is the maximum uptake described by the rectangular hyperbola, K_{LT} its half-saturation constant, and V_D is the dark uptake rate. Alternatively, another half-saturation constant, $K_{LT}"$, can be calculated by substituting V for one-half the velocity of N uptake at saturating PPFD. Both of these derived half-saturation constants will generate values that are more realistic measures of the PPFD at one-half the actual maximum N uptake taking place in the phytoplankton community as they include dark uptake (Table 2.4).

The values of the half-saturation constant for NO_3^- uptake in the present study range from 0-14% I_o, which is consistent with previously published values for marine and freshwater natural phytoplankton assemblages (Table 2.5). The K_{LT} values for urea uptake are similar (0-13% I_o). Previously published kinetic studies for urea are few. Webb and Haas (1976) report a K_{LT} of ca. 0.01 langleys$\cdot$min^{-1} (35$\cdot\mu$E$\cdot$m$^{-2}\cdot$s^{-1}) for phytoplankton from the York River estuary in Virginia during the summer, although values in the autumn ranged from 0.02 to 0.12 langleys$\cdot$min^{-1} (69-418 μE$\cdot$m$^{-2}\cdot$s^{-1}). A similar summer K_{LT} value was reported by Mitamura (1986) for urea uptake by phytoplankton from oligotrophic Lake Biwa in Japan (2.44 Klux = 39 μE$\cdot$m$^{-2}\cdot$s^{-1}). They also reported a similar value K_{LT} for NH_4^+ uptake (28 μE$\cdot$m$^{-2}\cdot$s^{-1}) and a greater K_{LT}

Table 2.4 Indices of N uptake dependency on PPFD for phytoplankton in the Strait of Georgia: the ratio of dark to light-saturated uptake rate (V_D:V_L), the PPFD at which half of total N uptake occurs (K_{LT}', K_{LT}'')*, ratio of uptake under 1% I_o to 55% I_o ($V_{1\%}$:$V_{55\%}$). The K_{LT} values are expressed as PPFD values and as a percentage of surface PPFD (I_o) which is shown in parentheses.

Station	Nitrogen substrate	Depth (m)	V_D:V_L	K_{LT}' ($\mu E \cdot m^{-2} \cdot s^{-1}$)	K_{LT}''	$V_{1\%}$:$V_{55\%}$
T14	NO_3^-	0	0.28	36 (3.2)	43 (3.9)	0.38
		8	0.25	24 (2.1)	28 (2.5)	0.40
A5	NO_3^-	0	0.51	0 -	1.6 (0.1)	0.60
		15	0.49	3.0 (0.3)	15 (1.3)	0.56
	Urea	0	0.77	0 -	0 -	0.80
		15	0.15	33 (3.0)	39 (3.5)	0.33
T8	NO_3^-	0	0.48	1.9 (0.2)	3.1 (0.3)	0.60
		15	0	0 -	0 -	0.37
	Urea	0	0.58	0 -	0 -	0.65
		15	0.16	39 (4.5)	52 (5.8)	0.28

*Definitions given in text.

Table 2.5 Comparison of half-saturation constants (K_{LT}) for inorganic nitrate transport in various aquatic ecosystems.

Region	Area	Depth (% I_o)	$K_{LT}(NO_3^-)$ % surf light range (mean)	$K_{LT}(NO_3^-)$ $\mu E \cdot m^{-2} \cdot s^{-1}$ range (mean)	Reference
Oceanic	E. Tropical Pacific	25	14.0	-	MacIsaac & Dugdale (1972)
Upwelling	Peru	100	0.9 - 12.7 (5.4)	14 - 108 (63)*	MacIsaac & Dugdale (1972)
		10	0.9 - 13.3 (8.9)	7 - 199 (122)*	
Upwelling	N.W. Africa	50	1.5 - 7.0 (5.4)	-	MacIsaac et al. (1974)
Upwelling	N.W. Africa	50-0.1	5.5 - 6.2 (5.9)	-	Nelson & Conway (1979)
Upwelling	Baja Calif., Mexico	50-3	3.3 - 32.4 (16.1)	-	Nelson & Conway (1979)
Upwelling	Antarctic	50-25	1.1 - 2.3 (1.7)	2.3 - 4.4 (3.3)**	Slawyk (1979)
		7	1.3	2.8**	
Coastal	Peru	100	4.4	45	MacIsaac & Dugdale (1972)
		10	1.0	14	
Coastal	Strait of Georgia	100	4.6 - 8.2 (6.5)	45 - 91 (70)	Present study
		ca. 1	1.8 - 14 (6.9)	18 - 156 (76)	
Freshwater	Toolik L., Alaska	ca. 10-15	6 - 31 (15)	7 - 29 (16)	Whalen & Alexander (1984b)
Freshwater	L. Kinneret, Israel			77	Berman et al. (1984)
Freshwater	L. Biwa, Japan	100	4.29	70.8***	Mitamura (1986)
Freshwater	Castle L.,	ca.50	2.6 - 2.7 (2.65)	15.1 - 16.2 (15.7)+	Priscu (1984)
	Calif., U.S.A.	ca. 1	0.6 - 3.7 (1.55)	4.6 - 25.5 (10.7)+	
Freshwater	L. Vanda, Antarctica	10.2	0.5 - 2.0	2.4 - 9.5	Priscu (1989)
		1.0	0.4 - 5.1	1.7 - 24.9	
Freshwater	L. Fryxell, Antarctica	0.4	0.08	0.04 (?)	Priscu (1989)

Table 2.5 Continued

* Values calculated by converting from $ly \cdot min^{-1}$ using 1 $ly \cdot min^{-1}$ = 3485 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Richardson et al., 1983)

** Values calculated by converting from $quanta \cdot m^{-2} \cdot h^{-1}$ using 1 $quanta \cdot m^{-2} \cdot h^{-1}$ = 4.614×10^{-22} $\mu E \cdot m^{-2} \cdot s^{-1}$ (Lüning, 1981)

*** Values calculated by converting from klux using 1 klux = 16.5 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Richardson et al., 1983)

\+ Values calculated from total PPFD during incubation periods (ca. 12 h)

? Probable error, correct value from data given in Priscu (1989) is 0.4 $\mu E \cdot m^{-2} \cdot s^{-1}$

value for nitrate uptake (67 $\mu E \cdot m^{-2} \cdot s^{-1}$). Interpretation of the small differences in the K_{LT}'s of the present study (less than 50 $\mu E \cdot m^{-2} \cdot s^{-1}$), either between N substrates or the communities taken from different depths, is rather difficult. However this kinetic parameter has been included for literature comparative purposes. A simpler and more straightforward index to assess the effect of PPFD on N uptake can be determined by comparing N uptake at low ($1\%I_o$) and saturating ($55\%I_o$) PPFD (Conway and Whitledge, 1979); lower percentages represent greater PPFD dependency. At the frontal station (T14) both surface and deep chlorophyll maximum (DCM) communities have the same PPFD dependency for NO_3^- uptake (38-40%); which is likely a reflection of the similarity in both the species composition and the physiological state of these two N-replete communities. At the shallow stratified station (A5), the two phytoplankton communities are very similar with respect to NO_3^- uptake response (60, 56%) but there is a substantial difference between the surface and DCM urea uptake dependency: 80 and 33%, respectively. Similar large differences were found for both NO_3^- (60, 37%) and urea (65, 28%) in the two communities of the deeply stratified station (T8). It appears that uptake of the regenerated N source, urea, has a greater dependence on PPFD in the NO_3^-- replete DCM community which was effectively isolated from the well-lit surface layers by the strong pycnocline present and only normally received <u>ca</u>. 1-3% I_o. The lesser PPFD dependency of the surface phytoplankton may be a consequence of their N-

depleted physiological state, which could explain the decrease in PPFD dependency of NO_3^- uptake in the surface populations of the stratified waters (60%) relative to the frontal waters (38%). Alternatively it may be attributed merely to an accumulation of stored energy and C skeletons produced during photosynthesis. One cannot directly compare the uptake responses of urea and nitrate in the surface waters due to differences in ^{15}N-enrichment (saturating versus trace). At the DCM, however, saturating NO_3^- conditions were present during all the uptake experiments. It appears that the phytoplankton in the DCMs had a similar degree of PPFD dependency for NO_3^- uptake which was similar to that for urea in the stratified DCM communities.

Dark nitrogen uptake

Nitrate and urea uptake occurred in the dark in both frontal and stratified waters (Table 2.3) In the stratified surface waters (A5, T8), the relative contribution of dark NO_3^- uptake to total NO_3^- uptake under saturating PPFD was ca. 50% while in the surface waters of frontal stn (T14) the dark uptake contribution was only 28% (Table 2.4). At the deeply stratified stn (T8), there was no dark NO_3^- uptake by the DCM population whereas the relative dark NO_3^- uptake at the shallow stratified (A5) and frontal (T14) DCM communities were similar to their respective surface phytoplankton communities (49 and 25%). Dark uptake of urea was also a substantial portion of total urea uptake averaging 16 and 68% for the DCM and surface communities, respectively. Dark N uptake by

phytoplankton is not uncommon and a summary of literature values of the ratio of dark to light N uptake rates (V_D:V_L) for natural phytoplankton assemblages is shown in Table 2.6. A review of the literature permits two generalizations to be made concerning dependence of light for N uptake: 1) in N-impoverished waters, the V_D:V_L ratio is greater (approaching unity) than in N-replete waters, suggesting the enhancement of dark uptake by nutrient stress, and 2) V_D:V_L ratio is generally greater (closer to unity) in samples collected from and incubated under lower PPFD, suggesting a lesser dependence of light for N uptake with increasing depth in the euphotic zone. The first suggestion is not new as many laboratory experiments have shown that N deprivation enhances to a greater degree the uptake of N in the dark than in the light. (e.g., Syrett, 1962; Thacker and Syrett, 1972; Harrison, 1976; Rees and Syrett, 1979). The ability to take up nitrogen in the dark may be, however, species dependent; for example Eppley et al. (1971b) showed that although a somewhat N-depleted tropical oceanic coccolithophorid (*Emiliana huxleyi*) took up nitrate in the dark, a similarly N-depleted coastal diatom (*Skeletonema costatum*) did not. Also whether or not a species is able to take up a significant amount of nitrogen at night or in the dark may depend on its degree of N depletion. This is suggested for nitrate-limited continuous cultures of *Chaetoceros* spp. (Malone et al., 1975); at the three lower dilution rates, nitrate uptake was continuous and independent of the natural light/dark cycle, but there was diel

Table 2.6 Summary of literature values of dark:light nitrogen specific (V_D/V_L) or absolute (ρ_D/ρ_L) uptake rates, determined during daytime, in natural phytoplankton communities.

Area	Ambient NO_3^- conc (μg-at $N \cdot L^{-1}$)	ρ_D/ρ_L or V_D/V_L NO_3 range (mean)	NH_4 range (mean)	Urea range (mean)	Reference
Oceanic					
N. Atlantic Gyre (Sargasso Sea)	-	(0.30)	- (0.59)	-	[a]Dugdale & Goering (1967)
N. Pacific Central Gyre					
- 50°N, 155°W	>10	0.0 -0.63 (0.30)	0.38- 2.0 (0.83)	-	Hattori & Wada (1972)
- 40°N, 150°W	1.0	0.92	0.78- 1.5 (1.2)		
N. Pacific Ocean					
- northern (J1-J7)	>2	0.0 - 9.7 (2.4)	5.7 -27.4 (16.9)	0.0-18.5 (9.3)	Kanda et al. (1985)
- tropical/subtropical (J9-J23)	<0.1	7.5 -31.9 (22.4)	16.6 -52.9 (34.0)	12.2-49.8 (25.7)	"
N.E. Pacific Ocean	12	0.08-1.01 (0.09)[g]	-	-	Cochlan (Chap. 1)
Upwelling					
N.W. Africa	>10	0.05-0.57 (0.36)	0.00-0.02 (0.07)		Nelson & Conway (1979)
Baja Calif., Mexico	>10	0.10-1.46 (0.49)	0.01-0.67 (0.16)	-	Nelson & Conway (1979)
Baja Calif., Mexico*	-	0.34	0.02	-	[b]MacIsaac (1978)

Table 2.6 continued

Area	Ambient NO_3^- conc (μg-at N·L^{-1})	ρ_D/ρ_L NO_3 range (mean)	V_D/V_L NH_4 range (mean)	Urea range (mean)	Reference
Polar					
Scotia Sea	>20	0.30-0.47 (0.38)	0.88-1.2 (1.0)	-	[c,g]Glibert et al. (1982a)
Scotia Sea	>20	13.0-75.0	0.27-1.0	-	[c]Rönner et al. (1983)
Barents Sea	0-1.5	-	-	0.3-0.5	[c]Kristiansen & Lund (1989)
Coastal					
Oslofjord (Norway)	>2	0.06-0.57 (0.17)	-	-	[a]Paasche & Erga (1988)
	<1	0.18-1.7 (0.47)			
New York Bight	ca. 0.1	0.2 -1.0 (0.7)	0.5 -1.3 (0.7)	-	Conway & Whitledge (1979)
Gulf of Maine	ca. 1-2	0.00-1.00 (0.26)	0.00-0.20 (0.10)	-	[a,b]Dugdale & Goering (1967)
Peru*	0.09	0.60-0.86 (0.73)	-	-	Dortch & Maske (1982)
Strait of Georgia, B.C.					
- Frontal	3.0-4.6	0.00-0.08 (0.03)	0.37-0.39 (0.38)	0.00-0.81 (0.36)	Price et al.
- Stratified	<0.05	0.00-0.18 (0.09)	0.52-0.58 (0.55)	0.06-0.66 (0.36)	(1985)

Table 2.6 continued

Area	Ambient NO_3^- conc (μg-at N·L^{-1})	ρ_D/ρ_L NO_3 range	(mean)	or V_D/V_L NH_4 range	(mean)	Urea range	(mean)	Reference
Strait of Georgia, B.C.								
- Frontal	6-15	0.25-0.28	(0.27)	-		-		Present study
- Surface stratified	<0.05	0.48-0.51	(0.50)	-		0.58-0.77	(0.68)	"
- Bottom stratified	7-20	0.00-0.49	(0.25)	-		0.15-0.16	(0.16)	"
Washington coast	-	-	(0.21)	-	(0.43)	-	(0.38)	Dortch & Postel (1989)
Western Irish Sea								
- Surface stratified	ca. 2.5	-		-		0.47-1.1	(0.67)	[h]Turley
- Mixed & bottom strat.	ca. 4.5	-		-		0.37-1.3	(0.72)	(1985)
Estuarine								
Pamlico River, N.C.		-		0.71-0.82		-		Fisher et al.,
South River, N.C.		-		0.18-1.01	(0.57)	-		(1982)
Neuse River, N.C.		-		0.04-0.95	(0.61)	-		"
Newport River, N.C.		-		0.02-0.11	(0.06)	-		"
Delaware Bay		0.00-0.09		0.06-1.02		-		"
Chesapeake Bay		-		0.26		-		"
Freshwater								
L. Kinneret (Israel)	ca. 0.10	0.40-0.91	(0.56)	0.29-1.0	(0.60)	0.13-0.67	(0.34)	McCarthy et al.
	0.2-0.6	0.16-0.33	(0.22)	0.53	(0.53)	0.33-0.44	(0.38)	(1982)
L. Kinneret (Israel)	<0.05	0.32		0.59		-		Berman et al. (1984)
L. Nakanuma (Japan)	-	-		0.21-1.1	(0.57)	-		[d]Miyazaki et al. (1985)

Table 2.6 continued

Area	Ambient NO_3^- conc (μg-at N·L^{-1})	ρ_D/ρ_L NO_3 range (mean)	V_D/V_L NH_4 range (mean)	Urea	Reference
L. Biwa (Japan)	-	0.26	0.78	0.51	Mitamura and Saijo (1986)
L. Kasumigaura** (Japan)	≤0.07	0.18-0.27	0.60-0.90	0.71	Takamura et al. (1987)
Shagawa L. (Minnesota, U.S.A.)	-	-	0.27-2.3 (1.0)	-	[a,e] Toetz & Cole (1980)
Toolik L. (Alaska, U.S.A.)	ca. 1.0	0.05-0.31 (0.15)	0.27-0.57 (0.41)	-	Whalen & Alexander (1984b)
L. Vanda (Antarctica)	0.0-0.7				
sfc pop'n		0.41	0.47	-	[f] Priscu (1989)
deep-chl pop'n		0.24	0.83		
Czechoslavakian reservoirs	>35	0.05-0.38 (0.11)	-	-	Procházková et al. (1970)
L. Calado (Brazil)	ca. <0.1	0.00-0.32 (0.16)	0.11-1.0 (0.49)	-	Fisher et al. (1988)
Amazon River (Brazil)	11.1	0.20	0.54	-	Fisher et al. (1988)
Castle L., (California, U.S.A.)	0-ca. 2.5	- (0.55)	- (0.50)	-	[c] Priscu (1984)
L. Ontario (Ontario, Canada)	0-15	0.02-0.30 (0.14)	0.30-0.60 (0.40)	-	[g] Liao and Lean (1978)

Table 2.6. Superscripts.

[a]Values calculated as $1/(V_L/V_D)$ or $1/(\rho_L/\rho_D)$ from reported values of V_L/V_D or ρ_L/ρ_D.

[b]Values estimated from figures.

[c]Values reported in text, no data available.

[d]Values calculated from turnover times.

[e]Light rates determined at ambient N conc., dark rates determined at saturating N conc.

[f]Values are $\rho_d/N_m{}^b$, where $N_m{}^b$ is the chlorophyll specific transport rate at optimal PPFD level.

[g]Experiments utilized 24 h incubations over natural light/dark cycle.

[h]Average values reported.

*Dinoflagellate bloom.

**_Microcystis_ bloom

periodicity in nitrate uptake at the highest dilution rate. It should be noted that the dark uptake rates reported in the present study and used in the ratios of Table 2.6 were determined during daylight and may not necessarily reflect the uptake rates observed during the night. During 24 h time course experiments conducted in the same waters as the present study I observed a constancy in $V_D:V_L$ for NH_4^+ uptake in frontal waters (Chapter 1) which suggests that NH_4^+ uptake is circadian. This conclusion is supported by Goering et al. (1964) who found rhythmic variation in both NH_4^+ and NO_3^- uptake by Sargasso Sea phytoplankton incubated under continuous light. However, in the stratified waters of the Strait of Georgia, $V_D:V_L$ for NO_3^- and urea demonstrated both diel and diurnal variability. Diurnal (daytime) variability in the $V_D:V_L$ of NH_4^+ uptake by freshwater phytoplankton assemblages of Lake Calado (Fisher et al., 1988) and the South River estuary (Fisher et al., 1982) have also been observed. In the present study, all the experiments were conducted at approximately the same time of the day thereby compensating for any diurnal variability in N uptake, (either independent or dependent of the daily light cycle) and thus permitting comparisons between stations.

An unknown portion of the dark uptake in the present experiments may also be attributed to marine heterotrophic bacteria. Wheeler and Kirchman (1986), using metabolic inhibitors, size-fractionation and ^{15}N methodology, estimated that 78% of the ammonium uptake in the surface waters off

Sapelo Island, Georgia and the Gulf Stream off Georgia was due to bacteria. Brown et al. (1975) reported NO_3^- uptake and reduction and NH_4^+ uptake by batch cultures of a marine pseudomonad and Remsen et al.(1972) have demonstrated competition for urea among both bacteria and phytoplankton of the estuaries/coastal waters of Georgia. During the experiments reported in this study Whatman GF/F filters were used to collect the particulate material after incubation with ^{15}N-labelled urea and NO_3^-; these filters do not discriminate completely between bacteria and phytoplankton and can capture 40-50% of the bacteria in marine systems (R. Keil, pers. comm.). In the present study, the proportion of uptake of inorganic and organic N which may be attributed to bacteria is unknown; previous studies in shallow sea frontal systems have reported both greater bacterial biomass and relative heterotrophic activity (as determined by glucose uptake) on the stratified side of a front in Saanich Inlet (Parsons et al., 1983), in Liverpool Bay (Floodgate et al., 1981), and the Irish Sea (Egan and Floodgate, 1985; Lochte, 1985).

Summary

The N uptake response to PPFD of the phytoplankton in the frontal and stratified communities of the Strait of Georgia can be described by the Michaelis-Menten formulation. Dark uptake of nitrate and urea is a substantial portion of the total uptake in these phytoplankton communities, and should not be overlooked. In the frontal waters, the dependency on PPFD for NO_3^- uptake is similar for both surface and DCM

communities, whereas in the stratified waters, the surface phytoplankton exhibit less PPFD dependency than those from the DCM, particularly for urea uptake. The dramatic change in species composition of the phytoplankton communities from one dominated by large, chain-forming diatoms in the N-replete frontal waters to one composed primarily of microflagellates in the N-depleted stratified waters probably contributed to the observed variability in their PPFD response and precludes a simple explanation of PPFD effect(s) on N uptake based merely on phytoplankton N status. Clearly more detailed studies on the response of N uptake to PPFD in unialgal (and axenic) phytoplankton cultures, at various degrees of N deficiency, need to be conducted before the effect(s) of N limitation on the N uptake response to PPFD can be adequately explained.

CHAPTER THREE

NITROGEN UPTAKE BY THE EUCARYOTIC PICOPLANKTER, *MICROMONAS PUSILLA* AND THE EFFECTS OF N DEPRIVATION ON UPTAKE RESPONSE

INTRODUCTION

It is well established that the availability of nitrogen is the dominant nutritional factor regulating phytoplankton growth in coastal (e.g., Ryther and Dunstan, 1971) and oceanic waters (e.g., Thomas, 1966, 1969; Goldman et al., 1979). The focus of many investigations has been on the kinetics of nitrogen uptake by marine phytoplankton in order to understand how phytoplankton respond and adapt to nitrogen limitation. Dugdale (1967) first proposed relating nitrogen uptake rates of phytoplankton to the external N concentration by a hyperbolic function which was similar to the Michaelis-Menten equation for enzyme kinetics, $V = V_{max}\ [S/(K_s+S)]$, where V is the uptake velocity (h^{-1}), V_{max} the maximal uptake velocity, S the concentration of limiting substrate and K_s the half-saturation constant representing the value of S at which V is equal to half the maximum uptake rate (i.e.,$V = V_{max}/2$). Measurement of uptake rates by natural phytoplankton assemblages (e.g., MacIsaac and Dugdale, 1969; Probyn, 1985; Kanda et al., 1985; Whalen and Alexander, 1986) and numerous culture studies (e.g., Eppley and Coatsworth, 1968; Eppley and Thomas, 1969; Eppley et al., 1969) have demonstrated that the uptake of nitrogenous nutrients can be described by this hyperbolic function which relates uptake rate to the limiting nutrient concentration.

The species specific nutrient uptake kinetic parameters, V_{max} and K_s may be used to explain species competition involving the limiting nutrient (Dugdale, 1967; Tilman, 1977; Button, 1985). It is now realized that V_{max} is frequently variable over time, and for some nutrients it may be elevated initially over the first few minutes following enrichment of the limiting nutrient with uptake proceeding at a slower, more constant rate later. Several studies have demonstrated that N-deficient or N-starved phytoplankton have the ability to rapidly take up ammonium initially upon exposure to an elevated NH_4^+ concentration in the culture medium (Conway et al., 1976; Conway and Harrison, 1977; McCarthy and Goldman, 1979; Dortch et al., 1982; Goldman and Glibert, 1982; Horrigan and McCarthy, 1982; Parslow et al., 1984a b) and in the field (Glibert and Goldman, 1981; Wheeler et al., 1982; Harrison, 1983a; Priscu and Priscu, 1984, Priscu, 1987; Suttle and Harrison, 1988). In contrast, after a nitrate addition to a N-starved culture there is often, but not always, a lag whose duration is quite variable, before nitrate uptake is observed at either elevated, normal or reduced velocities (Dortch et al., 1982; Collos, 1983; Parslow et al., 1984 b). Nitrogen starvation may (Rees and Syrett, 1979; Horrigan and McCarthy, 1981; Syrett et al., 1986; Price and Harrison, 1988), or may not (Bekheet and Syrett, 1979) increase urea uptake rates although only three phytoplankters have been tested to date.

Uptake interactions between inorganic nitrogen sources, and more particularly ammonium and nitrate, have been the

subject of many culture studies (see reviews by Morris, 1974; McCarthy, 1981; Syrett, 1981, Collos, 1989, Dortch, in press). These studies have revealed a variety of responses depending on species and their nutritional state, but one of the main tenets still held in phytoplankton ecology is that the uptake of nitrate stops when the ambient NH_4^+ concentration exceeds a certain threshold and resumes when algal uptake causes the ambient NH_4^+ concentration to decrease below this threshold value. The negative effect of NH_4^+ on NO_3^- uptake is often not that severe and there are numerous examples of simultaneous and equal rates of uptake of both NO_3^- and NH_4^+, mostly in N-deficient or N-limited cells (e.g., Caperon and Ziemann, 1976; Conway, 1977; DeManche et al., 1979) and in the field (e.g., Conover, 1975; McCarthy et al., 1977; Maestrini et al., 1982, 1986; Price et al., 1985; Collos et al., 1989).

The literature is replete with studies on many aspects of phytoplankton nitrogen utilization, including: 1) the estimation of kinetic parameters, 2) their transient nature under conditions of physiological stress and 3) the interference/interaction of multiple nitrogen sources. However, there are few observations of nitrogen utilization by picoplankton (0.2 μm - <2.0 μm, Sieburth et al., 1978) and there does not appear to be any kinetic studies on N uptake by marine picoplankters. The ubiquitous and usually abundant presence of both cyanobacteria and eucaryotic algae in this size class has been reported in offshore and nearshore waters (see reviews by Fogg, 1986; Joint, 1986; Stockner and Antia,

1986; Mikheyeva,1988). Their importance as photoautotrophs has been demonstrated in certain environments, particularly oligotrophic, oceanic regions, where picoplankton are responsible for the majority of photosynthetic production (e.g., Li et el., 1983; Platt et al, 1983; Takahashi and Bienfang,1983; and above reviews). Studies with size-fractionation techniques and nitrogen tracers have confirmed that picoplankton nitrogen uptake is also substantial, averaging 10-30% and 30-70% of the total N uptake of the natural communities of coastal and oceanic waters, respectively (Glibert,1982; Nalewajko and Garside, 1983; Probyn, 1985; Probyn and Painting, 1985; Harrison and Wood, 1988).

In the present study the utilization of nitrate, ammonium and urea by the prasinophyte, picoflagellate *Micromonas pusilla* (Butch.) Manton et Parke was demonstrated by using N-replete and N-starved cells and measuring the uptake rate of NO_3^- in the presence of potential competitors, ammonium and urea. Therefore, the objective of this research was to determine the basic aspects of nitrogenous nutrition of this ubiquitous, eukaryotic picoflagellate. This study is the first to report on the nitrogen utilization of a cultured picoplankter.

MATERIALS AND METHODS

Culturing

Stock cultures of *Micromonas pusilla* (culture NEPCC 29-1 Northeast Pacific Culture Collection, Department of Oceanography, University of British Columbia) were maintained on filter-sterilized (0.22 μm Millipore) nutrient-enriched artificial seawater based on ESAW (Harrison et al., 1980). Modifications to ESAW included replacing ferrous ammonium sulfate ($FeNH_4(SO_4)_2 \cdot 6H_2O$) and sodium glycerophosphate with equimolar concentrations of ferric chloride ($FeCl_3 \cdot 6H_2O$) and sodium phosphate (Na_2HPO_4), respectively (Parslow et al., 1984a). Sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$) was prepared and added as described by Suttle et al. (1986) and 10 nM Se was added as selenite (Na_2SeO_3) according to Harrison et al. (1988). Nitrate, the sole nitrogen enrichment, was reduced from 550 to 50 μg-at $N \cdot L^{-1}$. Reagent grade chemicals were used in preparing salt and nutrient enrichment solutions in deionized, distilled water (DDW). Glass and polycarbonate flasks used for culturing algae and storing ESAW were soaked in freshly-made 10% HCl (v/v) for at least 2-3 days, rinsed thoroughly with DDW, and autoclaved prior to use.

All cultures (i.e. stock and experimental) were continuously illuminated from two sides by six Vita-Lite^R UHO fluorescent tubes (3 on either side of culture vessels). The light was filtered through 3 mm thick blue Plexiglas^R (No. 2069, Rohm and Haas) and the irradiance, measured with a 2π collector (LiCor LI-192SB) from the centre position of the

culture vessels, was ca. 120 $\mu E \cdot m^{-2} \cdot s^{-1}$ (saturating for growth of *M. pusilla*, see Appendix 2). Temperature was maintained at 17°C (± 0.5°C) in a temperature regulated water bath and cultures continuously stirred by teflon-coated magnetic stir bars at 60 rpm. Cultures were unialgal and sterile technique was employed to minimize bacterial contamination. Cell growth was monitored by in vivo chlorophyll a fluorescence measured by a Turner Designs model 10 fluorometer.

Analytical methods

Cell counts were measured with a Coulter Counter^R model TA II electronic particle counter with the population accessory (enabling cells to be counted into 16 channels based on their volume) and equipped with a 30 μm aperture. The Coulter Counter^R was calibrated with latex microspheres of 2.02 μm in diameter. Culture samples were diluted (1:20) with freshly filter-sterilized, unenriched ESAW (gravity filtration, Whatman GF/F) and gently homogenized (i.e. mixed) prior to counting. Average cell volumes were computed from the particle size distribution based on equivalent spherical diameter.

Concentrations of dissolved NO_3^- + NO_2^- and NH_4^+ were measured with a Technicon AutoAnalyzer^R II following the procedures outlined in Wood et al., (1967) and Slawyk and MacIsaac (1972), respectively. Samples for nutrient analyses were filtered through precombusted (460°C for 4 h) Whatman GF/F filters into previously acid-washed, DDW-rinsed polypropylene bottles. Ammonium concentrations were always

determined immediately while NO_3^- + NO_2^- concentrations were occasionally measured later after frozen storage (-20°C).

Duplicate samples (20-30 ml) for particulate organic carbon and nitrogen (POC and PON) were collected at the start and end of experimentation on precombusted Whatman GF/F filters and stored frozen in desiccators. After thawing/drying (<60°C for 24 h) samples were analyzed by the dry combustion method of Sharp (1974) with either a Control Equipment Corp. model 240-XA (remanufactured Perkin-Elmer model 240) or a Carlo Erba model 1106 elemental analyzer. Both instruments were calibrated with acetanilide standards.

Samples for ^{15}N analysis were collected on precombusted Whatman GF/F filters, folded, placed into acid-washed, petri-dishes, and immediately frozen for later isotopic analyses. Nitrogen in the particulate samples was converted to dinitrogen gas (N_2) by the micro-Dumas dry combustion technique (LaRoche,1983) and subsequently analyzed for ^{15}N enrichment with a JASCO model N-150 emission spectrometer (Fiedler and Proksch, 1975) as outlined in Chapter 1.

Experimental procedures

Kinetic parameters for N uptake

The kinetic parameters of nitrate, ammonium and urea uptake were determined with duplicate NO_3^--replete cultures of *M. pusilla* grown in 2 L Pyrex flat-bottom boiling flasks, fitted with silicone stoppers. Prior to experimentation, ambient NO_3^- + NO_2^- was measured at 0.5 - 1 h intervals. The experiments were initiated immediately after the nitrate

concentration was <0.05 μg-at $N \cdot L^{-1}$ in the culture medium. Less than 2 h elapsed between the time ambient concentrations were considered saturating to growth (>2.5 μg-at $N \cdot L^{-1}$) and the time they had decreased to detection limits (0.05 μg-at $N \cdot L^{-1}$). The timing was critical, because nitrate in the medium must be depleted, but the condition of the cells had to be nearly N replete to minimize non-linearity in uptake during experimentation. Immediately following ambient NO_3^- depletion, 60 ml subsamples were transferred to a series of sterile, 85 ml polycarbonate Oak Ridge tubes (Nalgene^R); alternatively 200 ml subsamples to 250 ml polycarbonate Erlenmeyer flasks (Nalgene^R), and inoculated with ^{15}N-labelled NO_3, NH_4 or urea (Kor Isotopes, 99 atom %) at a range of initial substrate concentrations (0.2, 0.4, 0.8, 1.6, 2.4, 4.2 and 10 μg-at $N \cdot L^{-1}$). Incubations were conducted under the same conditions as those under which the cells were grown and incubations terminated after 10 min by filtration (pressure differential ≤ 80 mm Hg). The fitration period was always less than 30 s. The 4.2 and 10 μg-at $N \cdot L^{-1}$ enrichment experiments were allowed to incubate for an additional 50 min to determine if uptake rates were constant with incubation time. Previously acid-washed polypropylene bottles, were rinsed once with filtrate and then used to collect the filtrate. The filtered samples were immediately analyzed for ambient NH_4^+ and $NO_3^- + NO_2^-$ concentrations. The initial substrate concentrations at time zero (T_0) were calculated by adding known volumes of $^{15}NO_3^-$ and $^{15}NH_4^+$ to culture filtrate

and then measuring ambient concentrations. Specific uptake rates (N taken up per unit PON) were calculated according to a constant specific uptake model (Dugdale and Wilkerson, 1986 ; equation 6 of Appendix 1).

Substrate interaction

Two series of substrate interaction experiments were performed with NO_3^--replete cultures. In the first series, ambient inorganic N (NO_3^- + NO_2^- and NH_4^+) levels were monitored every 30 min for 3 h before the nitrate concentration decreased to 15 μg at $N \cdot L^{-1}$. A single 4-L culture was divided and poured into four 1 L Pyrex flat-bottomed boiling flasks 1 h prior to N substrate enrichment. The experiments were initiated when 10 μg-at $N \cdot L^{-1}$ of ^{15}N labelled NO_3^- (enriched control), NH_4^+ and urea (99 atom %) were added to three of the flasks and the remaining flask was not enriched (undisturbed control). Filtered samples were then collected at 20-30 min intervals for 4 h for immediate NO_3^- + NO_2^- and NH_4^+ analyses following previously outlined procedures. Nitrate and ammonium uptake rates were calculated from the slope of separate linear regressions of substrate concentration in the medium plotted against time. These uptake rates are termed (absolute or transport rates) and are expressed as μg-at $N \cdot L^{-1} \cdot h^{-1}$. Specific uptake rates were obtained by dividing the absolute rates by the exponential average concentration of PON (geometric mean) over the duration of the incubation period.

The specific uptake of urea was determined from a

constant specific uptake model (Dugdale and Wilkerson,1986; equation 8 of Appendix 1). This equation compensates for the effect of simultaneous uptake of unlabelled substrate by utilizing independent estimates of the absolute uptake of unlabelled nitrate provided from NO_3^- disappearance measurements thus correcting for the isotope dilution in the particulate matter originating from the ^{14}N in the unlabelled source (Collos, 1987; Lund, 1987). It should be noted that in Dugdale and Wilkerson (1986) the equation for constant specific uptake (V_c), when unlabelled sources are present, is incorrectly written and is correctly reported in Appendix 1. The absolute (transport) rate of urea was calculated using equation 10 of Dugdale and Wilkerson (equation 7 of Appendix 1) which compensates for the simultaneous uptake of unlabelled NO_3^- and the change in concentration of PON during the incubation period.

Effect of NH_4^+ concentration on NO_3^- uptake rate

In the second series of experiments, the effect of NH_4^+ concentration on the uptake of NO_3^- was examined. A NO_3^--replete culture was monitored for the decline of $NO_3^- + NO_2^-$ in the medium and four 250 ml subsamples were transferred to polycarbonate (Nalgene^R) Erlenmeyer flasks (fitted with silicone stoppers and sampling tubes) prior to the ambient NO_3^- concentration reaching 15 μg-at $N \cdot L^{-1}$. $^{15}NH_4Cl$ (99 atom %) was added to the flasks to bring the initial enrichment NH_4^+ concentration to 5, 2, and 1 μg-at $N \cdot L^{-1}$. Fifty ml subsamples were removed every 20-30 min for 2 h and ^{15}N

incubations terminated by filtration. Filtration, collection of filtrate, storage and analyses were conducted as previously outlined. Samples for PON analysis were collected at the beginning, middle and end of the incubations. The disappearance rate of NO_3^- was calculated from the slope of external NO_3^- + NO_2^- concentration against time; division of this rate by the exponential average PON concentration provided an estimate of the specific NO_3^- uptake rate. The specific NH_4^+ uptake rates were calculated using the constant specific uptake model (Dugdale and Wilkerson, 1986; equation 6 of Appendix 1) with the atom % ^{15}N excess ($^{15}N_{ex}$) in the particulate matter estimated from the slope of the least-squares linear regression of $^{15}N_{ex}$ versus time, prior to isotope depletion.

Uptake of nitrogen by NO_3^--starved cells

Two series of experiments were conducted, both in duplicate, to assess the effect of nitrogen deficiency on the uptake of NH_4^+ or urea by NO_3^--starved cultures of *Micromonas pusilla*. In each series nitrogen-starved cells were obtained from duplicate batch cultures, started with 50 μg-at N-$NO_3 \cdot L^{-1}$ as the initial concentration and N source and allowed to remain in nitrogen-free medium for 2 d after the external nitrogen was depleted. After the cultures became N-starved, they were split into separate flasks and either 15 μg-at $N \cdot L^{-1}$ of $^{15}NH_4Cl$ or $CO(^{15}NH_4)_2$ (both 99 atom %) was added to each subculture. The ambient NH_4^+ concentration in the medium and the ^{15}N accumulation in the cells were measured at time

intervals of 5-15 min for 3 h according to procedures outlined above.

In the second series of experiments, duplicate NO_3^- starved cultures were enriched with $Na^{15}NO_3$ (99 atom %) and samples collected for measurement of ambient NO_3^- concentration and ^{15}N accumulation at time intervals of 5-30 min for 6 h. Uptake rates, estimated from ^{15}N accumulation in the particulates, were calculated acording to the constant, specific uptake model (V_C) of Dugdale and Wilkerson (1986) (equation 6 of Appendix 1) from measurements of atom % excess of ^{15}N in successive samples during the time intervals Uptake rates, estimated from disappearance of NO_3^- or NH_4^+ in the medium, were calculated by dividing the difference in nutrient concentration in successive samples by the length of the time interval; specific rates were calculated by dividing this value by the estimated exponential average PON concentration during that time assuming that all the nutrient removed from the medium was incorporated into the particulate fraction (see Appendix 4).

Estimation of kinetic parameters

The kinetic parameters, K_s and V_{max} were obtained in two ways: a direct fit of the data to the Michaelis-Menten hyperbola using a computerized, iterative, non-linear, least-squares regression technique (Labtec Notebook Curvefit^R, Laboratory Technologies Corp.) and a least-squares linear regression analysis of Hanes-Woolf linear transformation (S/V vs S) of the data. In the latter method, the standard errors

of the kinetic parameters were estimated using the Delta method of variances (Bishop et al., 1975). The Hanes-Woolf transformation was used in preference to other linear transformations as it gave a better spread of the data points and generally provided the most accurate determinations of K_s and V_{max} (Dowd and Riggs, 1965). However, any linearization of the Michaelis-Menten equation violates a basic assumption of least-squares regression analysis, lack of error in the independent variable, S (Zar, 1974). Although in practice this assumption is sufficiently met if errors in the independent variable are small relative to errors in the dependent variable. The problem of utilizing unweighted, transformed data (Dowd and Riggs, 1965) and the inevitable correlation between variables (measured variable S appears in both dependent and independent variables) makes a linear transformation statistically inferior to direct, non-linear fitting of data to the Michaelis-Menten equation (Li, 1983; Robinson and Characklis, 1984). Since earlier investigators did not always have the same accessibility to non-linear fitting by computers as we enjoy today, the parameters, estimated by both methods, have been included in Table 3.1 for literature comparative purposes. The kinetic parameters have been calculated separately for the individual cultures as well as for the data treated together.

RESULTS

Uptake kinetics

Nitrogen specific uptake rates are plotted versus the average ambient nitrogen concentration experienced by the cells during the 10 min incubation period (Fig. 3.1). A list of the half saturation constants (K_s) and maximum uptake velocities (V_{max}) are presented in Table 3.1 along with their estimated standard errors. Generally the values for separate cultures agreed well, but poorer agreement was found between estimates of urea-V_{max} and NH_4-K_s values. The discrepancy between the latter estimate can be attributed partly to the paucity of uptake values from low substrate enrichments in the second culture (substrate exhaustion occurred during the incubation for 0.1, 0.2, and 0.4 μg-at $N \cdot L^{-1}$ enrichment). The values of V_{max} of NO_3^- and urea agreed well and are about half the V_{max} for NH_4^+. *Micromonas pusilla* demonstrated the same affinity for each N substrate as the K_s values for NO_3^-, NH_4^+, and urea were within ± 0.1 μg-at $N \cdot L^{-1}$ of each other.

An underlying assumption in the use of the Michaelis-Menten equation for the estimation of kinetic uptake parameters is that uptake remains constant over the duration of the experimental incubation. In the present kinetic experiments, it is unlikely that the non-linearity that has been reported for nitrogen uptake by N-deficient cells would occur because in this study N-replete cells were utilized. Nitrogen-replete cultures (4.2 and 9.9 μg-at $N \cdot L^{-1}$) were incubated for both 10 and 60 min to determine if V_{max}

Figure 3.1. Nitrogen specific uptake rates (V) determined over 10 min after the addition of 0.2, 0.4, 0.8, 1.6, 2.4, 4.2 and 10 μg-at $N \cdot L^{-1}$ of NO_3^- (A), urea (B) or NH_4^+ (C) to duplicate nitrate-replete cultures (○ , ●) of *Micromonas pusilla*. Rates (h^{-1}) are plotted versus the average substrate concentration during the 10 min interval. Curve calculated by computer programme (see text for details).

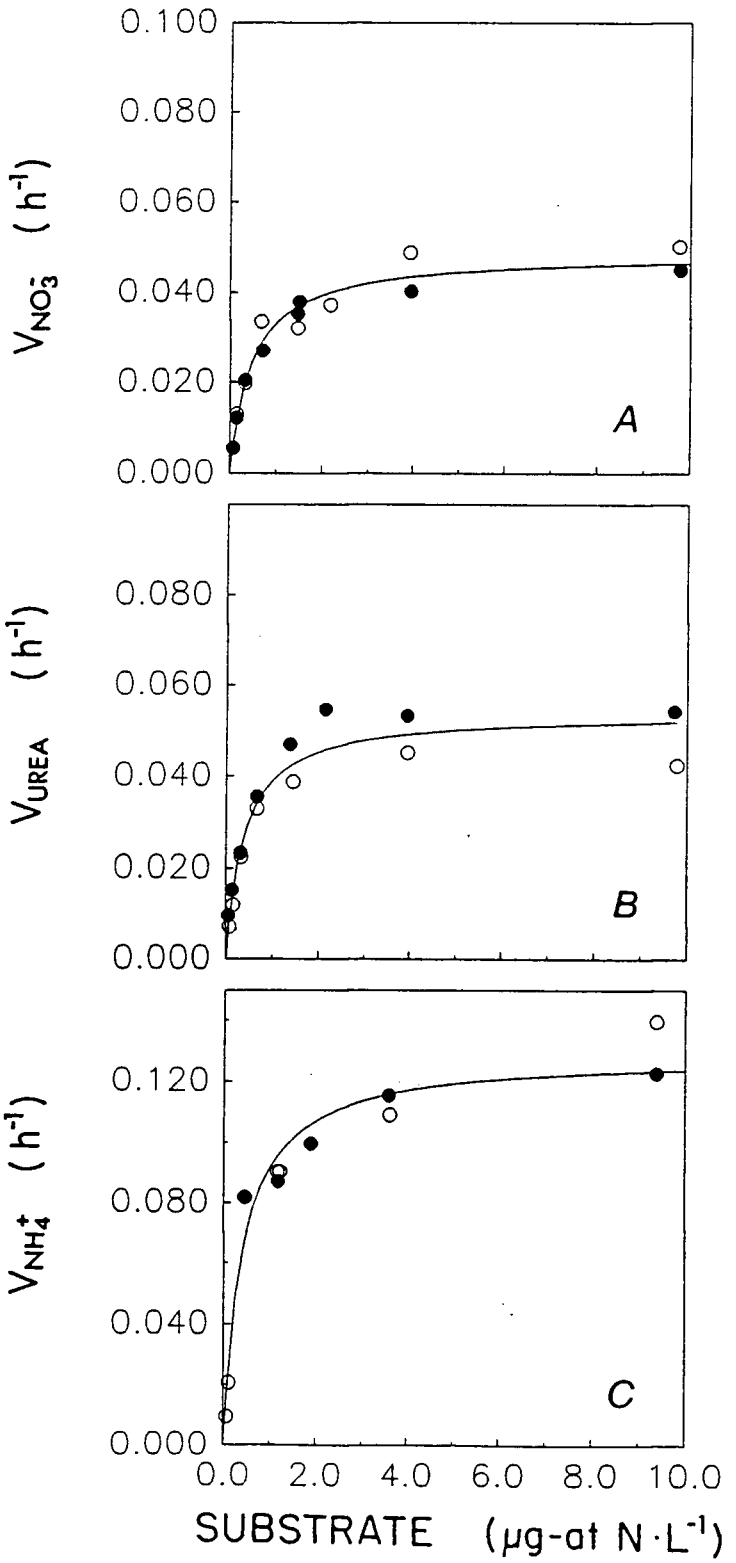


Table 3.1 Kinetic parameters for nitrate, urea and ammonium uptake of N-replete *Micromonas pusilla*. Michaelis-Menten parameters, K_s (half-saturation constant) and V_{max} (maximum uptake velocity) were estimated from a direct non-linear curve fitting model[1] and Hanes-Woolf linear transformation[2] of the data obtained from replicate cultures (1 or 2) and the cultures treated together (1 + 2).

Substrate	Culture	$V_{max}{}^1$ ($\times 10^{-2} h^{-1}$)	$K_s{}^1$ (μg-at N·L^{-1})	$V_{max}{}^2$ ($\times 10^{-2} h^{-1}$)	$K_s{}^2$ (μg-at N·L^{-1})
Nitrate	1	4.64 (0.118)	0.44 (0.044)	4.70 (0.057)	0.49 (0.050)
"	2	5.07 (0.361)	0.50 (0.014)	5.32 (0.176)	0.60 (0.022)
"	1 + 2	4.86 (0.183)	0.47 (0.069)	4.99 (0.125)	0.54 (0.108)
Urea	1	4.70 (0.176)	0.35 (0.051)	4.42 (0.098)	0.27 (0.093)
"	2	5.93 (0.211)	0.40 (0.059)	5.64 (0.082)	0.30 (0.059)
"	1 + 2	5.38 (0.257)	0.38 (0.073)	4.95 (0.219)	0.26 (0.181)
Ammonium	1	13.6 (0.99)	0.49 (0.142)	14.8 (0.56)	0.76 (0.165)
"	2	12.1 (0.74)	0.28 (0.104)	12.8 (0.25)	0.43 (0.098)
"	1 + 2	12.9 (0.61)	0.40 (0.087)	13.8 (0.44)	0.62 (0.145)

decreased with increased incubation time. The V_{max} values for $V^{0-10min}$ and $V^{0-60min}$ are presented in Figure 3.2. and there appears to be no significant difference between the two rates, although duplicate measurements were not always possible. An average of 30, 40 and 60% of the available isotope was utilized during the 60 min incubations of the urea, NO_3^- and NH_4^+ enriched cultures, respectively.

Substrate interaction

The addition of 10 μg-at $N \cdot L^{-1}$ of nitrate to a NO_3^--replete culture of *M. pusilla* did not alter the disappearance rate of $NO_3^- + NO_2^-$ (0.0441 h^{-1}) from that observed for 3 h prior to N enrichment (0.0446 h^{-1}, 3 d monitoring). There was, however a 10% difference between the undisturbed control and the NO_3^--enriched culture (enriched control = 0.0492 h^{-1}) during the 3 h experiment (Fig. 3.3). The addition of 10 μg-at $N \cdot L^{-1}$ of [^{15}N] urea resulted in a 28% decrease in the disappearance rate of $NO_3^- + NO_2^-$ and a specific urea uptake rate of 0.0260 h^{-1}. The total nitrogen uptake (0.0579 h^{-1}) increased by ca. 30% over the nitrate enrichment alone. Ammonium addition (10 μg-at $N \cdot L^{-1}$) resulted in the complete cessation of $NO_3^- + NO_2^-$ disappearance from the medium and a NH_4^+ specific uptake rate of 0.0732 h^{-1}.

The second series of substrate interaction experiments, designed to determine the effect(s) of NH_4^+ concentration on NO_3^- uptake, were conducted on a NO_3^--replete culture (preconditioned growth rate = 0.0484 h^{-1}, 4 d monitoring). With no enrichment (control), the $NO_3^- + NO_2^-$ depletion rate

Figure 3.2. Comparison of nitrogen specific uptake rates for nitrate-replete cultures of *Micromonas pusilla* determined over 10 and 60 min incubation periods. Cultures are numbered and values are the mean (n = 2) of duplicate incubations, * designates no replicate. Bar represents ± 1 S.D.

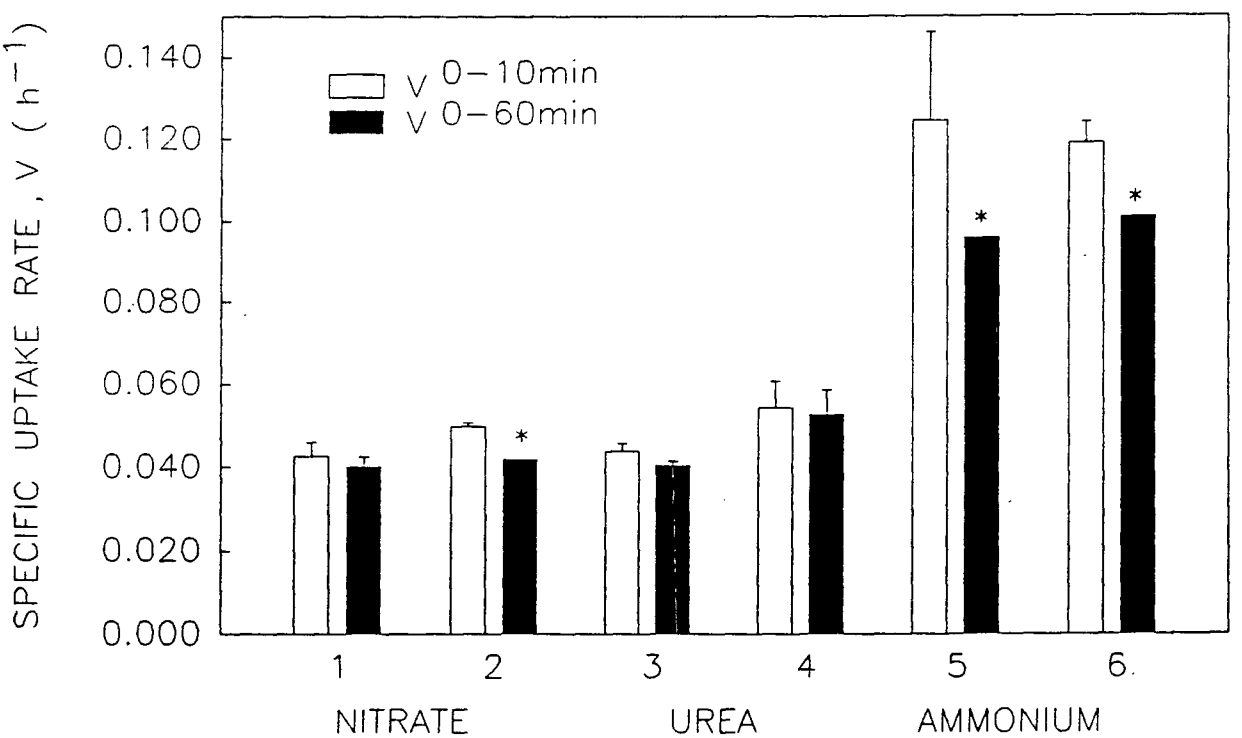
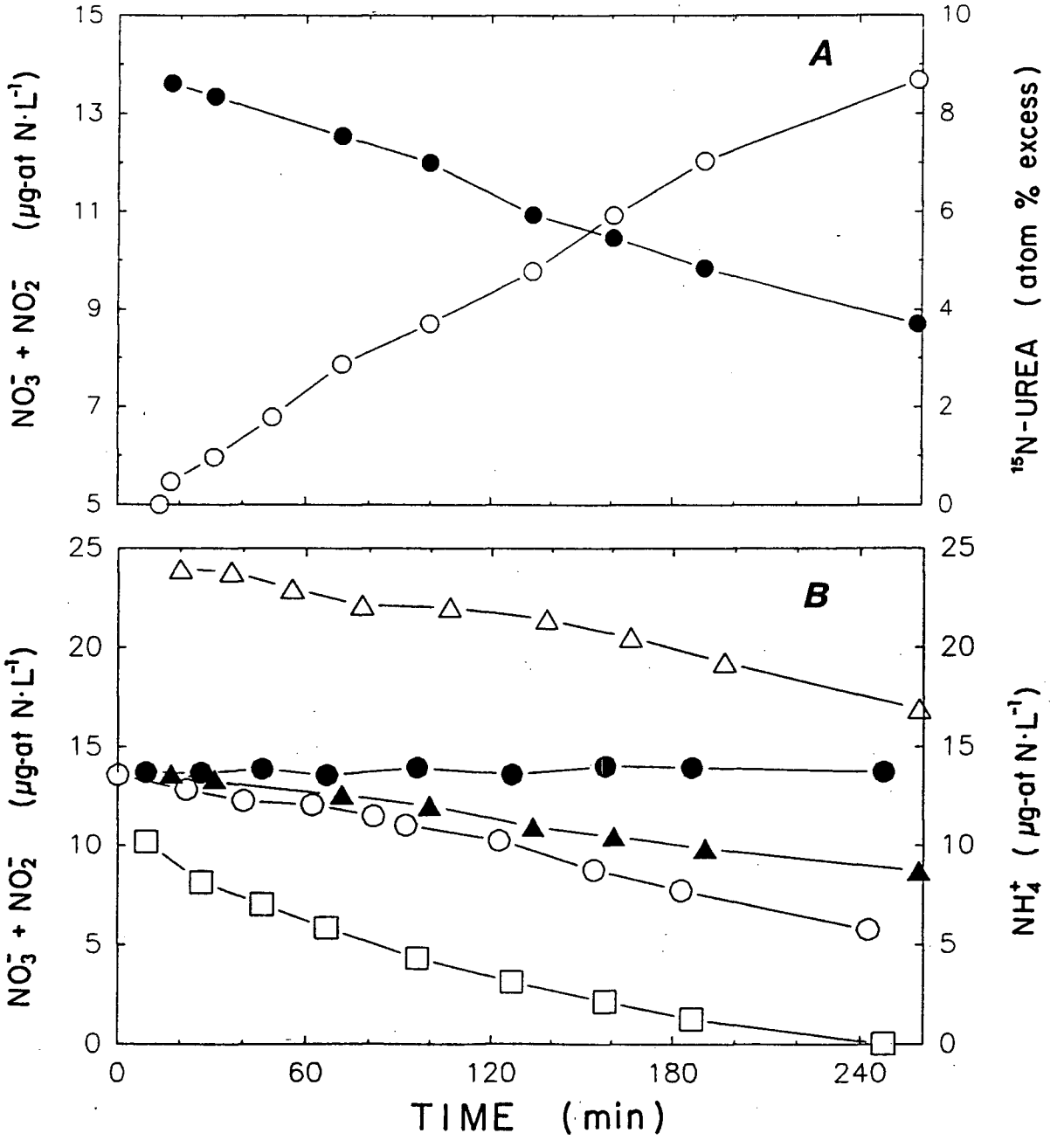


Figure 3.3. Nitrogen uptake by replicate cultures of nitrate-replete *Micromonas pusilla* over a 4 h incubation period. A. Dissolved NO_3^- + NO_2^- (●) concentration and ^{15}N-atom % excess (○) after 10 μg-at N-urea·L^{-1} addition. B. Dissolved NO_3^- + NO_2^- concentration (○ , △) after no and 10 μg-at N- NO_3^-·L^{-1} addition, respectively. Dissolved NO_3^- + NO_2^- concentration (● , ▲) after 10 μg-at N·L^{-1} addition of NH_4^+ and urea, respectively. Dissolved NH_4^+ concentration (□) after addition of 10 μg-at N-NH_4^+·L^{-1}.



averaged 0.0500 h^{-1} over the 2 h duration of the experiment. All the $^{15}NH_4^+$ enrichments (5, 2, and 1 μg-at $N \cdot L^{-1}$) caused cessation of NO_3^- + NO_2^- disappearance (< 0.1 μg-at $N \cdot L^{-1} \cdot h^{-1}$) when the $^{15}NH_4^+$ isotope was still available in the medium; the concentration of external NO_3^- + NO_2^- did not measurably decrease until the $^{15}NH_4^+$ was exhausted (Fig. 3.4). It should be mentioned that the uptake of NO_3^- was estimated from the disappearance of NO_3^- + NO_2^- in the medium. Although other investigators (e.g., Serra et al., 1978a; Olson et al., 1980; Parslow et al., 1984b) have recorded substantial excretion of NO_2^- by marine diatoms and natural assemblages (Harrison and Davis, 1977), *M. pusilla* demonstrated no excretion of NO_2^- and only trace levels of NO_2^- were found in the medium during exponential growth on NO_3^- (Appendix 5). Excretion of NO_2^- by *M. pusilla* would contribute to a reduction in any measurable decline of NO_3^- + NO_2^- in the medium and thus enhance the apparent inhibitory effect of NH_4^+ or urea on NO_3^- uptake.

Nitrogen-starved cells

The exponential growth rate of *M. pusilla* was 1.11 d^{-1} (0.0463 h^{-1}) prior to the depletion of nitrate in the medium, a value which agrees well with the V_{max} for NO_3 calculated previously (0.0486 ± 0.0018 h^{-1}). The specific uptake of NO_3^- by NO_3^--starved cultures, estimated from both the accumulation of $^{15}NO_3$ into the particulate material and the disappearance of NO_3^- + NO_2^- from the medium, averaged 0.0238 h^{-1} over the 5-6 h incubation period (Table 3.2). This rate was roughly constant over the incubation (Fig. 3.5) although it appears

Figure 3.4. Dissolved NO_3^- + NO_2^- concentration without (O), and with (●), 5(A), 2(B), and 1(C) μg-at $N \cdot L^{-1}$ [^{15}N]-NH_4^+ enrichment; $^{15}NH_4^+$ atom % excess in particulates (■) plotted versus time (min). Arrows designate time of NH_4^+ addition.

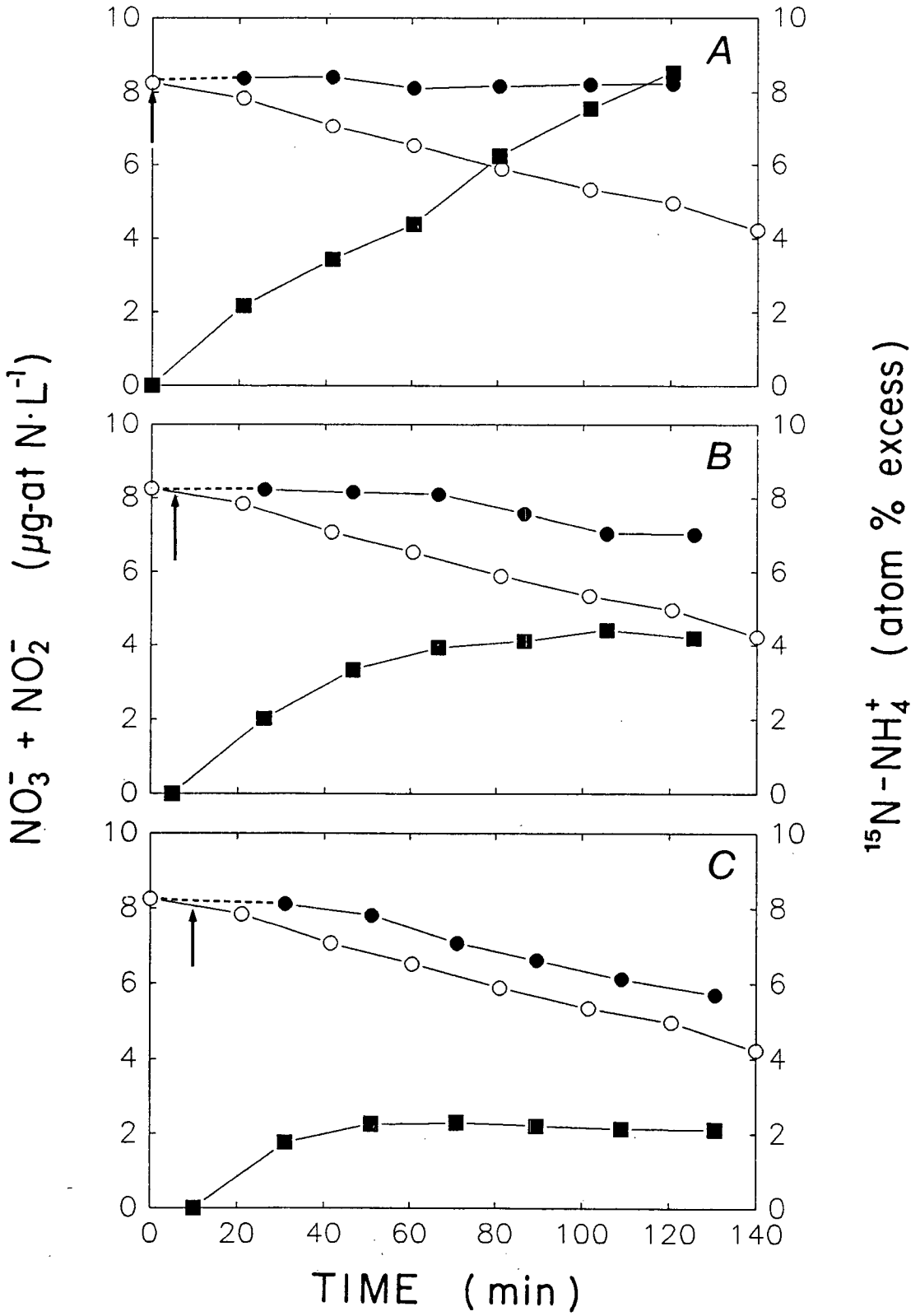


Table 3.2 Average nitrate uptake rates (h^{-1}) for NO_3^--starved *Micromonas pusilla*. Rates determined from least-squares linear regression of particulate ^{15}N enrichment or the decrease in the external concentration of NO_3^- + NO_2^- versus time and reported as ± 1 standard deviation (in parentheses) of the mean of duplicate cultures.

Time Interval	Nitrate Uptake ($\cdot 10^{-2} h^{-1}$)	
(h)	$^{15}NO_3^-$	NO_3^-disappearance
0 - 1	2.22 (0.0071)	1.77 (0.537)
1 - 2	2.10 (0.184)	1.86 (0.233)
2 - 3	2.34 (0.014)	2.94 (0.170)
3 - 4	2.56 (0.417)	2.80 (0.629)
4 - 5	2.44 (0.099)	2.66 (0.438)
5 - 6*	2.12	2.73

(* = uptake rates calculated from one culture)

Table 3.3 Average N uptake rates V (h^{-1}) for NO_3^--starved *Micromonas pusilla*. Rates determined from least-squares linear regression of particulate ^{15}N enrichment or the decrease in the external concentration of dissolved nitrogen versus time and reported as ± 1 standard deviation (in parentheses) of the mean of duplicate cultures.

N Substrate	N Uptake ($\cdot 10^{-2}$ h^{-1})		
	$V^{0-60min}$	$V^{60-120min}$	$V^{120-180min}$
Urea	4.80 (0.129)	3.72 (0.149)	3.32 (0.158)
$^{15}NH_4^+$	7.05 (0.0120)	5.59 (0.0559)	4.23 (0.0288)
NH_4^+	6.75*(0.739)	6.76 (0.449)	4.85 (0.600)

(* = uptake rate calculated from 2.5 - 60 min)

that there was elevated uptake (0.0386 ± 0.0012 h^{-1}) during the first 5 min after ^{15}N enrichment. Elevated uptake rates were not observed in the NO_3^- + NO_2^- disappearance measurements, possibly due to the reduced sensitivity of colourimetric analysis at elevated (> 15 μg-at $N \cdot L^{-1}$) nitrate concentrations. The average rate of 0.0238 h^{-1} is ca. 25% less than the nitrogen specific rate (0.0308 h^{-1}) calculated from NO_3^- + NO_2^- disappearance (1.54 μg-at $N \cdot L^{-1} \cdot h^{-1}$) and the average concentration of PON (50.0 μg-at $N \cdot L^{-1}$) during the 4 h monitoring period prior to N depletion. This reduction is probably only a minimum estimate as uptake rate before depletion may have already begun to decrease as a consequence of low NO_3^- concentrations in the medium. The average uptake rate after starvation is only half the N demand calculated from either the pre-conditioned growth rate or V_{max}. However there was, no lag period in NO_3^- uptake by the previously starved cells (Fig. 3.5).

The exponential growth rate of duplicate cultures used in the second series of NO_3^--starved uptake experiments (NH_4^+ and urea) averaged 1.11 d^{-1} (0.0463 h^{-1}) prior to depletion of nitrate in the medium. Maximum [^{15}N] urea uptake rate (0.105 ± 0.016 h^{-1}) occurred during the 0-5 min interval and subsequently decreased rapidly in the next 10-20 min to a roughly constant rate of 0.0349 ± 0.0016 h^{-1} (Fig. 3.6) The average hourly urea uptake rates, calculated from the slope of particulate APE versus time, are presented in Table 3.3. Average uptake rates during the second hour of incubation were

Figure 3.5. Nitrate uptake by nitrate-starved *Micromonas pusilla* after the addition of 15 μg-at $N\text{-}NO_3^- \cdot L^{-1}$ to duplicate cultures. A. Dissolved $NO_3^- + NO_2^-$ (□,■) in the culture medium; $^{15}NO_3^-$ atom % excess in particulate matter (O,●). B. Nitrate uptake rate determined from $NO_3^- + NO_2^-$ disappearance technique. C. [^{15}N] nitrate uptake rate. Values in A are plotted against elapsed time measured after enrichment and uptake rates (B,C) are plotted against average incubation time.

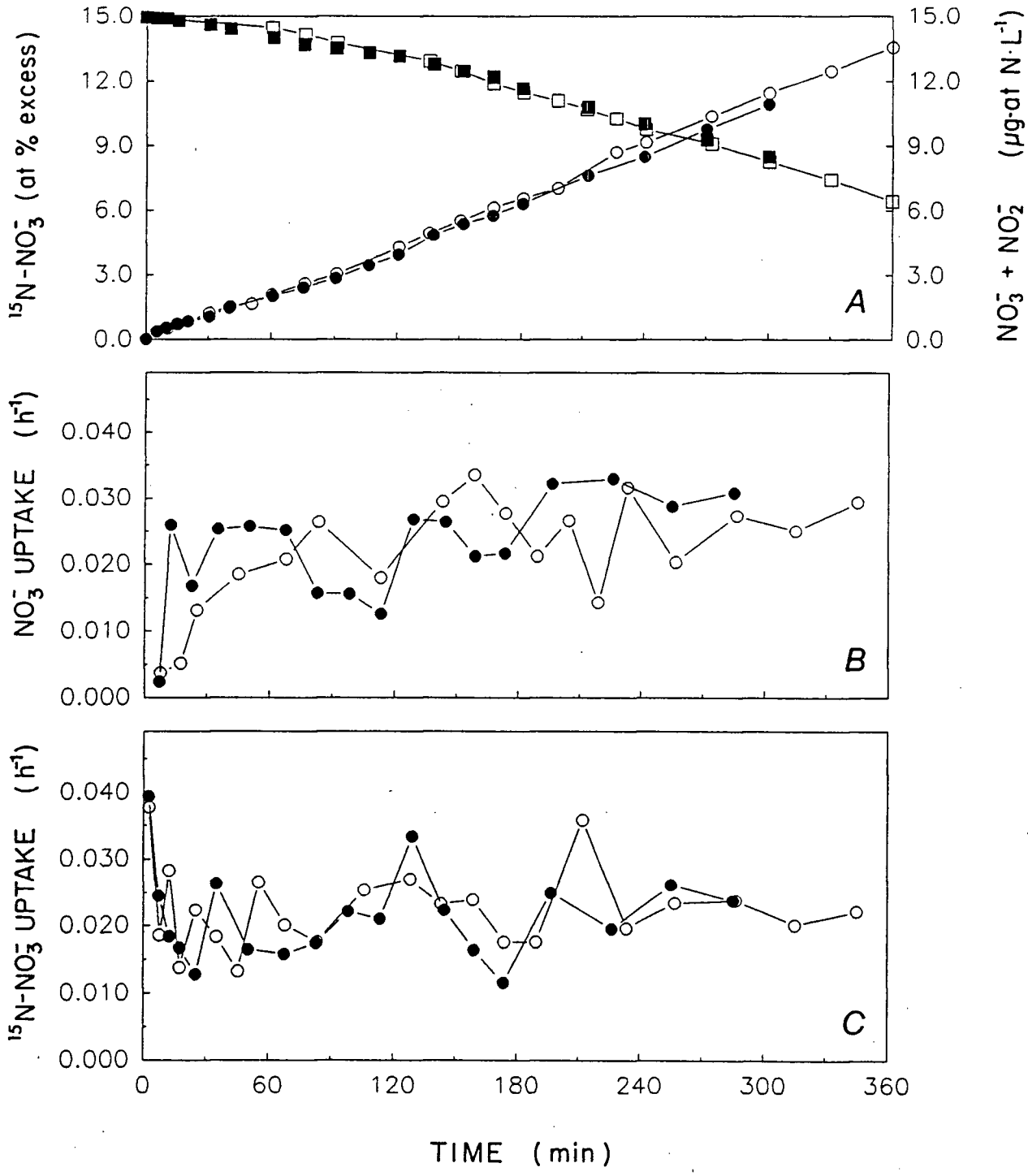
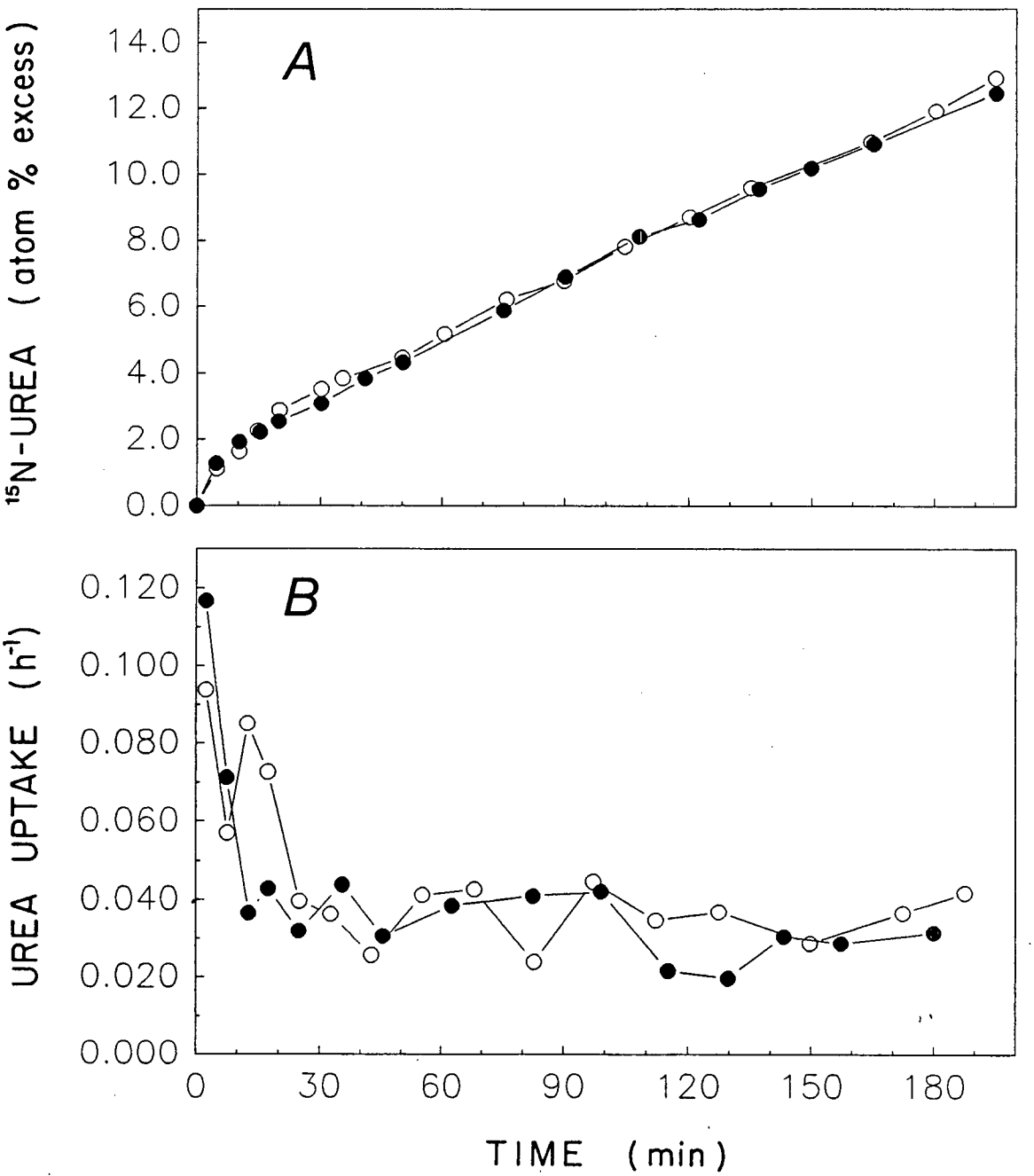


Figure 3.6. Urea uptake by nitrate-starved *Micromonas pusilla* after the addition of 10 μg-at N-urea·L^{-1} to duplicate cultures (O,●). A. ^{15}N-urea atom % excess in particulate matter is plotted against elapsed time measured after addition of urea. B. [^{15}N] urea uptake rate plotted against average incubation time.

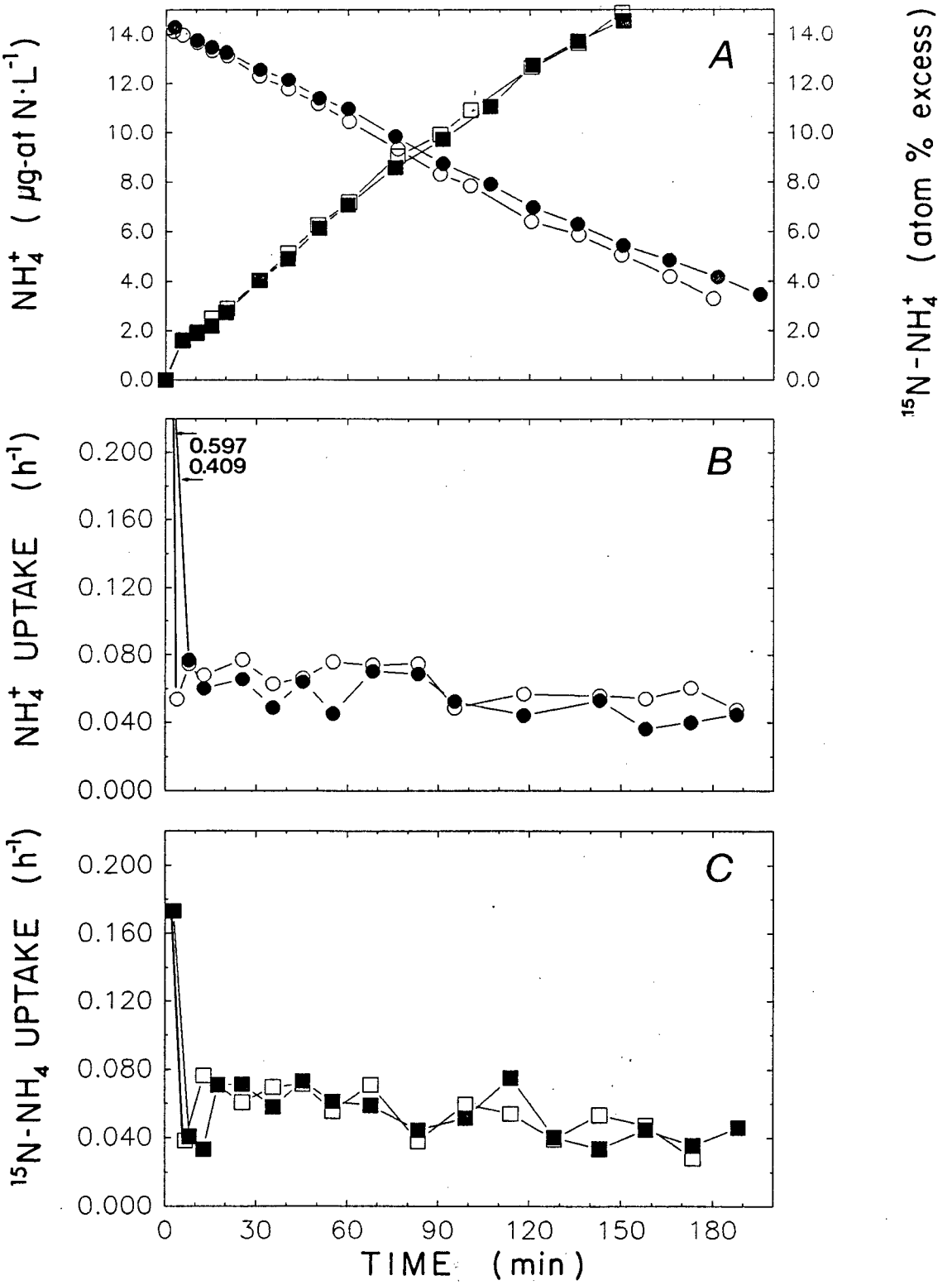


78% of the initial hourly rates and during the third hour, uptake merely decreased an additional 11%. The uptake rates during 30-60, 60-120 and 120-180 min intervals were < 40% of the maximal rate and 70-80% of the nitrogen uptake rate needed to support exponential growth observed prior to N-depletion.

Maximum $^{15}NH_4$ uptake rate (0.175 ± 0.0024 h^{-1}) occurred during the initial 0-5 min interval, followed by a rapid, but short-term decrease (ca. 80%) in uptake before it reached a roughly constant rate of 0.0668 ± 0.0073 h^{-1} for the remainder of the first hour of incubation (Fig. 3.7 C). The average hourly rate declined by 20-25% per hour over the 3 h of monitoring and averaged 150, 120 and 90% of the nitrogen uptake, needed to support exponential growth, during the 0-60, 60-120, and 120-180 min incubation periods respectively.

The rate of NH_4^+ disappearance in the medium (0.0612 ± 0.0060 h^{-1}) (excluding the 0-2.5 min interval), is in good agreement with the $^{15}NH_4$ uptake rate (0.0561 ± 0.0010 h^{-1}), and also declined over the 3 h incubation period (Table 3.3). Maximal NH_4^+ disappearance rates (0.503 ± 0.133 h^{-1}) during the 0-2.5 min interval were 7-9 times greater than the average rate and nearly 3 times greater than the elevated $^{15}NH_4^+$ uptake rates reported for the 0-5 min interval (Fig. 3.7 B). However, unlike the ^{15}N-tracer technique, where an accurate measurement of $^{15}N_{ex}$ in the unenriched particulates can be obtained, the concentration of NH_4^+ at time-zero for the NH_4^+ disappearance technique can only be estimated from a cell-free sample, and may be subject to measurement error.

Figure 3.7. Ammonium uptake by nitrate-starved *Micromonas pusilla* after the addition of 15 μg-at N- $NH_4^+ \cdot L^{-1}$ to duplicate cultures. A. Dissolved NH_4^+ concentration in the culture medium (●,○); ^{15}N-NH_4 atom % excess in particulate matter (■,□) plotted against elapsed time after enrichment. B. Ammonium uptake rate, determined by NH_4^+ dissappearance technique. C. [^{15}N] NH_4 uptake rate. Values in B and C plotted against average incubation time.



A summary of the culture conditions at the beginning of each series of experiments is presented in Table 3.4.

Table 3.4 Summary of culture conditions at the beginning of each experiment.

Culture description	Experiment and culture number	$NO_3^- + NO_2^-$ (μg-at N·L^{-1})	PON (μg-at C·L^{-1})	POC (μg-at C·L^{-1})	Cell density (10^9·L^{-1})	Total cell Volume* (μL·L^{-1})	Cell Quota (fg-at N·cell^{-1})
NO_3^- sufficient	A-1	<0.05	49.2	382.1	5.71	13.2	8.6
"	A-2	<0.05	51.9	382.7	6.08	13.3	8.5
NO_3^- sufficient	B-1	13.6	35.2	302.3	-	-	-
"	C-1	8.3	32.5	295.3	4.72	9.64	6.9
NO_3^- starved	D-1	<0.05	50.8	546.7	10.38	14.1	4.9
"	D-2	<0.05	54.8	549.6	-	-	-
NO_3^- starved	E-1	<0.05	59.3	504.8	9.28	12.3	6.4
"	E-2	<0.05	47.1	526.7	10.00	12.8	4.7

A: NO_3^-, NH_4^+ and urea uptake kinetic exp.

B: NH_4^+ and urea inhibition exp. (Series 1).

C: NH_4^+ inhibition exp. (Series 2).

D: NH_4^+ and urea uptake exp.

E: NO_3^- uptake exp.

*: L cell volume per liter of culture.

DISCUSSION

Uptake kinetics

Over the last two decades numerous investigators have determined the kinetics of nitrogen uptake in both cultured and natural assemblages of phytoplankton (see reviews by McCarthy, 1981; Goldman and Glibert, 1983; Dortch, in press). It is often difficult to compare and interpret the results of the various studies due to the variety of techniques, experimental incubation periods and the physiological condition of the phytoplankton (e.g., Harrison et al., 1989). Generally the values of the half-saturation constant (K_s) for N-uptake are lower for oligotrophic oceanic natural assemblages (e.g., MacIsaac and Dugdale, 1969; Kanda et al., 1985) and isolated oligotrophic clones (e.g., Eppley et al., 1969; Carpenter and Guillard, 1971) than half-saturation values from eutrophic, and neritic areas of the ocean. Eppley et al. (1969) demonstrated a direct correlation between cell size and the K_s value, although no such correlation has been observed for freshwater phytoplankters (Halterman and Toetz, 1984). It also appears that V_{max} for nitrate is lower than the V_{max} for reduced N forms such as NH_4^+, except during spring blooms or in upwelling areas where V_{max}-NO_3^- equals or exceeds that of ammonium (e.g, Dugdale, 1976; Dortch, in press). This general observation is consistent with the hypothesis that the large planktonic forms that bloom during such conditions (i.e., NO_3^- is abundant in the euphotic zone) depend primarily on nitrate (Malone, 1980). However, since

nutrient uptake and growth processes are not necessarily coupled (i.e., balanced and equal) and algal physiology and chemical composition are adaptable (see reviews by Dugdale, 1977; McCarthy, 1981) phytoplankton generally have the ability to grow equally well on NO_3^-, NH_4^+ and urea (eg., Syrett, 1981). To date, only a few studies (Paasche, 1971; Ward and Wetzel 1980; Rhee and Lederman, 1983; Thompson et al., 1989) have provided good evidence for an increase in growth rate of cells growing on NH_4^+ versus NO_3^- under saturating growth PPFD.

In the present work, the V_{max}-NH_4^+ values are twice those of NO_3^- and urea, although their K_s values are all similar (0.28 - 0.50 μg-at $N \cdot L^{-1}$) and fall within the range reported by Eppley et al. (1969) for small, oceanic diatoms (0.1-0.7 μg-at $N \cdot L^{-1}$). The lowest K_s values to date have been reported by Koike et al. (1983) for two microflagellates isolated from the oligotrophic North Pacific Ocean; *Platymonas* and *Mantoniella* sp. with K_s-NH_4^+ values of 50 and 2.9 ng-at $N \cdot L^{-1}$, respectively, suggesting that these two phytoflagellates are well adapted to their extremely low ammonium environment. The affinity for a given nutrient at low concentrations can be best estimated from the initial slope (α) of the Michaelis-Menten plot (i.e. $\alpha = V_m/K_s$, Healey, 1980; Parslow et al., 1985). In the present work, the values of α are 10.3 ± 1.9, 14.2 ± 3.4 and 32.3 ± 8.5 for NO_3^-, urea and NH_4^+, respectively. These results suggest that *M. pusilla* can utilize low concentrations of NH_4^+ more effectively than

equivalent concentrations of urea and NO_3^-. While actual kinetic studies of other picoplankters have not been conducted, related studies on natural assemblages utilizing size-fractionation techniques and nitrogen tracers or analogues have demonstrated that picoplankton, including microheterotrophs (Wheeler and Kirchman (1986), generally prefer the reduced forms of nitrogen and use relatively higher proportions of these reduced forms for growth than the larger phytoplankton (Nalewajko and Garside, 1983; Probyn, 1985; Probyn and Painting, 1985; Sahlsten, 1987; Harrison and Wood, 1988). Similar fractionation studies on net (> 20 μm) and nanoplankton (< 20 μm) have often found this distinction not to be as clear cut: the partitioning between "new" and "regenerated" nitrogen uptake is nearly equally distributed between the two size-fractions in various marine habitats (Sherr et al., 1982; Furnas, 1983; Rönner et al., 1983) while the results of Koike et al., (1986) for Antarctica phytoplankton confirmed the former pattern.

Cellular physiological state

The effects of cellular physiological state on nitrogen uptake rates by phytoplankton were first demonstrated by Syrett (1953) and Harvey (1953). They showed that NH_4^+ and NO_3^- uptake by batch cultures of "nitrogen-starved" cells was much more rapid than by "normal" cells that were nitrogen-replete. Fitzgerald (1968) noted that these rates were not sustainable and decreased quite rapidly once the nitrogen deficit was overcome. Conway et al. (1976) described the

response in considerably more detail in marine diatoms and distinguished three phases of uptake of the limiting nutrient: a short-lived period of very high uptake, termed "surge uptake" (V_s), a longer, sustainable phase characterized as "internally" (cellularly) controlled uptake (V_i), and "externally" (ambient limiting nutrient concentration) controlled uptake (V_e). More recent studies have also demonstrated "surge" or "enhanced" NH_4^+ uptake capabilities under conditions of N deprivation in numerous culture studies (e.g., McCarthy and Goldman, 1979; Dortch et al., 1982; Goldman et al., 1981; Goldman and Glibert, 1982; Parslow et al., 1984a,b; Syrett and Peplinska, 1988) and natural communities (Glibert and Goldman, 1981; Wheeler et al., 1982; Harrison, 1983a; Priscu and Priscu, 1984:,Priscu, 1987; Suttle and Harrison, 1988). The results of the present study with NO_3^--starved *M. pusilla* are similar to those described above with N-starved cultures; the initial surge NH_4^+ uptake rate was several fold greater (V_s^{0-5min} = 2.5-4 times, $V_s^{0-2.5min}$ = 7-9 times uptake rates obtained by ^{15}N and nutrient disappearance, respectively) than the internally controlled uptake rate and the uptake rate necessary to maintain the preconditioned growth rate observed before N depletion. The magnitude of surge uptake response is both species specific (e.g., Conway and Harrison, 1977) and a function of the duration of N deprivation (e.g., Parslow et al., 1984a). These elevated transients have been hypothesized to be an ecological adaptation that allows phytoplankton to rapidly

sequester ephemeral micropatches of N (McCarthy and Goldman, 1979; Glibert and Goldman, 1981; Goldman and Glibert, 1982) and maintain high growth rates in oligotrophic environments (Goldman et al., 1979; Goldman and Glibert, 1982). However this concept is not without controversy as both Jackson (1980) and Williams and Muir (1981) contend that the molecular diffusion of those patches would be so rapid as to prevent them from existing long enough to be exploited by phytoplankton. Due to technical limitations, nutrient data on micropatches are few. Collos (1986) suggested that the values reported in micropatches by Shanks and Trent (1979) (maximum values of ca. 500, 300 and 60 μM for NH_4^+, NO_2^- and PO_4^{3-}, respectively) may have to be revised upwards as they were measured on sample volumes which are too large to be considered relevant to phytoplankton spatial scales (Allen, 1977; Harris, 1980; McCarthy; 1980). This, together with empirical data showing that phytoplankton can utilize phosphorus patches produced by zooplankton (Lehman and Scavia, 1982a,b) lends credence to the original suggestion of Conway and Harrison (1977) that elevated uptake rates may be important in dictating competitive advantage in oligotrophic areas of the ocean; however, this topic is still a subject of controversy.

A rare, but significant finding in the present work is the appearance of a rapid, but short-term decrease in $^{15}NH_4$ uptake following surge uptake. This response has only been documented for freshwater phytoplankton (Suttle and Harrison

1988) and has not been reported in previous time course studies of rapid NH_4^+ uptake in marine phytoplankton. Suttle and Harrison (1988) suggested this temporary decrease in uptake may be the result of a short lag before NH_4^+ can be processed into amino acids. Alternatively, they suggest it could be the result of a sudden loss of membrane potential due to the influx of cations, a phenomenon observed when N-starved *Lemna gibba* (duckweed) was pulsed with NH_4^+ (Ullrich et al., 1984).

The response of NO_3^--starved *Micromonas pusilla* to urea enrichment was similar to the $^{15}NH_4^+$ uptake response; the elevated surge uptake during the first 5 min was 2-3 fold greater than the internally controlled rate and the preconditioned growth rate. Price and Harrison (1988b) also found a similar increase in [^{15}N] urea uptake in NO_3^--starved cultures of *Thalassiosira pseudonana* after a short lag period (5 min). They contend that the subsequent elevated [^{15}N] urea uptake rate was the combined result of concomitant [^{15}N] urea uptake and the rapid reabsorption of previously released $^{15}NH_3/^{15}NH_4^+$. The internally controlled rate of urea uptake in *M. pusilla* was slightly lower than the rate needed to support the maximum growth observed prior to N depletion. Others (Rees and Syrett, 1979; Horrigan and McCarthy, 1981; Price and Harrison, 1988b) have found N deprivation increased urea uptake in marine diatoms relative to N-replete cells. Rees and Syrett (1979) suggested that ammonium formed from urea in the growth medium may partially suppress formation of

the urea uptake mechanism and that this repression is removed during nitrogen deprivation. Price and Harrison (1988b) contend that the increased nitrogen-specific urea uptake was caused by a reduction in the cell N quota and retention of all the urea-N by the nitrate-starved cells.

The importance of the "enhanced" or "surge" uptake to phytoplankton growth depends on the coupling of the uptake of nitrogen to the incorporation into new cellular material (Collos, 1986). If uptake rates respond rapidly to brief pulses of ambient nitrogen but the cells are not able to incorporate that nitrogen on a similar time scale, then growth may be limited by the rate at which cellular metabolism can incorporate dissolved nitrogen into macromolecules (Wheeler et al., 1982,1983; Zar,1988).

Nitrate was not taken up as readily by the NO_3^--starved culture of *Micromonas pusilla* as the reduced N forms, NH_4^+ and urea. However, unlike many NO_3^--starved phytoplankton (review by Collos, 1983; Dortch et al., 1982; Parslow et al., 1984b) no previous exposure to NO_3^- was required before NO_3^- uptake commenced (i.e. there was no lag period).

The maximum nitrate uptake rate attained, which was measured during the first 5 min after enrichment, was considerably lower than the NO_3^- rates measured in NO_3^--replete cultures and the elevated rates of NH_4^+ and urea observed in the starved cultures. The lower, sustainable internally controlled rate over the next 5-6 h was only half that required to support the pre-conditioned growth rate and

comparable to internally controlled reduced N uptake rates. This reduction in NO_3^- uptake capability after N deprivation is not uncommon (e.g., Collos, 1980; Dortch et al., 1982), although others (e.g., Morris and Syrett, 1965; Thacker and Syrett, 1972a; Harrison, 1976) have reported increased ability to take up nitrate. It appears that although *M. pusilla* still retained NO_3^- uptake ability it may require an "acclimation period" before maximal NO_3^- uptake can be attained, a response observed by many others (e.g., see review by Collos, 1983). It is possible that the physiological stress experienced by the cells during the 48 h of NO_3^- starvation reduced their viability and hence their nitrogen uptake capability. The relative "health" of the N-starved cells was assessed by microscopic examination of cellular colour and motility. The decline in NO_3^- uptake ability during starvation could also be due to the loss of an active uptake system (Falkowski, 1975a) or to inactivation of nitrate reductase (e.g., Syrett, 1981). However, inactivation of nitrate reductase alone need not prevent enhanced initial NO_3^- uptake as transient internal nitrate pools are often observed in phytoplankton (e.g., Dortch, 1982; Dortch et al., 1984).

Substrate interaction

Uptake interactions between inorganic N sources, particularly nitrate and ammonium, have been the subject of many studies (see reviews by Guerrero et al., 1981; McCarthy, 1981; Syrett, 1981; Ullrich, 1983, Dortch, in press) which reveal a variety of responses depending on the phytoplankton

species and its nutritional state. Nitrate uptake has been reported to be inhibited to different degrees by ammonium ranging from total suppression (e.g., Syrett and Morris, 1963; McCarthy and Eppley, 1972; Cresswell and Syrett, 1979) to simultaneous and comparable rate of NH_4^+ and NO_3^- uptake in cultures (e.g., Caperon and Ziemann, 1976; Conway, 1977; Dortch and Conway, 1984; DeManche et al., 1979) and natural communities (e.g., Conover, 1975; McCarthy, 1977; Maestrini et al., 1982; 1986; Price et al., 1985; Quéguiner et al., 1986; Collos et al., 1989). There are even a few reports of stimulation of NO_3^- uptake at low NH_4^+ concentrations even though higher concentrations inhibit NO_3^- uptake (Conover, 1975; Caperon and Ziemann, 1976; Glibert et al., 1982c; Yin, 1988; Dortch et al., submitted). The present study demonstrates that NO_3^- uptake by *M. pusilla* cannot proceed in the presence of NH_4^+ concentrations as low as 1.0 μg-at $N \cdot L^{-1}$. Only after NH_4^+ is exhausted from the external medium does this phytoplankter resume its uptake of nitrate.

The mechanism of depression of nitrate utilization is not well understood. There is evidence for regulatory action at both the level of nitrate uptake (e.g., Eppley and Rogers, 1970; Cresswell and Syrett, 1979; Serra et al., 1978b; Tischner and Lorenzen, 1979) and nitrate reduction (e.g., Syrett and Morris, 1963; Amy and Garrett, 1974; Hipkin et al., 1980) and to a certain extent the effect of ammonium on both mechanisms may be independent (Blasco and Conway 1982, Ullrich 1987). It is now generally accepted that the primary, and

rapidly acting effect of NH_4^+ on nitrate utilization is due to an inhibition of nitrate uptake which may be followed by the effects on nitrate metabolism through inhibition of nitrate reductase activity, from either irreversible proteolytic breakdown (e.g., Hipkin et al., 1980), reversible inactivation (e.g., Pistorius et al., 1978) or suppression of its synthesis (e.g., Morris and Syrett, 1963; Amy and Garrett, 1974). Although some (e.g., Florencio and Vega, 1982) argue that NH_4^+ per se inhibits NO_3^- assimilation most evidence suggests that the rate of NO_3^- uptake is modulated in response to changes in pools of some organic product of ammonium assimilation (e.g., Syrett, 1981,; Guerrero et al., 1981).

There are only a few reports of simultaneous uptake of urea and other N sources by natural phytoplankton communities (McCarthy and Eppley, 1972; Price et al., 1985). It is however, generally believed that urea suppresses the uptake of NO_3^- but at a lower level than ammonium (Grant et al., 1967; McCarthy and Eppley, 1972; Molly and Syrett, 1988b). An unchanged rate of NO_3^- uptake in the presence of 10 μg-at $N \cdot L^{-1}$ of urea has, however, been reported in the marine diatom, *Skeletonema costatum* (Lund, 1987). In the present study the rate of NO_3^- uptake was lower in the presence of urea, but in terms of total N taken up it was approximately 30% greater than the unaltered rate of NO_3^- uptake alone and the uptake rate necessary to maintain the pre-conditioned growth rate.

Although not measured in the present study, it is

possible that NH_4^+ was excreted by the cells and was the causative factor for reduced NO_3^- uptake rates after the addition of urea. Price and Harrison (1988b) reported the release of NH_3/NH_4^+ into the medium by axenic cultures of *Thalassiosira pseudonana* following addition of 10 μg-at $N \cdot L^{-1}$ of urea. Uchida (1976) observed that the red-tide dinoflagellate *Prorocentrum minimum* excreted NH_3/NH_4^+ when grown in urea-enriched culture medium and Rees (1979) also reported NH_3/NH_4^+ release by urea-grown *Phaeodactylum tricornutum*.

Ecological significance

As Dortch et al. (1982) have pointed out previously, the reduction in NO_3^- uptake capability and an enhanced ability to take up NH_4^+ by N-deprived phytoplankton may be an adaptive response to the patterns of nitrogen availability in oligotrophic areas of the ocean. Ammonium and urea are both recycled rapidly within the euphotic zone and may be added sporadically by animal excretion (e.g., Dugdale, 1967). Phytoplankton which have the ability to assimilate NH_4^+ or urea rapidly after starvation may have a selective advantage in areas where N limitation is the major environmental stress. Phytoplankton which maintain the ability to take up nitrate rapidly during N starvation confer little competitive advantage because nitrate is recycled on a much longer time scale and usually supplied continuously at low rates by eddy diffusion from deeper nitrate-rich layers (e.g., Dugdale, 1967; Eppley et al., 1979; King and Devol, 1979). Physical

events such as upwelling (e.g., Walsh et al., 1977, 1978) frontal mixing (e.g., Pingree et al., 1978; Parsons et al., 1981) internal waves (e.g., McGowan and Hayward, 1978; Cullen et al., 1983) may supply NO_3^- at elevated concentrations to the euphotic zone at intervals of days, weeks or longer. However, as Parslow et al. (1984b) pointed out these physical mechanisms of sporadic NO_3^- supply also dilute the phytoplankton concentrations in the euphotic zone, thereby decreasing the demand for the nutrient, increasing the lifetime of the pulse and consequently reducing the benefits of transient elevated uptake rates; hence the metabolic cost of maintaining high uptake capability for nitrate during starvation (between pulses) may outweigh the benefits.

CHAPTER FOUR

EFFECTS OF IRRADIANCE AND DIEL PERIODICITY ON NITROGEN UTILIZATION IN *MICROMONAS PUSILLA*

INTRODUCTION

In order to describe the mechanisms involved in phytoplankton ecology there is a need to first understand the significance of biological interactions with environmental parameters. In most marine environments, light and the availability of nitrogen have been shown to be the factors which primarily regulate phytoplankton productivity (e.g., Dugdale and Goering, 1967; Ryther and Dunstan, 1971; MacIsaac and Dugdale, 1972).

In 1967, Dugdale and Goering partitioned oceanic primary production according to its nitrogen source: "new" production is fuelled by allochthonous N sources, principally nitrate mixed into surface waters from deep ocean reserves, and secondarily, N_2-fixation, riverine inputs and rainfall; "regenerated" production is fuelled by autochthonous N-sources, principally ammonium and urea, derived from biological processes (nutrient recycling) occurring *in situ*. Changes in nitrogen concentrations in the euphotic zone are usually the result of physical mechanisms, such as upwelling and vertical mixing (e.g., Codispoti, 1983; Takahashi et al., 1986; Platt et al., 1989), which increase "new" nitrogen concentrations, or alternatively, biological processes, such as animal excretion (e.g., McCarthy and Goldman, 1979;) which increase "regenerated" nitrogen concentrations.

The light environment shows extreme variation and in a periodic fashion during 24 h, (except at times in high latitude areas). Diel physiological rhythms, in response to fluctuations in light intensity, have been detected in cultures and natural phytoplankton communities and include processes such as photosynthesis (e.g., MacCaull and Platt, 1977; Prézelin and Ley, 1980; Harding et al., 1981, 1983; Putt and Prézelin, 1988); cellular pigment content (e.g., Sournia, 1974; Owens et al., 1980; Harding et al., 1983; Kohata and Watanabe, 1989); *in vivo* chlorophyll *a* fluorescence (e.g., Owens et al., 1980); carbohydrate and protein content (e.g., Ricketts, 1977; Hitchcock, 1980; Terry et al., 1985); cell division (e.g., Chisholm et al., 1980; Chisholm, 1981; Sournia, 1974) enzyme activities (e.g. Eppley et al., 1970; Packard and Blasco, 1974; Martinez et al., 1987) and nitrogen uptake (e.g., Eppley et al., 1970 1971a,b; MacIsaac, 1978; Terry et al., 1985).

Previous studies of cultures and natural phytoplankton communities suggest that N starvation (e.g., Harrison, 1976; Bhovichitra and Swift, 1977) or N limitation (e.g. Malone et al., 1975; Eppley et al., 1971b) may dampen diel periodicity of NO_3^- uptake and assimilation by a relative enhancement of NO_3^- uptake during the night. To date our knowledge of nitrogen uptake by picoplankton have been derived from tracer studies of size-fractioned natural phytoplankton communities (e.g., Probyn, 1985; Probyn and Painting, 1985; Nalewajko and Garside, 1983) and the effects of nutrient limitation,

irradiance, or light periodicity on their N uptake abilities have not been addressed.

The objective of this study was to determine the effect(s) of N limitation on the periodicity of *in situ* and potential N uptake by the ubiquitous, picoflagellate *Micromonas pusilla* (Butch.) Manton et Parke. Nitrate, the N substrate which supports the most productive areas of the world's oceans (e.g., Eppley, 1981; Eppley and Peterson, 1979; Harrison et al., 1987) was the principal focus of the present study. However, the diel periodicity of potential uptake rates of urea and NH_4^+ were also examined in view of the importance of regenerated nitrogenous nutrition of phytoplankton in oligotrophic, oceanic areas (e.g., Harrison, 1980); environments where picoplankton have been demonstrated to be responsible for the majority of the photosynthetic production (e.g., Li et al., 1983; Platt et al., 1983; see revievs by Fogg, 1986; Joint, 1986; Stockner and Antia, 1986).

MATERIALS AND METHODS

Culturing

Continuous and batch cultures of *Micromonas pusilla* (culture NEPCC 29-1, Northeast Pacific Culture Collection, Department of Oceanography, University of British Columbia) were grown on filter-sterilized (0.22 μm Millipore) nutrient-enriched artificial seawater based on ESAW (Harrison et al., 1980). The modifications to the medium and the details of its preparation and storage are described in Chapter 3. Cultures were maintained in an air-cooled, walk-in, growth chamber at 17 ± 0.5°C and illuminated from two sides by eight Vita-Lite^{R} UHO fluorescent tubes (4 on either side of culture vessels) set on a 14 h light and 10 h dark cycle. The light was filtered through 3 mm thick blue Plexiglas^{R} (No. 2069, Rohm and Haas) and the irradiance adjacent to the centre of the culture vessels was ca. 120 $\mu E\ m^{-2}s^{-1}$, as measured with a Li-Cor LI-185 light meter equipped with a LI-190S quantum sensor (2π).

Continuous cultures of *M. pusilla* were maintained in chemostats similar to those described by Davis et al. (1973). Constant flow piston pumps (Fluid Metering Inc., New York) were used to pump the medium from 20 L Pyrex carboys through borosilicate glass or silicone rubber tubing into the reactor vessels. The reactor vessels consisted of 3 L, borosilicate, flat-bottom, boiling flasks (Pyrex) sealed with a silicone stopper so that a constant volume of 2.5 L (accurately measured before each experiment) was maintained inside the

reactor. Cultures were stirred by teflon-coated magnetic stir bars at 60 rpm. Cultures were unialgal but not axenic, however attempts were made to minimize bacterial growth in the medium and cultures by using aseptic techniques and scrupulously cleaning all glassware, pump fittings and tubing with 10% HCl acid (v/v), rinsing with distilled, deionized water (DDW) and autoclaving prior to use. Difficulties in establishing a defined medium after autoclaving and in keeping a continuous culture axenic for extended periods is a common problem (e.g., Goldman, 1977). However, it was felt that by using the above precautions any bacterial effects would be minimized to the point where their effects would be negligible relative to the response(s) of *Micromonas pusilla*. Cultures were allowed to grow as batch cultures for several days (4-5) before continuous addition of the inflow medium containing 50.6 ± 0.9 μg-at $NO_3^- \cdot L^{-1}$ and 0.18 ± 0.08 μg-at $NO_2^- \cdot L^{-1}$ was initiated. Dilution rates, calculated from the volume of effluent collected daily, were 0.24, 0.49 and 0.74 d^{-1} and varied less than 0.01 d^{-1}. Culture samples were withdrawn daily by syringe at the mid-point of the light period for monitoring of <u>in vivo</u> fluorescence, cell counts and particle size distribution. Experiments were not initiated until steady-state had been achieved within each continuous culture. Steady-state was assumed when cell counts were constant to within ±10% for a minimum of three consecutive days in the highest growth culture and ±5% for the two lower growth rate cultures.

Analytical procedures

Cell concentrations were measured with a Coulter Counter^{R} model TA II electronic particle counter according to the procedures outlined in detail in Chapter 3. Average cell volumes were computed from the particle size distribution based on equivalent spherical diameter.

Replicate (2-4) samples for the measurement of particulate organic carbon (POC) and particulate organic nitrogen (PON) were filtered onto precombusted (460°C, 6 h) Whatman GF/F glass fiber filters, stored frozen at -20°C in desiccators and analyzed on a CHN elemental analyzer (Control Equipment Corp. model 240-XA) using acetanilide as the calibration standards. External (ambient) and internal (cellular pools) concentrations of nitrate and nitrite were measured with a Technicon AutoAnalyzer^{R} II following the procedures outlined in Wood et al. (1967). The precision of the above analytical techniques are presented in Appendix 6. Samples for the measurement of external nutrient concentrations were filtered through precombusted Whatman GF/F filters into previously acid-washed, DDW-rinsed polypropylene bottles after an initial rinse with filtrate. Samples were stored frozen (-20°C) until analyses. Internal dissolved nitrogen pools were determined after extraction with boiling DDW (method C-2, Thoresen et al., 1982). Phytoplankton cells (20-50 ml of culture) were filtered onto combusted glass fiber filters (Whatman GF/F, 25 mm, previously washed with 2N HCl and subsequently rinsed with 50 ml boiling and 50 ml cool DDW)

with a low differential pressure (< 80 mm Hg). After filtration, the filter was rinsed with 5-10 ml of culture medium containing no detectable inorganic nitrogen (obtained from gravity filtration of N-starved cultures described in Chapter 3). The filter and cells were extracted with boiling DDW, collected directly into polypropylene bottles, and stored frozen (-20°C) until analyses. Blanks were filters without cells on them and they were treated the same as samples. The filtration apparatus was acid-washed, DDW-rinsed and dried prior to collection of samples.

Samples for ^{15}N analysis were collected on precombusted Whatman GF/F filters and stored frozen in desiccators. The ^{15}N enrichment of samples was assayed by emission spectrometry (JASCO model N-150) after conversion of the particulate N to N_2 gas by the micro-Dumas dry combustion technique. All ^{15}N analyses were conducted in duplicate according to the procedures described in detail in Chapter 1.

Experimental procedures

Diel cycles of uptake and growth

The concentration of cells and inorganic nitrogen in the three continuous cultures and batch cultures was monitored over a 24 h period beginning at the start of the light period. These experiments were conducted with duplicate cultures which were originally inoculated from the same stock culture. At two hour intervals, samples were drawn by syringe directly from the reactor vessels for determination of cell concentration and volume and the ambient and internal NO_3^- and

NO_2^- concentrations. Small volumes (25-60 ml) were withdrawn from the continuous cultures to minimize the perturbation to their steady-state condition. The total volume withdrawn from the cyclostats was between 1 and 2.5% of the culture volume. During the time that the cyclostats were refilling, the outflow was closed. The mathematical equations governing the rate of change of substances in the culture vessels are virtually identical to those describing overflow conditions, because the increase in culture volume due to the input of fresh medium dilutes the culture in a manner similar to that resulting from overflow under constant volume conditions (Laws, 1985; DiTullio and Laws, 1986). Therefore, as long as the volume of sample withdrawn is small compared to the volume of the growth chamber it can be assumed that sampling will not significantly perturb the steady state condition.

Rates of nitrate uptake in the continuous cultures were calculated using the equations of Caperon and Ziemann (1976). The rate of change of nitrate concentration was assumed to be described by the equation:

$$dS/dt = D\cdot(S_i-S) - U/V$$

Where S is the concentration of NO_3^- in the chemostat, S_i is the NO_3^- concentration of the inflow medium, U is the total NO_3^- uptake rate, V is the volume of the growth chamber and D is the dilution rate. The integrated solution of this equation has been given by Caperon and Ziemann (1976) and the nitrate uptake during the interval (t) between two sampling periods is:

$$nX = DS_i + D \cdot \left[\frac{e^{-Dt} \cdot S_o - S_t}{1 - e^{-Dt}} \right]$$

where S_o and S_t are NO_3^- concentrations at times 0 and t, respectively, n is the PON concentration of the culture (assumed to be constant at steady state) and X is the specific uptake rate with nX = U/V. This equation assumes that uptake rates are constant during the sampling intervals, which is not necessarily always true, but the calculated rates are useful as estimates of average uptake rates during these periods.

Uptake rates in the batch cultures, estimated from the disappearance of NO_3^- in the medium, were calculated by dividing the difference in the nutrient concentration in successive samples by the length of the time interval; specific rates were calculated by dividing this value by the estimated exponential average PON concentration during that time. It was assumed that all the NO_3^- removed from the medium was incorporated into the particulate fraction and that none of the NO_3^- taken up was excreted as dissolved organic nitrogen (DON). This situation was observed in other experiments for *M. pusilla* cultures growing under continuous light (Appendix 4).

Diel variation in maximum N uptake rates

At 8 separate times over a 17 d period, experiments were conducted to determine the saturated uptake rates of NO_3^-, NH_4^+ and urea from the continuous cultures grown at 0.74, 0.49, and 0.24 d^{-1}. These experimental times corresponded to

the beginning, middle and end of the 14 h light and 10 h dark periods and are given in Table 4.3. The experimental procedure at each time was as follows: duplicate samples were first collected for the measurement of POC, PON, ambient nitrate and nitrite and cell concentrations. Thirty ml samples were transferred to sterile, 50 ml borosilicate glass test tubes with teflon-lined caps and duplicates inoculated with 16.6 μg-at $N \cdot L^{-1}$ of $^{15}NH_4Cl$, $Na^{15}NO_3$ or $CO(^{15}NH_4)_2$ (Kor Isotopes, 99 atom %). Incubations were conducted in the dark and at the same PPFD as previously grown (ca. 120 $\mu E\ m^{-2}s^{-1}$) and then terminated after 2 h by filtration (pressure differential $\leq$ 80 mm Hg). The N specific saturated uptake rates were determined from the constant specific uptake model of Dugdale and Wilkerson (1986) (equation 6 of Appendix 1). Although the total sample volume withdrawn represents only 10-15% of the growth chamber volume, the cultures were sampled at intervals never less that 1 d^{-1}. Cell concentration was monitored over the 16 d experimental period by withdrawing samples at the mid-point of the light-dark cycle (Fig. 4.1). Cell concentrations were relatively stable over the course of the study and daily variations averaged 8.4 (±7.3), 3.7 (±3.2) and 4.6 (±3.7)% in the 0.73, 0.49 and 0.24 d^{-1} cyclostats, respectively,

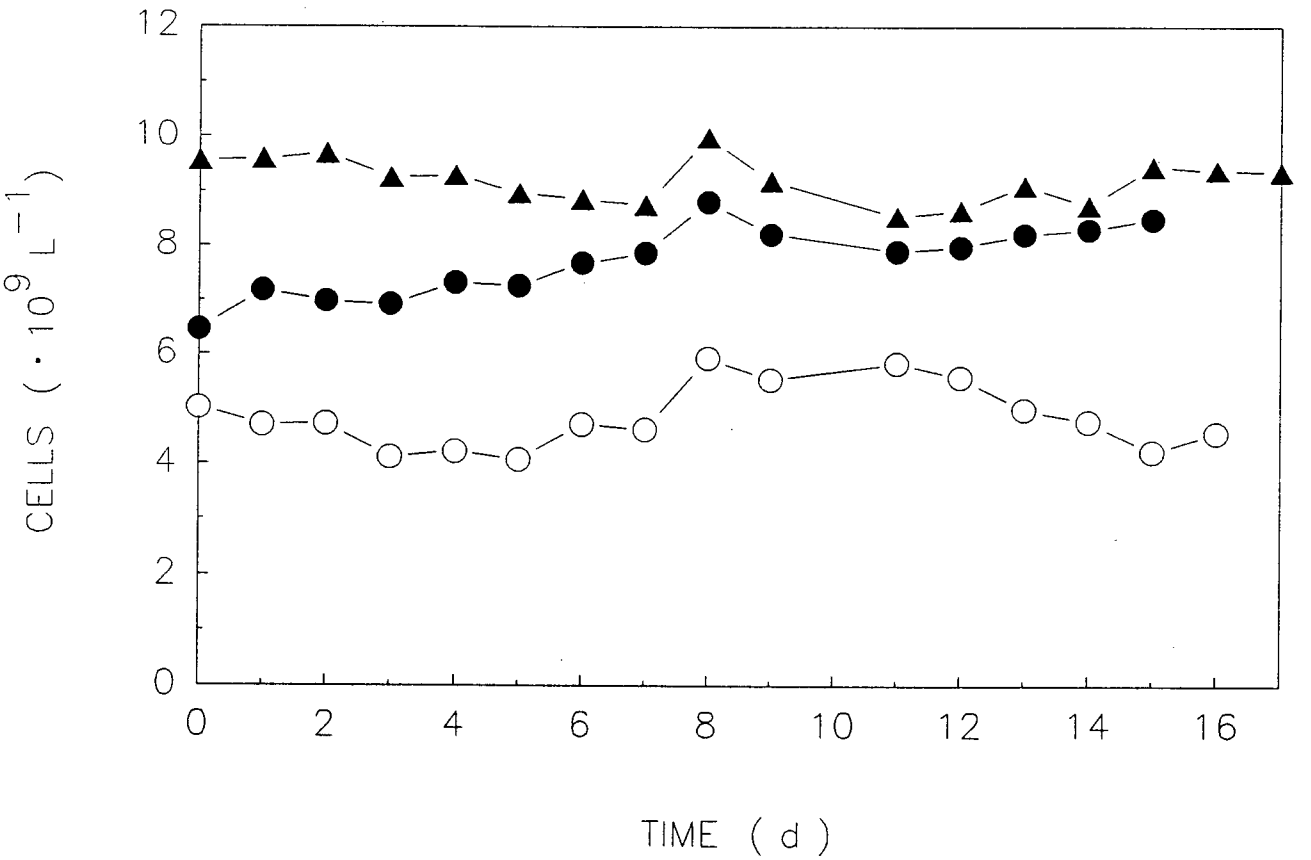
Effect of PPFD on NO_3^- and NH_4^+ uptake

Three continuous cultures (0.77, 0.52 and 0.24 d^{-1}) were harvested just prior to the middle of the light period (6 h

light) to determine the effect(s) of PPFD on NO_3^- and NH_4^+ uptake rate. Duplicate (3 or 4) samples were initially collected for concentration measurements of PON, POC, ambient NO_3^- and NO_2^- and phytoplankton cells. The remainder of the culture was split in half and inoculated with 15 μg-at $N \cdot L^{-1}$ of $Na^{15}NO_3^-$ or $^{15}NH_4Cl$ (Kor Isotopes, 99 atom %). Forty ml samples were immediately transferred, under reduced light conditions, to sterile 50 ml borosilicate glass test tubes, with teflon-lined caps, and placed within neutral density screening to simulate a range of PPFDs from 140 to 3.5 $\mu E\ m^{-2}s^{-1}$ and darkness. Irradiances, achieved by attenuation due to distance and screening, were measured with a Biospherical Instruments QSL-100 4π sensor placed within the screening in the incubation position. Incubations were conducted at the growth temperature and terminated after 2 h by low vacuum filtration ($\leq$ 80 mm Hg) for collection of ^{15}N-labelled particulate material. Duplicate cultures enriched with ^{15}N-labelled NO_3^- and NH_4^+ were also incubated for 4 and 6 h in the dark.

Nitrogen uptake rates were calculated according to the equations described previously for the saturated N uptake experiments. Kinetic constants for NO_3^- and NH_4^+ uptake with respect to PPFD were obtained by a direct fit of the data to a modified Michaelis-Menten hyperbola using the non-linear least-squares technique and formulation described earlier in Chapter 2.

Figure 4.1. Cell concentration as a function of time for nitrate-limited cyclostat cultures of *Micromonas pusilla* grown in a 14h:10h L:D cycle at (○) 0.74, (●) 0.49 and (▲) 0.24 d^{-1} dilution rates. Experiments were conducted on days 2,7,8,11,13 and 16.



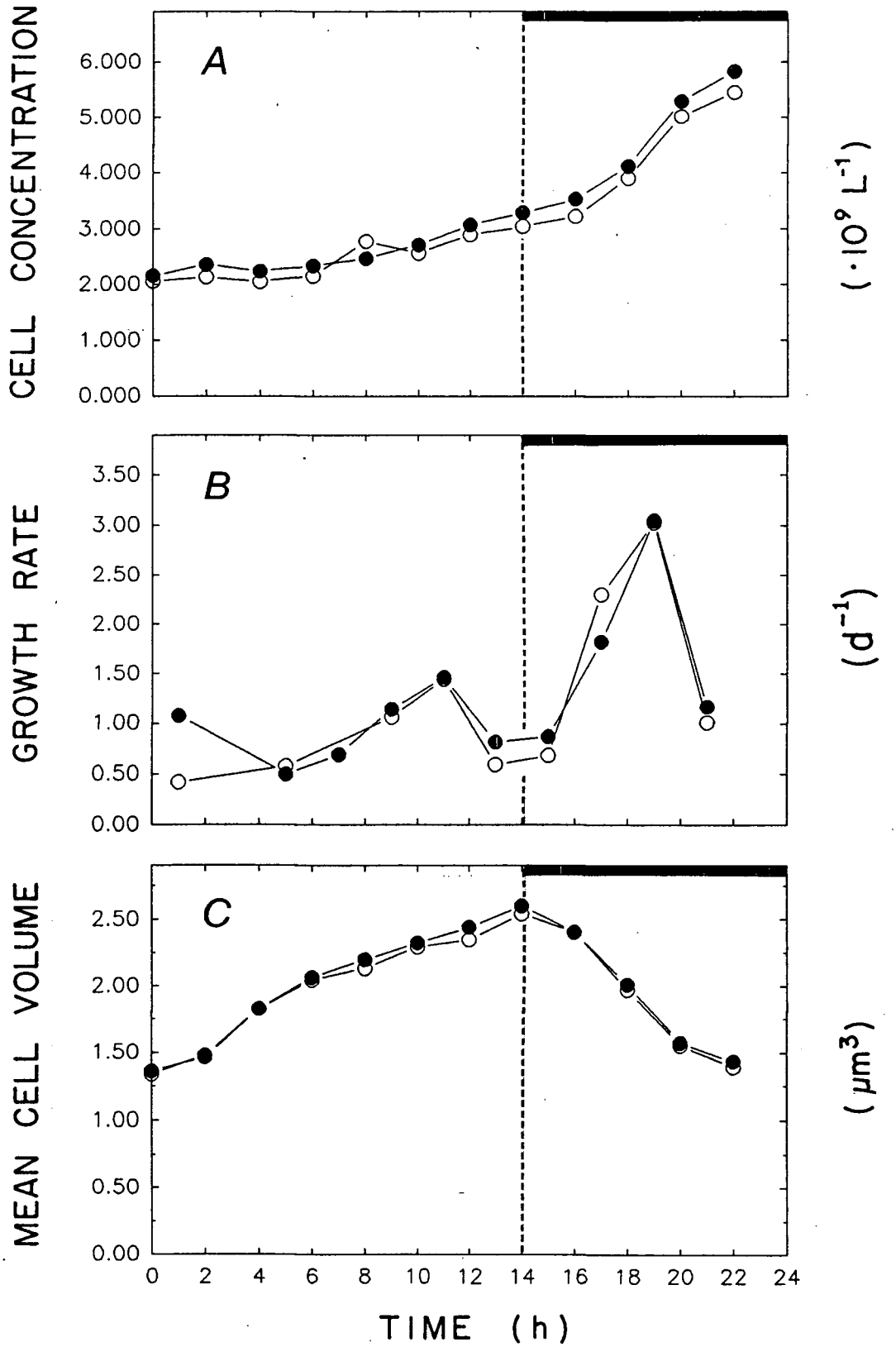
RESULTS

Nitrate-replete cultures

The specific growth rate μ of duplicate NO_3^--replete cultures of *Micromonas pusilla* averaged 1.08 ± 0.013 d^{-1} and 1.13 ± 0.022d^{-1} based on cell concentration and total cell volume, respectively, over the 22 h monitoring period. This rate is consistent with the average growth rate, calculated by the increase in *in vivo* fluorescence, measured prior to experimentation (1.03 d^{-1}) and is not different from the growth rate measured under continuous light (1.11 d^{-1}, Chapter 3). Total cell volume (μL cell volume per L culture medium) was divided by the cell concentration (cells·L^{-1}; Fig. 4.2A) to estimate mean cell volume ($\mu m^3 \cdot cell^{-1}$; Fig. 4.2C) at each 2 h interval during the light-dark illumination cycle. Cell division of *M. pusilla* occurred during the late light period (>10 h light) and primarily throughout the dark period and resulted in a ca. two-fold increase in cell concentration (Fig. 4.2A). The increase in cell concentration by cell division was accompanied by a reduction in cell size and resulted in decreased mean cell volume during the dark period (Fig. 4.2C). The mean cell volume of duplicate NO_3^--replete cultures grown on a 14:10 L:D cycle was 1.96 ± 0.02 μm^3; mean cell volume of *M. pusilla* increased 86% during the light period from 1.35 μm^3 to 2.57 μm^3 at the onset of the dark period.

Nitrate uptake and intracellular pool accumulation showed marked diel variations in the exponentially growing cultures,

Figure 4.2. Cell concentration (A), growth rate (B) and mean cell volume (C) versus elapsed time since lights on in duplicate (O,●) batch cultures of *Micromonas pusilla* grown on a 14h:10h L:D illumination cycle. Dashed line indicates onset of dark period denoted by dark bar. Growth rate plotted against average time between sampling.



although there was considerable variability in internal NO_3^- concentration between replicate cultures. Specific NO_3^- uptake rates averaged 0.0360 ± 0.0010 h^{-1} during the 14 h light period and decreased to 0.0168 ± 0.0028 h^{-1} during the dark (Fig. 4.3B). Rates of NO_3^- uptake normalized to total cell volume showed the same diel variation as N specific rates; daytime values were ca. 2-fold greater than nighttime values. The internal concentrations of NO_3^- (mg-at N·litre cell volume^{-1}; Fig. 4.3C) were greatest at the beginning of the light period in parallel with the most active phase of uptake and decreased throughout the remainder of the light period. The lowest internal nitrate concentrations were during the first 4 h of darkness and subsequently increased markedly (ca. 7-fold) in one culture and only slightly in the other before the commencement of the light period.

Nitrate-limited cultures

Cell division (cytokinesis) showed a strong diel periodicity in the NO_3^--limited cyclostat cultures of *M. pusilla* (Fig. 4.4). In continuous culture the rate of change in cell concentrations with time (dx/dt) is given by:

$$d \ln x/dt = \mu(t) - D$$

where $\mu(t)$ is the instantaneous population growth rate and D is the dilution rate of the culture, which is expressed in the same units of time as $\mu(t)$. An increase in cell numbers with time during the 14h:10h L:D photoperiod, i.e. a positive

Figure 4.3. Dissolved nitrate concentration in culture medium (A), specific nitrate uptake rate (B), and intracellular nitrate concentration (C) of duplicate, batch cultures of *Micromonas pusilla* grown on a 14h:10h L:D illumination cycle. Dashed line indicates onset of dark period denoted by dark bar. Nitrate concentrations plotted against elapsed time since lights on; nitrate uptake plotted against average time between sampling.

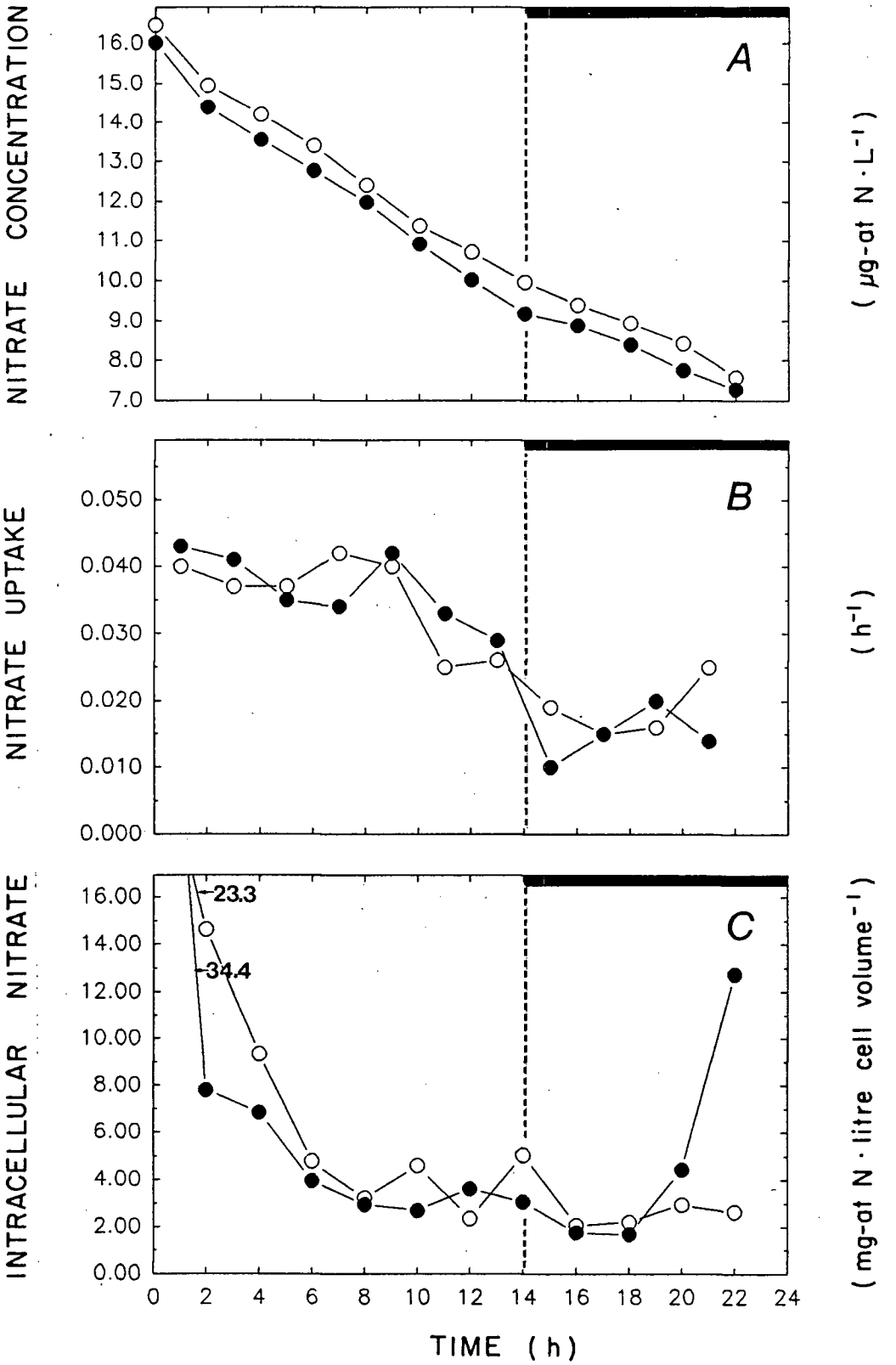
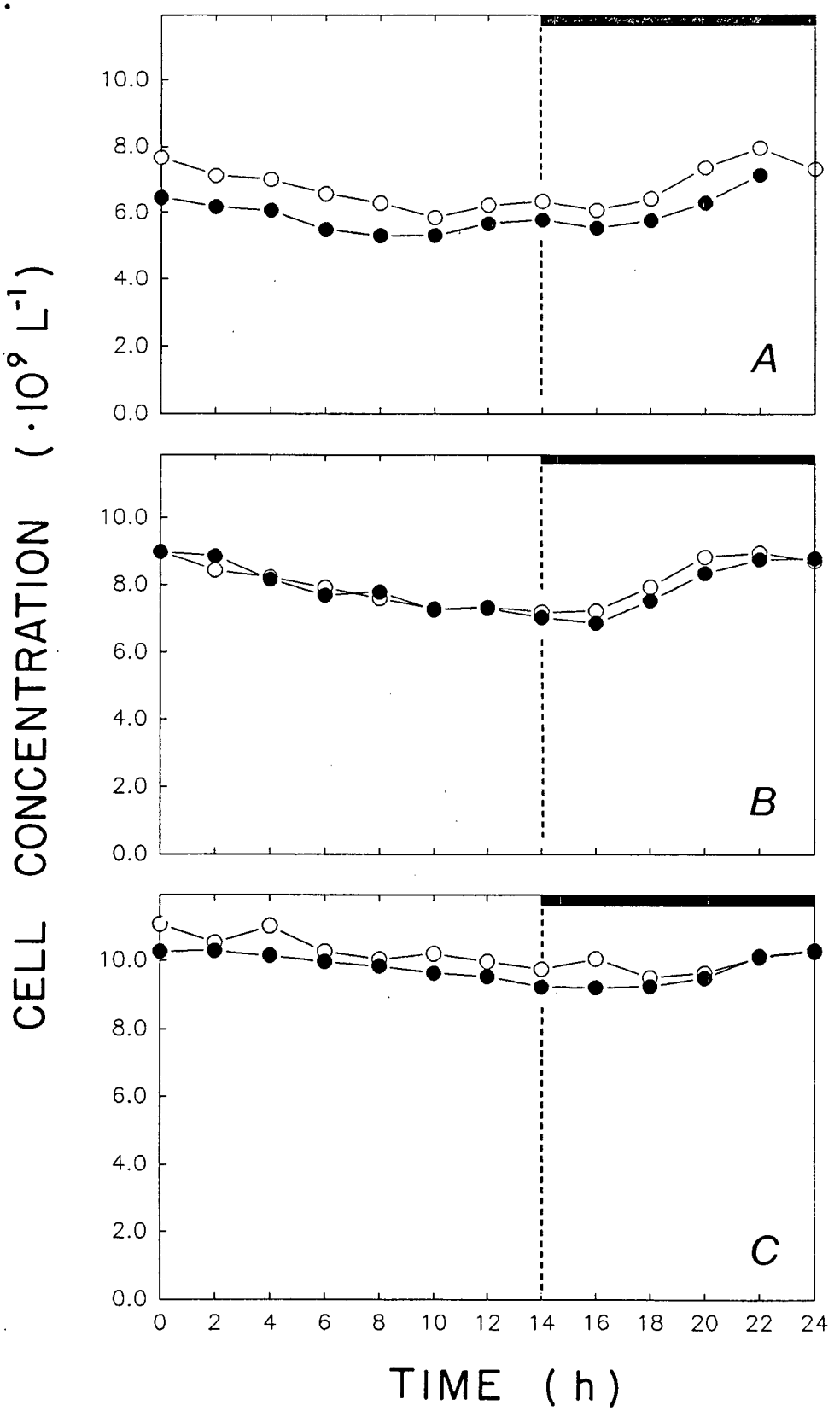


Figure 4.4. Cell concentrations of duplicate nitrate-limited cyclostats of *Micromonas pusilla* grown in a 14h:10h L:D cycle at 0.74 d^{-1} (A), 0.48 d^{-1} (B), and 0.24 d^{-1} (C) dilution rate. Concentration plotted against elapsed time since lights on. Dashed line indicates onset of dark period denoted by dark bar.



dlnx/dt, indicates that $\mu(t) > D$. Thus the time interval during which dlnx/dt is both positive and greatest is the period of maximum cell division. For the cultures grown at the dilution rates 0.73 and 0.75 d^{-1} (hereafter referred to as 0.74 d^{-1}) maximum cell division occurred during the mid-dark period (4-8 h after onset of darkness) although lower positive values of dlnx/dt occurred during late light (10-14 h after lights on) and 2-4 h after darkness (Fig. 4.5). The period of maximum cell division occurred earlier in the dark period (1-6 h after onset of darkness) for the continuous cultures grown at 0.48 d^{-1} dilution rate, although again there were lower positive values of dlnx/dt during the late light (10-12 h after lights on). In the slowest growing continuous cultures (0.24 d^{-1}) the period of maximum cell division occurred 6-8 h after the onset of darkness; in one culture lower positive values of dln/dt were observed throughout the dark period, including the first 2 h of darkness, while in the replicate culture positive values were not observed until after 2 h of darkness and continued throughout the night and the first hours of light.

Mean cell volume ($\mu m^3 \cdot$cell) showed marked diel variation in all cyclostat cultures (Fig. 4.6). In the cultures grown at a dilution rate of 0.74 d^{-1} the cell volume increased by an average of 85% during the light period (0800 h - 2200 h) from 1.12-1.20 μm^3 at the beginning of the light period to a maximum size of 2.08 μm^3, achieved after 6 h of light, and then decreased during the night (2200 h to 0800 h) to the

Figure 4.5. Cell division rate of duplicate nitrate-limited cyclostats of *Micromonas pusilla* grown in a 14h:10h L:D cycle at 0.74 d^{-1} (A), 0.48 d^{-1} (B), and 0.24 d^{-1} (C) dilution rates. Cell division plotted against average time between sampling, dashed horizontal line indicates dilution rate in h^{-1}. Dashed vertical line indicates onset of dark period denoted by dark bar.

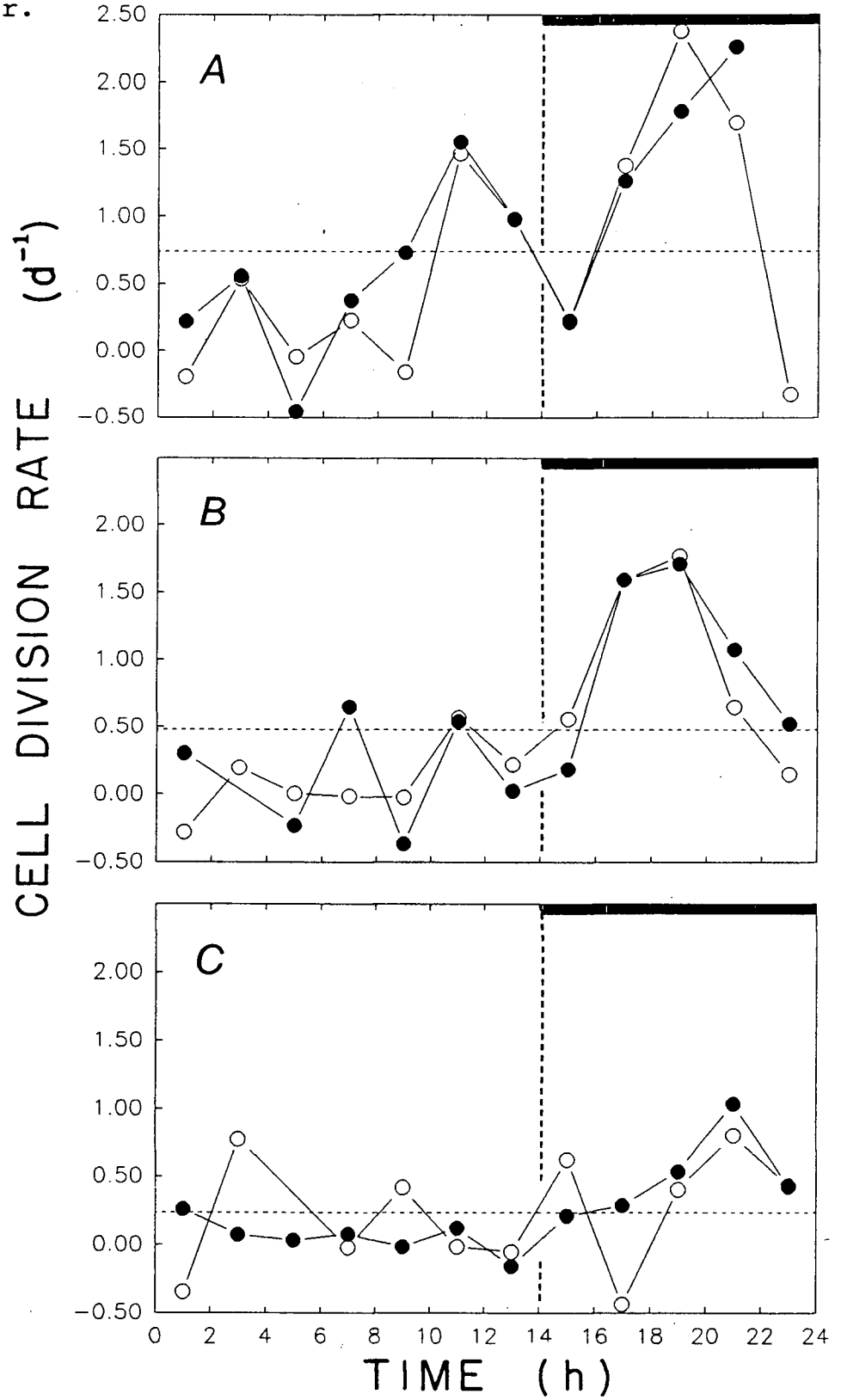
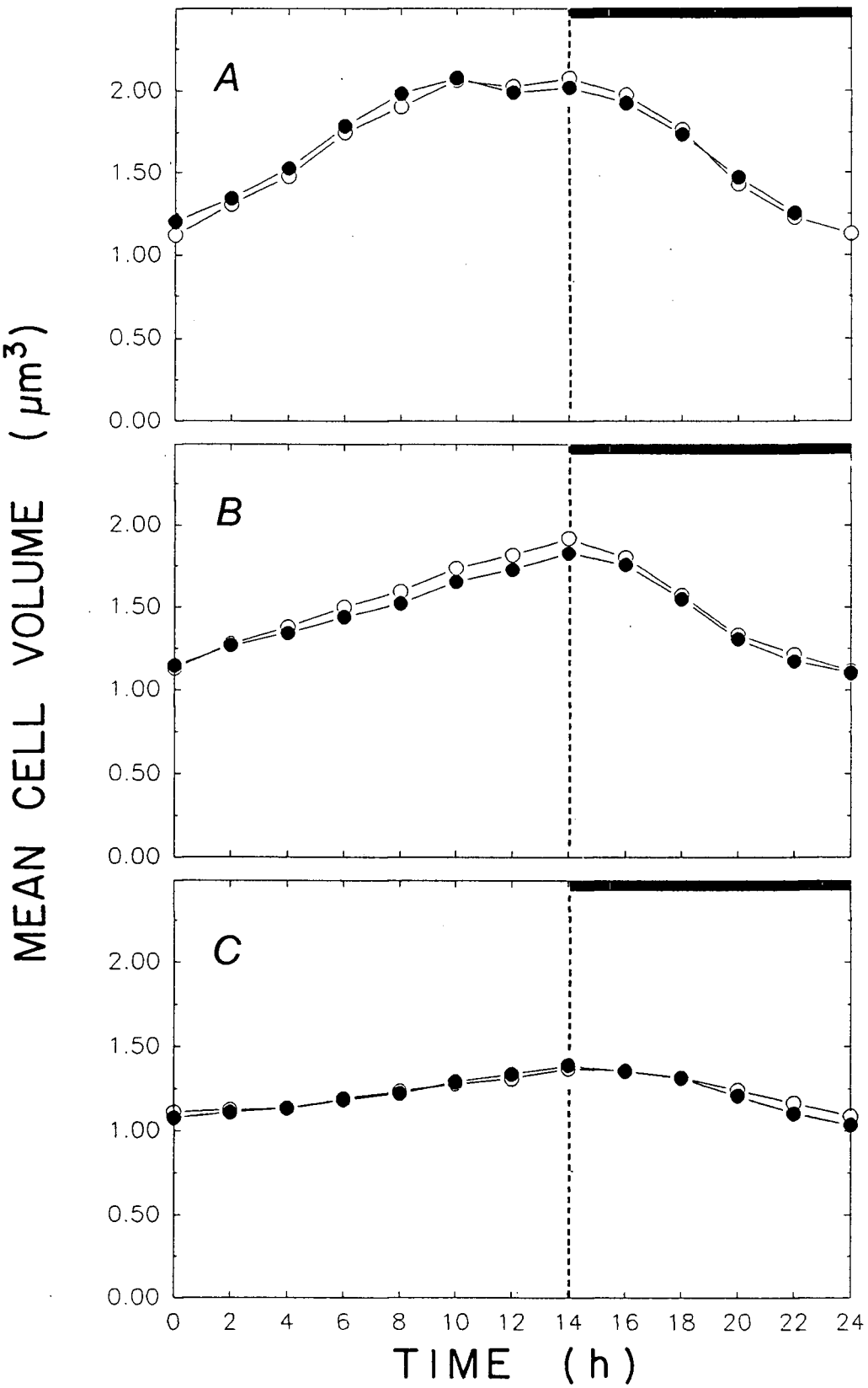


Figure 4.6. Mean cell volume of duplicate nitrate-limited cyclostats of *Micromonas pusilla* grown in a 14h:10h L:D cycle at 0.74 d^{-1} (A), 0.48 d^{-1} (B), and 0.24 d^{-1} (C) dilution rates plotted against elapsed time since lights on. Dashed line indicates onset of dark period denoted by dark bar.



original minimum size by the start of the light period. This diurnal enlargement and nocturnal reduction in cellular volume was also observed in the 0.48 d^{-1} and 0.24 d^{-1} grown cultures where mean cellular volume increased an average of 67% (1.11-1.15 μm^3 to 1.83-1.92 μm^3) and 26% (1.08 to 1.38 μm^3), respectively, over the course of the illumination cycle (Fig. 4.6). The mean (daily) cell volume of duplicate cultures was 1.69 ± 0.01, 1.50 ± 0.03 and 1.23 ± 0.01 μm^3 grown at dilution rates of 0.74, 0.48 and 0.24 d^{-1}, respectively. Cellular nitrogen, estimated from the mass balance of external NO_3^- + NO_2^- concentration in the reactor chamber and the NO_3^- + NO_2^- concentration of incoming medium, showed this rhythmic diel variation of steadily increasing values during the light period and reduction during the night for all the continuous cultures.

The 0.74 d^{-1} dilution rate resulted in diel variations in external NO_3^- concentration in the culture medium, beginning with the rapid reduction during the first half of the light period to minimal concentrations (0.2-1.0 μg-at $N \cdot L^{-1}$ NO_3^-) for the second half of the light period (Fig. 4.7A). With the onset of darkness the external concentration immediately started to steadily increase to a "sunrise" maximum (ca. 7.0 μg-at $N \cdot L^{-1}$). A similar diel trend was observed for NO_2^- concentrations: a decrease during the first half of the light period and subsequent steady increase during the dark period. The 0.48 d^{-1} dilution rate cultures also demonstrated diel variation in external NO_3^- and NO_2^- concentration beginning

with rapid reduction during the first 2 h of light to concentrations at or near the levels of detection (NO_3^-: 0.01; NO_2^- 0.01 μg-at $N \cdot L^{-1}$). With the commencement of darkness, external NO_3^- concentrations increased steadily to a "sunrise" maximum (ca. 1.7 μg-at $N \cdot L^{-1}$) while NO_2^- concentrations reached a maximal concentration (0.30 μg-at $N \cdot L^{-1}$) 2 h prior to lights on (Fig. 4.7B). No diel variation in the external concentration of NO_3^- or NO_2^- was observed for either of the 0.24 d^{-1} dilution rate cultures and ambient concentrations remained at or near the limits of analytical detection throughout the light-dark cycle (Fig. 4.7C).

Rates of nitrate uptake were calculated using the equations of Caperon and Ziemann (1976); specific rates (normalized to average PON during time interval) showed the same diel trends as rates normalized to total cellular volume of culture, and are plotted against time in Figure 4.8. Specific nitrate uptake rates (h^{-1}) of the light period were greater than those of the dark period and demonstrated both nocturnal and diurnal variation in uptake velocities for the 0.74 d^{-1} and 0.48 d^{-1} dilution rate cultures. In the 0.74 d^{-1} (0.0309 h^{-1}) dilution rate cultures, maximal uptake rates of 0.0530 ± 0.0007 h^{-1} were obtained 4-6 h after the lights came on and steadily declined to half this value by the end of the light period. An additional 50% reduction in uptake rate occurred during the first 2 h of darkness with rates subsequently increasing during the remaining dark period to maximal dark uptake rates of 0.0183 ± 0.0003 h^{-1} during 20-22

Figure 4.7. Dissolved nitrate (O,●) and nitrite (Δ,▲) concentrations in the medium of duplicate nitrate-limited cyclostats of *Micromonas pusilla* grown in a 14h:10h L:D cycle at 0.74 d^{-1} (A), 0.48 d^{-1} (B), and 0.24 d^{-1} (C) dilution rates plotted against elapsed time since lights on. Dashed line indicates onset of dark period denoted by dark bar.

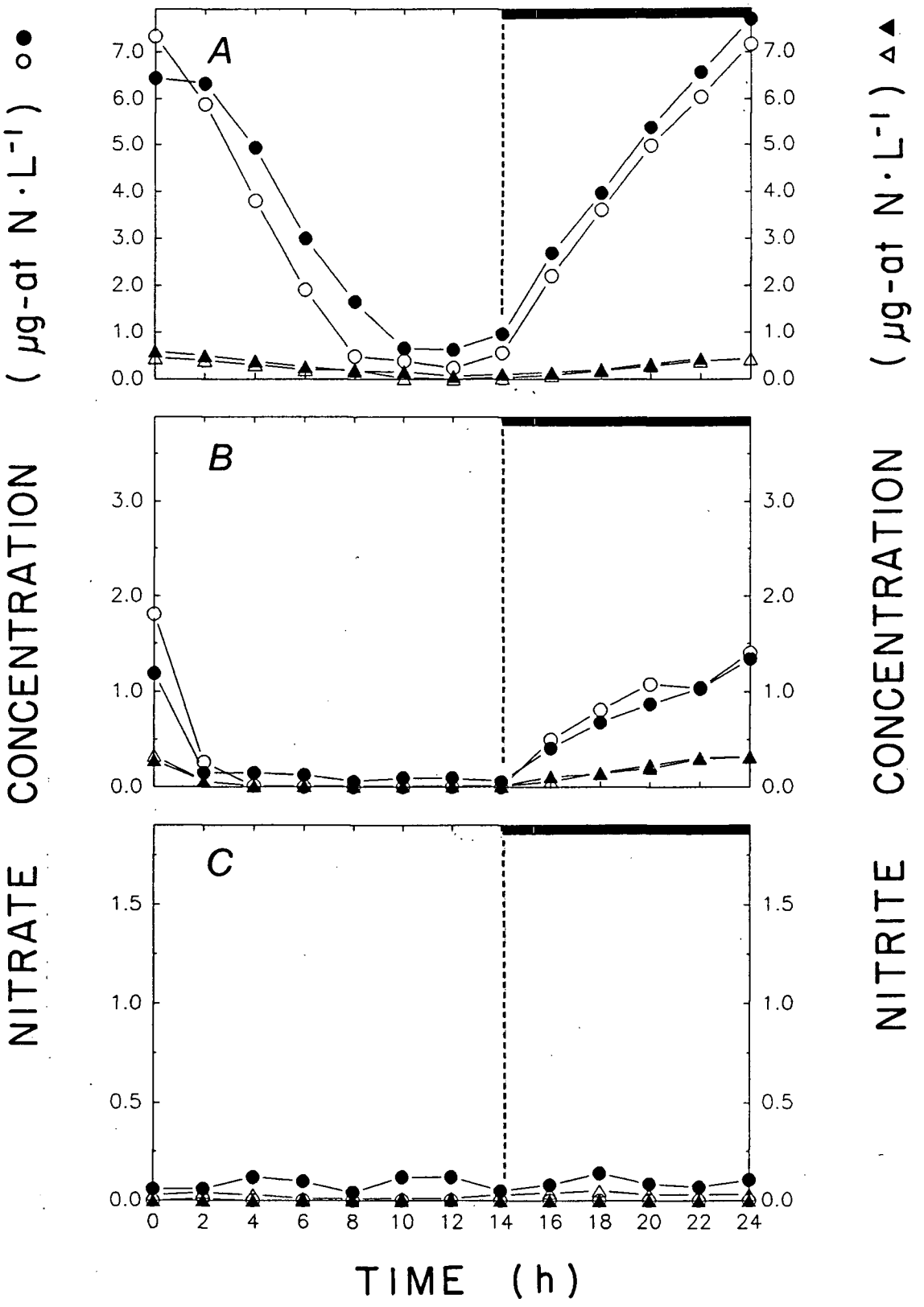
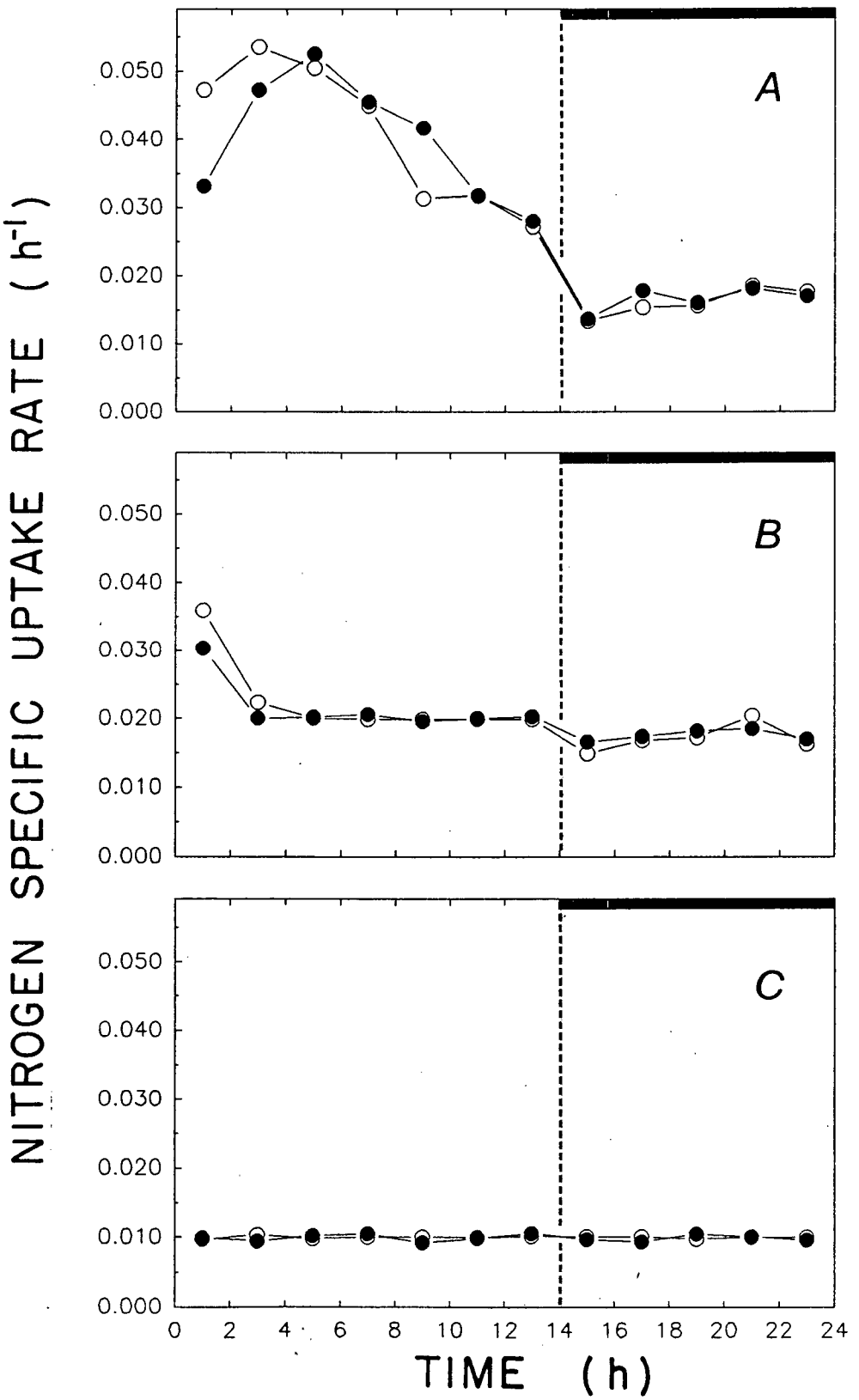


Figure 4.8. Specific nitrate uptake rates of duplicate nitrate-limited cyclostats of *Micromonas pusilla* grown in a 14h:10h L:D cycle at A: 0.74 d^{-1} (0.031 h^{-1}); B: 0.48 d^{-1} (0.020h^{-1}); and C: 0.24 d^{-1} (0.010h^{-1}) dilution rates. Rates plotted against average time between sampling. Dashed line indicates onset of dark period denoted by dark bar.



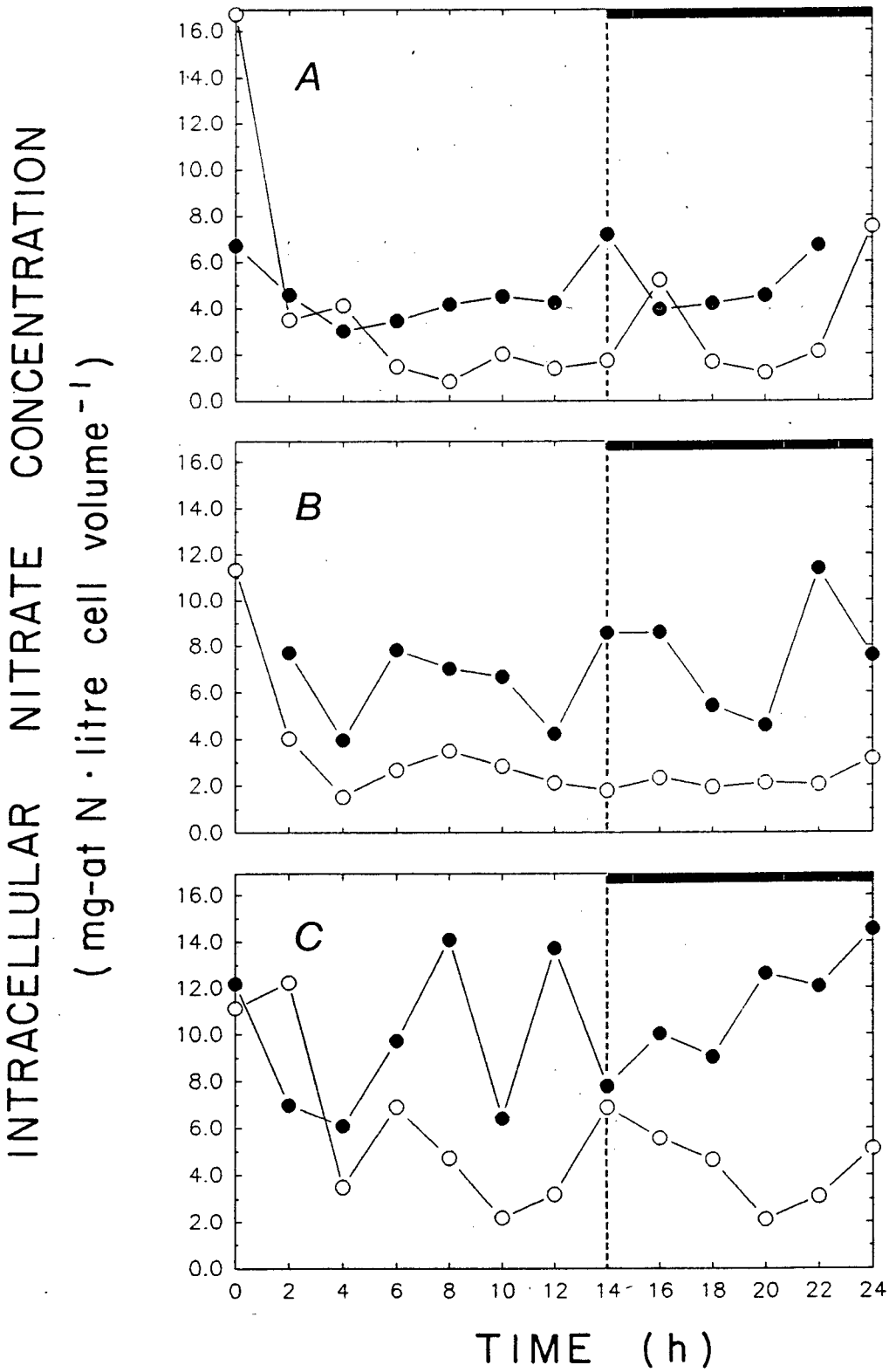
h internal. A very similar pattern of diurnal decline and nocturnal increase occurred in the 0.48 d^{-1} (0.0200 h^{-1}) dilution rate cultures. The maximal light uptake rate of (0.0331 ± 0.0040 h^{-1}) occurred earlier (during the first 2 h of light) and declined over the next 2-4 h to a relatively constant light value of 0.202 ± 0.0001 h^{-1} over the remainder of the light period. During the first 2 h of darkness the uptake rate decreased by an average of 22% and then steadily increased during the remainder of the dark period reaching a maximum dark uptake rate (0.0195 ± 0.0013 h^{-1}) during the 20-22 h interval. The uptake rates of the 0.24 d^{-1} (0.0100 h^{-1}) dilution cultures were constant throughout the light-dark cycle and averaged 0.0100 ± 0.0001 h^{-1} for duplicate cultures. The average light and dark uptake rates for the cyclostat cultures are presented in Table 4.1. The ratio of dark:light uptake rate increased from an average of 0.40 for the fastest growing cultures (D=0.74 d^{-1}) to 0.78 for the 0.40 d^{-1} dilution rate cultures to a value of unity for the 0.24 d^{-1} cultures.

Internal pools of NO_3^- + NO_2^- increased during the last part of the night (t = 20-24 h) in all the cyclostat cultures attaining maximal size at the beginning of the light period (t = 0-2 h; Fig. 4.9). Although there was considerable variability in the magnitude of pool sizes between replicate cultures both the 0.74 d^{-1} and 0.48 d^{-1} dilution rate cultures, which also demonstrated diel variability in NO_3^- uptake, showed a ca. 2-fold increase in NO_3^- + NO_2^- pool size

Table 4.1 Mean light and dark specific nitrate uptake rates (h^{-1}) and their ratios (dark:light) for *Micromonas pusilla* grown on a 14:10 light-dark cycle in batch (*) and cyclostat cultures. The standard deviations of separate (5-7) rate measurements during the light or dark period are given in parentheses.

Growth rate (h^{-1})	NO_3^- uptake rate (h^{-1}) Light	Dark	Dark:Light
0.0470 *	0.0353 (0.0071)	0.0187 (0.0046)	0.53
0.0449 *	0.0366 (0.0053)	0.0149 (0.0042)	0.41
0.0314	0.0400 (0.0092)	0.0161 (0.0020)	0.39
0.0304	0.0409 (0.0106)	0.0164 (0.0020)	0.41
0.0200	0.0226 (0.0060)	0.0171 (0.0020)	0.76
0.0200	0.0216 (0.0039)	0.0175 (0.0008)	0.81
0.0100	0.0100 (0.0002)	0.0100 (0.0001)	1.00
0.0100	0.0100 (0.0001)	0.0099 (0.0004)	0.99

Figure 4.9. Intracellular nitrate concentrations of duplicate nitrate-limited cyclostats of *Micromonas pusilla* grown in a 14h:10h L:D cycle at 0.74 d^{-1} (A), 0.48 d^{-1} (B), and 0.24 d^{-1} (C) dilution rates plotted against elapsed time since lights on. Dashed line indicates onset of dark period denoted by dark bar.



after the first 2 h of darkness in parallel with the rapid reduction in NO_3^- uptake during this time.

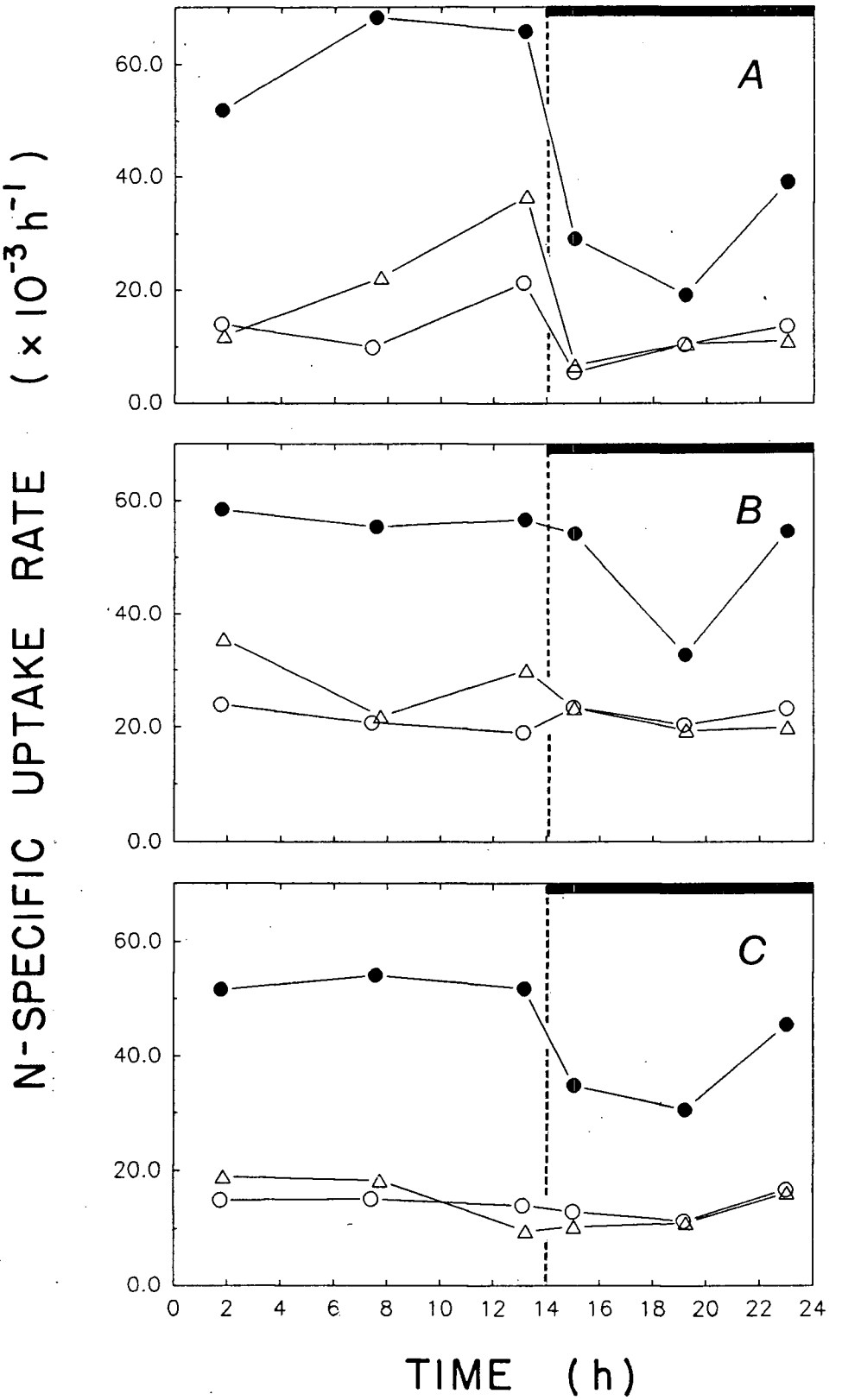
Potential nitrogen uptake rates

At the beginning, middle, and end of the 14 h light and 10 h dark periods saturated uptake rates of NO_3^-, NH_4^+ and urea were determined, from 2 h incubations in the dark or light, for samples collected from the 0.74, 0.49, and 0.24 d^{-1} dilution rate cyclostat cultures. The initial conditions of the cultures used for these batch style incubations are presented in Table 4.2. Diel variability in the initial cell concentration, mean cell volume and cellular N quota of the three cultures were similar to the cyclic patterns discussed earlier for the replicate cyclostats sampled at 2 h intervals over the 14 h light:10 h dark illumination cycle. In only the fastest grown culture ($D = 0.74\ d^{-1}$) was diel variation in external $NO_3^- + NO_2^-$ concentration observed. The N specific uptake rates of NH_4^+, NO_3^- and urea are plotted versus the mid-point of their incubation periods in Figure 4.10. Rates of ammonium uptake were consistently 2-3 fold greater than NO_3^- and urea uptake rates which were similar to each other. It should be noted that the urea uptake rates of the 0.74 d^{-1} culture, estimated using the ^{15}N technique, may be slightly underestimated due to simultaneous uptake of unlabeled substrate (Collos, 1987; Lund, 1987). During 5 of the 6 sampling periods, NO_3^- concentrations saturating for uptake by *M. pusilla* (Chapter 3) were present in the external medium; the potential uptake of unlabeled NO_3^- will dilute the

Table 4.2 Summary of cyclostat culture conditions at the beginning of each experiment.

Day	Culture description	Dilution rate (d^{-1})	$NO_3^- + NO_2^-$ (μg-at N·L^{-1})	PON (μg-at C·L^{-1})	POC	Cell density (10^9·L^{-1})	Average cell Volume (μm^3)	Cell Quota (fg-at N·$cell^{-1}$)
16	initial light	0.76	19.4	31.6	244	4.56	1.4	6.9
2	mid-light	"	9.8	45.5	321	4.73	1.8	9.6
11	end light	"	0.5	50.6	406	5.84	2.0	8.7
13	initial dark	"	3.1	46.2	425	5.04	2.2	9.2
7	mid-dark	"	9.9	41.2	340	4.86	1.8	8.5
8	end dark	"	11.9	38.1	273	5.87	1.3	6.5
16	initial light	0.49	0.3	53.1	386	10.14	1.1	5.2
2	mid-light	"	0.3	49.4	419	6.98	1.5	7.1
11	end light	"	0.3	52.5	429	7.90	1.6	6.6
13	initial dark	"	0.3	48.7	513	7.65	1.8	6.4
7	mid-dark	"	0.3	51.5	468	8.13	1.5	6.3
8	end dark	"	0.4	53.2	399	9.52	1.1	5.6
16	initial light	0.24	0.3	51.1	411	9.86	1.1	5.2
2	mid-light	"	0.2	50.3	443	9.68	1.2	5.2
11	end light	"	0.3	51.6	416	8.55	1.3	6.0
13	initial dark	"	0.2	47.1	468	8.70	1.3	5.4
7	mid-dark	"	0.2	53.8	470	8.69	1.3	6.2
8	end dark	"	0.3	51.9	429	9.56	1.1	5.4

Figure 4.10. Maximum specific uptake rates (h^{-1}) of nitrate (Δ), urea (O), and ammonium (●) determined in 2 h incubations of samples from nitrate-limited cyclostat cultures of *Micromonas pusilla* (14h:10h L:D cycle) grown at 0.74 d^{-1} (A), 0.49 d^{-1} (B), and 0.24 d^{-1} (C) dilution rates. Specific rates are plotted against average time of incubation period. Dashed line indicates onset of dark period denoted by dark bar.



isotopic ratio in the particulate matter and thus decrease the uptake rates. In NO_3^--replete cultures of *M. pusilla*, grown under continuous light, a saturating addition of urea resulted in a 28% decrease in NO_3^- uptake (Chapter 3), however, the degree of urea suppression of NO_3^- uptake in N-limited chemostat cultures is unknown. It is unlikely that NH_4^+ uptake rates were affected by the presence of unlabeled NO_3^- in the medium. The presence of ammonium, at concentrations as low as 1.0 μg-at $N \cdot L^{-1}$, completely inhibited NO_3^- uptake in N-replete *M. pusilla* (Chapter 3) thus, the possibility of simultaneous uptake of labelled NH_4^+ and unlabeled NO_3^- is unlikely.

A marked diel variability in the uptake of all three N substrates was observed for the samples collected from the 0.74 d^{-1} dilution rate culture, average (n = 3) potential dark uptake rates (V_d) of urea, NH_4^+ and NO_3^- were 65, 52, and 40%, respectively, of the mean light uptake rates (Table 4.3). In the more N-deficient cultures (0.49 and 0.24 d^{-1} dilution rates) dark uptake rates were generally very similar to light uptake rates (V_{LT}). In the 0.49 d^{-1} dilution rate cultures, V_d values of urea, NH_4^+ and NO_3^- averaged 106, 83, and 72%, respectively of the mean light values and in the 0.24 d^{-1} dilution rate cultures V_d values were 93, 71, and 80%, respectively of urea, NH_4^+ and NO_3^- potential light uptake rates. Although in both the slower growing cyclostat cultures the mean light and dark rates were of a comparable magnitude, a diurnal variation in V_{LT} was apparent for NO_3^- and urea;

Table 4.3 Nitrogen specific uptake rates (h^{-1}), determined over 2 h in light and darkness, and the their ratios (D/L) for *Micromonas pusilla* previously grown at 0.24, 0.49, 0.74 d^{-1} in NO_3^--limited cyclostat cultures on a 14 h light:10 h dark illumination cycle (lights on: 0800 h, lights off: 2200 h).

Starting time of Incubation Day:time (h)	N specific uptake rate (h^{-1}) 0.74 d^{-1}			0.49 d^{-1}			0.24 d^{-1}		
	L	D	D/L	L	D	D/L	L	D	D/L
Urea									
16: 0845	0.0140	0.0019	0.13	0.0239	0.0058	0.24	0.0150	0.0076	0.51
2: 1425	0.0099	0.0030	0.30	0.0208	0.0025	0.12	0.0151	0.0054	0.36
11: 2007	0.0213	0.0029	0.14	0.0191	0.0103	0.54	0.0140	0.0057	0.41
13: 2200	0.0155	0.0055	0.35	0.0314	0.0234	0.75	0.0242	0.0129	0.53
7: 0210	0.0137	0.0104	0.75	0.0209	0.0203	0.97	0.0142	0.0113	0.80
8: 0615	0.0160	0.0136	0.85	0.0313	0.0232	0.74	0.0263	0.0168	0.64
Ammonium									
16: 0847	0.0520	0.0133	0.26	0.0584	0.0247	0.42	0.0516	0.0277	0.54
2: 1435	0.0682	0.0142	0.21	0.0554	0.0181	0.33	0.0541	0.0234	0.43
11: 2010	0.0658	0.0280	0.43	0.0566	0.0329	0.58	0.0517	0.0298	0.58
13: 2202	0.0555	0.0291	0.53	0.0718	0.0542	0.75	0.0550	0.0348	0.63
7: 0212	0.0394	0.0291	0.74	0.0534	0.0327	0.61	0.0369	0.0306	0.83
8: 0616	0.0668	0.0390	0.58	0.0735	0.0546	0.74	0.0616	0.0455	0.74
Nitrate									
16: 0851	0.0119	0.0037	0.31	0.0355	0.0028	0.08	0.0190	0.0056	0.30
2: 1445	0.0223	0.0036	0.16	0.0219	0.0018	0.08	0.0183	0.0019	0.10
11: 2014	0.0356	0.0068	0.19	0.0300	0.0110	0.37	0.0096	0.0076	0.79
13: 2203	0.0214	0.0067	0.32	0.0456	0.0234	0.51	0.0300	0.0103	0.34
7: 0215	0.0160	0.0105	0.66	0.0292	0.0194	0.66	0.0212	0.0110	0.52
8: 0618	0.0270	0.0109	0.40	0.0490	0.0199	0.41	0.0383	0.0162	0.42

maximal rates were observed in the samples collected at the beginning of the light period, while NH_4^+ potential uptake rates were relatively constant throughout the light period. Dark uptake rates were consistently lower for samples collected during the night, however light uptake rates from both light and dark periods were generally equitable.

Influence of light on nitrate and ammonium uptake rates

Nitrogen specific uptake rates, determined over 2 h, were plotted versus the PPFD experienced by the cells previously grown in 0.24, 0.52 and 0.77 d^{-1} dilution rate cyclostats (Fig. 4.11). Dark uptake (V_D), the half-saturation constant (K_{LT}'), and maximum nitrogen uptake velocity V'_{max} for light dependent NH_4^+ and NO_3^- uptake are summarized in Table 4.4. It is important to note that these Michaelis-Menten parameters are derived from data obtained from the hyperbolic (or light) portion of the PPFD response curve, thus K_{LT} values reported are the PPFD at which 0.5 V'_{max} occurs where V'_{max} is the maximal velocity in the light. The half-saturation constant (K_{LT}') which is the PPFD when one-half the total maximum N-uptake of the cells is achieved, $(V'_{max} + V_D)/2)$, can be calculated by simple rearrangement of the Michaelis-Menten equation (see Chapter 2 for details). Another similar half-saturation constant ($K_{LT}"$) can be calculated for half the total N-uptake achieved at the growth PPFD (120 $\mu E\ m^{-2}s^{-1}$). These two latter half-saturation constants include the substantial dark uptake velocities which appear to vary inversely with dilution rate (Table 4.5). The dark NO_3^-

Figure 4.11. Nitrogen specific uptake rates, determined over 2 h, after saturating enrichment of $^{15}NH_4^+$ (●) or $^{15}NO_3^-$ (○) to nitrate-limited cyclostat cultures of *Micromonas pusilla* (14h:10h L:D cycle) previously grown at 0.77 d^{-1} (A), 0.52 d^{-1} (B), and 0.24 d^{-1} (C) dilution rates. Uptake rates (h^{-1}) are plotted against incident PPFD, curved plots are fitted directly to the Michaelis-Menten equation by computer programme (see text for details).

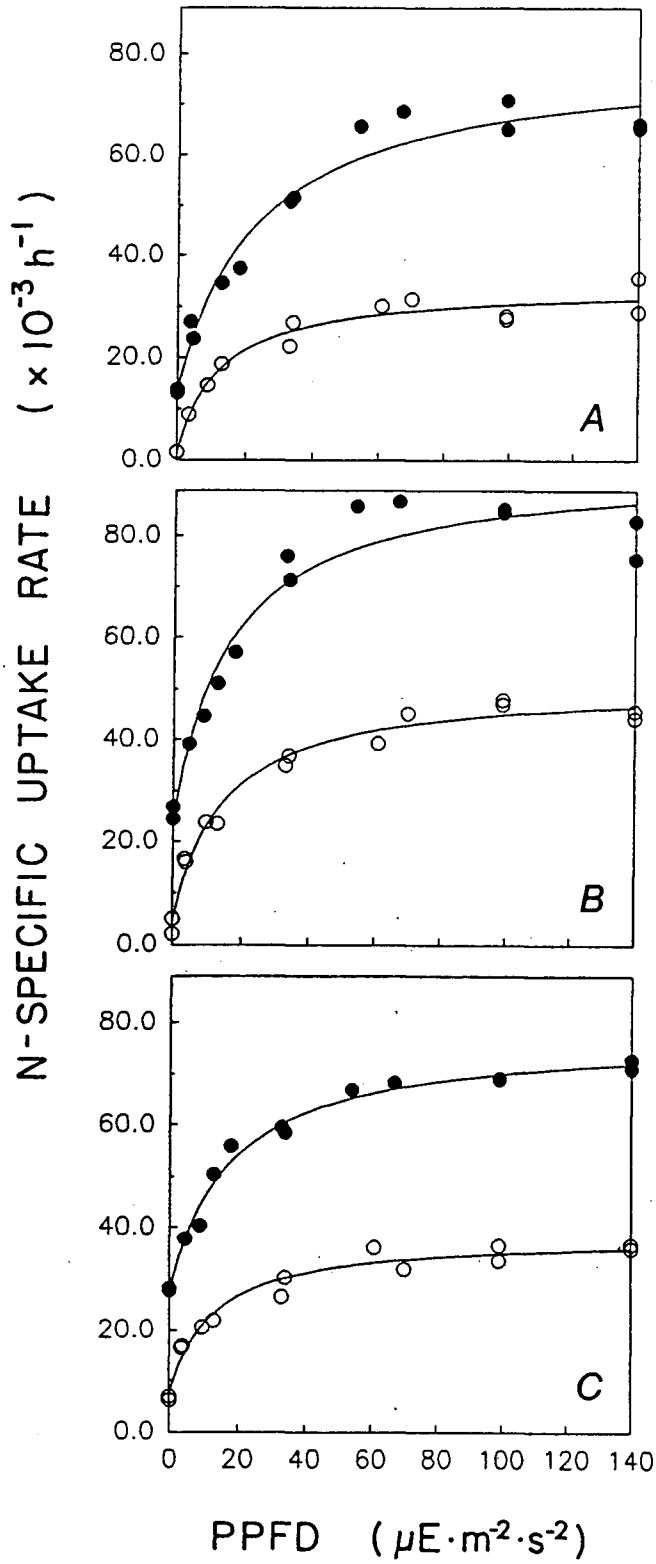


Table 4.4 Parameters describing the characteristics of N specific uptake (h^{-1}), as a function of PPFD for cyclostat cultures of *Micromonas pusilla* (Fig. 4.11). Dark uptake (V_D), maximum specific light uptake (V'_{max}), the half-saturation constant (K_{LT}), and the slope of initial portion of N uptake vs PPFD curve ($\alpha = V'_{max}/K_{LT}$). Estimated standard errors of parameters are given in parentheses.

Dilution rate (d^{-1})	PON conc. (μg-at $N \cdot L^{-1}$)	Nitrogen substrate	V_D (h^{-1})	V'_{max} (h^{-1})	K_{LT} ($\mu E\ m^{-2}s^{-1}$)	α
0.77	28.0	NO_3^-	0.0017 (0.00160)	0.0323 (0.00203)	13 (3.3)	2.6
		NH_4^+	0.0137 (0.00255)	0.0656 (0.00411)	26 (5.8)	2.8
0.52	53.5	NO_3^-	0.0048 (0.00155)	0.0460 (0.00198)	15 (2.7)	3.2
		NH_4^+	0.0243 (0.00370)	0.0693 (0.00496)	16 (4.5)	4.2
0.24	47.8	NO_3^-	0.0076 (0.00134)	0.0310 (0.00164)	13 (2.9)	2.5
		NH_4^+	0.0276 (0.00126)	0.0499 (0.00173)	18 (2.4)	2.8

Table 4.5 Indices of N uptake dependency on PPFD for cyclostat cultures of *Micromonas pusilla*: the ratio of dark to light-saturated uptake rate (V_D:V_L), the PPFD at which half the total N uptake occurs (K_{LT}', K_{LT}'')* and the ratio of N uptake at 1% I to N uptake at 100% I ($V_{1\%}$:$V_{100\%}$). Saturated PPFD and I are the growth PPFD (120 $\mu E\ m^{-2}s^{-1}$).

Dilution rate (d^{-1})	Nitrogen Substrate	V_D:V_L	K_{LT}'	K_{LT}''	$V_{1\%}$:$V_{100\%}$
0.77	NO_3^-	0.05	11.3	9.6	0.15
0.77	NH_4^+	0.20	15.4	11.3	0.25
0.52	NO_3^-	0.10	11.9	9.8	0.18
0.52	NH_4^+	0.29	7.9	7.3	0.34
0.24	NO_3^-	0.21	7.6	6.4	0.29
0.24	NH_4^+	0.39	5.1	3.5	0.43

* Definitions given in text

uptake rate is 5, 10, and 21% of the total uptake for the 0.77, 0.52, and 0.24 d^{-1} dilution rate cultures, respectively. Dark NH_4^+ uptake rates, consistently greater than dark NO_3^- uptake rates, are 20, 29 and 39% of the total (light + dark) uptake for the same cultures. The slope (α) of the initial portion of the PPFD response curve, calculated by division of V_{max} by K_{LT} (Healey, 1980; Parslow et al., 1985) are similar for NH_4^+ and NO_3^- uptake by the cells from the 0.77 and 0.24 d^{-1} dilution rate cultures, but substantially greater for NH_4^+ uptake by the 0.52 d^{-1} dilution rate culture. These results suggest similar light uptake response of both N-substrates to increasing subsaturating PPFDs by fast and slow growing cultures whereas the intermediately growing cultures appear most capable of increasing their uptake ability of NO_3^-, and particularly NH_4^+, in response to increasing, but subsaturating PPFDs.

The effects of PPFD on total N uptake (light + dark) can be estimated by a comparison of uptake at 1 and 100% of the growth irradiance; lower percentages represent greater PPFD dependency (Table 4.5). The effect of PPFD on total N uptake increases with increasing dilution rate, which suggests that increased N limitation lessens the light dependency of NO_3^- and NH_4^+ by *Micromonas pusilla*. Although this light effect was observed for both N substrates, NH_4^+, which was consistently taken up at greater rates than NO_3^- in both light and the dark, demonstrated ca. 40% less dependency on PPFD than NO_3^-.

DISCUSSION

The cell division cycle of most phytoplankton cells is partially synchronized or phased by the environmental light:dark cycle. Evidence of phasing in both algal cultures and natural communities has shown that, in most species grown on light:dark cycles, the instantaneous population division rate, μ, varies with a 24 h periodicity and that the timing of division depends on both experimental conditions and species (see reviews by Sournia, 1974; Chisholm et al., 1980; Sweeney, 1983). In both N-replete (batch) and N-limited (cyclostat) cultures of *Micromonas pusilla*, grown in a 14:10 L:D cycle, partial phasing of cell division was evident. Maximum division rates occurred at the end of the light period and the middle to late dark period. With increased nitrate limitation (i.e. decreased dilution rate) the importance of the light division appeared to decrease and the timing of the dark division was shifted slightly later in the night in the 0.24 d^{-1} dilution rate cultures. Nitrogen and carbon uptake rates are often presented on a normalized cellular basis, but any co-occurrence of synchronous cell division can seriously modify the apparent periodicity in uptake rates. For this reason the majority of the results in the present study have been normalized to total cellular volume or particulate N. The synchrony of cell division was reflected in the mean cell volume (or size) of the *M. pusilla* cells. Cell volume steadily increased during the light period attaining maximal size at the onset of the dark period and subsequently declined

steadily throughout the night attaining minimal size at the onset of the light period. However, with increasing NO_3^- limitation, the increase in average cell volume during the light period was reduced; maximal cell volumes attained by the 0.24 d^{-1} dilution rate cultures were only ca. 50% of the maximal size attained by the NO_3^--replete batch cultures. The mean (daily) cell volume of *M. pusilla* increased significantly ($r = 0.99$, $P \leq 0.01$) with increasing steady-state dilution rate (increasing μ and improved NO_3^- supply). Similar reduction in cell volume with increasing nutrient limitation has been reported for P-limited (Burmaster, 1979) and NH_4^+-limited chemostat cultures of *Pavlova lutheri* (Caperon and Meyer, 1972), and *Chaetoceros debilis, Skeletonema costatum,* and *Thalassiosira gravida* (Harrison et al., 1977). However, others have documented either no cell volume variability with dilution (or growth) rate in N-limited chemostat cultures of *Dunaliella tertiolecta, Thalassiosira pseudonana* and *Coccochloris stagnina* (Caperon and Meyer, 1972) or an increase in cell volume with increasing P-limitation in chemostat cultures of *T. pseudonana* (Fuhs et al., 1972). Using cyclostat cultures of four different clones of *Thalassiosira weisflogii*, Chisholm and Costello (1980) studied the effect of average cell size on growth rate and concluded that growth rate was an increasing function of average cell volume. Munk and Riley (1952) state that the larger the surface area to volume ratio (SA/V) the greater the cell's capacity to absorb nutrients. The reduction in mean cell volume of *M. pusilla*

and the subsequent increase in relative surface area available for NO_3^- uptake is perhaps an adaptive response to NO_3^- limitation.

In the nitrate-replete batch cultures and the nitrate-limited cyclostat cultures, where growth rate is limited by the rate of supply of NO_3^-, obvious diel patterns for NO_3^- uptake were observed for *M. pusilla* in all but the most NO_3^--deficient cyclostat populations ($D = 0.24\ d^{-1}$). The nitrate uptake rates, for the cultures grown in batch or in cyclostats at 0.74 and 0.42 d^{-1} dilution rates, were maximal during the light period and decreased during the dark period. In addition to the diel pattern, the early light nitrate uptake maximum (early morning) and increased dark uptake during late night (pre-dawn) suggests diurnal and nocturnal cycles for the 0.74 and 0.42 d^{-1} dilution rate cultures. Malone et al. (1975) found similar cyclic variations in NO_3^- uptake by an outdoor culture of *Chaetoceros* sp. grown at high dilution rate (2.0 d^{-1}) under natural sunlight, but uptake independence of the light-dark cycle for three slower dilution rates (0.5, 1.0 and 1.5 d^{-1}); absence of diel periodicity in NO_3^- uptake was also observed in axenic cyclostat cultures of the same *Chaetoceros sp.* (STX-105) grown at 6 low dilution rates (0.3 - 1.2 d^{-1}) by Picard (1976). Dark NO_3^- uptake capacity of cyclostat cultures of the marine prymnesiophyte, *Pavlova lutheri* and chlorophyte, *Dunaliella tertiolecta* was exceeded by the supply rate of NO_3^- at high dilution rates (> ca. 0.5 d^{-1}), but no diel periodicity was observed at lower dilution

rates (Laws and Caperon, 1976; Laws and Wong, 1978) although the diatom, *Thalassiosira allenii* never showed diel periodicity in NO_3^- uptake at 6 dilution rates from 0.1-1.4 d^{-1} (Laws and Wong, 1978). Eppley et al. (1971b) found a similar lack of diel periodicity in nitrogen-(NO_3^- + NH_4^+) limited cyclostat (0.78 d^{-1}) cultures of *Emiliana huxleyi*, but greater NO_3^- and NH_4^+ uptake rates in the light period and lesser rates in the dark period for a similarly grown cyclostat (0.73 d^{-1}) culture of *Skeletonema costatum*. It appears that the ability to take up NO_3^- during the night may be species dependent and that the degree of N limitation affects dark uptake capacity and hence the presence or lack of diel periodicity in NO_3^- uptake rates. A dampening effect on diel periodicity of NO_3^- uptake by nitrogen stress is suggested by the results of numerous field studies of natural phytoplankton communities (see Chapter 1). For example, in extensive dinoflagellate blooms dominated by *Gymnodinium splendens* (= *G. sanguineum*) in the nitrate-depleted (<0.1 μg-at $N \cdot L^{-1}$) waters off Peru, nighttime NO_3^- uptake rates were ca. 50% of daylight NO_3^- uptake rates (Dortch and Maske, 1982). In contrast, MacIsaac (1978) reported that uptake at night averaged only 10-20% of the daylight rates for a *Gonyaulax polyedra* bloom off Baja California. However, here the ambient NO_3^- concentrations in the surface waters were generally ca. 1 μg-at $N \cdot L^{-1}$ and the nitracline was much shallower than off Peru. Similar results were observed by Harrison (1976) for N-sufficient cultures of *Gonyaulax*

polyedra; nighttime NO_3^- uptake was ca. 20% of daytime uptake, but dark uptake increased to ca. 40% of daytime values in N-starved cultures and similarly NO_3^--depleted red tide populations dominated by *G. polyedra*. It is difficult to compare the results of field studies to laboratory cyclostat studies as the uptake rates reported in field investigations (e.g., Harrison, 1976; MacIsaac, 1978) are generally substrate saturated or considerably enhanced relative to the ambient concentration due to the concentration of ^{15}N added. Diel field studies of in situ NO_3^- uptake in marine (e.g., Cochlan 1982, 1986; Koike et al., 1986) and freshwater environments (e.g. Whalen and Alexander, 1984) are relatively few and no clear evidence of diel periodicity in either NO_3^- or NH_4^+ uptake rates were observed when concentrations of ambient inorganic nitrogen are low (see Chapter 1 Discussion).

Intracellular nitrate concentrations demonstrated a marked nocturnal increase during the latter portion of the night and attained maximal concentrations at the beginning of the light period for N-sufficient and N-limited cultures of *M. pusilla*. Raimbault and Mingazzini (1987) also observed an early morning maximum in NO_3^- intracellular pool size for N-sufficient cultures of *Phaeodactylum tricornutum* and *Skeletonema costatum* and a minimal internal NO_3^- pool in their singular nighttime measurement taken in the first half of the dark period. Only in the highest dilution rate cyclostat culture of *Chaetoceros* sp. did Malone et al. (1975) observe diel periodicity of internal NO_3^- pool size, while internal

NO_3^- concentrations remained low and constant in the lower dilution rate cultures which also demonstrated NO_3^- uptake independence of light-dark cycle. No clear diel trends in internal pools were observed by Picard (1976) in similar cyclostats with *Chaetoceros* grown at low dilution rates. In shipboard cultures of a natural phytoplankton community from an upwelled region, Collos and Slawyk (1976) observed maximal intracellular NO_3^- concentrations at the beginning of the light period and minimal values during the night. In Dabob Bay, Washington, Dortch et al. (1985) reported diel variability in intracellular nitrate pools for surface communities during July, similar to my results, but variable results with no clear diel rhythm apparent for deepwater communities in May and July and the surface community during May.

The increase in internal NO_3^- observed in the *Micromonas pusilla* cultures demonstrates an uncoupling between NO_3^- uptake and reduction during this time; if NO_3^- intracellular pools were constant then rates of uptake and reduction would be equal (or both zero), and if intracellular pools decreased the rate of reduction of NO_3^-would be greater than rate of uptake (Collos and Slawyk, 1976). Clearly during the late night and early light periods, the processes of NO_3^- uptake and reduction are not in phase, and are likely the result of diel cycles of nitrate reductase (NR) activity.

Early studies of the Peru (Eppley et al., 1970; Packard et al., 1974) and northwest African (Packard and Blasco 1974)

upwelling system have suggested diel cycles of NR activity and in a similar, but more detailed study of the northwest African upwelling system, Martinez et al. (1987) observed diel cycles in NR activity that clearly followed the diel variation in light intensity: a daytime maximum, nighttime minimum and the onset of NR activation coincident with dawn. They failed to observe a pre-dawn rise in NR activity that had been previously reported in upwelling areas (Eppley et al., 1970; Packard and Blasco, 1974), but suggested that the sampling frequency around dawn in those earlier studies was insufficient to determine conclusively the presence of such a pre-dawn rise. Diel periodicity has also been observed in unialgal cultures, such as *Amphidinium carterae* and *Cachonina niei* (Hershey and Swift, 1976), *Emiliana huxleyi* (Eppley et al., 1971b) with minimal activity reported during late night. A similar rhythm in NR activity in *M. pusilla* could account for the accumulation of internal NO_3^- observed during late night and early morning. Alternatively, the absence of diel NR activity with little or no variation during the light-dark cycle, such as in N-starved natural populations of *Gonyaulax polyedra* (Eppley and Harrison, 1975; Harrison, 1976), combined with the increased NO_3^- uptake during the late night and early morning observed in the two faster grown cyclostat cultures (D = 0.74 and 0.48 d^{-1}) , could lead to accumulation of NO_3^- internally. A reduction in nitrite reductase (NiR) activity during late night (e.g., Eppley et al., 1971b) and subsequent leakage of NO_3^- + NO_2^- from the cells may account for the

increase in external NO_2^- concentrations during late night for the 0.74 and 0.48 d^{-1} dilution rate cultures. Alternatively, the rise in external NO_2^- concentration may be explained as resulting from cellular loss during this period of maximal cell division.

A pronounced diel rhythm in the potential uptake capacity of NO_3^-, NH_4^+ and urea was observed in the fastest growing cyclostat cultures of *Micromonas pusilla* (D = 0.74 d^{-1}), whereas the more N-limited cultures (D = 0.48 and 0.24 d^{-1}) had similar night and day N uptake capacity. Laws and Wong (1978) also observed that the relative importance of potential nighttime uptake decreased relative to potential daylight uptake with increasing dilution rate (0.00573-0.02464 h^{-1}) in cyclostat cultures of *Pavlova lutheri*, although they observed no such trend for *Thalassiosira allenni* over a range of dilution rates from 0.00474 to 0.05937 h^{-1}, in fact nighttime uptake averaged 120% of the respective daylight NO_3^- uptake. Although there are no other comparable diel uptake data for cyclostat cultures growing at various degrees of N limitation a revealing comparison can be made between my results and previous work with N-replete and N-starved cultures. For example, the dinoflagellate *Gyrodinium aureolum* does not take up NO_3^- in the dark when in a state of nitrogen sufficiency, but when N-starved for 24 h nighttime uptake became about half of light uptake (Paasche et al., 1984). They found that the ability to take up NO_3^- and NH_4^+ during the nighttime varied considerably among N-sufficient dinoflagellates, but that

relative dark uptake of NH_4^+ was generally greater than that of NO_3^-, similar to that observed here for *M. pusilla*. Similarly, Bhovichitra and Swift (1977) showed that the NO_3^- uptake capacity of N-starved oceanic dinoflagellates, *Pyrocystis noctiluca* and *Dissodinium lunula*, were virtually independent of the light-dark cycle.

It appears that N starvation or limitation enhances potential dark uptake more than light uptake of nitrogen resulting in a more or less continuous uptake of nitrogen in N-depleted phytoplankton, including the picoplankter *Micromonas pusilla*.

The decreasing light dependence with increasing N limitation is also suggested by the results of the N uptake versus irradiance experiments conducted during mid-day. With increased N limitation (decreased dilution rate) the relative dark uptake capacity increased four-fold from 5 to 21% of total NO_3^- uptake and two-fold from 20 to 39% of total NH_4^+ uptake. A comparison of calculated total uptake (light + dark) of samples incubated at 1 and 100% of the growth irradiance (120 $\mu E \cdot m^{-2} \cdot s^{-1}$) reveals an approximate two-fold reduction in light dependence of NO_3^- and NH_4^+ uptake with increasing N limitation in cyclostat cultures of *M. pusilla*. The decreased light dependence of N uptake is perhaps an adaptive response to N limitation which allows the cell to optimize its uptake capability at low PPFDs without incurring the relatively high metabolic costs of migration for a picoflagellate (Raven, 1986) to a more suitable light

environment saturating to N uptake.

Despite its pronounced phototaxis (Manton and Parke, 1960; Throndsen, 1973) and its relatively good swimming ability (Knight-Jones and Walne, 1952; Throndsen, 1973) *Micromonas pusilla* has been found deeper in the sea than flagellates in general, and it is often found well below the euphotic zone (e.g., Manton and Parke, 1960; Throndsen, 1976). *Micromonas pusilla* can swim at ca. 90 $\mu m \cdot s^{-1}$ (75-100 $\mu m \cdot s^{-1}$, Throndson, 1973) which would enable this picoflagellate to achieve meaningful changes in its incident photon flux density (PPFD) during daytime vertical migration in a stratified water column. In the Fraser River plume, in the southern portion of the Strait of Georgia, British Columbia, *M. pusilla* can be numerically the most abundant phytoplankter in the euphotic zone (Clifford et al., 1989), but it only accounts for <7% of the phytoplankton biomass (Harrison et al., submitted). From Beer's law one can calculate that the 3.9 m that *M. pusilla* could move vertically in these N-replete waters during a day's (12 h) swimming (attenuation coefficient of ca. 0.42 m^{-1}) would increase the cell's mean incident PPFD by 5 times the original value. This could be advantageous, in terms of increased specific nutrient and growth rate, if the initial PPFD was limiting. Conversely, downward swimming could take a cell from a region of high, inhibitory PPFD to one with lower PPFD but still saturating for nitrogen uptake.

CONCLUSIONS

This dissertation examined the uptake of nitrogenous nutrients by both natural assemblages of marine phytoplankton and unialgal cultures of the picoflagellate, *Micromonas pusilla* as a function of light and nutritional history. The specific findings of the research are summarized below.

1. The uptake of NO_3^-, NH_4^+ and urea by coastal and oceanic phytoplankton communities demonstrated pronounced diel periodic rhythms, with minimal uptake rates at night and maximal uptake during the daytime. The amplitude of uptake periodicity appeared to be influenced by a number of factors besides light intensity and these included the phytoplankton species composition, the ambient nitrogen concentrations, the actual N substrate utilized, and the depth in the water column.

2. In both the frontal and stratified waters of the Strait of Georgia the dependence of nitrate and urea uptake upon irradiance could be described by a rectangular hyperbola similar to the Michaelis-Menten formulation. The light dependency of NO_3^- uptake was the same for both the surface and DCM communities of the frontal water, whereas in the stratified waters surface phytoplankton showed less light dependence for NO_3^-, and particularly for urea uptake, than those from the DCM.

3. Uptake rates of NO_3^-, NH_4^+, and urea in the night and artificial darkness were a substantial portion of total uptake by coastal phytoplankton communities; under conditions of nitrogen limitation (low ambient N concentrations) dark uptake increased relative to light uptake.

4. Ammonium completely suppressed the uptake of NO_3^- by N-replete cultures of *Micromonas pusilla* whereas the addition of urea only resulted in partial inhibition of NO_3^- uptake. The inhibitory effect of NH_4^+ on NO_3^- uptake was complete even at NH_4^+ concentrations as low as 1 μg-at $N \cdot L^{-1}$.

5. Uptake kinetic experiments showed that *M. pusilla* can take up NH_4^+ at twice the rates of NO_3^- or urea uptake. Although the half-saturation constants are similar for the three substrates (0.3-0.5 μg-at $N \cdot L^{-1}$), the greater initial slope of the Michaelis-Menten plot for NH_4^+ uptake suggests that *M. pusilla* can utilize low concentrations of NH_4^+ more effectively than equivalent concentrations of urea and NO_3^-

6. Transient elevated (surge) rates of NH_4^+ and urea uptake were observed after N enrichment of N-starved cultures of *M. pusilla*. Nitrate uptake was slower (25-50%) in N-starved cells than N-replete cells, but there was no lag in uptake after the initial NO_3^- enrichment of starved cells; uptake commenced immediately.

7. Diel patterns were observed in both continuous and batch cultures of *M. pusilla* grown on a L:D illumination cycle. Diel periodicity in cell division, mean cell volume, N uptake and internal pools of NO_3^- were observed. With decreased

dilution rate (decreased μ and slower NO_3^- supply) in situ NO_3^- uptake periodicity was absent. Potential rates of NH_4^+ were consistently 2-3 fold greater than NO_3^- or urea uptake rates regardless of the degree of N limitation. A marked diel variability in potential uptake rates of all three N substrates was apparent in the fastest grown cyclostat culture (0.74 d^{-1}), but not in the slower grown cyclostats (0.49 and 0.24 d^{-1})

8. The effect of irradiance on the uptake of NH_4^+ and NO_3^- by *M. pusilla* could be described by Michaelis-Menten kinetics. Dark uptake rates of NH_4^+ were a greater portion of total uptake than dark NO_3^- rates, and the relative importance of dark N uptake increased with increased N limitation. With increased N limitation the light dependency of NO_3^- and NH_4^+ uptake was lessened.

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APPENDIX 1

Equations used to calculate ^{15}N uptake rates.

Once the percentage of ^{15}N (specific activity) in the particulate material ($^{15}N_s$) has been experimentally determined by emission spectrometry (procedures reviewed by Fiedler and Proksch, 1975; Harrison, 1983a) the appropriate equation to calculate nitrogen uptake must be chosen to correspond with the experimental protocol employed.

The atom % ^{15}N excess ($^{15}N_{xs}$) of the particulate material is calculated by subtracting the natural abundance of ^{15}N (F) which is generally taken as ca. 0.365% (natural atmospheric enrichment of ^{15}N) for field samples and can be measured directly in culture samples:

$$^{15}N_{xs} = {}^{15}N_s - F \qquad (1)$$

The specific uptake rate (N taken up per unit particulate N) is calculated as V_t and arises from a constant transport model based on the isotopic ratio of the particulate sample taken at the end of the incubation:

$$V_t = \frac{^{15}N_{xs}}{(R - F) \cdot T} \qquad (2)$$

where T is the incubation time and R is the initial atom % enrichment of the N substrate calculated as

$100 \cdot [(S_i \cdot A + S_a \cdot F)/(S_i + S_a)]$ where S_i is the concentration of added substrate, A the specific activity of the isotope (always 99 atom % in present study), and S_a the ambient concentration of unenriched N substrate. Careful chemistry and accurate determination of ambient N concentration are essential for an accurate determination of R.

The absolute (or transport) rate (ρ_t) is calculated by multiplication of V_t and the PON collected at the end of the incubation period (PON_t):

$$\rho_t = V_t \cdot PON_t \tag{3}$$

Another equation for the calculation of specific uptake rate (V_t) arises from the constant transport model based on PON concentration collected at the beginning of the incubation period (PON_o):

$$V_o = \frac{^{15}N_{xs}}{(R - {}^{15}N_s) \cdot T} \tag{4}$$

Alternatively an equivalent expression replaces the denominator with $[(R - F) - {}^{15}N_{xs}] \cdot T$. The absolute uptake rate ρ_o is calculated by multiplication of V_o by PON_o:

$$\rho_o = V_o \cdot PON_o \tag{5}$$

Since both equations 2 and 4 are derived from a constant transport model they do not allow for changes in PON

concentration during the course of the incubation, thus yielding an underestimate (V_t) and an overestimate (V_i) of the mean specific uptake rate during the incubation period. Dugdale and Wilkerson (1986) suggest the most obvious way to achieve a better estimate is to use the mean of the two values:

$$V_m = (V_o + V_t) \ /2 \qquad (5')$$

The constant specific uptake model assumes that each new cell added during incubation contributes as equally to the sum of uptake as each pre-existing cell is contributing and V_c can be calculated as:

$$V_c = \frac{1}{T} \cdot \ln \left[\frac{(R - F)}{(R - {}^{15}N_{xs})} \right] \qquad (6)$$

Alternatively the denominator can be substituted with the equivalent expression $[(R - F) - {}^{15}N_{xs}]$. Equation 6 is equivalent to the uptake rate calculated as $\ln(PON_t/PON_o)/T$ in Collos (1987) and shown in his Fig. 3 (Eq. 8').

The uptake of unlabelled N forms during the course of an incubation can result in a reduction of the nitrogen specific uptake rate of the ^{15}N-labelled compound determined when only the ^{15}N-labelled compound is being taken up (Collos, 1987; Lund, 1987). Equation 3 yields an accurate estimate of transport rate (P_t) of the labelled compound since V_t and PON_t are estimated from the same sample (Dugdale and Wilkerson,

1986; Collos, 1987). The dilution effect of the uptake of unlabelled substrate can be compensated for V_o, provided independent estimates of the transport rate for unlabelled sources (ρ_i) are available (i.e. ambient nutrient disappearance over time):

$$V_o = \frac{^{15}N_{xs}}{(R - {}^{15}N_s) \cdot T} + \text{sum} \left[\frac{\rho_i}{PON_o}\right] \cdot \left[\frac{^{15}N_{xs}}{R - {}^{15}N_s}\right] \quad (7)$$

The equation for the constant specific uptake model compensated for uptake of unlabelled N is:

$$V_c = \frac{1}{T} \cdot \ln\left[\frac{R - F}{R - {}^{15}N_s}\right] \cdot \left\{1 + \left[\frac{\text{sum } \rho_i \cdot T}{PON_o}\right]\right\} \quad (8)$$

It should be noted that in Dugdale and Wilkerson (1986) this equation is incorrectly written; the placement of the left curly parenthesis should not be placed before the ln term but as shown above. Both equations 7 and 8 (equivalent to Eq. 10 and 11 of Dugdale and Wilkerson, 1986) yield increased estimates of V according to the proportion of initial PON concentration added from unlabelled sources during the incubation.

APPENDIX 2

Growth-irradiance curve of *Micromonas pusilla*.

Objective: The experiment was conducted to obtain a growth-irradiance curve for *M. pusilla* for determination of the PPFD necessary to sustain maximal growth in subsequent nitrogen utilization studies.

Methods: Cultures of *M. pusilla* were grown in 40 ml of medium in 50 ml borosilicate glass test tubes with teflon-lined caps. The medium and its preparation are described in the *Culturing* section of Chapter 3. Sterile techniques were used for all culturing work; the absence of bacteria was only confirmed by microscopic examination. All cultures were grown at 17°C (± 0.5°C) at the following PPFDs: 145, 120, 89, 71, 55, 44, 34, 27, 19 and 15 $\mu E \cdot m^{-2} \cdot s^{-1}$ (2 - 13 cultures per PPFD). Continuous light was provided by Vita-Lite^R UHO fluorescent tubes filtered through blue Plexiglas^R (No. 2069, Rohm & Haas) and attenuated by distance and neutral density screening. Incident irradiation was measured with a LiCor model LI 185 quantum meter (Lambda Instruments) with a 2π collector and the screening calibrated with a Biospherical Instruments QSL-100 4π sensor placed in the same position as the culture tubes. Determinations of biomass (in vivo fluorescence) were made at ca. 12 or 24 h intervals by inserting the whole tube into a Turner Designs model 10 fluorometer, after mixing by multiple inversions.

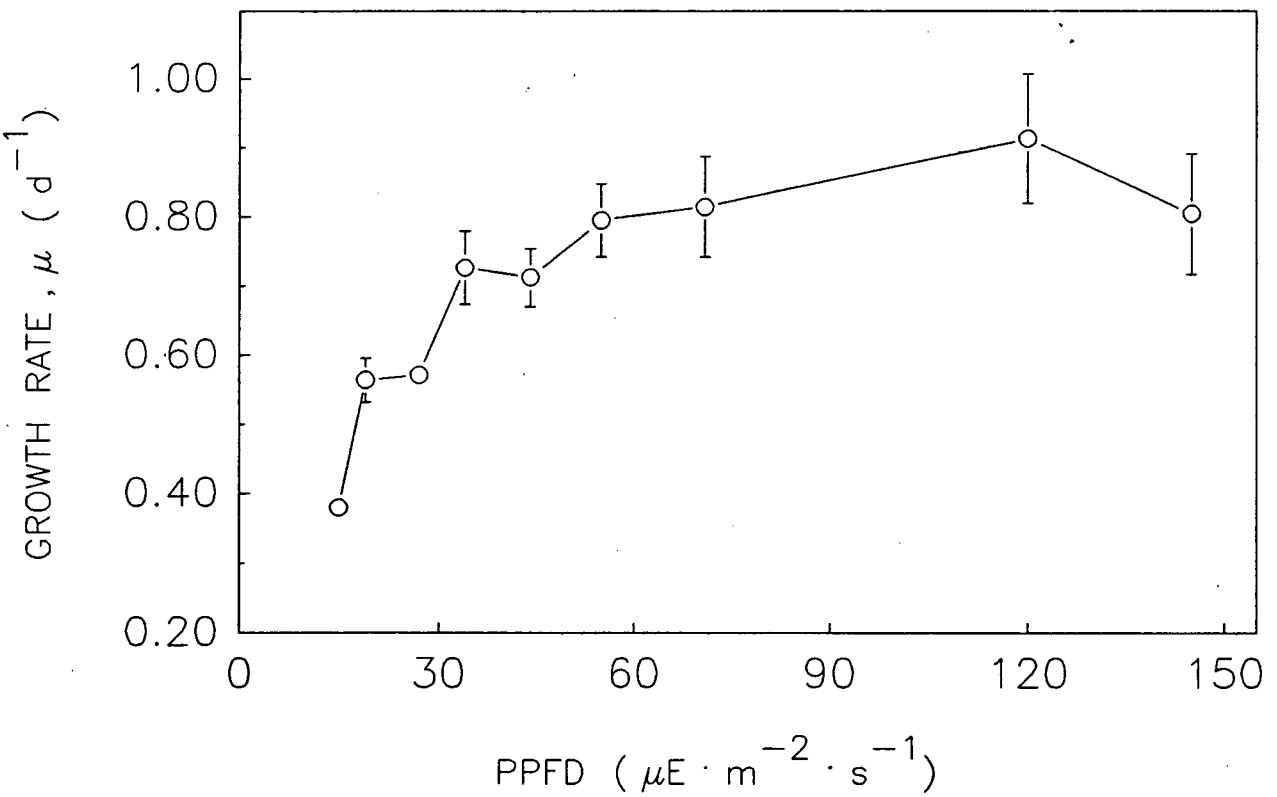
Cultures were transferred prior to N depletion so that they were never nutrient-limited. One estimate of the growth rate was obtained per transfer by calculating the growth rate between each successive measure of fluorescence and averaging over the 4-6 day period. Growth rates (μ) were calculated as:

$$\mu = \ln (F_2/F_1)/(t_2-t_1)$$

where F_1 and F_2 are fluorescence at time 1 (t_1) and time 2 (t_2), respectively.

Results and Conclusions: Specific growth rates (d^{-1}) were calculated from increases in <u>in vivo</u> fluorescence and are plotted against PPFD in Figure A.1. These results indicate that growth of *M. pusilla* saturated at <100 $\mu E \cdot m^{-2} \cdot s^{-1}$, with no photoinhibition apparent at the greatest PPFD examined (145 $\mu E \cdot m^{-2} \cdot s^{-1}$). It was concluded that a PPFD of 120 $\mu E \cdot m^{-2} \cdot s^{-1}$ would be employed exclusively throughout the current research as it was saturating for growth and could be achieved without difficulty in both the continuous light (Chapter 3) and the 14:10 light-dark (Chapter 4) experimental chambers.

Figure A.1. Specific growth rate (μ) in d^{-1} as a function of PPFD for *M. pusilla* grown on NO_3^-. Bars represent ± 1 S.D. (n = 2-13). Error bars are smaller than symbols where not visible.



APPENDIX 3

Comparison of the increases in *in vivo* fluorescence and cell concentration during exponential growth of *Micromonas pusilla*.

Objective: This experiment was conducted to determine if in vivo fluorescence could be used routinely to monitor the growth of batch cultures of *M. pusilla*.

Methods: Cultures of *M. pusilla* were grown in duplicate according to the conditions and procedures outlined in the *Culturing* section of Chapter 3. At 12 h intervals samples were collected for determination of both cell concentration, with a Coulter Counter^R model TA II equipped with the population accessory, and in vivo fluorescence, with a Turner Designs model 10 fluorometer (see Chapter 3, Materials and Methods for additional details). Specific growth rates (d^{-1}) were calculated between each successive measure of fluorescence and cell concentration as:

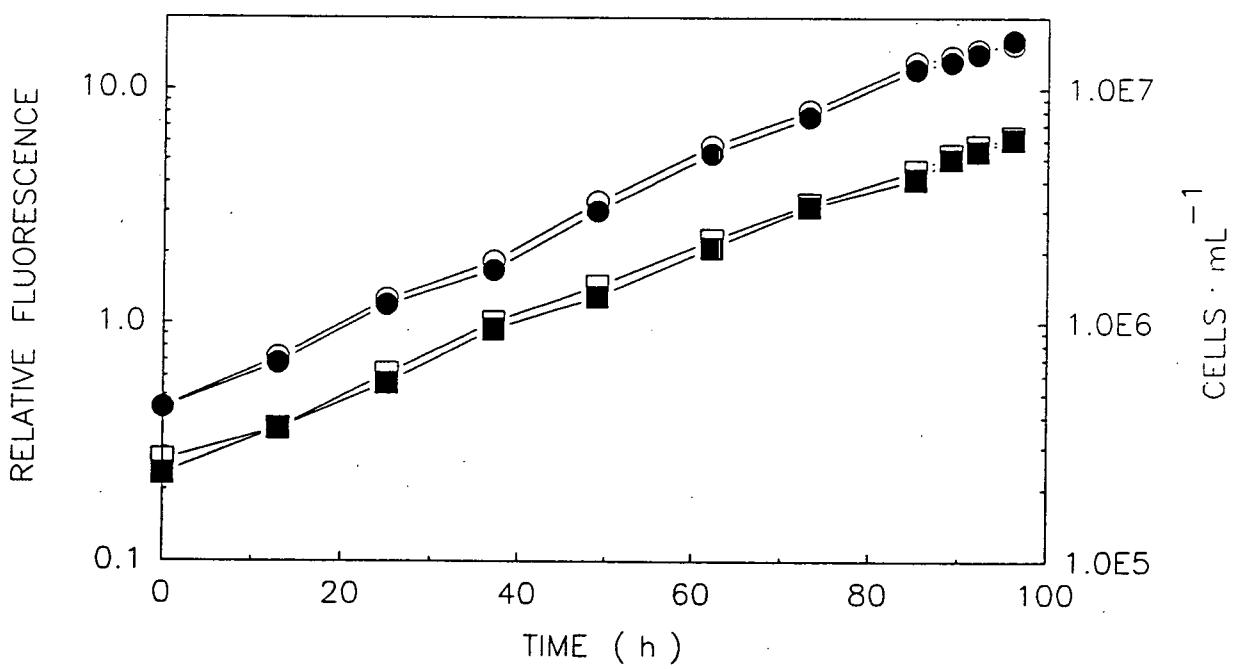
$$\mu = \ln (F_2/F_1)/(t_2 - t_1)$$

where F_2 and F_1 are the fluorescence or cell concentration at time 2 (t_2) and time 1 (t_1), respectively and are reported as the mean (n = 2) ± 1 S.D. of duplicate cultures.

Results & Conclusions: The increases in in vivo fluorescence

and cell concentration of *M. pusilla* as a function of time are plotted in Figure A.2. At time zero (t = 0) the NO_3^- + NO_2^- concentration in the cultures averaged 50.1 ± 0.5 μg-at $N \cdot L^{-1}$ and decreased to < 1.0 μg-at $N \cdot L^{-1}$ after 90 and 95 h of exponential growth in cultures 1 and 2, respectively. During N-replete conditions the growth rate, averaged 0.835 ± 0.011 d^{-1} and 0.832 ± 0.001 d^{-1} (n = 2) from in vivo fluorescence and cell count measurements, respectively. This result indicates that both methods measure the increase in biomass of *M. pusilla* to a similar degree of accuracy. It was decided that in vivo fluorescence can be used to accurately measure cell growth of *M. pusilla* and would be employed to routinely monitor the growth of batch cultures prior to experimentation.

Figure A.2. Growth curves of duplicate batch cultures of *M. pusilla* grown on NO_3^- under saturating PPFD. Semi-log plots of relative <u>in vivo</u> fluorescence (O,●) and cell concentration (□,■) versus time.



APPENDIX 4

Comparison of the rates of particulate nitrogen production and inorganic nitrogen disappearance.

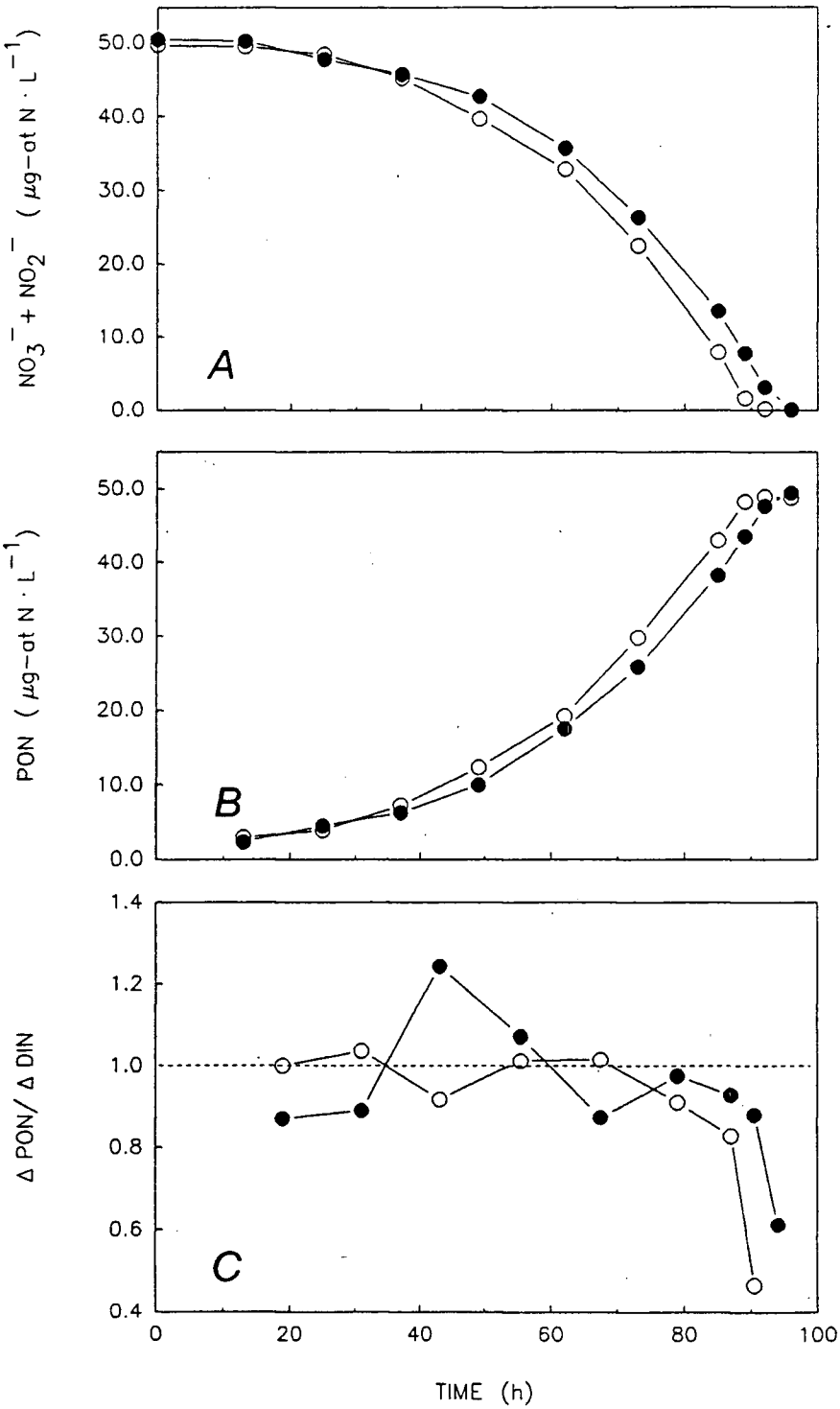
Objective: Two measures of nitrate uptake, the production of particulate organic nitrogen (PON) and the disappearance of NO_3^- + NO_2^- over time, were compared to determine the efficiency of conversion of inorganic N to particulate organic N and thus estimate the extent of dissolved organic N (DON) loss during exponential growth of *M. pusilla*.

Methods: Duplicate batch cultures of *M. pusilla* were grown according to the conditions and procedures outlined in the *Culturing* section of Chapter 3. At 12 h intervals, 50 ml samples were collected by low vacuum filtration (≤ 80 mm Hg) onto precombusted (460°C for 4 h) Whatman GF/F filters for measurement of PON concentration with a Control Equipment Corp. model 240-XA elemental analyzer. The filtrate was analyzed for NO_3^- + NO_3^- with a Technicon AutoAnalyzer^R II. Details of the filtration procedure and analytical techniques are described in the Materials and Methods of Chapter 3.

Results & Conclusions: The disappearance of NO_3^- + NO_2^- from the medium, the increase in PON concentration and the ratio of these two measures of NO_3^- uptake over successive sampling intervals are presented in Figure A.3. These results indicate

that during the period of N-replete growth (0-ca. 90 h) neither measure of NO_3^- uptake was consistently greater or less than the other; the ratio of the change in concentration of PON to NO_3^- + NO_2^- averaged 0.96 (± 0.08) and 0.97 (± 0.13) for culture 1 and 2, respectively. In both N-replete cultures there was no significant difference in rates of NO_3^- + NO_2^- disappearance and PON accumulation (paired *t*-test, $P \geq 0.05$, n = 7 pairs in culture 1, n = 8 pairs in culture 2) but when the cells became N-limited (< 1 μg-at $N \cdot L^{-1}$ of NO_3^- + NO_2^-) an average of 54% of the nitrate removed from the medium was accounted for in the PON. These results suggest that only during conditions of N-limitation were the decreases in NO_3^- + NO_2^- from the medium not reflected in similar increases in PON concentration, suggesting excretion of DON to the medium. Past estimates of DON excretion typically ranged from 20-40% of the assimilated nitrogen in N-limited cells and 5-10% in healthy phytoplankton cells (e.g., Sharp, 1977). However, it is possible that much of the DON excretion measured in these early experiments may have resulted from cell breakage (e.g., Fuhrman and Bell, 1985; Goldman and Dennett, 1985) or cell passage (e.g., Li, 1986; Stockner et al., in press) during filtration.

Figure A.3. Nitrate uptake by duplicate batch cultures of *Micromonas pusilla*. A. Decrease in dissolved NO_3^- + NO_2^- concentration in the culture medium. B. Accumulation of particulate organic nitrogen. C. Ratio of NO_3^- uptake rate calculated from PON accumulation to rate calculated from NO_3^- + NO_2^- disappearance from the medium. Nitrogen concentrations are plotted against elapsed time measured after culture initiation; uptake rate ratios are plotted against average elapsed time between successive sampling periods.



APPENDIX 5

Dissolved inorganic nitrogen disappearance curve during growth of M. pusilla.

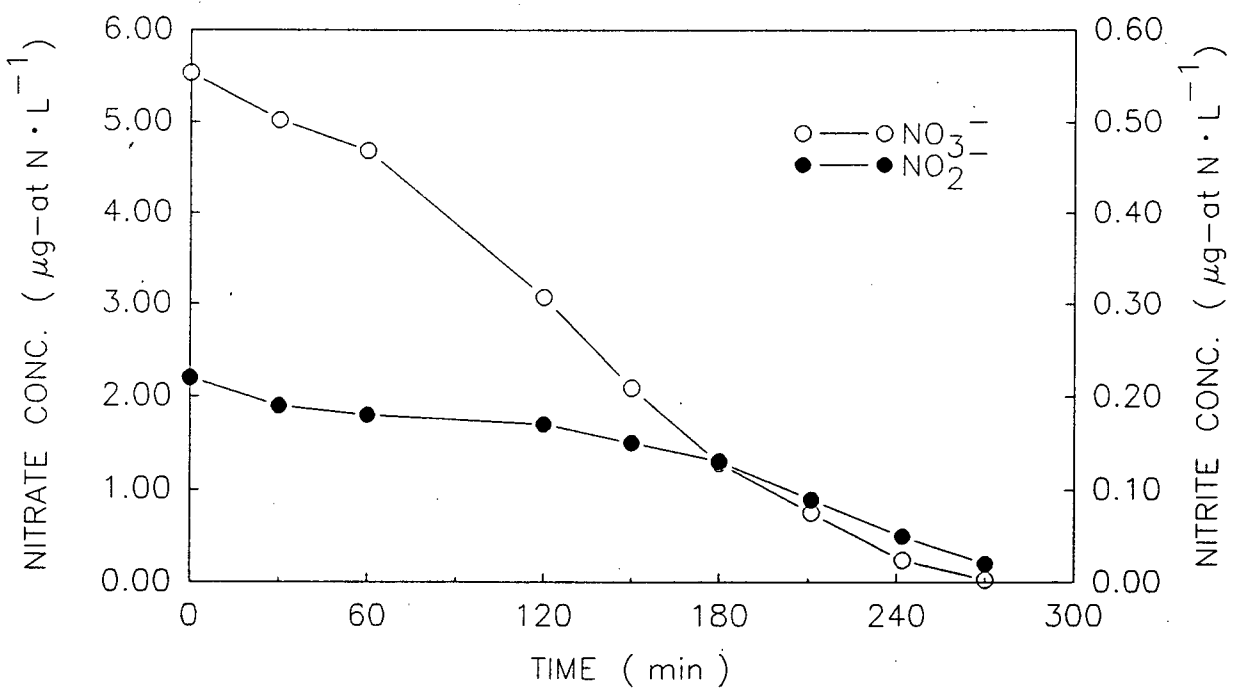
Objective: To determine if nitrite is released into the culture medium by *M. pusilla*, during growth on nitrate, as the ambient concentration of nitrate approaches zero.

Methods: A batch culture of *M. pusilla* was grown, under constant blue light at a saturating PPFD, according to the conditions and procedures outlined in the *Culturing* section of Chapter 3. At 30 min intervals, filtered samples were analyzed for nitrate (NO_3^-) and nitrite (NO_2^-) with a Technicon AutoAnalyzer^R II following the techniques outlined in Wood et al. (1967) and Chapter 3. Sampling continued until the ambient concentrations of NO_3^- and NO_2^- reached their respective analytical limits of detection (NO_3^-: 0.03 μg-at $N \cdot L^{-1}$; NO_2^-: 0.02 μg-at $N \cdot L^{-1}$).

Results & Discussion: The ambient concentrations of NO_3^- and NO_2^- are plotted as a function of time in Figure A.4. Over a period of 4.5 h the total concentration of $NO_3^- + NO_2^-$ declined from 5.76 μg-at $N \cdot L^{-1}$ to <0.05 μg-at $N \cdot L^{-1}$ with concomittant decreases in the ambient concentration of both NO_3^- and NO_2^-. No evidence of NO_2^- excretion by *M. pusilla* was observed with increasing NO_3^- limitation. The ambient

concentration of NO_2^- in five separate 20 L reservoirs of 50 μg-at $NO_3^- \cdot L^{-1}$ enriched ESAW averaged < 0.05% of the total NO_3^- + NO_2^- concentration (0.20 ± 0.07 μg-at $NO_2^- \cdot L^{-1}$, n = 5) which is consistent with the initial values reported here during the first hour of sampling. It is concluded that *M. pusilla*, unlike other phytoplankton species such as *Thalassiosira pseudonana* (e.g., Olson et al., 1980; Parslow et al., 1984b), does not release NO_2^- during growth on NO_3^- under saturating PPFD.

Figure A.4. Dissolved NO_3^- and NO_2^- concentration in the culture medium, during batch growth of *M. pusilla*, plotted against time of sampling.



APPENDIX 6

Precision of analytical techniques.

The precision of the analytical techniques employed throughout this study are reported as the mean coefficient of variation (C.V. = S.D./$\bar{x}$ · 100) of replicate samples (n) collected and processed according to the procedures outlined in Chapters 1-4. The estimated error includes both collection (i.e. filtration) and analytical errors, except in the atom % excess ($^{15}N_{ex}$) measurements of ^{15}N in particulates. Here duplicate measurements were determined from the same original sample (collected by filtration), but evacuated, combusted and analyzed by emission spectrometry separately (n = 150 pairs).

Measure	Number of Replicates (n)	Coefficient of Variation (%)
$NO_3^- + NO_2^-$	2-3	1.2
NH_4^+	2-3	1.5
Urea	2	0.3
Chl *a*	2	4.4
POC	2-3	5.2
PON	2-3	3.8
cell concen.	2	3.5
$^{15}N_{ex}$	2	1.5

Refereed Publications

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Cochlan, W.P., P.J. Harrison, P.A. Thompson and T.R. Parsons. 1986. Preliminary observations of the summer production of three British Columbian coastal inlets. Sarsia 71: 161-168.

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