THE ROLE OF PICOPLANKTON IN THE UPTAKE OF INORGANIC NITROGEN IN OCEANIC WATERS

by

Sophie Carler

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
November, 1995

ISBN 0-612-15861-6

Name SOPHIE CARLER

Dissertation Abstracts International is arranged by broad, general subject categories Please select the one subject which most nearly describes the content of your dissertation Enter the corresponding four digit code in the spaces provided

BIOLOGY
SUBJECT TERM

0416
SUBJECT CODE

U·M·I

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS

Architecture	0729
Art History	0377
Cinema	0900
Dance	0378
Fine Arts	0357
Information Science	0723
Journalism	0391
Library Science	0399
Mass Communications	0708
Music	0413
Speech Communication	0459
Theater	0465

EDUCATION

General	0515
Administration	0514
Adult and Continuing	0516
Agricultural	0517
Art	0273
Bilingual and Multicultural	0282
Business	0688
Community College	0275
Curriculum and Instruction	0727
Early Childhood	0518
Elementary	0524
Finance	0277
Guidance and Counseling	0519
Health	0680
Higher	0745
History of	0520
Home Economics	0278
Industrial	0521
Language and Literature	0279
Mathematics	0280
Music	0522
Philosophy of	0998
Physical	0523
Psychology	0525
Reading	0535
Religious	0527
Sciences	0714
Secondary	0533
Social Sciences	0534
Sociology of	0340
Special	0529
Teacher Training	0530
Technology	0710
Tests and Measurements	0288
Vocational	0747

LANGUAGE, LITERATURE AND LINGUISTICS

Language	
General	0679
Ancient	0289
Linguistics	0290
Modern	0291
Literature	
General	0401
Classical	0294
Comparative	0295
Medieval	0297
Modern	0298
African	0316
American	0591
Asian	0305
Canadian (English)	0352
Canadian (French)	0355
English	0593
Germanic	0311
Latin American	0312
Middle Eastern	0315
Romance	0313
Slavic and East European	0314

PHILOSOPHY, RELIGION AND THEOLOGY

Philosophy	0422
Religion	
General	0318
Biblical Studies	0321
Clergy	0319
History of	0320
Philosophy of	0322
Theology	0469

SOCIAL SCIENCES

American Studies	0323
Anthropology	
Archaeology	0324
Cultural	0326
Physical	0327
Business Administration	
General	0310
Accounting	0272
Banking	0770
Management	0454
Marketing	0338
Canadian Studies	0385
Economics	
General	0501
Agricultural	0503
Commerce Business	0505
Finance	0508
History	0509
Labor	0510
Theory	0511
Folklore	0358
Geography	0366
Gerontology	0351
History	
General	0578
Ancient	0579
Medieval	0581
Modern	0582
Black	0328
African	0331
Asia, Australia and Oceania	0332
Canadian	0334
European	0335
Latin American	0336
Middle Eastern	0333
United States	0337
History of Science	0585
Law	0398
Political Science	
General	0615
International Law and Relations	0616
Public Administration	0617
Recreation	0814
Social Work	0452
Sociology	
General	0626
Criminology and Penology	0627
Demography	0938
Ethnic and Racial Studies	0631
Individual and Family Studies	0628
Industrial and Labor Relations	0629
Public and Social Welfare	0630
Social Structure and Development	0700
Theory and Methods	0344
Transportation	0709
Urban and Regional Planning	0999
Women's Studies	0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES

Agriculture	
General	0473
Agronomy	0285
Animal Culture and Nutrition	0475
Animal Pathology	0476
Food Science and Technology	0359
Forestry and Wildlife	0478
Plant Culture	0479
Plant Pathology	0480
Plant Physiology	0817
Range Management	0777
Wood Technology	0746
Biology	
General	0306
Anatomy	0287
Biostatistics	0308
Botany	0309
Cell	0379
Ecology	0329
Entomology	0353
Genetics	0369
Limnology	0793
Microbiology	0410
Molecular	0307
Neuroscience	0317
Oceanography	0416
Physiology	0433
Radiation	0821
Veterinary Science	0778
Zoology	0472
Biophysics	
General	0786
Medical	0760

EARTH SCIENCES

Biogeochemistry	0425
Geochemistry	0996
Geodesy	0370
Geology	0372
Geophysics	0373
Hydrology	0388
Mineralogy	0411
Paleobotany	0345
Paleoecology	0426
Paleontology	0418
Paleozoology	0985
Palynology	0427
Physical Geography	0368
Physical Oceanography	0415

HEALTH AND ENVIRONMENTAL SCIENCES

Environmental Sciences	0768
Health Sciences	
General	0566
Audiology	0300
Chemotherapy	0992
Dentistry	0567
Education	0350
Hospital Management	0769
Human Development	0758
Immunology	0982
Medicine and Surgery	0564
Mental Health	0347
Nursing	0569
Nutrition	0570
Obstetrics and Gynecology	0380
Occupational Health and Therapy	0354
Ophthalmology	0381
Pathology	0571
Pharmacology	0419
Pharmacy	0572
Physical Therapy	0382
Public Health	0573
Radiology	0574
Recreation	0575
Speech Pathology	0460
Toxicology	0383
Home Economics	0386

PHYSICAL SCIENCES

Pure Sciences

Chemistry	
General	0485
Agricultural	0749
Analytical	0486
Biochemistry	0487
Inorganic	0488
Nuclear	0738
Organic	0490
Pharmaceutical	0491
Physical	0494
Polymer	0495
Radiation	0754
Mathematics	0405
Physics	
General	0605
Acoustics	0986
Astronomy and Astrophysics	0606
Atmospheric Science	0608
Atomic	0748
Electronics and Electricity	0607
Elementary Particles and High Energy	0798
Fluid and Plasma	0759
Molecular	0609
Nuclear	0610
Optics	0752
Radiation	0756
Solid State	0611
Statistics	0463

Applied Sciences

Applied Mechanics	0346
Computer Science	0984
Engineering	
General	0537
Aerospace	0538
Agricultural	0539
Automotive	0540
Biomedical	0541
Chemical	0542
Civil	0543
Electronics and Electrical	0544
Heat and Thermodynamics	0348
Hydraulic	0545
Industrial	0546
Marine	0547
Materials Science	0794
Mechanical	0548
Metallurgy	0743
Mining	0551
Nuclear	0552
Packaging	0549
Petroleum	0765
Sanitary and Municipal	0554
System Science	0790
Geotechnology	0428
Operations Research	0796
Plastics Technology	0795
Textile Technology	0994

PSYCHOLOGY

General	0621
Behavioral	0384
Clinical	0622
Developmental	0620
Experimental	0623
Industrial	0624
Personality	0625
Physiological	0989
Psychobiology	0349
Psychometrics	0632
Social	0451

Till Osvaldo och mina föräldrar

TABLE OF CONTENTS

Chapter 3: Bacterial uptake of inorganic nitrogen in coastal and oceanic waters of the subtropical North Atlantic

LIST OF TABLES

LIST OF FIGURES

Figure 2.8. Depth of the nitracline versus longitude at all oligotrophic stations (data from Dr. Harrison). The range for each station represents the difference between the two depths at which the variation in the nitrate concentration was ≥ 100 nM.

Figure 2.9. *f*-ratios of the <2-μm fraction versus *f*-ratios of the >2-μm fraction, (a) at all stations, (b) at the oligotrophic stations, and (c) at the upwelling stations The *f*-ratios were calculated as in Fig. 2.7

Figure 2.10. New and regenerated production in the <2-μm fraction, integrated over the euphotic zone, along the transect (based on profiles of unfractionated production rates from Dr. Harrison). Regenerated production represents the uptake of ammonium only.

Figure 2.11. Integrated values of f' for the <2-μm fraction versus f' for the >2-μm fraction (based on profiles of nitrogen uptake rates from Dr. Harrison), (a) at all stations and (b) at the oligotrophic stations. $f' = p\mathrm{NO_3^-} / [p\mathrm{NO_3^-} + p\mathrm{NH_4^+}]$.

Figure 3.1. Bacterial production rates (based on leucine incorporation rates and thymidine incorporation rates) and primary production rates (based on ^{14}C uptake rates in the <2-μm fraction), in (a) the mixed layer and (b) the DCM layer.

Figure 3.2. Ratios of bacterial nitrogen demand, D_b (based on leucine incorporation rates) to picophytoplankton nitrogen demand D_p, in the mixed layer, and in the DCM layer. DCM samples from stations 17 and 54 showed ratios >>1 (14, 6, respectively) and were not included

Figure 3.3. Estimates of bacterial uptake rates of inorganic nitrogen ($NH_4^+ + NO_3^-$) in the <2-μm fraction and estimates of bacterial nitrogen demand in (a) the mixed layer and (b) the DCM layer; error bars are standard deviations. The upwelling stations are indicated by an arrow above the corresponding

station numbers. In (b) the upwelling stations are equally spaced only for clarity.

Figure 3.4. Relative errors in the estimates of Up and U_b due to uncertainties associated with diel periodicity in protein synthesis, uncoupling of C and N uptake, the case in which <85% of the N uptake goes into proteins, and the measurements of U_t. The estimation of the relative errors in Up and U_b is detailed in Appendix 2 and 3 (see also Discussion). Error bars indicate the range of uncertainties.

Figure 3.5. Major pathways of N in the upper water column showing the production that is exported out of the euphotic layer. Nitrogen uptake, which is measured by the uptake of $^{15}NO_3^-$ and $^{15}NH_4^+$, is represented by solid lines and includes both phytoplankton and bacterial uptake. Pathways not estimated in the present study are represented by dashed lines. New production is the total microbial uptake of nitrate under the assumptions that: 1) NO_3^- represents new inputs only, *i.e.*, does not include NO_3^- produced by nitrification within the euphotic zone, and 2) other external sources of nitrogen such as nitrogen fixation are not significant. Likewise, regenerated production is the total microbial uptake of NH_4^+.

ABSTRACT

The abundance, distribution, and nitrogen productivity of picoplankton were studied in upwelling and oceanic waters of the subtropical North Atlantic. The oceanic community was dominated by picoplankton, within which prochlorophytes and, to a lesser extent, heterotrophic bacteria accounted for most of the estimated carbon biomass. Based on size-fractionated (<2 μm) measurements of nitrogen uptake ($^{15}NO_3^-$, $^{15}NH_4^+$), picoplankton were shown to be the major contributors to new and regenerated production (>80%) at the oligotrophic stations, and to be responsible for a significant fraction (~70%) at the upwelling site. Given that prochlorophytes accounted for most of the picophytoplankton biomass at the oligotrophic stations, these results suggest that prochlorophytes accounted for most of the new and regenerated production in these waters.

Bacterial uptake rates of inorganic nitrogen were estimated as the difference between total uptake rates of inorganic nitrogen (using ^{15}N) and phytoplankton uptake rates of nitrogen (based on protein synthesis). Bacteria contributed 25% on average to the total uptake of inorganic nitrogen. The errors in new production resulting from this bacterial uptake were ~30% for NO_3^- uptake rates, and ~10% for the *f*-ratio. These errors were small compared with some of the uncertainties associated with the ^{15}N technique. However, with the recent improvements in the ^{15}N measurement, bacterial utilization of nitrate and ammonium is likely to be one of the major problems associated with the estimation of autotrophic new and regenerated production. By not correcting new production estimates for a bacterial uptake of inorganic nitrogen, this may result in the C:N assimilation ratio being lower than the Redfield ratio. This can lead, in turn, to an overestimation of carbon-based new production when computed as the product of nitrate uptake and Redfield ratio, as is conventionally done.

ABBREVIATIONS AND SYMBOLS

B_p Picophytoplankton carbon biomass (μg C L^{-1})

B_b Bacterial carbon biomass (μg C L^{-1})

E_f Absolute error in f due to bacterial uptake of [$NH_4^+ + NO_3^-$]

f Ratio of pNO_3^- to [$pNO_3^- + pNH_4^+ + p$N-Urea]

f' Ratio of pNO_3^- to [$pNO_3^- + pNH_4^+$]

D_b Bacterial nitrogen demand (μg-at N L^{-1} h^{-1})

D_p Picophytoplankton nitrogen demand (μg-at N L^{-1} h^{-1})

K_s Half-saturation constant for pNO_3^- and pNH_4^+ (nM)

pNO_3^- Uptake rate of $^{15}NO_3^-$ (ng-at L^{-1} h^{-1})

pNH_4^+ Uptake rate of $^{15}NH_4^+$ (ng-at L^{-1} h^{-1})

pN-Urea Uptake rate of ^{15}N-Urea (ng-at L^{-1} h^{-1})

P_b Bacterial production (μg C L^{-1} h^{-1})

P_e Incorporation rate of ^{14}C into the protein fraction (μg C L^{-1} h^{-1})

P_p Picophytoplankton production as estimated by ^{14}C uptake (μg C L^{-1} h^{-1})

p_{max} Maximum uptake rate of $^{15}NO_3^-$ and $^{15}NH_4^+$ (ng-at L^{-1} h^{-1})

U_p Picophytoplankton uptake rate of inorganic nitrogen based on P_e (μg-at N L^{-1} h^{-1})

U_t ^{15}N uptake rate in the <2 μm-fraction [$^{15}NO_3^- + ^{15}NH_4^+$], including photosynthetic and bacterial uptake (μg-at N L^{-1} h^{-1})

U_b Bacterial uptake rate of inorganic nitrogen [$NH_4^+ + NO_3^-$] estimated by the difference between U_t and U_p (μg-at N L^{-1} h^{-1})

$U_{b(a)}$ Bacterial uptake rate of NH_4^+ estimated as 90% of U_b (μg-at N L^{-1} h^{-1})

$U_{b(n)}$ Bacterial uptake rate of NO_3^- stimated as 10% of U_b (μg-at N L^{-1} h^{-1})

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Glen Harrison, for his advice and encouragement during the course of this thesis. I would also like to thank the members of my supervisory committee: Dr. Bill Li, Dr. Robert Brown, and Dr. Marlon Lewis; a special thanks to Dr. Bill Li for his continuous advice and guidance. My sincere gratitude to Dr. Shubha Sathyendranath and Dr. Trevor Platt for their advice, encouragement, and moral support.

Financial support during the course of this work was provided by the Natural Sciences and Engineering Research Council through an operating grant to Dr. Glen Harrison (three years). I am also indebted to Dr. Trevor Platt for providing financial support (six months), as well as to the Patrick Lett Foundation (8 months).

I am grateful to all the scientific and technical staff at the Biological Oceanographic Division of the Bedford Institute of Oceanography, and in particular to Paul Dickie and Les Harris, for their help at sea and in the laboratory.

Many thanks to all the friends in Halifax who made my experience as a graduate student in Canada so enjoyable. A special thanks to all the friends who let me stay in their home during the last two years while Osvaldo was away. I also want to express my gratitude to Vivian Lutz (Manana) and Margareth Kyewalyanga (Sunshine) for so many precious moments shared together and so much laughter. Most of all, I want to thank Osvaldo for his love, support, patience and understanding. I am deeply grateful to my parents who transmitted their spirit of adventure to me and always supported me with so much love so that I would fulfill my aspirations.

General Introduction

The discovery, in the last decade, of abundant, microbial-sized plankton in marine waters (Waterbury *et al.*, 1979; Johnson and Sieburth, 1979; Johnson and Sieburth, 1982; Chisholm *et al.*, 1988; Fuhrman *et al.*, 1989) has changed our view of the structure and functioning of the marine pelagic ecosystem (Azam *et al.*, 1983; Cho and Azam, 1990; Fuhrman *et al.*, 1989). These minute cells can account for a significant fraction of the biomass (Li *et al.*, 1983; Azam *et al.*, 1983; LeBouteiller *et al.*, 1992) and carbon production (Li *et al.*, 1983; Li and Platt, 1987) of the pelagic communities, particularly in the open-ocean oligotrophic waters. Most of these planktonic cells range in diameter from 0.2 to 2.0 μm and have been named picoplankton (Sieburth *et al.*, 1978; Stockner and Antia, 1986); they include photosynthetic cells (picophytoplankton) and heterotrophic bacteria. Because picophytoplankton are important contributors to primary production, and they are ubiquitous (Stockner, 1988; Yentsch, 1990; Fogg, 1995), this suggests that the global primary production of picoplankton is significant and raises the question, what is the role of picoplankton in the pelagic food webs, particularly in the transfer of energy and materials to higher trophic levels?

Primary production can be partitioned based on the nitrogen form used (Dugdale and Goering, 1967) into new production which is fueled by newly-available nitrogen, mainly nitrate from below the photic zone, and into regenerated production which is fueled by recycled nitrogen, mainly ammonium. At steady-state, new production represents the fraction of production that can be exported out of the photic zone without modifying the balance of the system (Dugdale and Goering, 1967; Eppley and Peterson, 1979; Platt *et al.*, 1992). Given that picoplankton generally predominate in oligotrophic waters (Li *et al.*, 1983), and that new production in these open-ocean areas represent 50% of the global new production (Berger *et al.*, 1989), this suggests that picoplankton must contribute significantly to new production on a global scale.

New and regenerated primary production rates are most often estimated by the uptake rates of $^{15}NO_3^-$ and $^{15}NH_4^+$, respectively, assuming that only phytoplankton are responsible for this production (Dugdale and Goering, 1967). In the recent years, however, it has been recognized that heterotrophic bacteria may also consume inorganic nitrogen such as nitrate and ammonium (Laws *et al.*, 1985; Wheeler & Kirchman, 1986). This could have important implications for the estimation of new and regenerated primary production by ^{15}N tracer methods, particularly in the picoplankton fraction Therefore, it is important to know if the bacterial uptake of inorganic nitrogen represents a significant fraction of the total microbial uptake of inorganic nitrogen

The high numerical abundance of picoplankton (10^6 L ^{1} to 10^8 L ^{1}) was first reported in the 1970's (Stockner, 1988) with the discovery of chroococcoid cyanobacteria (Johnson and Sieburth, 1979; Waterbury *et al* , 1979), picoeukaryotes (Johnson and Sieburth, 1982), and, more recently, the prochlorophytes (Chisholm *et al* , 1988); numerous reports have also shown heterotrophic bacteria to be even more abundant than picophytoplankton (Fuhrman *et al* , 1989; Cho & Azam, 1990, Li *et al* , 1992), reaching concentrations of 10^9 L ^{1} At the present time, field observations of picoplankton are too scarce to enable a generalization of the relative contributions from each of the different picoplanktonic groups to the total, global abundance and biomass

In this thesis. I examine the role of picoplankton in the nitrogen dynamics of a pelagic ecosystem based on measurements made along a transect across the North Atlantic and at an upwelling site off the North-West coast of Africa. The main objectives of this thesis are:

1) to estimate the contribution of picoplankton to total microbial biomass (taking into account the different picoplanktonic groups), and to new and regenerated production, and

2) to estimate the heterotrophic bacterial uptake of inorganic nitrogen ($NH_4^+ + NO_3^-$).

In chapter 1, I describe the community structure of picoplankton by estimating the abundance and biomass (chlorophyll *a* biomass and carbon biomass) of this size-fraction; I determine the contribution of the <2-μm fraction, relative to unfractionated water, and the contribution from the different groups comprising the picoplankton fraction (cyanobacteria, picoeukaryotes, prochlorophytes, and heterotrophic bacteria). It is concluded that picophytoplankton dominate the total biomass in oceanic waters and that prochlorophytes account for most of the picophytoplankton biomass there.

In chapter 2, I estimate the contribution of picoplankton to new and regenerated production and show that picoplankton account for most of the new and regenerated production, both in oligotrophic waters and at the upwelling site. Under the steady-state assumption, I discuss the possible mechanisms by which picoplankton are exported out of the photic zone. The food web structure consistent with a significant export of picoplankton production is also discussed.

In chapter 3, I address the problem of bacterial uptake of inorganic nitrogen by estimating bacterial uptake rates, using the methods of DiTullio and Laws (1983) and Laws *et al.* (1985) Bacterial uptake of inorganic nitrogen is estimated from the difference between total microbial uptake and phytoplankton uptake These values are compared with an independent estimate of total bacterial nitrogen demand, the discrepancy observed between the two estimates is discussed. It is concluded that bacteria take up a significant fraction of inorganic nitrogen, relative to phytoplankton I discuss the implications of these results for estimating new and regenerated production and I interpret the results in the context of possible interactions between bacteria and phytoplankton

Chapter 1

Abundance and biomass of picoplankton in coastal and oceanic waters of the subtropical North Atlantic

1.1. Introduction

Over the last fifteen years, the study of marine food webs has rapidly widened to encompass minute autotrophic cells and heterotrophic bacteria (Williams, 1981; Stockner, 1988). These microbial cells, which are mostly <2 μm, have been recognized as key components of the marine microbial food web (Pomeroy, 1974; Azam *et al.*, 1983; Sherr & Sherr, 1984). In fact, these planktonic cells contribute significantly to the living biomass (Fuhrman *et al.*, 1989; Cho & Azam, 1990; Li *et al.*, 1983; Herbland *et al.*, 1985; Weber & El-Sayed, 1987; Chavez, 1990; Hall & Vincent, 1990; Peña *et al.*, 1990). Moreover, the small photosynthetic cells, as picophytoplankton (<2 μm), can account for a substantial fraction of the primary productivity worldwide (Li *et al.*, 1983; Platt *et al.*, 1983; Stockner, 1988). This suggests that the picoplankton, including photosynthetic cells and heterotrophic bacteria, play an essential role in the production (picophytoplankton) and turnover (bacteria) of organic matter in the pelagic ecosystem. In order to understand the role of picoplankton in the material cycling and energy flow, it is necessary to determine first their contributions to the total biomass.

The composition of picophytoplankton has been described in recent years for various water regimes (Olson *et al.*, 1990a, b; Li *et al.*, 1992, 1993) through the application of flow cytometry in biological oceanography (Chisholm *et al.*, 1988; Olson *et al.*, 1993; Li & Platt, 1987). Three main groups have been identified: cyanobacteria (belonging mainly to the genus *Synechococcus*), prochlorophytes (*Prochlorococcus spp.*), and eukaryotes (*e.g.* prasinophyceae, chlorophyceae). Improvements in the sensitivity of

flow cytometers have enabled the detection of prochlorophytes throughout the upper water column (Olson *et al.*, 1990a; Campbell & Vaulot, 1993). Although these cells were initially detected only in the deeper parts of the euphotic zone, at lower concentrations (Chisholm *et al.*, 1988; Li & Wood, 1988), some recent field observations have shown that prochlorophytes can dominate the numerical abundance and biomass of picophytoplankton (<2 μm) in the euphotic zone of open-ocean waters (Veldhuis *et al.*, 1993; Campbell *et al.*, 1994). In fact, Olson *et al.* (1990a) reported high prochlorophyte concentrations for the Sargasso Sea, similar to those observed by Campbell *et al.* (1994) in the oligotrophic Pacific. Campbell & Vaulot (1993) reviewed data on the composition of picophytoplankton in different oceanic waters (Subtropical Pacific, Sargasso Sea, Mediterranean Sea), and concluded that the depth-integrated abundances of prochlorophytes and cyanobacteria showed an opposite trend, *i.e.* prochlorophyte abundance was higher in the Pacific compared with the Atlantic Ocean and the Mediterranean Sea, while cyanobacterial abundance was lower. Picoeukaryote abundance did not vary significantly among the different oceans.

Dortch & Packard (1989) measured the ratio of chlorophyll *a* to protein as a relative index of phytoplankton to total biomass, and reported that in oligotrophic areas, most of the biomass consisted of bacteria and zooplankton, while in eutrophic areas the living biomass was mainly comprised of phytoplankton. Other workers have estimated the contribution of phytoplankton to total biomass by bulk chlorophyll *a* measurements (Cho & Azam, 1990) and by microscopic counts of cyanobacteria and photosynthetic nanoplankton (Fuhrman *et al.*, 1989, where the average cell size of nanoplankton was 3-4 μm), and both concluded that heterotrophic bacteria dominated the total biomass in oligotrophic environments. Based on bacterial counting and flow cytometry measurements, Li *et al.* (1992) found that phytoplankton and bacteria co-dominated the living biomass of the Sargasso Sea. In eutrophic waters, on the other hand, bacteria were shown to compare with approximately

50% of the phytoplankton biomass (Li *et al.*, 1993). Thus, reports on the partitioning of living biomass into autotrophic cells and heterotrophic cells do not show any systematic trend; this can be explained, on the one hand, by the uncertainties associated with the methods of estimation (Turley and Hughes, 1992; Monger and Landry, 1993), and on the other, by a real seasonal or spatial variation.

In the present study, I describe the composition of picoplankton along an East-West transect in the North Atlantic, including an upwelling site off the North West coast of Africa. I assess the contribution of prochlorophytes, cyanobacteria, picoeukaryotes, and bacteria to picoplankton abundance and biomass. Results from this Chapter will also provide the necessary community structure information required for interpreting results in the following Chapters.

1.2 Materials and methods

1.2.1. Location of stations and depths sampled

Data were collected during a five-week cruise (CSS *Hudson*, 92-037, 16 Sept to 21 Oct, 1992) across the North Atlantic, from Halifax (Eastern Canada) to the Moroccan upwelling region (North West Africa), covering a distance of ~ 3000 nautical miles (60° W-10° W). Sixteen stations were occupied on the transect, and eight stations were occupied at the upwelling site, off the North-West coast of Africa (Fig. 1.1, Table 1.1). Two stations from the transect were located in slope waters near the Scotian shelf (#85, 82). At 10 out of 24 stations, one depth was sampled, and at the remaining stations, two depths were sampled: one of the depths represented the upper mixed layer, and the other the portion of the euphotic zone below the mixed layer coinciding with the subsurface chlorophyll *a* maximum layer (DCM layer) when possible. The mixed layer was considered to be the upper portion of the water column in which temperature was homogenous (±3°C) as

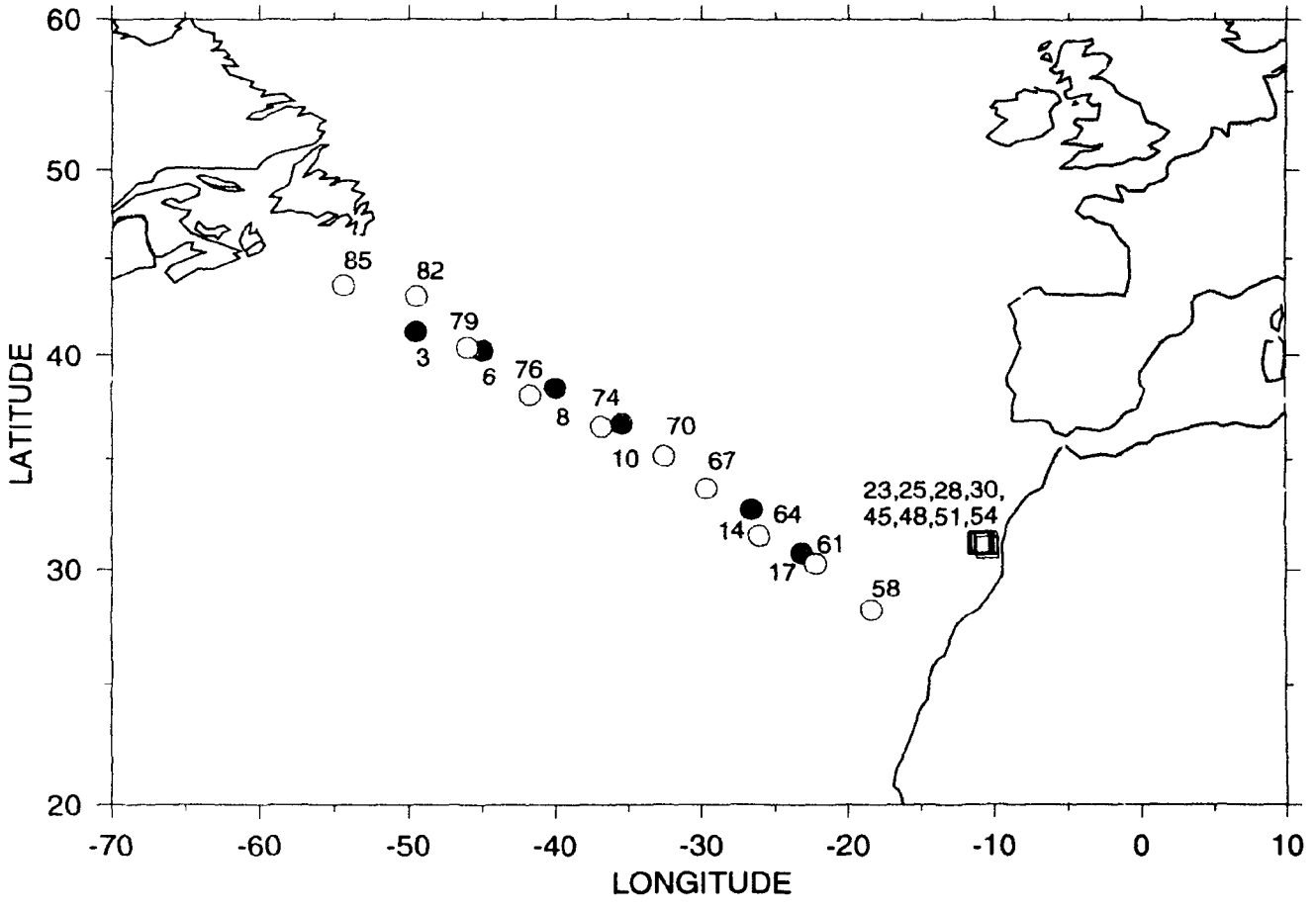


Figure 1.1 Location of the stations sampled along the East-West transect from the Scotian Shelf (Eastern Canada) to the Moroccan upwelling region (North-West coast of Africa). Dark-filled circles represent the Eastward transect, squares represent the upwelling site.

Table 1.1 Location, station number, date, depth, and water type (OLIGO=oligotrophic, UW=upwelling, SL=slope waters) of all stations sampled. DCM layer=deep chlorophyll-maximum layer.

Longitude °W	Station #	Date	Depth (m)		Water type
			Mixed layer	DCM layer	
54.35	85	19 Oct	10	60	SL
49.55	3	19 Sep	40		OLIGO
49.50	82	18 Oct	1	20	SL
46.02	79	17 Oct	20	60	OLIGO
45.00	6	20 Sep		70	OLIGO
41.73	76	16 Oct	20	60	OLIGO
39.97	8	21 Sep	30		OLIGO
36.87	74	15 Oct	20	60	OLIGO
35.48	10	22 Sep		70	OLIGO
32.57	70	14 Oct	20	60	OLIGO
29.67	67	13 Oct	20	60	OLIGO
26.65	14	24 Sep	20		OLIGO
26.10	64	12 Oct	20	60	OLIGO
23.20	17	25 Sep		80	OLIGO
22.27	61	11 Oct	20	50	OLIGO
18.44	58	10 Oct	20	80	OLIGO
11.10	54	7 Oct	20	60	UW
10.98	51	6 Oct	20	60	UW
10.86	48	5 Oct	10	50	UW
10.78	30	2 Oct		40	UW
10.78	45	4 Oct	20	60	UW
10.61	28	1 Oct	1		UW
10.53	25	30 Sep		25	UW
10.47	23	29 Sep	5		UW

determined from temperature profiles with a CTD (Conductivity-Temperature-Depth). The subsurface chlorophyll-max layer was determined from *in vivo* fluorescence profiles. A pump sampler system (Herman *et al.*, 1984) was used to collect seawater samples and to obtain profiles of temperature and *in vivo* fluorescence.

1.2.2. Chlorophyll *a*

Fluorometric measurements (Turner fluorometer 10-005R) of chlorophyll *a* (Holm-Hansen *et al.*, 1965), extracted (24 hours in 90% acetone, at -20°C) from samples which had been size-fractionated (vacuum pressure < 120 mmHg) through 2-μm Nuclepore filters and collected on glass-fiber filters (MFS, Multi Filtration Systems, nominal pore size of 0.7 μm), were made; total chlorophyll *a* was measured by filtering water directly through the glass fiber filters (MFS). To monitor this fractionation procedure, water samples were passed through glass-fiber filters and the filtrates analyzed by flow cytometry to measure the fraction of autofluorescent cells lost through the glass-fiber filters.

1.2.3. Picophytoplankton abundance

The different picophytoplanktonic groups were identified and counted at sea using a flow cytometer (FACSort instrument, Becton Dickinson). Samples (0.25-0.5 ml) of unfractionated water and of water screened through 2-μm Nuclepore filters were analyzed. Fluorescence emission (488 nm excitation) measured at wavelengths longer than 650 nm corresponded to chlorophyll *a*, and fluorescence emission measured at 585 nm represented phycoerythrin. Three photosynthetic groups were distinguished by their *in vivo* fluorescence and intensity of side-scattering (90° angle), using an ataxonomic approach (Li, 1989). Cyanobacteria fluoresced in the wavelengths corresponding to phycoerythrin and chlorophyll *a*, while eukaryotes and prochlorophytes fluoresced in the wavelengths

corresponding to chlorophyll *a* only. Eukaryotes and prochlorophytes were distinguished by their different intensity in side-scatter (an index of size). Moreover, eukaryotes could be divided into two different size categories, "picoeukaryotes" and "large eukaryotes": the former were included in the <2-μm fraction (*i.e.* passed through a 2-μm Nuclepore filter) while the latter were included in the unfractionated sample (*i.e.* retained on a 2-μm Nuclepore filter). Since the mean diameters of cyanobacteria and prochlorophytes are ~1.3 μm and ~0.7 μm, respectively, any cells from these groups found in the >2-μm fraction did not represent a significant fraction of the total (note that the upper size-limit of the flow cytometer is 5 μm).

1.2.4 Bacterial abundance

Samples (18 ml) were filtered through 2-μm Nuclepore filters, immediately preserved with 0.2-μm filtered formalin (2% final concentration) and kept in the dark at ~5°C until counted. In the laboratory, 4-6 weeks after collection, triplicates of each sample were collected onto 0.2-μm black Nuclepore filters, which in turn were supported by pre-wetted Sartorius filters to allow an even distribution of cells on the Nuclepore surface. Cells were stained for 10 min with DAPI (4.5 μg ml^{-1}) and then counted under an epifluorescence microscope with ultraviolet excitation from a mercury lamp (Porter & Feig, 1980). The reported bacterial concentration (cells L^{-1}) is the average of the triplicates measured (precision of ±15%). In order to estimate bacterial loss through the glass-fiber filters used, replicate samples (from stations #58 to #85) were filtered through glass-fiber filters (MFS) and the resulting filtrates were analyzed as explained above.

1.2.5. Picoplankton biomass

Biomass of picophytoplankton and heterotrophic bacteria (in units of $\mu gC\ L^{-1}$) were estimated from cell abundance (cells L^{-1}) using the following conversion factors: 250 fgC $cell^{-1}$ for cyanobacteria (Kana & Glibert, 1987); 84 fgC $cell^{-1}$ for prochlorophytes, assuming a mean diameter of 0.7 µm and 470 fgC μm^{-3} (measured for *Synechococcus* by Verity *et al.*, 1992); 389 fgC $cell^{-1}$ for picoeukaryotes <2 µm, assuming a mean diameter of 1.5 µm and using 220 fgC μm^{-3} (Booth, 1988 for cells <4 µm other than *Synechococcus*); 7258 fgC $cell^{-1}$ for eukaryotes ≥2 µm, assuming a mean diameter of 4 µm and using the equation of Strathmann (1967); 20 fgC $cell^{-1}$ for heterotrophic bacteria (Lee & Fuhrman, 1987).

Although many of the marine eukaryotic plankton are >2 µm (range of 1 to 4 µm) in size (Estep *et al.*, 1984), a significant fraction of eukaryotes were observed in the <2-µm fraction of the present study. Moreover, numerous studies have reported eukaryotic groups which are <2 µm such as the chlorophyceae and prasinophyceae (Guillard *et al.*, 1991; Hoeppfner and Haas, 1990; Eikrem and Throndsen, 1990). For these reasons, I chose 1.5 µm as a mean diameter for picoeukaryotes.

1.3. Results

1.3.1. Chlorophyll *a* concentration

Considering all stations, chlorophyll *a* values ranged from 0.03 to 0.5 $\mu g\ L^{-1}$ for the <2-µm size-fraction and from 0.05 to 2.3 $\mu g\ L^{-1}$ for total chlorophyll *a* (Figs. 1.2 a,b), with picophytoplankton accounting for between 14 and 83% of the total chlorophyll *a*. The percentages of picophytoplankton cells passing through the glass-fiber filters were 2% in

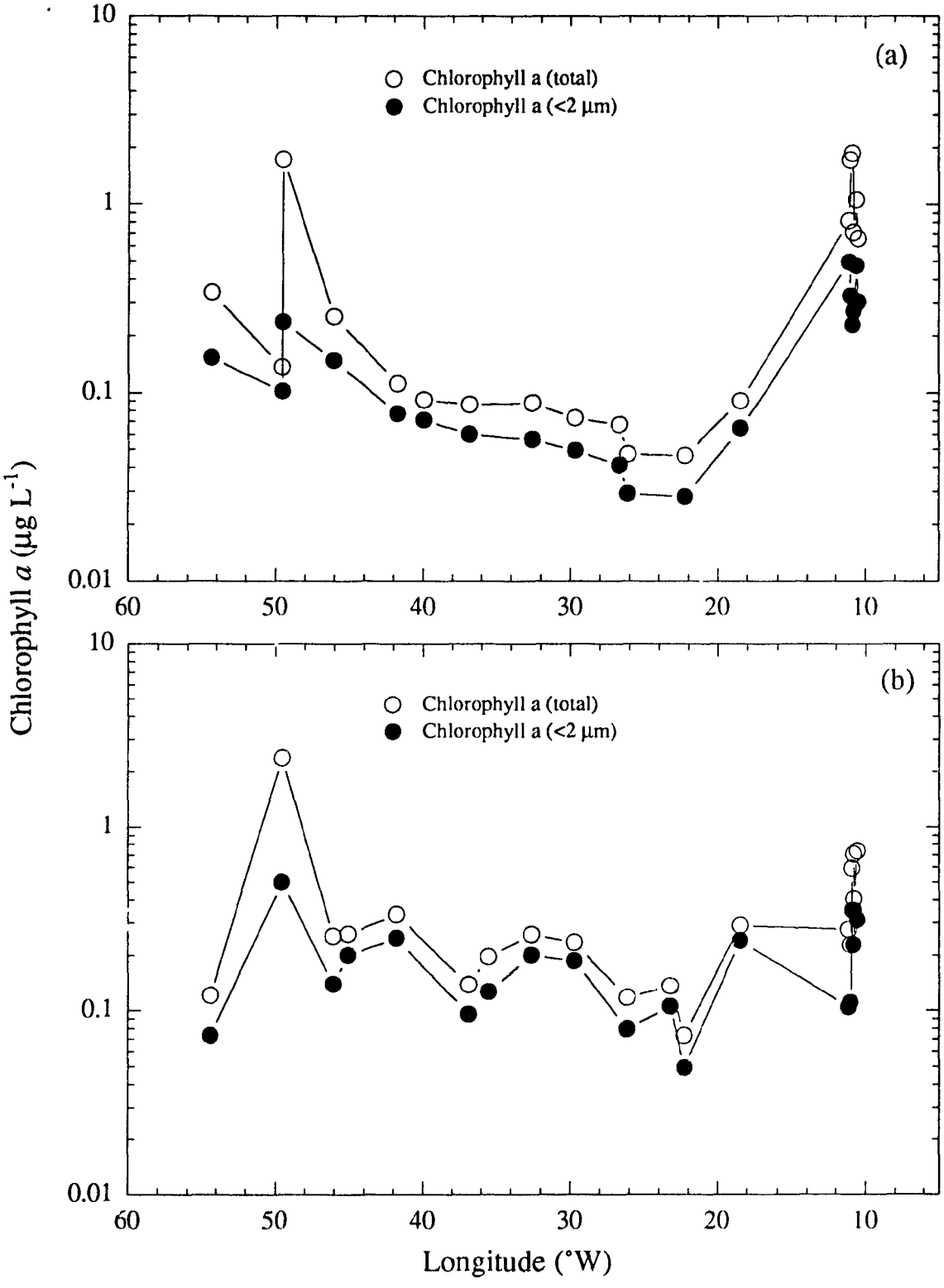


Figure 1.2 Average chlorophyll *a* concentration in the total fraction and in the <2-µm fraction for (a) the mixed layer and (b) the DCM layer.

oligotrophic waters and 4% in upwelling and slope waters, showing that there was an insignificant loss of chlorophyll *a*. At the oligotrophic stations, total chlorophyll *a* averaged 0.15 (±0.09) μg L^{-1}, while <2 μm chlorophyll *a* was 0.11 (±0.07) μg L^{-1}. At the upwelling and slope water stations, chlorophyll *a* averaged 0.89 (±0.67) μg L^{-1}, and the <2-μm chlorophyll *a* was 0.28 (±0.13) μg L^{-1}. Most chlorophyll *a* came from picophytoplankton at the oligotrophic stations (69%) while a less significant part of the chlorophyll *a* came from picophytoplankton in upwelling and slope waters (41%). For 2 of the upwelling stations (#48 and #51 in the mixed layer) and for one station located in slope waters (#82 in the mixed layer and in the DCM layer), total chlorophyll *a* values were high (1.7-2.3 μg L^{-1}) and picophytoplankton accounted for less than 20% of the chlorophyll *a*. At one of the upwelling stations (#48 in the mixed layer) diatoms >5 μm were present (HPLC measurements, E. Head, pers. comm.) whereas eukaryotes were absent in the 2-5 μm size-range (5 μm is the upper size-limit of the flow cytometer), suggesting that cells >5 μm were important. At the other upwelling station (#51 in the mixed layer), eukaryotes in the size-range 2-5 μm were dominant while picoeukaryotes <2 μm were absent, indicating that cells in the 2-5 μm size-range were important.

1.3.2. Picophytoplankton abundance

At the oligotrophic stations (Table 1.2), prochlorophytes were 1 to 2 orders of magnitude more abundant than picoeukaryotes and cyanobacteria (Figs. 1.3 a, b). In upwelling and slope waters, on the other hand, prochlorophytes and cyanobacteria both had lower concentrations than picoeukaryotes. On average, prochlorophytes accounted for 95% (81-98%) of picophytoplankton at the oligotrophic stations, while picoeukaryotes were the most abundant of the three groups at the upwelling and slope water stations representing, on average, 49% (6-74%) of the total picophytoplankton abundance (Table 1.2).

Table 1.2 Picophytoplankton abundance in absolute (10^7 L^{-1}) and relative (percentage of picophytoplankton abundance) terms for oligotrophic waters, and upwelling and slope waters. Absolute values are mean values with standard deviations; relative values are mean percentages with ranges.

Location	Cyanobacteria		Prochlorophytes		Picoeukaryotes	
	(10^7 L^{-1})	(%)	(10^7 L^{-1})	(%)	(10^7 L^{-1})	(%)
OLIGO	0.3±0.2	4 (2-15)	9.9±3.4	95(81-98)	0.15±0.1	2 (1-6)
UW+SL	0.9±1.1	27(16-40)	0.7±1.5	24 (0-71)	1.2±0.8	49 (6-75)

Figure 1.3 Abundance of prochlorophytes, cyanobacteria, and picoeukaryotes for (a) the mixed layer and (b) the DCM layer. No prochlorophytes were observed at station #82. Relative contribution of prochlorophytes, cyanobacteria, and picoeukaryotes to picophytoplankton biomass for (c) the mixed layer and (d) the DCM layer.

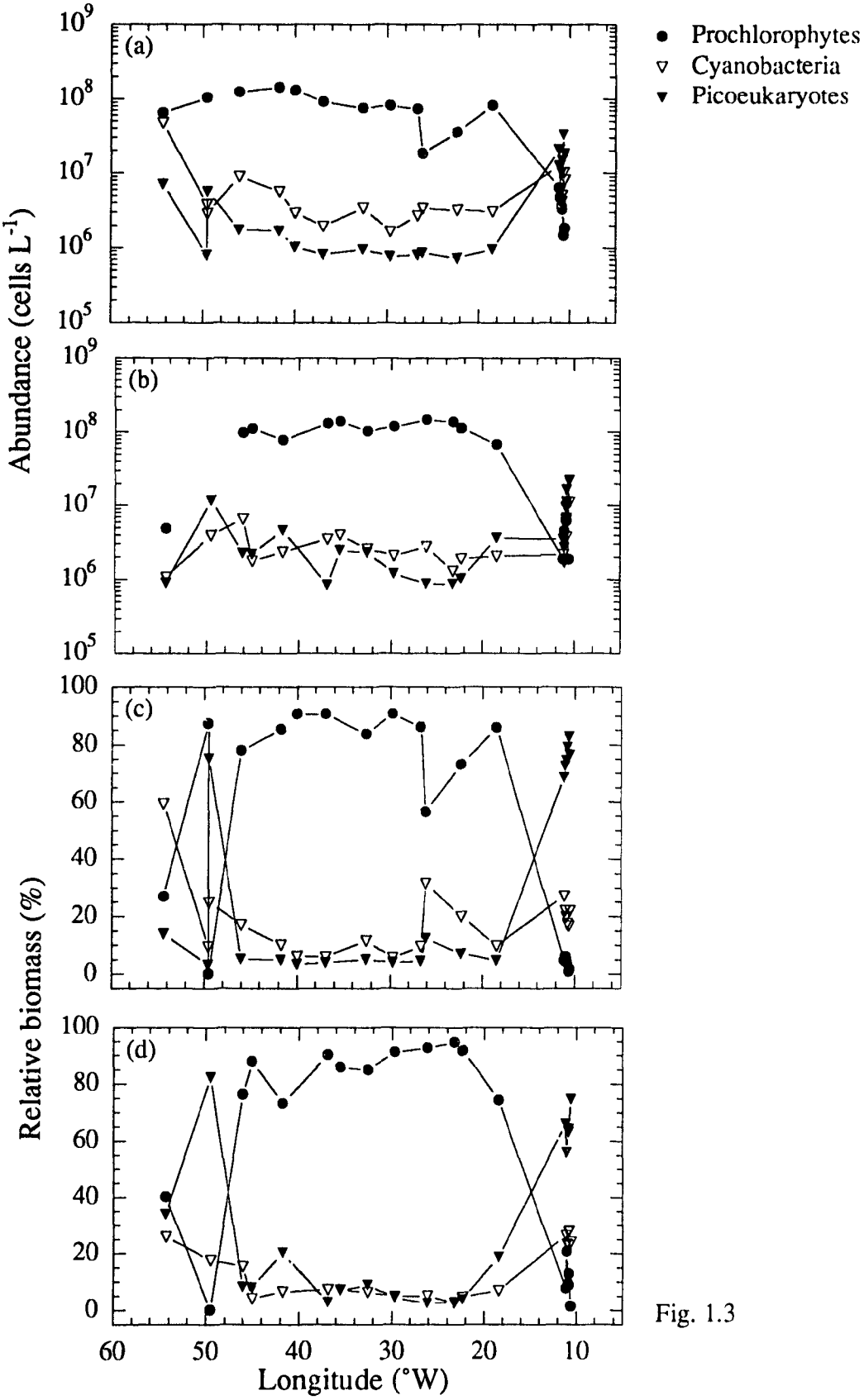

Prochlorophytes
Cyanobacteria
Picoeukaryotes
(a)
(b)
(c)
(d)
Abundance (cells L-1)
Relative biomass (%)
Longitude (°W)
10^9
10^8
10^7
10^6
10^5
100
80
60
40
20
0
60
50
40
30
20
10

Fig. 1.3

Flow cytometry indicated that most phytoplankton cells were found in the <2-μm fraction at both depths. In fact, picophytoplankton accounted for 99-100% of the phytoplankton abundance measured in unfractionated water (but note that 5 μm was the upper size limit of the flow cytometer, Li, pers. comm.). On average, 7% (<1-18%) of total phytoplankton (unfractionated water) were found in the 2-5 μm size range. However, at 3 stations located in upwelling and slope waters (#28, 51 in the mixed layer, and #85 in the DCM layer), 12-18% of the total phytoplankton community were large eukaryotes (≥2 μm). Results presented here include the picoplankton community, *i.e.* the <2-μm fraction (unless specified).

1.3.3. Picophytoplankton biomass

In oligotrophic waters, prochlorophytes dominated the picophytoplankton biomass (Figs. 1.3 c, d), accounting for 82% in the mixed layer (56-91%), and 86% in the DCM layer (73-95%). At the upwelling site, picoeukaryotes and cyanobacteria comprised 70% (56-83%) and 23% (17-28%), respectively, of the picophytoplankton biomass (averaged over both depths). Examination of unfractionated samples from the upwelling site indicated that large eukaryotes (2-5 μm) were more important than picoeukaryotes (<2 μm) at some stations (#28, 51 in the mixed layer and #54 in the DCM layer), equally important at one station (#54 in the mixed layer), but less important or absent at other stations (#23, 25, 30, 45, 48 at both depths).

1.3.4. Relative importance of picophytoplankton and bacteria

Bacterial concentrations reached ~10^8 cells L^{-1} in all water regimes (Table 1.3) which is of the same order of magnitude as picophytoplankton concentrations in oligotrophic waters, but higher than picophytoplankton concentrations (2.8×10^7 L^{-1}) in

Table 1.3 Abundance and carbon biomass of picophytoplankton and heterotrophic bacteria for oligotrophic waters, and upwelling and slope waters. Absolute values are means with standard deviation; relative values are means and ranges.

Location	Abundance				Carbon biomass			
	Picophytoplankton		Heterotrophic bacteria		Picophytoplankton		Heterotrophic bacteria	
	10^8 L^{-1}	(%)	10^8 L^{-1}	(%)	µg C L^{-1}	(%)	µg C L^{-1}	(%)
OLIGO	1.0±0.3	30(9-46)	2.5±0.7	70(54-91)	9.7±3.0	65(36-80)	5.0±1.5	35(20-64)
UW+SL	0.3±0.3	7(2-37)	4.5±1.2	93(63-99)	7.5±5.2	41(13-83)	9.0±2.3	59(17-87)

upwelling and slope waters (Table 1.2, 1.3). Overall, heterotrophic bacteria were numerically more abundant than autotrophic picoplankton (Table 1.3), contributing 70% (54-91%) to the picoplankton abundance in oligotrophic waters and 93% (63-99%) in upwelling and slope waters.

Relative to picoplankton biomass, the biomass of picophytoplankton was higher (65%) than the biomass of bacteria (35%) in oligotrophic waters; in upwelling and slope waters, on the other hand, the opposite trend was observed (Table 1.3, Fig 1.4 a, b). Prochlorophytes (55%) and bacteria (35%) were the main contributors to the carbon biomass of picoplankton in oligotrophic waters, accounting for 90% of the picoplankton biomass, and in the DCM layer, prochlorophytes accounted for 60% (47-72%), on average, of the total picoplankton biomass (not shown here). Moreover, for all oligotrophic stations but one, the ratio of prochlorophyte biomass to bacterial biomass was always greater or equal to 1, regardless of depth (not shown). Thus, in terms of picoplankton biomass, the community structure of open ocean waters was dominated by prochlorophytes, with a significant contribution by heterotrophic bacteria. In upwelling and slope waters, on the other hand, prochlorophytes did not account for a significant fraction of the biomass, instead bacteria represented most of the picoplankton biomass (average of 59%, range of 17-87%).

1.4. Discussion

1.4.1. Contribution of picophytoplankton to total chlorophyll *a*

In oligotrophic waters, picophytoplankton contribute most of the chlorophyll *a* (Li *et al.*, 1983; LeBouteiller *et al.*, 1992). In nutrient-rich areas of the oceans, on the other hand, *e.g.* in the Southern Ocean (Weber & El-Sayed, 1987), in the Equatorial Pacific

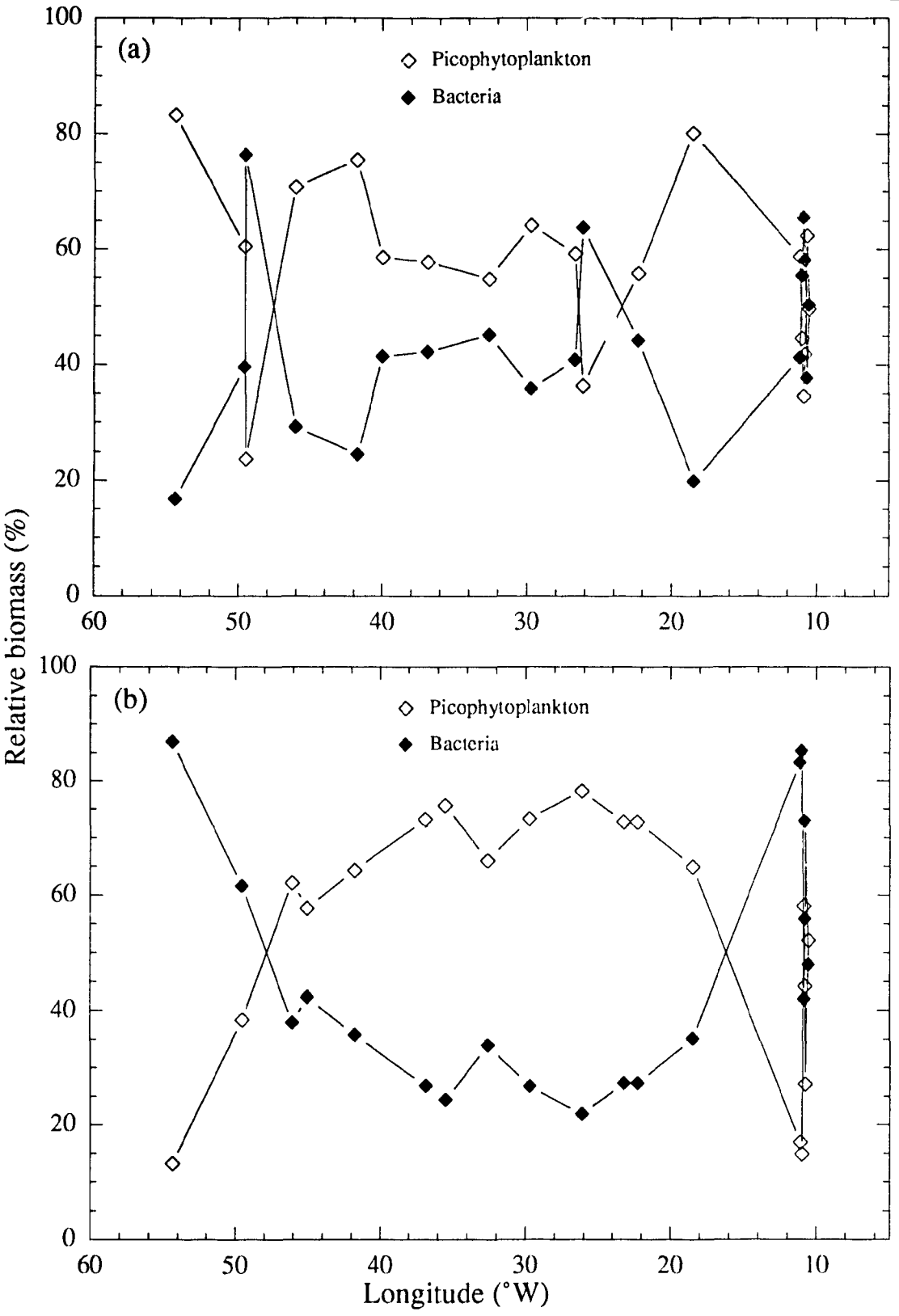


Figure 1.4 Relative contribution (% of picoplankton biomass) of picophytoplankton and bacteria (a) in the mixed layer and (b) in the DCM layer.

(Chavez *et al.*, 1990), in the Equatorial Atlantic (Herbland *et al.*, 1985), in coastal upwelling systems (Hall & Vincent, 1990), and in coastal waters (Søndergaard *et al.*, 1991), small-size phytoplankton <5 μm do not usually dominate the standing stock but can contribute significantly. Generally, the contribution of small size cells (*e.g.* picophytoplankton) to chlorophyll *a* increases as total chlorophyll *a* decreases, corresponding to the transition from eutrophic to oligotrophic waters (Chisholm, 1992). Results in the present study (Figs. 1.2 a, b) are in agreement with previous observations (Li *et al.*, 1983; LeBouteiller *et al.*, 1992): picophytoplankton dominated the total chlorophyll *a* in oligotrophic waters and contributed significantly to total chlorophyll *a* in upwelling and slope waters.

1.4.2. Picophytoplankton abundance

High concentrations of prochlorophytes have been observed in the North Atlantic using flow cytometry. Olson *et al.* (1990a) reported prochlorophyte concentrations (samples taken from the surface to 150-200 m) reaching 10^8 cells L^{-1} at the surface and at the subsurface chlorophyll-max in the Sargasso sea (May 1989); these high concentrations were not typical for all stations. In another study in the Sargasso Sea, Li *et al.* (1992) detected prochlorophytes only below 75 m at concentrations of $4x10^7$ cells L^{-1}. At 20°W in the subtropical North Atlantic, Veldhuis & Kraay (1990) and Veldhuis *et al.* (1993) did not detect prochlorophytes at all depths but showed them to be most abundant in the deep chlorophyll-maximum layer (maximum of $3\text{-}9.5x10^7$ cells L^{-1}). Using divinyl-chlorophyll-*a* as an absolute marker of prochlorophytes (high-performance liquid chromatography analysis), other workers (Veldhuis and Kraay, 1990; Goericke and Welschmeyer, 1993; Goericke and Repeta, 1993) have reported the presence of prochlorophytes throughout the water column for most of the year, and a maximum concentration at the subsurface chlorophyll maximum depth. At the oligotrophic stations of the present study,

prochlorophytes were detected at the two depths sampled, and they were present throughout the upper water column, down to 110 m (Li, 1995, during the same cruise). Moreover, the numerical dominance of prochlorophytes was observed at all oligotrophic stations, in agreement with the results of Olson *et al.* (1990a) and Li (1995). The numerical dominance of prochlorophytes was also observed when the abundances of prochlorophytes, cyanobacteria, and picoeukaryotes were integrated over the water column, down to 110 m (Fig. 1.6 based on data taken from Li, 1995). However, prochlorophyte concentrations (10^{13} cells m^{-2}) were higher by an order of magnitude than those reported by Olson *et al.* (1990a), which were integrated down to 150-200 m.

Concentrations of cyanobacteria and picoeukaryotes in the present study are similar to the concentrations reported in other studies for the North Atlantic (Veldhuis & Kraay, 1990; Olson *et al.*, 1990a; Li *et al.*, 1992). If integrated over the water column (Fig. 1.5), the abundance of cyanobacteria (10^{11} cells m^{-2}) was similar to the abundance reported by Olson *et al.* (1990a), except during the spring bloom off Bermuda where concentrations were ~10^{12} cells m^{-2}.

The large difference (1-2 orders of magnitude) observed between prochlorophyte and cyanobacterial concentrations (Figs. 1.3 a,b, 1.5) is in agreement with previous observations in the Sargasso sea (Olson *et al.*, 1990a) and in the subtropical North Pacific (Campbell and Vaulot, 1993). Since prochlorophytes and cyanobacteria are both photosynthetic prokaryotes and may occupy the same pelagic "niche" (Chisholm, 1992), these results suggest that prochlorophytes and cyanobacteria would not co-exist in high numbers (10^{8} L^{-1}). Does that signify that prochlorophytes are better adapted than cyanobacteria to grow under oligotrophic conditions? The higher surface-to-volume ratio of a prochlorophyte cell (8.5 μm^{-1}) compared with a cyanobacterial cell (4.6 μm^{-1}) may result in prochlorophytes taking up nutrients and capturing light more efficiently (Raven, 1986;

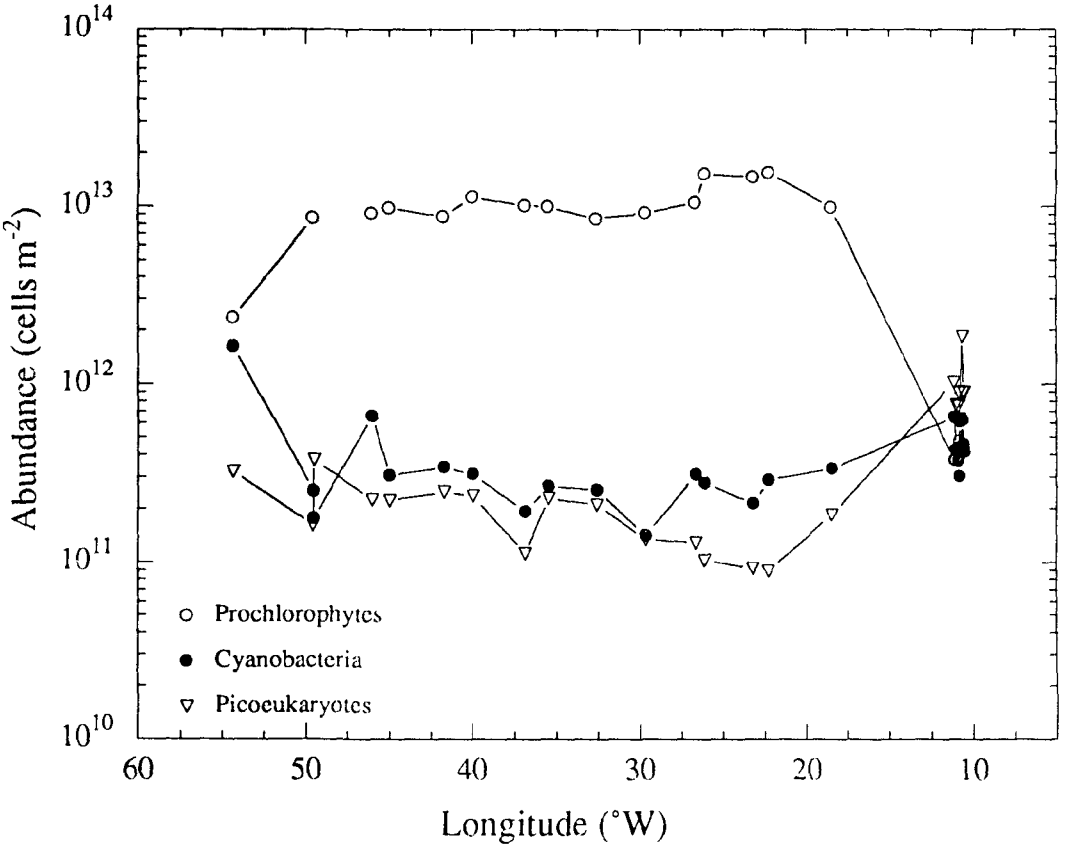


Figure 1.5 Cell concentrations (cells m^{-2}) of prochlorophytes, cyanobacteria, and picoeukaryotes integrated over the photic zone (data from Li, 1995). No prochlorophytes were detected at stations #82, 23, 25, and 28.

Moore *et al.*, 1995). On the other hand, Raven (1994) predicted, based on theoretical calculations, that 0.5 μm cells would have a lower specific growth rate compared with 5 μm cells, however, he found little experimental evidence to support this. Vaulot *et al.* (1995) recently measured growth of *Prochlorococcus* in the field and found growth rates (0.73-0.93 d^{-1}) close to the maximum rates measured in cultures, under optimum conditions. Cyanobacterial growth rates measured in the field are similar to these, *i.e.* 0.5-1.2 d^{-1} in the Sargasso Sea (Iturriaga and Marra, 1986), also close to maximum growth rates from culture studies (Kana and Glibert, 1987; Moore *et al.*, 1995). Thus, both prochlorophytes and cyanobacteria have been shown to grow at high rates in the field.

Temperature may also influence the distribution of prochlorophytes and cyanobacteria. Olson *et al.* (1990a) reported the presence of prochlorophytes only in waters warmer than 17°C; Moore *et al.* (1995), on the other hand, reported that the optimal temperature for growth of *Prochlorococcus marinus* (two clones) was 24°C. This suggests that the growth of prochlorophytes is dependent on warm temperatures. In agreement with these observations, higher concentrations of prochlorophytes (>$5x10^6$ cells L^{-1}) were generally found at temperatures above 17°C in the present study. At one of the slope water stations (#82), temperature was < 6°C, and no prochlorophytes were present at any depth; this could be due to the fact that the water originated from below the photic zone (source water), or that prochlorophytes could not grow at such low temperatures. A clear relationship was observed between prochlorophyte abundance in the mixed layer (down to 20 m in the water column) and the local temperature observed (Fig. 1.6 a): for a variation of 5°C in the mixed layer, prochlorophyte abundance varied by an order of magnitude. Prochlorophyte abundance increased from 18°C to 21°C, and remained more or less constant for temperatures greater than 21°C. On the other hand, cyanobacterial abundance varied inversely with temperature (Fig. 1.6 b). A correlation between cyanobacterial abundance and temperature has been reported, both over spatial (Waterbury *et al.*, 1986)

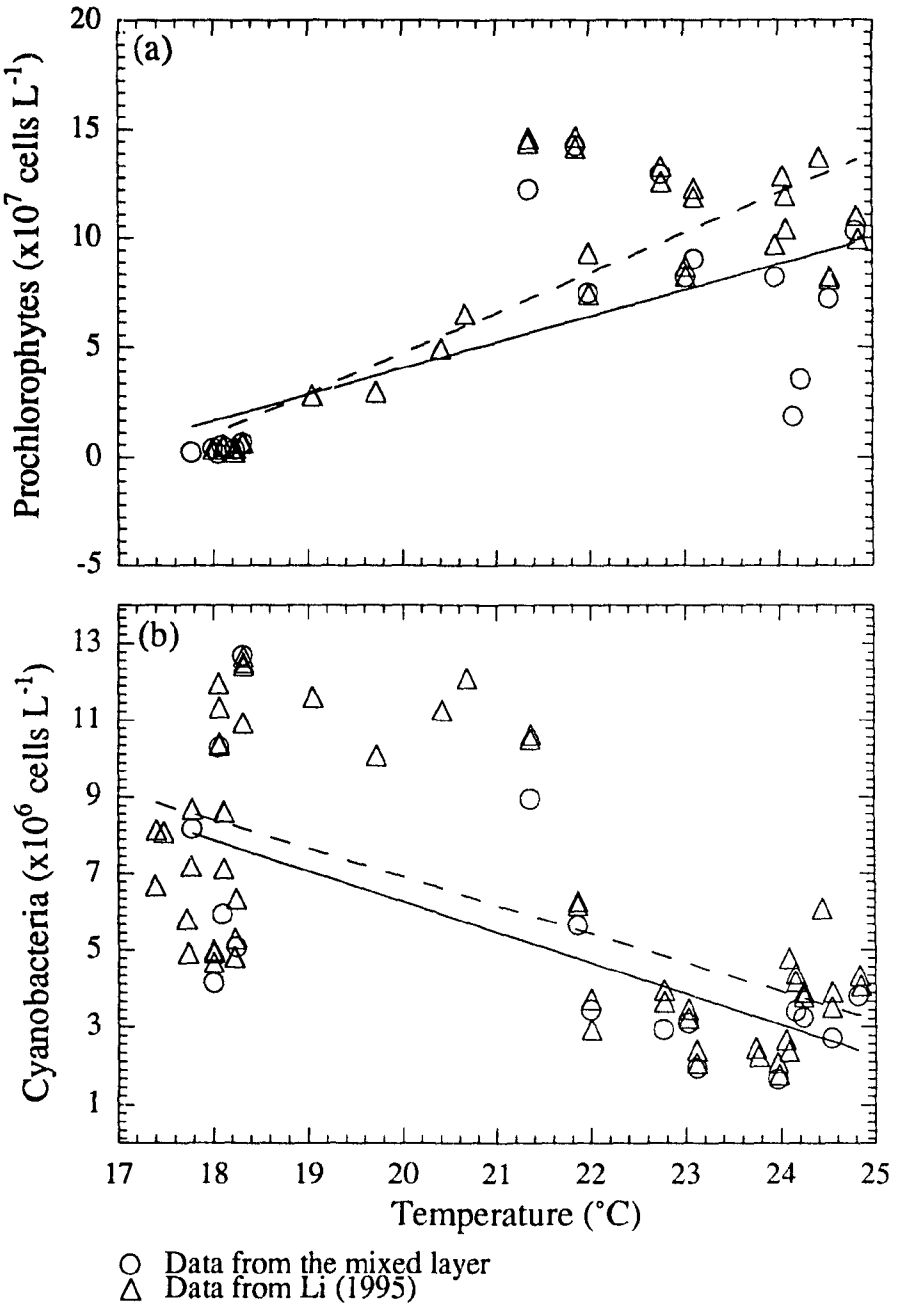


Figure 1.6 Prochlorophyte abundance (a) and cyanobacterial abundance (b) versus temperature for samples taken in the upper 20 m along the East-West transect and including the upwelling site. Dashed line represents linear regression (R^2= 0.86 for prochlorophytes, R^2= 0.62 for cyanobacteria) of data from Li (1995). Solid line represents linear regression (R^2= 0.64 for prochlorophytes, R^2= 0.69 for cyanobacteria) of data from the present study.

and temporal scales (El Hag & Fogg, 1986; Waterbury *et al.*, 1986). Moore *et al.* (1995) reported that both prochlorophytes and cyanobacteria cultivated in the laboratory were growth-limited under low temperatures (<15°C), however, prochlorophytes showed optimal growth at a lower temperature (24°C) than *Synechococcus* (28°C). In the present study, prochlorophytes showed maximum abundance at a higher temperature than cyanobacteria (Figs. 1.6 a, b). Note that the relationship between cyanobacterial abundance and temperature in the present study is less clear than between prochlorophytes and temperature, *i.e.* contains more scatter. The lowest abundance of prochlorophytes, observed at low temperatures (Fig. 1.6 a), was generally higher than the abundance of cyanobacteria, suggesting that temperature did not strongly affect the distribution observed of prochlorophytes and cyanobacteria.

The present study was carried out as part of the first extensive sampling of picophytoplankton in the Subtropical North Atlantic. Li (1995) studied the ultraphytoplankton and reported high concentrations of prochlorophytes (10^8 L^{-1}) at all oligotrophic stations. Such high concentrations were considered as "exceptional" in the North Atlantic by Campbell & Vaulot (1993) and more typical of the Subtropical Pacific (Station ALOHA, 22° N, 158° W). Similarly high prochlorophyte concentrations have been observed recently in the Central Equatorial Pacific (Landry *et al.*, in press). Thus, the high concentrations of prochlorophytes found in the tropical and equatorial Pacific (Campbell & Vaulot, 1993; Landry *et al.*, in press) are observed, at particular times of the year, in the subtropical North Atlantic over a large area. During a similar transect study (same location) carried out in the spring of 1993 (Li, 1995), the abundance of prochlorophytes was significantly decreased (by more than half) while the abundances of *Synechococcus* and eukaryotes were increased, compared with results from the fall of 1992. However, prochlorophytes numerically dominated the ultraplankton community <5 μm during both seasons (Li, 1995).

1.4.3. Bacterial abundance

Fuhrman *et al.* (1989) reported bacterial concentrations of 10^9 cells L^{-1} and 10^8 cells L^{-1} (Acridine Orange technique, Hobbie *et al.*, 1977) at the surface and at 150 m, respectively, at one station in the Sargasso Sea. Li *et al.* (1992) found bacterial concentrations of 3-4x10^8 cells L^{-1} in the upper 90 m, and 1-2x10^8 cells L^{-1} below 100 m at another station in the Sargasso Sea, using the DAPI technique (Porter & Feig, 1980). In the Central North Pacific gyre, Cho & Azam (1990) reported bacterial numbers of 2x10^8-2x10^9 cells L^{-1}, using the same technique as Li *et al.* (1992). The present study reports values comparable to those of Li *et al.* (1992). The higher values reported in the study of Fuhrman *et al.* (1989) could have resulted from the different staining technique used to count bacteria (the acridine orange technique as opposed to the DAPI technique), while in the case of Cho & Azam (1990), the difference could be attributed to a geographical difference (Pacific waters as opposed to North Atlantic waters). The acridine orange technique dies both the DNA and RNA, as well as the detrital components, whereas the DAPI technique is more specific of DNA (Porter and Feig, 1980). This could explain the much higher abundances of bacteria found by Fuhrman *et al.* (1989), compared with the present study. Note that Campbell *et al.* (1994) suggested that the abundances observed by Fuhrman *et al.* (1989) may have been an extreme case. Studies which use the DAPI or the Acridine orange technique may be overestimating bacterial counts by as much as 30% by counting photosynthetic bacteria (Campbell *et al.*, 1994). On the other hand, in the study of Li *et al.* (1992, 1993) and in the present study, the preservation technique used (2% formalin, refrigerated at 5°C) could have resulted in significant losses of bacteria (15-40%) and photosynthetic cells (~70%) with time (Turley and Hughes, 1992; Trousselier *et al.*1995). Thus, the error invoked by Campbell *et al.* (1994) would have been largely compensated for. In summary, bacterial concentrations measured in the present study were

comparable with bacterial concentrations previously reported (Li *et al.*, 1992; Davis *et al.*, 1985), but different from the studies of Fuhrman *et al.* (1989) and Cho & Azam (1990).

1.4.4. Picophytoplankton abundance and bacterial abundance

Bacterial abundance showed a positive correlation with chlorophyll *a* (Fig. 1.7 a), in agreement with previous studies (Bird and Kalff, 1984; Cole *et al.*, 1988; Cho and Azam, 1990). However, within the picoplankton community, bacterial abundance was inversely related to picophytoplankton abundance (Fig. 1.7 b), and the negative correlation corresponds to a transition from oligotrophic waters to upwelling and slope waters where bacterial abundance increased while picophytoplankton abundance decreased. Integrated abundances showed similar relative proportions (Table 1.4), however, no correlation was observed when all the stations were plotted. The difference observed between Fig. 1.7 a and b may be explained by the fact that phytoplankton >2 μm are only included in the first figure. In addition, the positive relationship observed in previous studies and in the present study is associated with a high degree of scatter (Li *et al.*, 1992).

1.4.5. Picophytoplankton biomass and bacterial biomass

Using 20 fgC cell^{-1} (Lee & Fuhrman, 1987) to convert bacterial abundance into carbon biomass for the Sargasso Sea, Fuhrman *et al.* (1989) found bacterial biomass values of ~20-40 μg C L^{-1} down to 150 m depth, while Li *et al.* (1992) found values of 6-8 μg C L^{-1} in the upper 90 m, and 2-4 μgC L^{-1} below 100 m. Using the same conversion factor for bacteria, the present study showed lower values of biomass in oligotrophic waters (average of 5 μg C L^{-1}, ranging from 1.2 to 8.5 μg C L^{-1}, see Table 1.4) than previous reports (Fuhrman *et al.*, 1989; Cho & Azam, 1990), except for Li *et al.* (1992) where biomass values were in a comparable range.

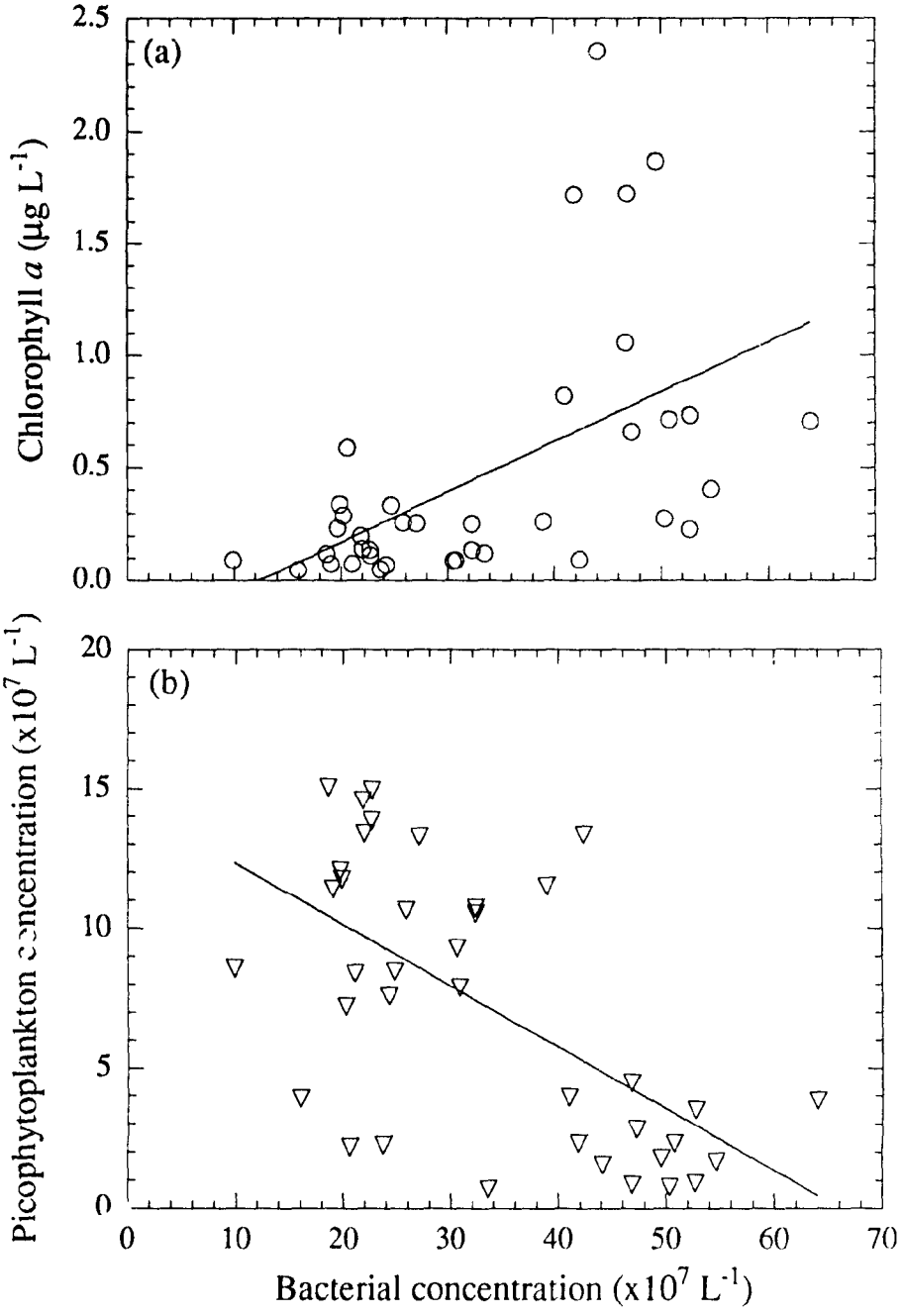


Figure 1.7 Chlorophyll *a* concentration in unfractionated water versus bacterial abundance in the mixed layer and in the DCM layer (a); R^2=0.53, n=38. Picophytoplankton abundance versus bacterial abundance in the mixed layer and in the DCM layer (b); R^2=0.61, n=38.

Table 1.4 Euphotic zone integrated abundance and biomass of picophytoplankton and bacteria for oligotrophic waters, and upwelling and slope waters. Values are means and standard deviations.

Location	Cyanobacteria		Prochlorophytes		Picoeukaryotes		Heterotrophic bacteria	
	10^{11} cells m^{-2}	mg C m^{-2}	10^{11}cells m^{-2}	mg C m^{-2}	10^{11} cells m^{-2}	mg C m^{-2}	10^{13} cells m^{-2}	mg C m^{-2}
OLIGO	2.9±1.2	74±29	110±24	905±206	1.7±0.6	155±54	3.1±1.0	632±210
UW+SL	5.6±4.0	141±99	8.0±8.7	67±73	8.4±4.2	775±391	4.4±0.9	880±174

At the oligotrophic stations, picophytoplankton accounted for most of the carbon biomass (65%) of picoplankton (Tables 1.3, 1.4; Fig. 1.4); at the upwelling and slope water stations, on the other hand, bacteria were the main contributors (59%) to picoplankton biomass. The ratio of picophytoplankton biomass to bacterial biomass (B_p:B_b) averaged 1.9 in oligotrophic waters and 0.5 in upwelling and slope waters (based on discrete depths). Note, however, that for the upwelling stations, picophytoplankton accounted for only ~40% of the total chlorophyll *a*, and other phytoplankton cells >2 µm were important (E. Head, pers. comm.). This suggests that the total autotrophic biomass may have dominated over heterotrophic biomass at the upwelling site as well, due to the importance of larger phytoplankton (>5 µm) and their higher carbon content. In fact, when plotting the water-column integrated values of picophytoplankton and bacterial biomass, the negative correlation was weak (R^2=0.34), and the degree of scatter was high. Thus, the relationship observed between picophytoplankton and bacterial abundance (Fig. 1.7 b), showing a clear transition between oligotrophic and upwelling and slope waters, was not observed for biomass.

The biomass proportions of picophytoplankton and bacteria of 2:1 at the oligotrophic stations (computed for discrete depths and for values integrated over the upper water column) contradict previous observations in the North Atlantic (Fuhrman *et al.*, 1989), and in the Central Pacific (Cho & Azam, 1990) where bacteria were reported to dominate the biomass. The differences reported are likely due to a difference in the observed bacterial concentrations (see section 1.4.3.). Li *et al.* (1992) found a co-dominance between phytoplankton (53%) and bacteria (47%) for the Sargasso sea, integrating over the same depth (110 m) as in the present study. These estimates were also based on DAPI counts; however, a different cell-to-carbon conversion factor was used for prochlorophytes (59 fgC $cell^{-1}$ instead of 84 fgC $cell^{-1}$ in the present study). Note that the conversion factor for prochlorophytes (59 fgC $cell^{-1}$) used by Li *et al.* (1992) was taken

from Booth (1988) as representative of phytoplankton cells <4 μm, whereas the conversion factor used in the present study (84 fgC cell^{-1}) was taken from Verity *et al.* (1992) and was measured for *Synechococcus*. Also, Li *et al.* (1992) assumed a diameter of 0.8 μm while I used a diameter of 0.7 μm. If the conversion factor used in Li *et al.* (1992) was applied to the present study (*i.e.* 59 fgC cell^{-1}) the resulting biomass of picophytoplankton represented 59% of the total biomass, while the bacterial biomass represented 41%, which is similar to Li *et al.* (1992). This suggests that the discrepancy between the present study and that of Li *et al.* (1992) is due to the use of different conversion factors. Note that even if the bacterial concentrations from the present study are adjusted for a 40% presumed bacterial cell loss (Turley & Hughes, 1992), the resulting picophytoplankton biomass would still represent 57% of the total picoplankton biomass (integrated value).

The higher values of picophytoplankton biomass relative to bacterial biomass are in agreement with recent results from Campbell *et al.* (1994) who used dual-beam flow cytometry (Monger & Landry, 1993) to monitor bacteria and picophytoplankton simultaneously in the Central North Pacific: they found photosynthetic biomass to be greater than bacterial biomass. Using the same technique as Campbell *et al.* (1994), and also working at station ALOHA (Central North Pacific), Christian & Karl (1994) found that heterotrophic bacteria represent a significant biomass, but do not necessarily dominate the microbial community. Based on a multiple linear regression between carbon-to-chlorophyll-*a* ratios, carbon-to-cell conversion factors and carbon-to-ATP ratios, Christian & Karl (1994) argued that the commonly-used conversion factor of 20 fg C cell^{-1} for bacteria may not be a realistic conversion factor, and that 10 fg C cell^{-1} should be used (Christian & Karl, 1994). If this is the case, the argument of picophytoplankton dominance in oligotrophic waters would be even stronger.

Previous measurements of divinyl-chlorophyll-*a* (absolute marker of prochlorophytes), as a fraction of total chlorophyll *a*, were shown to vary for different oligotrophic areas, from 25-35% (Goericke and Repeta, 1993; Goericke and Welshmeyer, 1993) to 40-60% (Veldhuis and Kraay, 1990; Letelier *et al.*, 1993; Suzuki *et al.*, 1995). Given that the relative contributions of bacteria and picophytoplankton to microbial biomass vary significantly for different oligotrophic areas, the previously held argument that heterotrophic bacterial biomass dominates over phytoplankton biomass cannot be supported.

The high proportion of picophytoplankton biomass in oligotrophic waters is due, for most part, to high values of prochlorophyte biomass (>80% of the picophytoplankton biomass). The biomass proportions observed in open-ocean waters were 1:3:8.5:12 for cyanobacteria, picoeukaryotes, bacteria, and prochlorophytes, respectively. Li *et al.* (1992) measured biomass proportions in the Sargasso sea of 1:4.4:8.7:2.5. The difference observed can be attributed to the fact that the study of Li *et al.* included cells >2 μm which were not taken into account in the present study. The dominance of prochlorophyte biomass in the present study was in agreement with Campbell *et al.* (1994) who reported *Prochlorococcus* to be the main contributors (67%) to the total photosynthetic biomass of the central North Pacific ocean. The high contribution of prochlorophytes to the biomass of picophytoplankton found at the oligotrophic stations (82-86%) is also supported by the independently measured ratio of divinyl-chlorophyll *a* to chlorophyll *a* in the <1 μm-fraction (E. Head, unpubl. data). This ratio varied between ~60% and 80% for two oligotrophic stations (#64, 70); note that most of the divinyl-chlorophyll *a* was in the <1 μm-fraction (94% on average). These results support the dominance of prochlorophyte biomass over the remaining picophytoplankton groups.

As previously mentioned, the abundance of the phytoplankton community can vary significantly over seasons, not only in eutrophic waters but also in open-ocean oligotrophic waters (Lohrenz *et al.*, 1992). In oligotrophic waters, where the phytoplankton population is dominated by small size phytoplankton *e.g.* picophytoplankton (Li *et al.*, 1983), this seasonal variability also implies changes in the proportions of the different photosynthetic groups. Such changes are shown for ultraphytoplankton by Li *et al.* (1992). Li (1995) showed, for the same stations sampled in the present study, that the partitioning of ultraphytoplankton into prochlorophytes, cyanobacteria, and eukaryotes was different between the fall of 1992 and the late spring of 1993: the high abundance of prochlorophytes observed in 1992 (Fig. 1.3) decreased in 1993, while the concentrations of cyanobacteria and eukaryotes increased over the same period of time. Li (1995) also estimated the relative contribution of the three groups to carbon biomass by estimating the ratio of the mean light scatter signal of each group to the sum of the mean light scatter signal for the three groups. These estimates showed eukaryotes to be the main contributors to the biomass of ultraphytoplankton for both 1992 and 1993. The biomass results in the present study, which are from 1992 only and based on cell abundances converted into biomass, showed prochlorophytes to be the main contributors to picoplankton biomass. Thus, results from the present study differed from the results of Li (1995) where data from 1992 and 1993 were taken together. It is difficult to compare the findings of Li (1995) with the present study for two reasons. First, the results of Li (1995) were reported for ultraphytoplankton (<5 μm), whereas results in the present study are reported for picophytoplankton (<2 μm); thus, eukaryotic cells in the 2-5 μm size-fraction may account for the difference. However, results from 1992 (taken from Li's data (1995)) that were integrated over the upper water column, *i.e.* 110 m (including the 2-5 μm fraction) also showed prochlorophytes to dominate the total biomass; this conclusion did not change whether I used 59 fg C $cell^{-1}$ (conversion factor used in previous studies of Li, 1992, 1993) or 84 fg C $cell^{-1}$ (conversion factor used in the present study) as conversion factor

for prochlorophytes. Second, comparison of a data set covering one season only (present study) with a data set that averages over the two seasons may be biased, since the seasonal variability is not accounted for in the present study. Thus, my conclusions are valid exclusively for one season of the year.

1.5. Conclusions

In the present study, picophytoplankton were shown to dominate the photosynthetic abundance and biomass in the oligotrophic waters of the subtropical North Atlantic (60°-10° W) during fall. Within the picophytoplankton community, prochlorophytes were the main contributors to abundance and biomass. On the other hand, at an upwelling site of the North West coast of Africa, picophytoplankton only contributed ~40% to the total phytoplankton biomass. Contrary to previous observations in oligotrophic waters of the North Atlantic, heterotrophic bacteria did not dominate phytoplankton biomass. In fact, picophytoplankton accounted for a higher fraction of the picoplankton biomass than bacteria.

Chapter 2

New and regenerated production of picoplankton in coastal and oceanic waters of the subtropical North Atlantic

2.1. Introduction

Photosynthetic picoplankton account for most of the phytoplankton abundance and biomass in oceanic waters of the subtropical North Atlantic (see Chapter 1). Picoplankton can also contribute significantly to the primary production in open-ocean oligotrophic waters (Li *et al.*, 1983; Platt *et al.*, 1983). Since most of the world's oceans (>80%) fall into this category (Berger *et al.*, 1989), primary production by picoplankton could contribute significantly to global carbon production.

Primary production is usually limited by the availability of nitrogen (Ryther and Dunstan, 1971) and can be partitioned based on the nitrogen source used (Dugdale and Goering, 1967). New production is based on the uptake of newly-introduced nitrogen, mainly nitrate, while regenerated production is based on recycled nitrogen, mainly ammonium. It has been estimated that open-ocean areas account for nearly 50% of global new production (Eppley and Peterson, 1979; Berger *et al.*, 1989). Since picoplankton predominate in these areas, it may be argued that picoplankton must contribute significantly to new production on a global scale. However, the conventional view has been that new production is mainly accounted for by large cells while small cells are responsible for most of the regenerated production (Malone, 1980; Legendre and Lefèvre, 1989). This view is based on the observation that small cells such as nanoplankton, ultraplankton, or picoplankton dominate the phytoplankton biomass in oligotrophic waters where regenerated production constitutes most of the total production, while large cells such as microplankton dominate the phytoplankton biomass in eutrophic waters where new production accounts

for most of the total production (Malone, 1980; Probyn, 1985; Probyn and Painting, 1985; Legendre and Lefèvre, 1989). These observations also led to the belief that large cells (microplankton) preferentially used nitrate as a nitrogen source, while small phytoplankton cells (nanoplankton) preferentially used ammonium as a nitrogen source (Malone, 1980; Probyn *et al.*, 1990). The question is therefore: how much do picoplankton contribute to new and regenerated production, particularly in open-ocean oligotrophic waters? Previous estimates of new and regenerated production in oligotrophic waters have mostly been made for the unfractionated phytoplankton community (Dugdale and Goering, 1967; Eppley *et al.*, 1973; Knauer *et al.*, 1990; Harrison *et al.*, 1992), and there are few measurements explicitly for the picoplankton fraction (but see Harrison and Wood, 1988).

Here I present the results of a study on new and regenerated production in the picoplankton fraction of the phytoplankton community along an east-west transect in the North Atlantic. New production here is measured by the uptake of nitrate (Dugdale and Goering, 1967), assuming that the input of new nitrogen is mainly from below the photic zone in the form of nitrate (Eppley and Peterson, 1979; Platt *et al.*, 1992). Results of the present study show picoplankton to be major contributors to both new and regenerated production in oceanic and upwelling waters, and thus challenge the conventional picture developed from coastal waters. The implications of equating new production to export production at steady-state are examined by considering the possible mechanisms for the export of picoplankton production out of the photic zone. I also discuss the type of food web structure that would be consistent with a significant export of this production.

2.2. Materials and methods

Data were collected during a fall cruise in the North Atlantic (16 Sept to 21 Oct, 1992), as described in Chapter 1. All data, except the unfractionated production, are from

two depths: one in the mixed layer and the other in the deep chlorophyll-maximum layer (DCM). Data of picoplankton abundance separated into prochlorophytes, cyanobacteria, and picoeukaryotes (data from Li, 1995) from 18 depths are also used here (see Chapter 1 for more details). A description of sampling locations, and date and depth of sampling is given in Table 1.1 (see also Fig. 1.1).

2.2.1. Nutrients and chlorophyll *a*

Nitrate concentrations (NO_3^-) were measured using the chemiluminescent technique (Garside, 1982), which has a detection limit of 2 ng at L^{-1}. Ammonium (NH_4^+) and urea concentrations were measured by the manual colorimetric methods of Solorzano (1969) and McCarthy (1970), respectively; the detection limits were 30 ng at L^{-1} for ammonium and 60 ng at L^{-1} for urea. Chlorophyll *a* concentration was measured by fluorometry (Holm-Hansen *et al.*, 1965), as described in Chapter 1.

Nitrate and chlorophyll *a* concentrations were plotted for all stations (Fig. 2.1). At the oligotrophic stations, chlorophyll *a* was <0.5 μg L^{-1} and nitrate concentrations were <100 ng at L^{-1}. At the upwelling stations, on the other hand, nitrate concentrations were ≥100 ng at L^{-1} and chlorophyll *a* concentrations were variable. At the slope water stations (#82, 85), chlorophyll *a* and nitrate concentrations showed a high variability.

2.2.2. Unfractionated production

Values of unfractionated production (^{13}C and ^{15}N) were measured at eight depths (W. G. Harrison, unpubl. data) by adding $^{13}HCO_3$, $^{15}NO_3^-$ and $^{15}NH_4^+$ to water samples (450 ml) and by incubating on deck (simulated *in situ* conditions) for three hours. The ^{15}N and ^{13}C isotopes were added at approximately 10% of the ambient concentration. When the

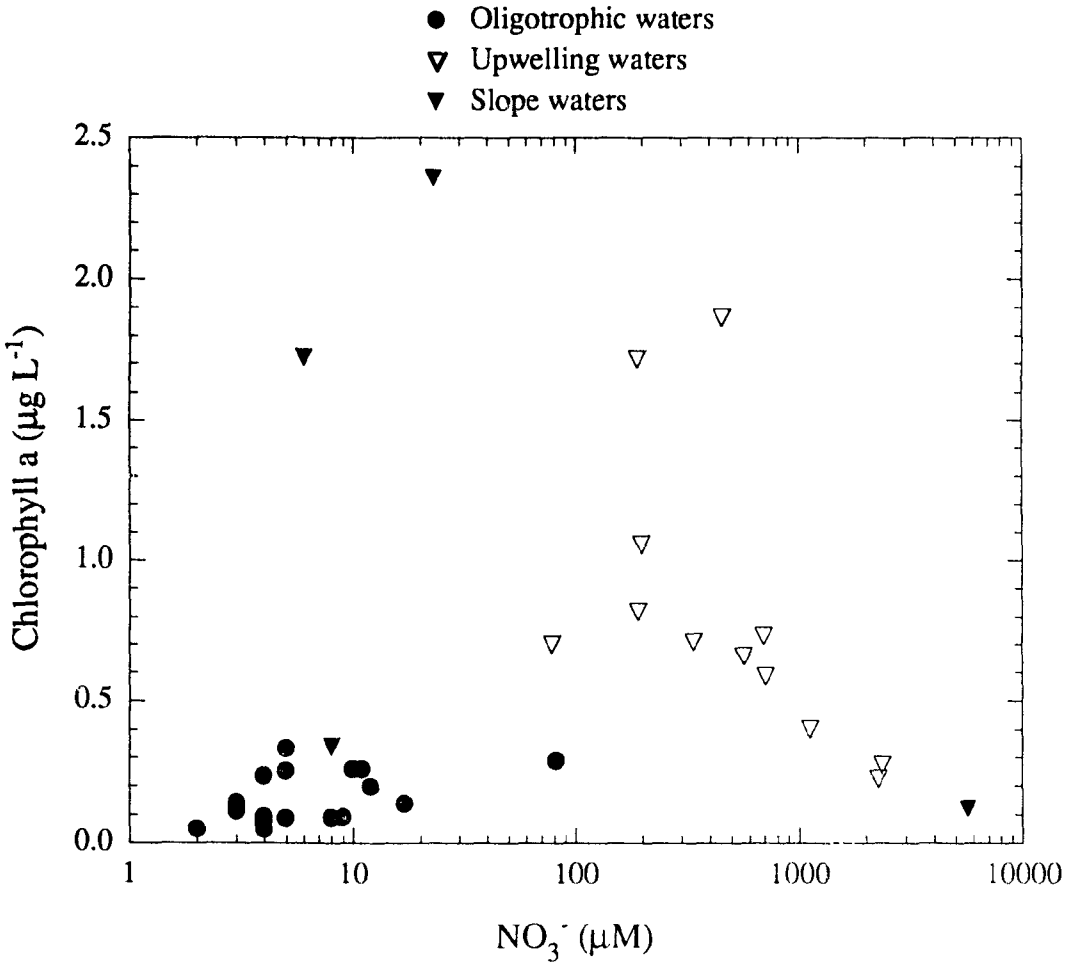


Figure 2.1 Average chlorophyll *a* concentration versus nitrate concentration in oligotrophic, upwelling, and slope waters, for the mixed layer depth and the DCM-layer depth.

ambient levels of nitrate and ammonium were below conventional detection limit (~50 ng at L^{-1}), isotopes were added to a final concentration of 10 ng at L^{-1}. The particulates were subsequently collected on precombusted glass-fiber filters (MFS, Multi Filtration Systems, nominal pore size of 0.7 μm). Light intensity was controlled by using neutral density filters to simulate the light level at each of the eight depths. This data set did not contain urea uptake measurements.

2.2.3. Size-fractionated production

Simultaneous nitrogen and carbon uptake rates were measured by the addition of ^{15}N (99% atom enrichment of $^{15}NO_3^-$, $^{15}NH_4^+$, and ^{15}N-Urea), and ^{13}C (99% atom enrichment of Na_2HCO_3) to water samples (2-L) and subsequent incubation for 3 hours on deck (simulated *in situ*). The ^{15}N and ^{13}C isotopes were added at approximately 10% of the ambient concentration, or at the concentration corresponding to the detection limit of the method used to measure the ambient concentration. The amount of nitrogen added ranged from 0.025 to 0.2 μM for $^{15}NO_3^-$, from 0.025 to 0.05 μM for $^{15}NH_4^+$, from 0.05 to 0.1 μM for ^{15}N-Urea, and from 0.1 to 0.2 mM for ^{13}C. Light intensities were controlled by using neutral screens for the samples in the mixed layer, and by using neutral screens combined with blue-colored filters (Rohm and Haas plexiglass filters no 2069) for the samples in the DCM layer in order to simulate the light level at the sampling depths. The light level at each depth was calculated, assuming an exponential decrease of light with depth, using the attenuation coefficient K which was estimated from Photosynthetically Available Radiation (PAR), measured using a PAR-meter (Licor) fixed onto the CTD. The estimated light levels were 20-60% of I_0 (light intensity at the surface) for the samples taken in the mixed layer, and 0.8-5% of I_0 for the samples taken in the DCM layer. Samples (200-500 ml) were size-fractionated after incubation (post-screening) through 2-μm Nuclepore filters (vacuum pressure < 120 mmHg) and the material was collected onto

precombusted glass-fiber filters (MFS). For each sample, the atom percent enrichment of ^{15}N and ^{13}C and the amount of PON and POC were measured on the same filter (two replicates of each sample) using an automated mass spectrometer (Europa Scientific). The analytical precision of the mass spectrometer was 9% for PON, 7% for POC, 0.003% for ^{15}N atom enrichment, and 0.004% for ^{13}C atom enrichment. Nitrogen uptake rates were computed using equations described by Dugdale and Goering (1967). Carbon uptake rates were computed according to Hama *et al.* (1983). Nitrogen uptake rates of the >2-µm fraction were obtained by subtraction of the >2-µm fraction from the total fraction

2.2.4. Production integrated over the euphotic zone

The relative production rates in the small size-fraction (<2 µm), compared with the total production rates (unfractionated water), that were measured in the mixed layer and in the DCM layer, were combined with the unfractionated production rates (measured at eight depths) in order to obtain values of production rates in the <2-µm fraction integrated over the water column as follows. First, the unfractionated uptake rates (eight depths) of ^{15}N ($^{15}NO_3^-$ and $^{15}NH_4^+$) and ^{13}C were integrated over the water column from the surface to the bottom of the photic zone (80-110 m for oligotrophic stations and 40-75 m for upwelling and slope water stations). At the upwelling site, 3 out of 6 stations were sampled at one depth only, but at these stations the picoplankton were distributed relatively homogenously over the depth of integration (40 m). Therefore, for each station, the percentage of uptake of NO_3^- and NH_4^+ in the <2-µm fraction was applied to the unfractionated uptake rate integrated over the water column. The same procedure was used at the oligotrophic stations for which only one depth was sampled (#3, 6, 8, 10, 14, 17). For the remaining oligotrophic, upwelling and slope water stations (#58, 61, 64, 67, 70, 74, 76, 79, 48, 51, 54, and 82), the two values of relative NO_3^- and NH_4^+ uptake in the <2-µm fraction (one corresponding to the mixed layer and the other corresponding to the

DCM layer) were applied to the absolute uptake rates of NO_3^- and NH_4^+ at the eight individual depths (the mixed layer value applied to the depths within the mixed layer, and the DCM value applied to the depth within the DCM layer). The resulting absolute uptake rates of NO_3^- and NH_4^+ in the <2-μm fraction were then integrated over the photic depth to give the water-column uptake rates of NO_3^- and NH_4^+. The same procedure was used to estimate the uptake rates of $^{13}CO_2$, POC, and PON integrated over the water column. Since urea uptake rates were only estimated at two depths, no water column values could be computed for urea.

The *f*-ratio was calculated as the ratio of nitrate uptake to the sum of nitrate, ammonium, and urea uptake. In the case of unfractionated production (eight depths), the *f*-ratio, *f'*, was computed as the ratio of nitrate uptake to the sum of nitrate and ammonium uptake (since no urea uptake data were available). Note that for the mixed layer and the DCM layer depths taken together, *f* and *f'* were similar, *i.e.* 0.06 and 0.04 for oligotrophic stations, and 0.30 and 0.28 for upwelling stations.

2.2.5. Uncertainties in calculating nitrogen uptake rates

In oceanic waters, ammonium and urea are often present in the nanomolar range (Garside, 1985; Brzezinski, 1988; Price and Harrison, 1988; McCarthy *et al.*, 1992). In the present study, the detection limit of ambient urea is 0.06 μg-at L^{-1}, *i.e.*, higher than typical concentrations in oceanic waters. This could lead to isotope enrichment of the media incubated, which in turn would result in overestimating the uptake rates of urea. Similar problems also exist for the estimates of ammonium uptake rates, since the detection limit for this nitrogen form (0.03 μg-at L^{-1}) is higher than typical levels expected in oceanic waters (Brzezinski, 1988). If all other errors associated with the estimation of urea uptake rates are ignored, and we assume that the ambient level of urea was actually lower by a

factor of 10, then the computed uptake rate of urea would be lower by 50-100%. If I accept that the urea uptake rate is overestimated by 50% (minimum error here), and therefore decrease the estimated uptake rate by 50%, it would still represent 15% of the total nitrogen uptake rate in oligotrophic waters, instead of 27% as estimated here. The *f*-ratios based on ammonium and urea uptake rates range from 0.02 to 0.06 (mean 0 04) in the mixed layer for both the total and the <2-μm fractions, and from 0.02 to 0.08 (mean 0 04) in the DCM layer for both the total and the <2-μm fractions. If the urea uptake rates were reduced by 50%, then the resultant *f*-ratios would range from 0.02 to 0.08 (mean 0.05) in the mixed layer for both the total and the <2-μm fractions, and from 0 02 to 0.09 (mean 0 05) in the DCM layer for both the total and the <2-μm fractions

In order to circumvent these errors in ammonium and urea uptake rates, the *f*-ratio can be computed as the ratio of nitrate uptake to the uptake of ^{13}C converted to total nitrogen uptake, using a C:N ratio (6.6). Note that the measured C:N uptake ratios were reasonably close to Redfield ratio, *i.e* 8.8 in the mixed layer and 5.9 in the DCM layer. The resulting *f*-ratios range from 0 03 to 0.17 (mean 0.07) and from 0.03 to 0.32 (mean 0.11) in the mixed layer for the total and the <2-μm fractions In the DCM layer, the *f*-ratios had a range of 0.05-0.11 (mean 0.07) for the unfractionated population, and of 0.06-0.23 (mean 0.09) for the <2-μm fraction (station #17 was excluded from these calculations since it had abnormal ^{13}C uptake rates) These *f*-ratios are, on average, higher than the *f*-ratios obtained using urea and ammonium uptake rates, suggesting that the ammonium and urea uptake rates are overestimated. However, these differences are fairly small, and therefore the potential errors in ammonium and urea uptake rates discussed here would not substantially modify the conclusions drawn on the role of picoplankton in new production.

2.2.6. Size-fractionated rates of nitrogen uptake normalised to chlorophyll *a* and specific rates of nitrogen uptake

Uptake rates of total nitrogen (nitrate+ammonium+urea) for the <2-μm and the >2-μm fractions were normalised to chlorophyll *a* concentrations of the corresponding size-fractions. Specific rates of nitrogen uptake for the <2-μm and the >2-μm fractions were calculated by dividing the normalized uptake rates of nitrogen by the ratio of nitrogen to chlorophyll *a* (PON:chlorophyll *a*) in the corresponding size-fraction. The PON:chlorophyll *a* ratio in each size fraction was computed as the slope of the linear fit to a plot of PON as a function of chlorophyll *a* for each size-fraction (R^2=0.66, slope=1.84 for the <2-μm fraction, R^2=0.94, slope=0.88 for the >2-μm fraction).

2.3. Results

2.3.1. Size-fractionated nitrogen uptake rates

The uptake rates of NO_3^- (Figs. 2.2 a, b) in upwelling and slope waters for the unfractionated water and for the <2-μm fraction (5.15 ng at L^{-1} h^{-1} and 3.67 ng at L^{-1} h^{-1}, respectively) were at least one order of magnitude higher compared with oligotrophic waters for the whole and for the <2-μm fractions (0.36 ng at L^{-1} h^{-1} and 0.32 ng at L^{-1} h^{-1}, respectively). Note that in oligotrophic waters, the uptake rates were more variable in the DCM layer (Fig. 2.2 b) than in the mixed layer (Figs. 2.2 a), as was observed for chlorophyll *a* concentrations (Fig. 1.2, Chapter 1).

In oligotrophic waters, picoplankton accounted for 87% (±7%) and 88% (±11%) of the NO_3^- uptake in the mixed layer and in the DCM layer, respectively (Table 2.1). Picoplankton also contributed 82% (±7%) to the NH_4^+ uptake and 93% (±7%) to the urea

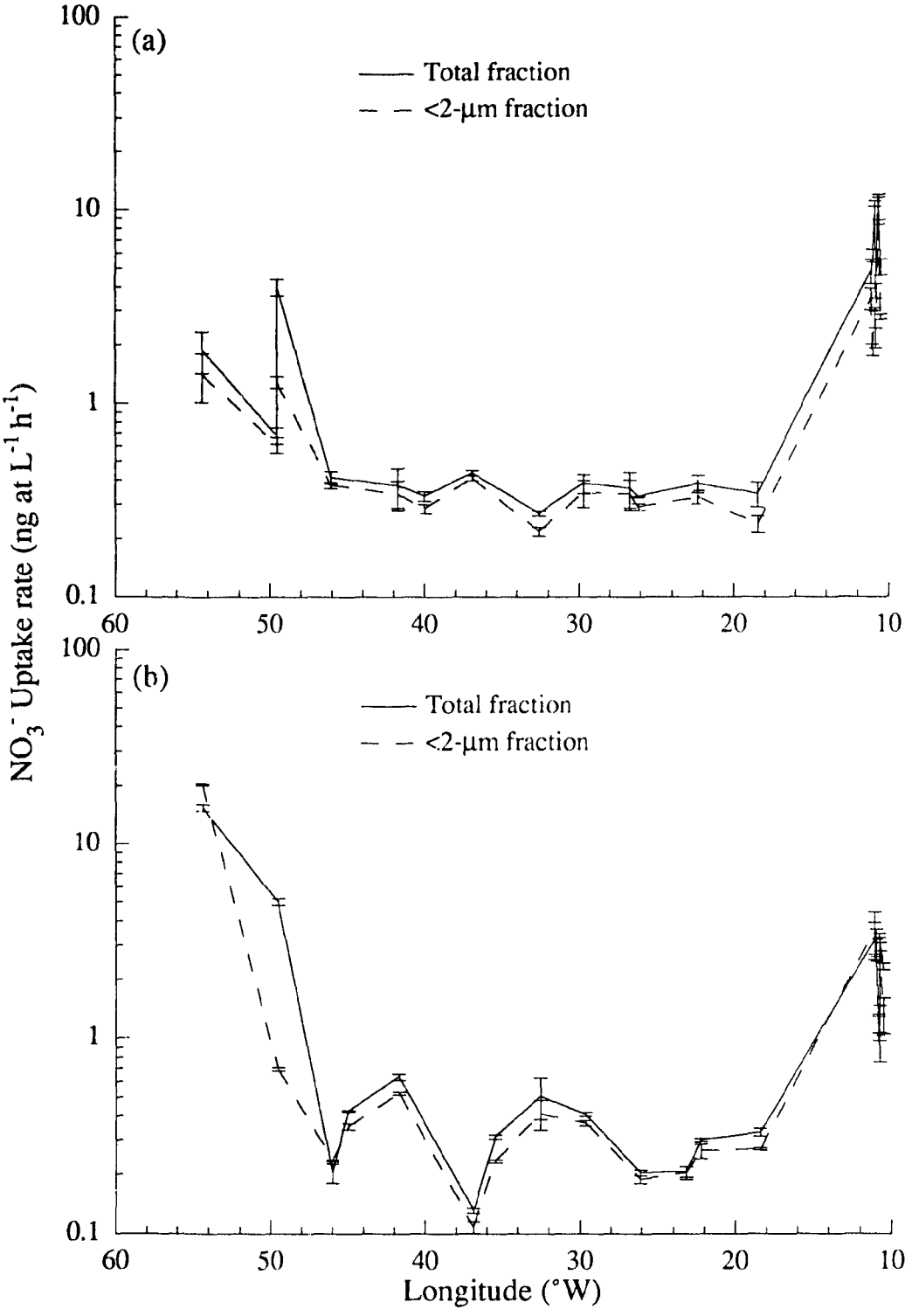


Figure 2.2 Average NO_3^- uptake rates measured for unfractionated water (Total) and for the <2-µm fraction, (a) in the mixed layer and (b) in the DCM layer. Error bars represent standard deviation based on two replicates.

Table 2.1 Average contribution of picoplankton (% of total) to the uptake of nitrate, ammonium, and urea for the major regions. The standard deviation (SD) is calculated for two replicates.

Regions	Depths sampled	$^{15}NO_3^-$ uptake <2 µm (±SD)	$^{15}NH_4^+$ uptake <2 µm (±SD)	^{15}N-Urea uptake <2 µm (±SD)
Oligotrophic	Mixed layer	87 (±7)	82 (±7)	93 (±7)
	DCM layer	88 (±11)	89 (±7)	89 (±6)
	Mean*	88 (±9)	86 (±8)	91 (±7)
Upwelling and slope waters	Mixed layer	53 (±20)	60 (±15)	58 (±16)
	DCM layer	86 (±37)	78 (±17)	73 (±17)
	Mean*	69 (±33)	69 (±18)	65 (±18)

* Values averaged over mixed layer depth and DCM-layer depth taken together.

uptake in the mixed layer, and 89% (±7%) to the NH_4^+ uptake and 89% (±6%) to the urea uptake in the DCM layer (Table 2.1). The variability around these averages was low, as indicated by the standard deviations. Nor was there any significant difference between the mixed layer and the DCM layer. In upwelling and slope waters, on the other hand, picoplankton contributed 53% (±20%) to the nitrate uptake in the mixed layer and 86% (±37%) in the DCM layer, with a higher variability among samples, compared with oligotrophic waters. Picoplankton accounted for 60% (±15%) of the NH_4^+ uptake and 58% (±16%) of the urea uptake in the mixed layer, and 78% (±17%) of the NH_4^+ uptake and 73% (±17%) of the urea uptake in the DCM layer. Note that the contribution of picoplankton to new and regenerated production was similar for upwelling waters, and for upwelling and slope waters taken together.

Nitrate uptake contributed 4-28% to the total nitrogen uptake, while ammonium uptake and urea uptake accounted for 63-69% and 9-27%, respectively, for both the unfractionated water and for the picoplankton fraction (Table 2.2 a) at all the stations. Nitrate represented only 4% of the total nitrogen present in oligotrophic waters (Table 2.2 b), which was consistent with the low contribution of nitrate uptake (Table 2.2 a), while ammonium accounted for 44% of total ambient nitrogen (Table 2.2 b) in accordance with high relative ammonium uptake rates (Table 2.2 a). The relative concentration of urea, however, was similar to that of ammonium in oligotrophic waters, and yet the contribution of urea uptake to total nitrogen uptake was lower, suggesting that ammonium is preferred over urea as the nitrogen source. In upwelling and slope waters, although nitrate was the predominant form of nitrogen present, nitrate uptake contributed only 27%, while ammonium uptake accounted for 63-64% of the nitrogen uptake, also suggesting a preference for ammonium over nitrate.

To quantitatively describe the preference of one nitrogen form over another, and its

Table 2.2 a Relative uptake of nitrate, ammonium, and urea (% of total) for the total and <2-µm fractions. The standard deviation (SD) is calculated on two replicates.

Regions	$^{15}NO_3^-$ uptake (±SD)		$^{15}NH_4^+$ uptake (±SD)		^{15}N-Urea uptake (±SD)	
	Total fraction	<2-µm fraction	Total fraction	<2-µm fraction	Total fraction	<2-µm fraction
Oligotrophic	4 (±2)	4 (±1)	70 (±10)	69 (±10)	26 (±9)	27 (±10)
Upwelling and slope waters	27 (±9)	27 (±25)	62 (±19)	63 (±21)	11 (±6)	10 (±6)

Table 2.2 b Relative proportions of nitrate, ammonium, and urea concentrations (% of total). The standard deviation (SD) is calculated on three replicates.

Regions	NO_3^- (±SD)	NH_4^+ (±SD)	N-Urea (±SD)
Oligotrophic	4 (±2)	44 (±17)	52 (±16)
Upwelling and slope waters	51 (±30)	35 (±19)	14 (±13)

relation to size, a relative preference index (RPI *sensu* McCarthy *et al.*, 1977) was calculated for nitrate and ammonium in the <2-µm fraction, and in the >2-µm fraction. This index has been defined (McCarthy *et al.*, 1977) as the ratio of the relative uptake rate of a given nitrogen form to the relative concentration of that nitrogen form in the water. RPI values of nitrate were >1 only at low levels of nitrogen, and showed a negative trend with respect to ambient nitrogen, in the <2-µm and the >2-µm fractions, for both depths (Figs. 2.3 a, b). In the DCM layer, the majority of RPI values for NO_3^- were lower than 1, indicating rejection of nitrate by phytoplankton in favor of other nitrogen forms. The same trend was observed when RPI values of nitrate were plotted against ambient ammonium levels (not shown here). Most importantly, the distribution of RPI values for NO_3^- as a function of total nitrogen concentration was similar for the <2-µm fraction and for the >2-µm fraction. It is noteworthy that the RPI values for NO_3^- are some of the highest values reported compared with previous measurements (McCarthy *et al*, 1977). Values of RPI for NH_4^+, on the other hand, were all distributed above unity for both the <2-µm fraction and for the >2-µm fraction (Figs. 2.4 a, b), indicating a preference for ammonium regardless of size and ambient nitrogen concentration.

2.3.2. Size-fractionated rates of nitrogen uptake normalized to chlorophyll *a* and specific rates of nitrogen uptake

Normalized values of nitrogen uptake rates for the <2-µm fraction were systematically higher than those for the >2-µm fraction, *i.e.* situated above the 1:1 line (Fig. 2.5). A linear regression (Model II) was fitted to the points and resulted in a slope equal to 2.3 (R^2=0.92). Moreover, specific rates of nitrogen uptake by picoplankton were in most cases higher for the <2-µm fraction than for the >2-µm fraction (Fig. 2.6) although the slope (1.04) resulting from fitting data to a linear regression was not significantly different from 1 (R^2=0.92). Note that both figures 2.5 and 2.6 include all stations (*i.e.*

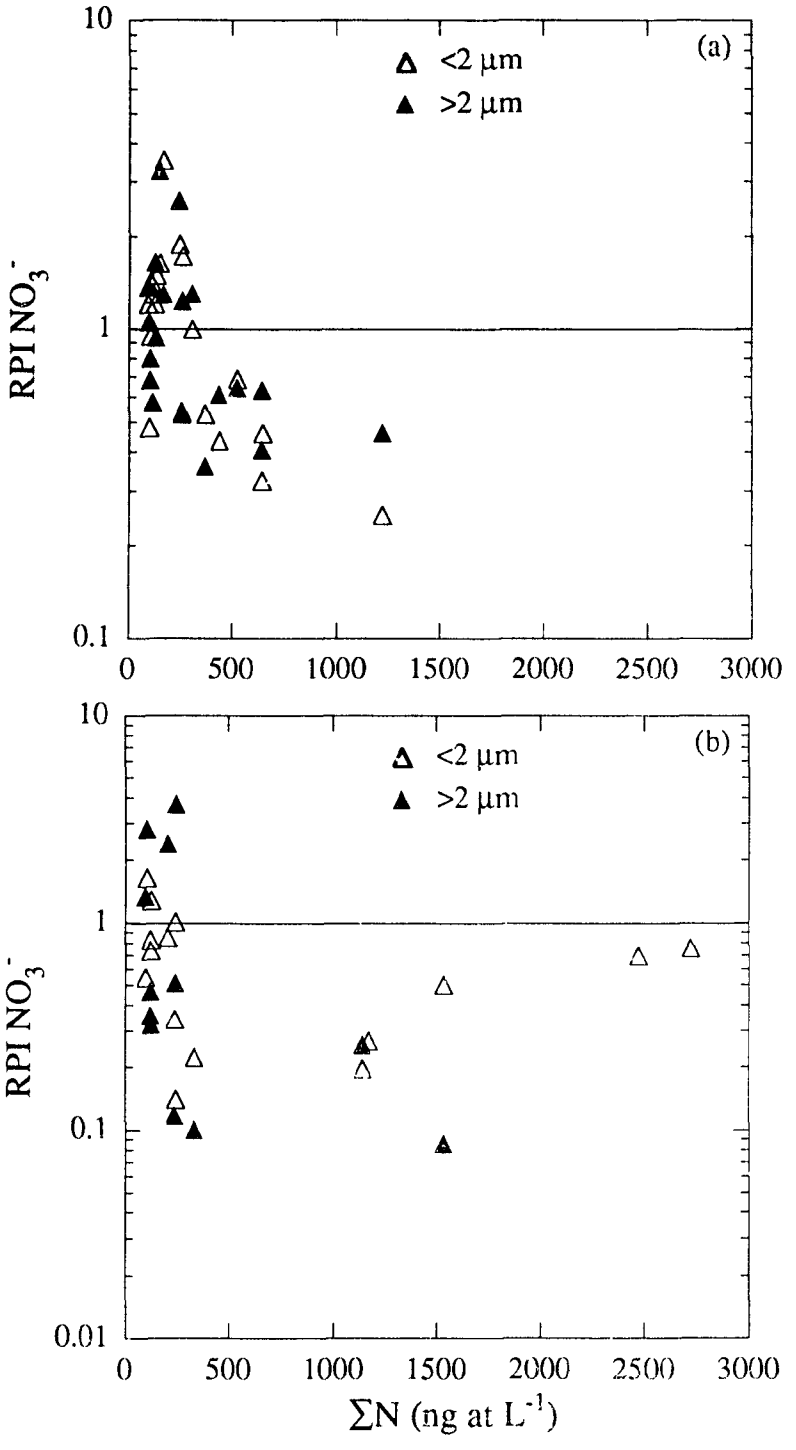


Figure 2.3 Relative preference index (RPI) of nitrate in the <2-µm fraction and in the >2-µm fraction versus total nitrogen concentration, (a) in the mixed layer and (b) in the DCM layer. Calculation of RPI values was based on nitrate, ammonium, and urea; total nitrogen concentration was the sum of all three nitrogen forms.

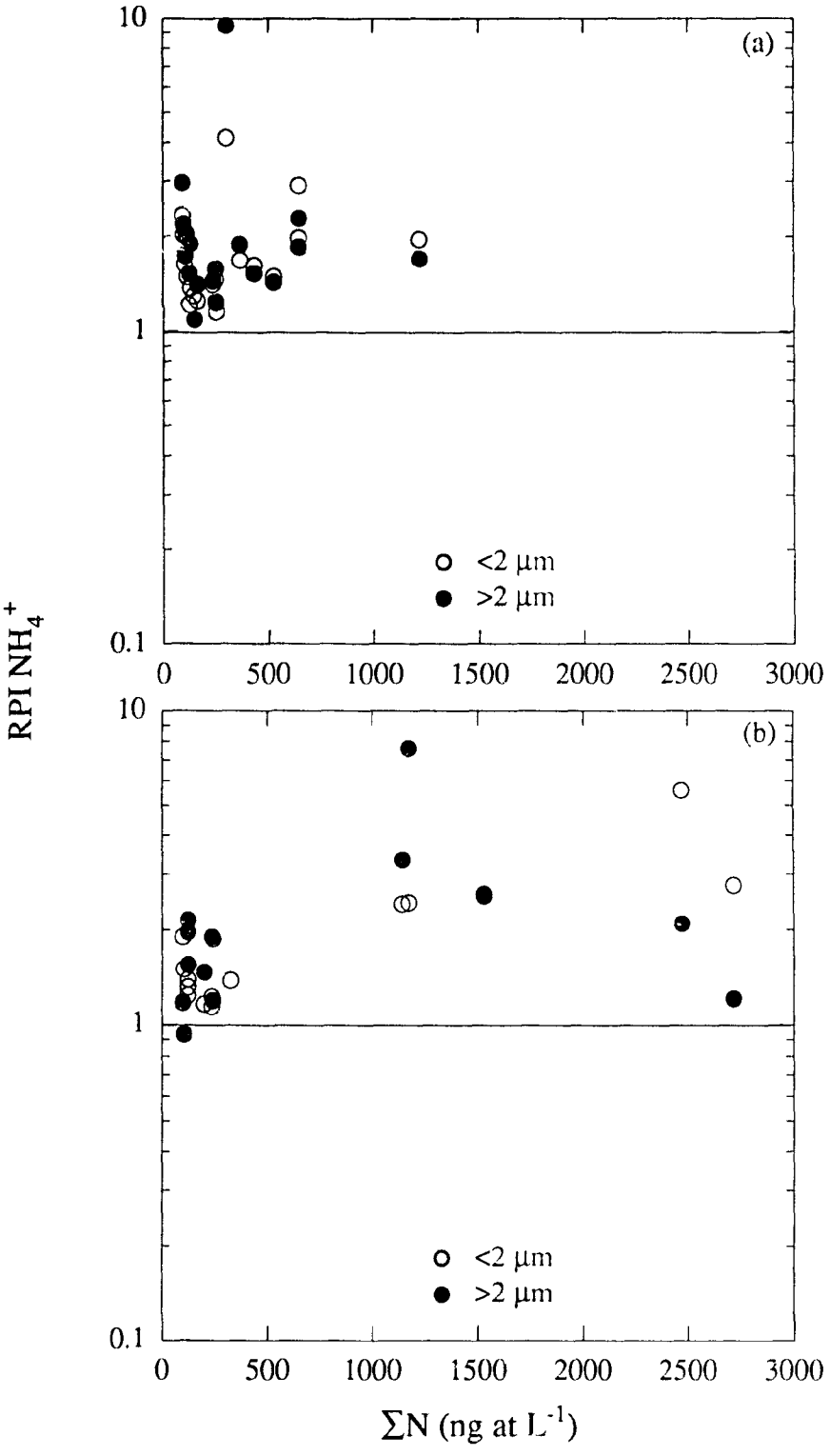


Figure 2.4 Relative preference index (RPI) of ammonium in the <2-μm fraction and in the >2-μm fraction versus total nitrogen concentration, (a) in the mixed layer and (b) in the DCM layer. Calculation of RPI values was based on nitrate, ammonium, and urea; total nitrogen concentration was the sum of all three nitrogen forms.

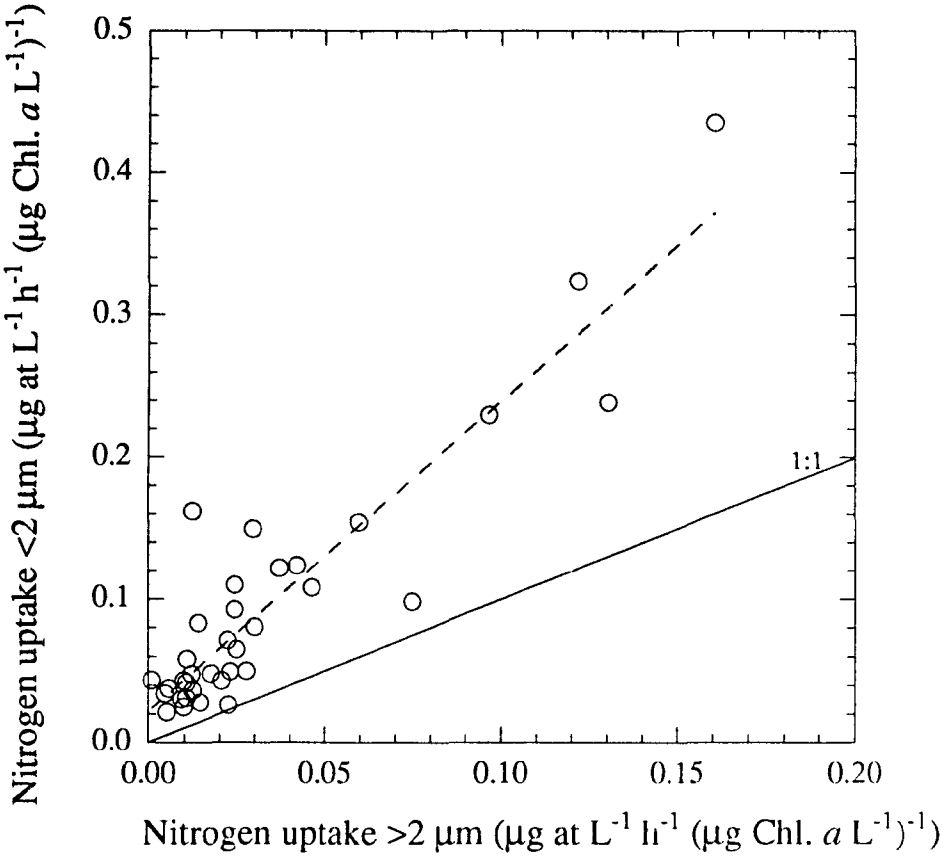


Figure 2.5 Nitrogen uptake rates, normalised to chlorophyll *a*, for all stations. Values in the <2-µm fraction are plotted versus values in the >2-µm fraction. Dashed line represents linear regression of Model II (R^2=0.92, slope=2.3).

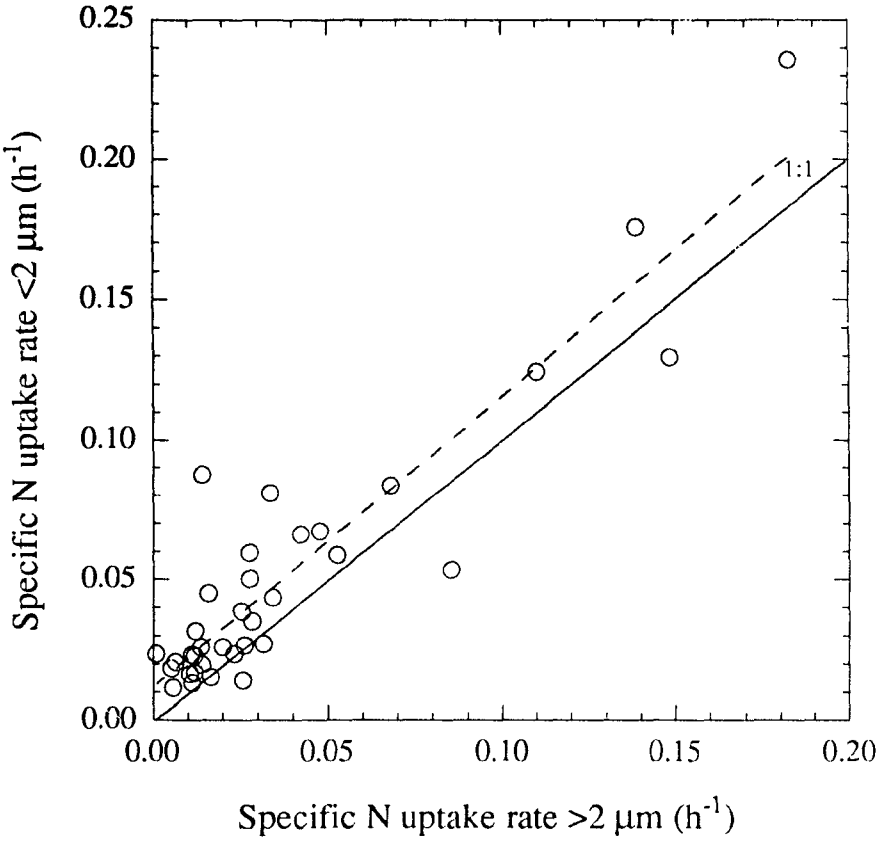


Figure 2.6 Specific rates of nitrogen uptake for the <2-µm fraction versus the >2-µm fraction. Values were obtained by dividing the normalised uptake rates of nitrogen (see Fig. 2.5) by the ratio of nitrogen to chlorophyll *a* in the corresponding size-fraction (R^2=0.92, slope=1.04).

oligotrophic, upwelling, and slope waters) in the mixed layer and in the DCM layer.

2.3.3. *f*-ratios

Values of *f*-ratios ($^{15}NO_3^-$ uptake / [$^{15}NO_3^-$ + $^{15}NH_4^+$ + ^{15}N-Urea] uptake) varied between 0.02 and 0.08 for the <2-μm fraction, and between 0.01 and 0.13 for the >2-μm fraction in oligotrophic waters, while it was in the range of 0.05-0.66 for the <2-μm fraction and 0.05-0.44 for the >2-μm fraction at the upwelling site (Fig. 2.7). On average, *f* equalled 0.04 and 0.05 in oligotrophic waters for the <2-μm and >2-μm fractions respectively, while the corresponding values in upwelling waters were 0.28 and 0.21, respectively. The plot of *f*-ratio as a function of longitude (Fig. 2.7) does not reveal any systematic differences between the picoplankton fraction and the large phytoplankton fraction. Nor can I distinguish patterns in mixed-layer samples from those in DCM-layer samples. However, *f*-ratios from the oligotrophic stations appear to decrease from west to east along the transect. This trend is also observed for water column values of *f*-ratios (not shown here). A coincident increase in the depth of the nitracline was observed (Fig. 2.8) which may explain the decrease in *f*-ratios computed for similar depths. Note that the corresponding change in latitude was 10°, which could have accounted for the variation in the depth of the nitracline (Fig. 2.8). For all the stations taken together, and for the oligotrophic stations, and upwelling stations, *f*-ratios calculated for the <2-μm fraction and for the >2-μm fraction were distributed more or less equally on either side of the 1:1 line (Fig. 2.9 a, b, c). Exceptions were one upwelling station (#45 at 60 m) in Figs. 2.9 a, c, and four stations (#6, 8, 10, 58) for oligotrophic waters (Fig. 2.9 b). The outliers in Fig. 2.9 b, which had *f*-ratios ≥ 0.1 in the >2-μm fraction, formed a distinct group and corresponded in three cases out of four to samples from the DCM layer. In the oligotrophic waters (Fig 2.9 b), it appears that the relationship between the two fractions may change depending on whether or not the *f*-ratio for the >2-μm fraction is less than or greater than

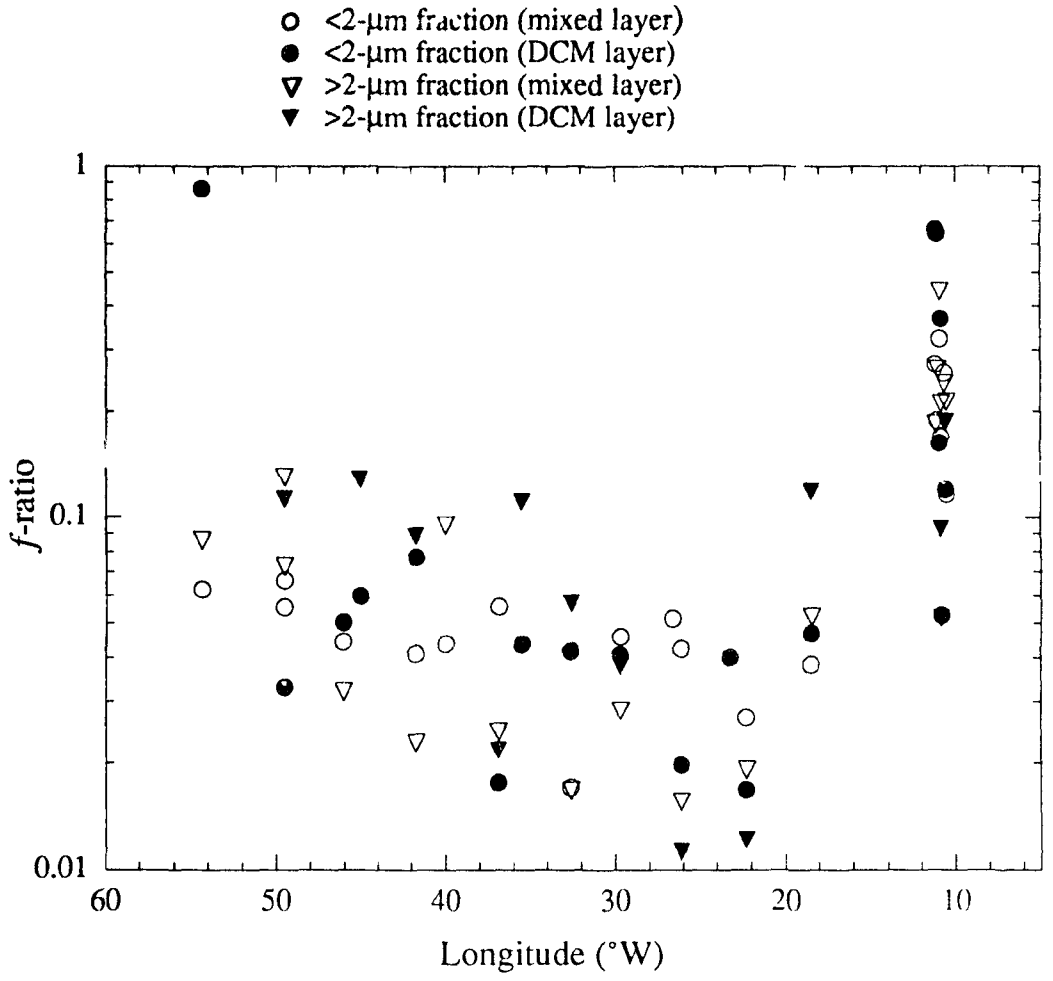


Figure 2.7 *f*-ratios of the <2-µm fraction and of the >2-µm fraction in the mixed layer and in the DCM layer. $f = pNO_3^- / [pNO_3^- + pNH_4^+ + p\text{N-Urea}]$.

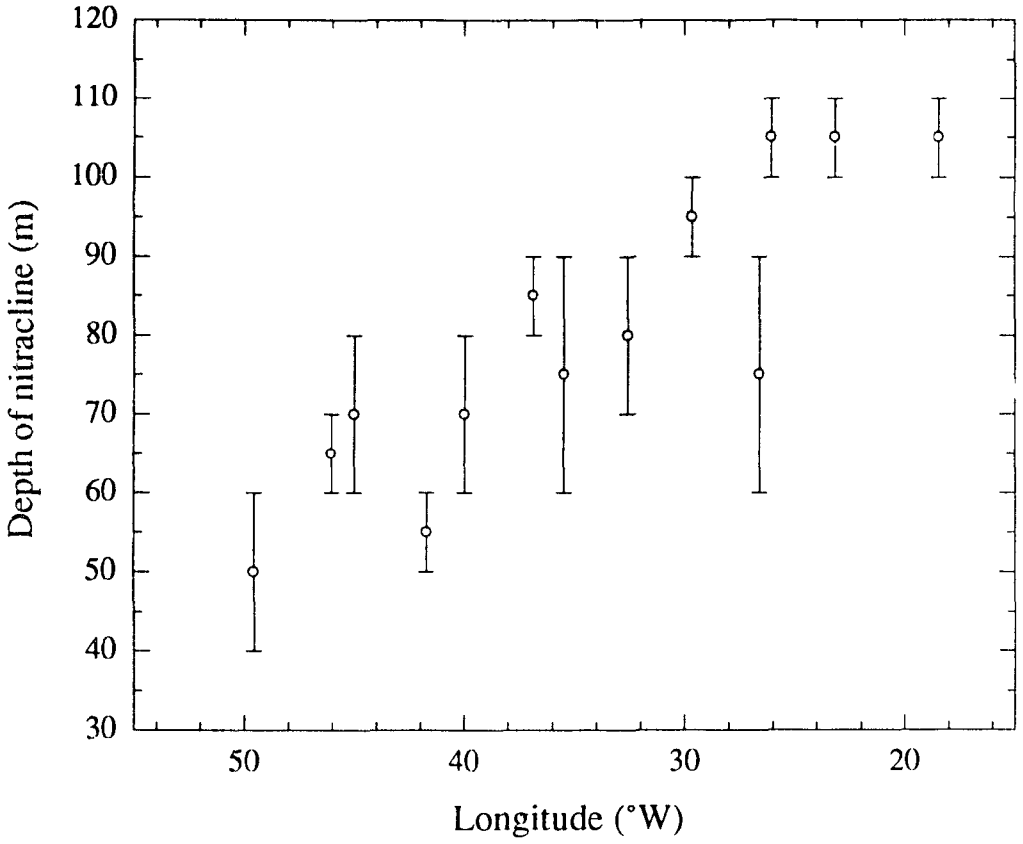


Figure 2.8 Depth of nitracline versus longitude at all oligotrophic stations (data from Dr. Harrison). The range for each station represents the difference between the first two depths at which the variation in nitrate concentration was ≥100 nM.

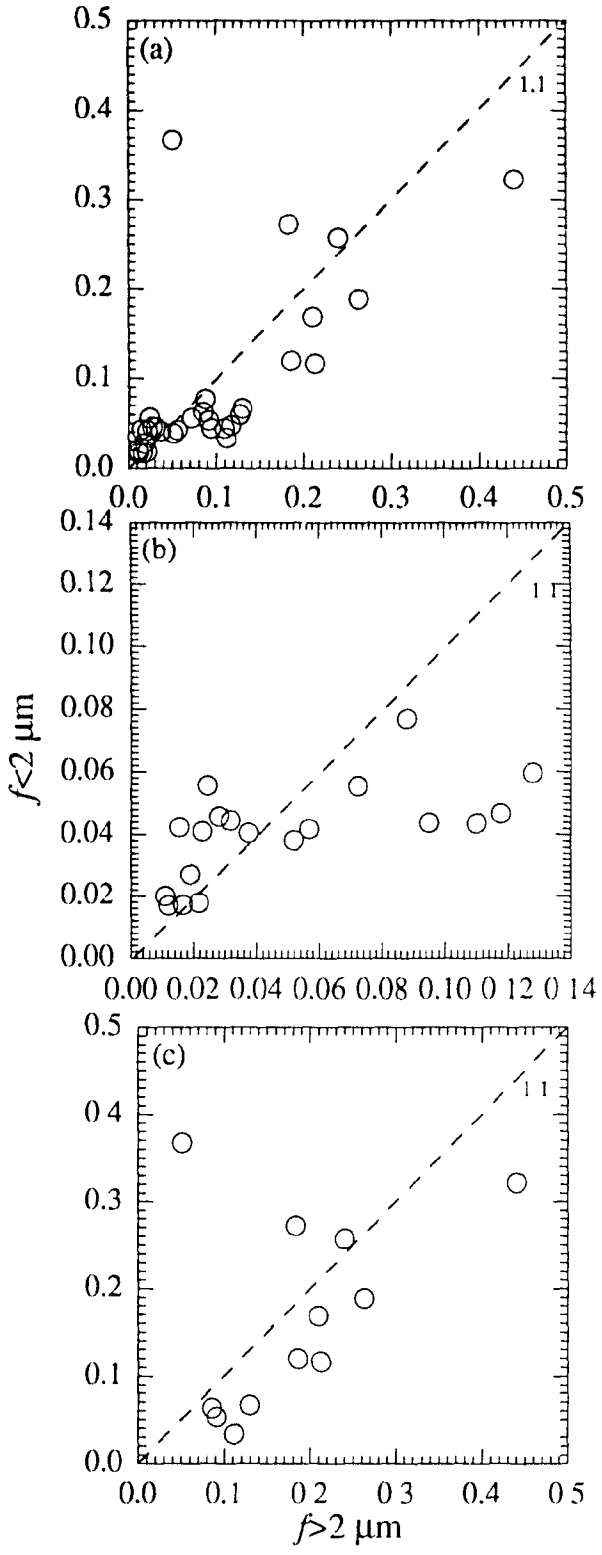


Figure 2.9 *f*-ratios of the <2-µm fraction versus *f*-ratios of the >2-µm fraction, (a) at all stations, (b) at the oligotrophic stations, and (c) at the upwelling stations *f*-ratios were calculated as in Fig. 2.7.

0.04: below that level, the *f*-ratios in the small size-fraction are higher than those for the large cells, while the inverse holds for larger *f*-ratios. However, if the four outliers are omitted in Fig. 2.9 b, this tendency is less apparent. In fact, for all stations taken together, or separated by regions, there was no significant statistical difference between *f*-ratios for the <2-μm and those for the >2-μm fraction (Mann-Whitney test: p=0.703 for all stations, p=0.859 for oligotrophic stations, p=0.753 for upwelling and slope water stations).

2.3.4. Euphotic zone integrated values of nitrogen uptake and *f*-ratios

Nitrate uptake rates for picoplankton were 3-59 μmol m^{-2} h^{-1} at the oligotrophic stations, and 58-287 μmol m^{-2} h^{-1} at the upwelling and slope water stations (Fig. 2.10). As in Figs. 2.2 a and 2.2 b, the nitrate uptake rates were substantially higher in the upwelling and slope waters compared with those in the oligotrophic waters. Ammonium uptake rates were 229-639 μmol m^{-2} h^{-1} at the oligotrophic stations, and 267-742 μmol m^{-2} h^{-1} at the upwelling and slope water stations (Fig. 2.10). This corresponded to an average contribution from picoplankton of 87% and 86% to new and regenerated production, respectively, in oligotrophic waters, and 58% and 59% to new and regenerated production, respectively, in upwelling and slope waters.

For all stations taken together, and for the oligotrophic stations, the integrated values of *f'* for the <2-μm fraction plotted against the integrated values of *f'* for the >2-μm fraction were distributed around the 1:1 line (Fig. 2.12 a, b), and did not show some of the trends observed for *f*-ratios at individual depths (Fig. 2.9 b). The *f'*-ratios based on water-column integrated uptake rates of nitrate and ammonium (Figs. 2.11 a, b) in the <2-μm fraction were not significantly different from those in the >2-μm fraction for all stations (Mann-Whitney test, p=0.953), or for the oligotrophic stations separately (Mann-Whitney test, p=0.885), or for the upwelling stations considered by themselves (Mann-Whitney

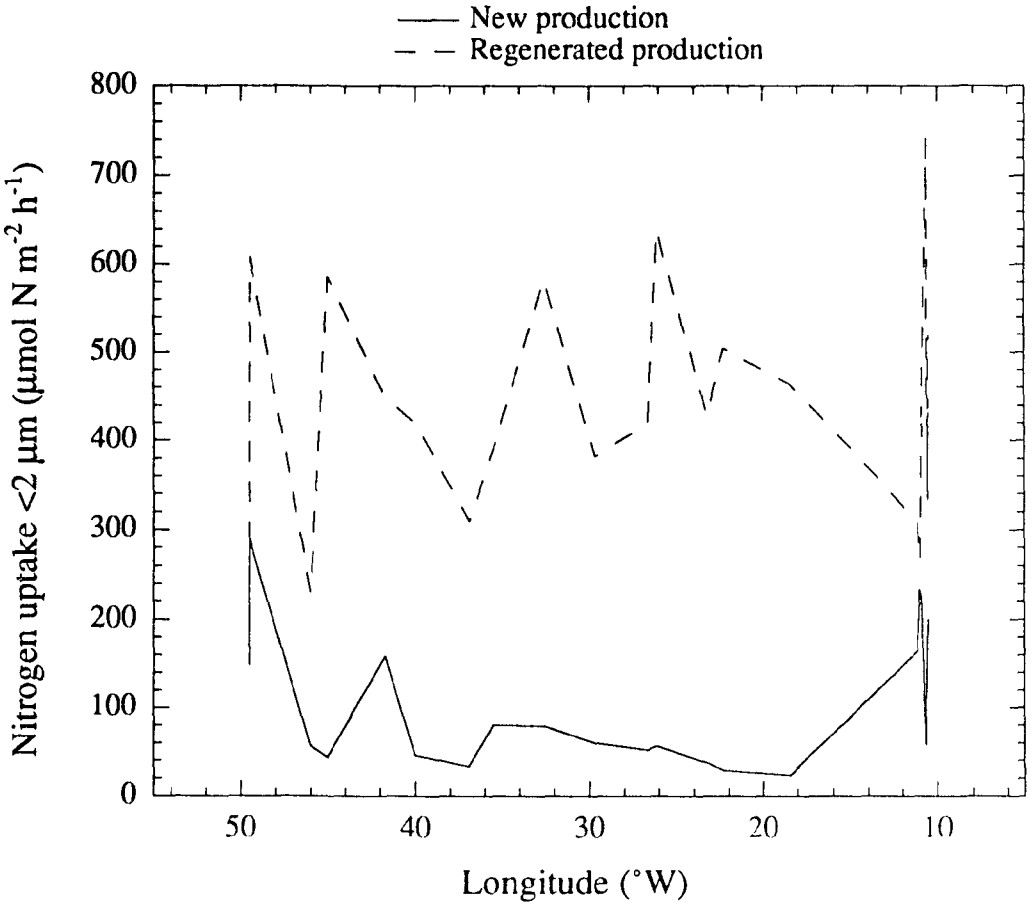


Figure 2.10 New production and regenerated production of the <2-µm fraction, integrated over the euphotic zone, along the transect (based on profiles of unfractionated production rates from Dr. Harrison). Regenerated production represents the uptake of ammonium only.

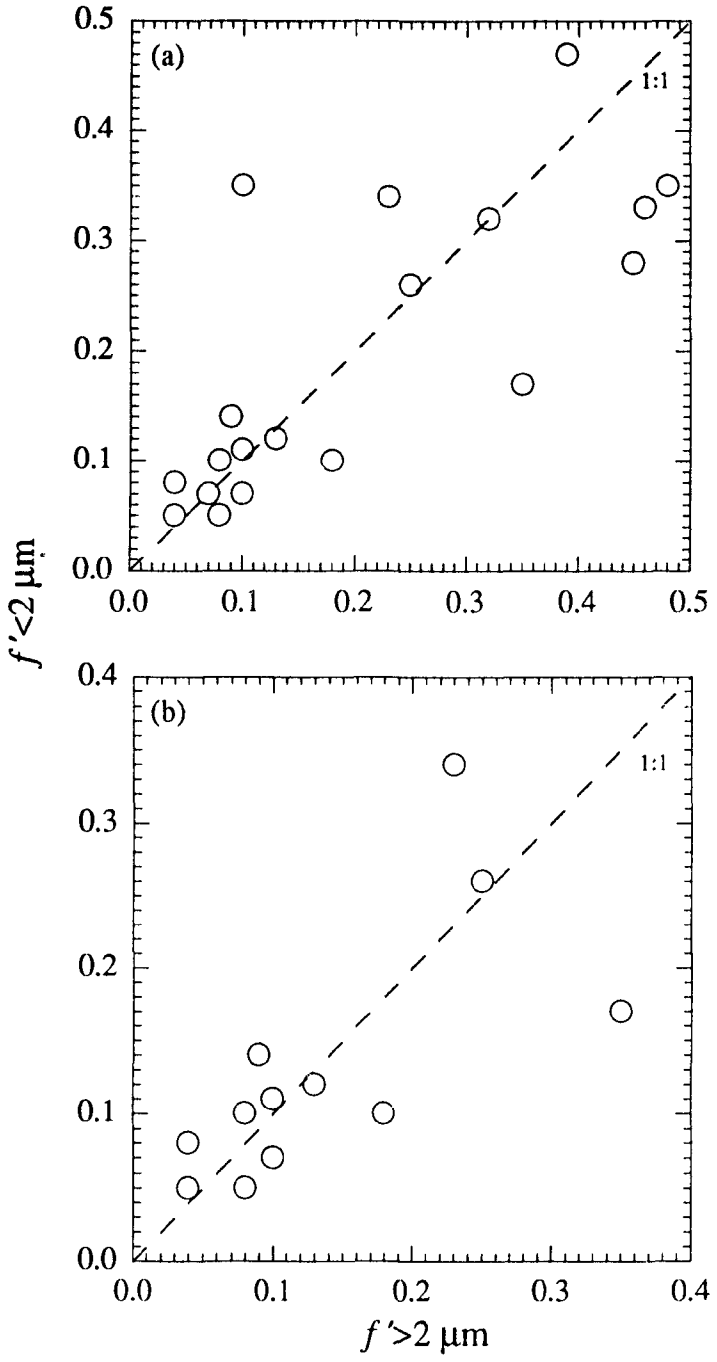


Figure 2.11 Integrated values of f for the <2-µm fraction versus f for the >2-µm fraction (based on profiles of nitrogen uptake rates from Dr. Harrison), (a) at all stations and (b) at the oligotrophic stations. $f' = pNO_3^- / [pNO_3^- + pNH_4^+]$.

test, p=0.863).

2.4. Discussion

2.4.1. Are new and regenerated production size-dependent?

It has generally been considered that small-size cells such as nanoplankton, ultraplankton, or picoplankton mainly contribute to regenerated production while the larger cells account for most of the new production (Malone, 1980; Goldman, 1988; Legendre and Lefèvre, 1989). This view is largely based on the observation that eutrophic areas, where new production is high, are populated primarily by larger cells while open-ocean productivity is mainly accounted for by regenerated production of nanoplankton and picoplankton (Malone, 1980; Bienfang and Ziemann, 1992). This has led to the hypothesis that small cells would preferentially take up ammonium while large cells would prefer nitrate (Malone, 1980; Probyn, 1992).

Many previous studies in coastal, frontal, and upwelling areas have supported the conventional view that the picoplankton fraction is largely responsible for regenerated production and larger cells account for most of the new production: it has been shown that picoplankton (*e.g.* <1 μm, <2 μm, or <3 μm) have higher ammonium uptake rates and lower nitrate uptake rates, compared with larger cells (Harrison and Wood, 1988; Nalewajko and Garside, 1983; Probyn *et al.*, 1990; Selmer *et al.*, 1993; Probyn, 1992), although evidence has not always been conclusive (Probyn, 1985; Probyn and Painting, 1985) and some observations to the contrary have also been reported (Chang *et al.*, 1989; Furnas *et al.*, 1983). In oceanic waters, these differences have been shown to be small (relative uptake rates of ammonium and nitrate equal to 43% and 36%, respectively) in the case of cells <1 μm (Harrison and Wood, 1988). Other studies in oceanic waters have reported that phytoplankton <3 μm assimilate ammonium at higher rates (Bienfang and

Takahashi, 1983; Le Bouteiller, 1986), and nitrate at lower rates (Le Bouteiller, 1986) compared with larger cells (>3 μm, or unfractionated water).

In the present study, the relative uptake rates of $^{15}NO_3^-$ and $^{15}NH_4^+$ in the <2-μm fraction were similar (Table 2.1), both for the oligotrophic stations and for the upwelling and slope stations. In absolute terms, these uptake rates were commensurate with previous studies in oceanic (Harrison and Wood, 1988) and coastal waters (Chang *et al.*, 1989; Probyn *et al.*, 1990; Nalewajko and Garside, 1983). Moreover, *f*-ratios for the <2-μm fraction and for the >2-μm fraction were not significantly different (Figs. 2.7, 2.9, 2.11). These results suggest that *f*-ratios are not size-dependent, and that picoplankton can contribute to the uptake of nitrate as much as the larger cells (in μg at N L^{-1} h^{-1}). In support of this conclusion, previously reported values of *f* from different locations did not show any significant differences between total and size-fractionated populations (Table 2.3, see also Chisholm, 1992). Although values of RPI for nitrate and ammonium indicated a higher preference for ammonium, this was independent of size (Figs. 2.3, 2.4). These results, therefore, do not support a size-dependence of new and regenerated nitrogen. It is noteworthy that, as in the case of Malone (1980), this conclusion is not based on a physiological data set. If there was a size-dependency in new and regenerated production, we would expect to observe differences in the relative uptake rates of nitrate and ammonium by small cells, or a size-dependence in the *f*-ratio, and this was not observed.

The increase in new production (pNO_3^-, *f*) from oligotrophic to upwelling waters was associated with a transition from a prokaryote-dominated community to a eukaryote-dominated community. In oligotrophic waters, prokaryotic picoplankton dominated the biomass and production, while at the upwelling site, eukaryotic cells, mainly >2 μm, represented most of the biomass and production. Thus, the community structure (prokaryotes versus eukaryotes), more than size, may be important in the differences

Table 2.3 Calculated *f*-ratios [pNO_3^- /pNO_3^- + pNH_4^+] from literature values. Values are from near surface or surface waters.

Location and water type	Size (μm)	*f*-ratio	Reference
Narragansett Bay (USA) "COASTAL"	Total <10	0.30 0.28	Furnas *et al.* (1983)
N.W. Atlantic "COASTAL" "OCEANIC"	Total <1 Total <1	0.48 0.21 0.02 0.02	Harrison and Wood (1988)
Antarctic "COASTAL"	Total <15 <1	0.62 0.55 0.57	Probyn and [illegible]anting (1985)
Antarctic "COASTAL"	Total <100 <10	0.28 0.19 0.21	Koike *et al.* (1981)
Antarctic "COASTAL"	Total <20 <10	0.26 0.14 0.22	Koike *et al.* (1986)
South Benguela Upwelling "COASTAL" "OCEANIC"	Total <10 <1 Total <10 <1	0.65 0.57 0.38 0.28 0.17 0.21	Probyn (1985)
Lake Kinneret (Israel)	Total <10	0.18 0.16	Sherr *et al.* (1982)

Table 2.3 cont.

off Westland (New Zealand) "inshore" "offshore"	Total <20 <2 Total <20 <2	0.72 0.57 0.69 0.73 0.77 0.67	Chang *et al.* (1989)
NW Mediterranean (Ligurian Sea)	1-10 <1	0.15 0.20	Selmer *et al.* (1993)

observed between the two regimes.

Yentsch (1990) describes the phytoplankton size distribution in the ocean as one where large-sized cells are mainly present only in nutrient-rich areas, while small phytoplankton cells are omnipresent throughout the world's oceans. Given that a size-dependence of new and regenerated production is not supported (the present study, see also Chisholm, 1992), how can we explain the fact that picoplankton are so widely distributed and that they contribute so substantially to new and regenerated production (Fig. 2.2, 2.7, Table 2.1)? One explanation may be that picoplankton are able to adapt more broadly than larger cells to different environments, ranging from nutrient-poor to eutrophic areas, for example by taking up nutrients more efficiently.

2.4.2. Nitrogen uptake by picoplankton and by cells >2 μm in different nutrient regimes

In the present study, nitrogen uptake rates normalized to chlorophyll *a* concentration were higher in the <2-μm fraction compared with the >2-μm fraction (Fig 2.5). Moreover, specific rates of nitrogen uptake by picoplankton were in most cases slightly higher than for the >2-μm fraction (Fig. 2.6). These results suggest that picoplankton are somewhat more efficient in the uptake of nitrogen than the larger size-fraction (μg at N L^{-1} h^{-1}), both in oligotrophic waters and in upwelling and slope waters. Note that the N uptake rates in the <2-μm fraction include bacteria, whereas the normalized uptake rates (<2 μm) do not. As a result, the specific rates of N uptake in the <2-μm fraction may be biased towards higher values compared with the rates in the >2-μm fraction, leading, in the worst case, to no difference observed in the specific rates of N uptake between the two size-fractions. A normalization of the N uptake that would take into account a bacterial uptake of N,

however, is not straightforward because the PON used for normalization already includes bacteria. Therefore this bias was not corrected for here.

For the <2-µm and the >2-µm fractions, the difference observed between the nitrogen uptake rates (Fig. 2.5) is larger than the difference observed between the specific nitrogen uptake rates (Fig. 2.6). This can be explained by the conversion factor (=PON:chlorophyll *a* ratio) used to transform nitrogen uptake rates into specific rates (Fig. 2.6) which is higher for the <2-µm fraction compared with the >2-µm fraction (1.8 and 0.9, respectively). This higher nitrogen to chlorophyll *a* ratio in the <2 µm-fraction (1.8) is similar to a value of 1.6 reported for a culture of *Synechococcus* WH7803 (Cuhel and Waterbury, 1984), suggesting that the higher PON: chlorophyll *a* ratio in the picoplankton fraction may be due to the presence of cyanobacteria which have accessory pigments rich in nitrogen, as shown by Kana and Glibert (1987) and Verity *et al.* (1992). However, since cyanobacteria represent a small fraction of the picoplankton biomass, they would not likely have influenced the overall PON:chlorophyll *a* ratio. On the other hand, it is possible that prochlorophytes also have a high N:C ratio (similar to the prokaryotic cyanobacteria and the heterotrophic bacteria), which in turn would result in a high PON:chlorophyll *a* ratio.

An increase in the rates of the <2-µm fraction also corresponded to an increase in the rates of the >2-µm fraction (Figs. 2.5, 2.6), indicating that the larger cells may also increase their uptake rates under certain conditions. Thus, the omnipresence of picoplankton in the world's oceans may not be due solely to a physiological advantage but to other physical factors as well, for example vertical mixing (Kiørboe, 1993). In conclusion, picoplankton are physiologically at an advantage compared with the larger cells for nitrogen uptake, and moreover are less dependent on physical factors to remain in the upper water column than are larger cells.

As an adaptation to nutrient-depleted waters, the small, neutrally buoyant cells (*e.g.* picophytoplankton, utraplankton or nanoplankton) have been shown to have a lower half-saturation constant, K_S (Eppley *et al.*, 1969; Parsons and Takahashi, 1973; Carpenter and Guillard, 1971; Raimbault and Gentilhomme, 1990; Garside and Glover, 1991) compared with larger cells. The cyanobacteria *Synechococcus* can mobilize nitrogen from their phycobilisome-bound phycobiliproteins under certain conditions of nitrogen deprivation and low-light, however, this process is not independent of photosynthesis (Kana and Glibert, 1987); small diatom species <10 µm can accumulate nitrogen in internal pools (Collos, 1986). These examples represent another adaptation of small cells to nutrient-poor waters. Small cells have also been shown to bloom as a result of rapid increases of nitrate in oceanic areas. Such a bloom was reported for *Synechococcus* in the oligotrophic Sargasso sea (Glover *et al* , 1988 a, b) In fact, high concentrations of picoplankton (up to 10^8 cells L^{-1}) have been reported in coastal and upwelling waters (Søndergaard *et al* , 1991; Hall and Vincent, 1990; Bienfang *et al.*, 1984), accounting for a significant fraction of the total chlorophyll *a*. Moreover, in high-nutrient-low-chlorophyll (HNLC) areas, *e g* the Equatorial Pacific, the autotrophic biomass is generally dominated by small cells (<5 µm) over most of the year (Chavez, 1989). Glibert *et al.* (1986) and Glibert and Ray (1990) studied two clones of *Synechococcus* and found them to have different patterns of growth and nitrogen uptake. One clone had the capacity to reduce its metabolic rates under low-level nutrient conditions without harming the cell, and was therefore adaptable to oceanic, nutrient-depleted conditions The other clone continued to divide even after nitrogen depletion, and was therefore adapted to a continuous supply of nitrate such as found in coastal waters. Thus, the capacity of picoplankton to grow under very different nutrient regimes is consistent with their broad distribution

2.4.3. Contributions of the different picophytoplankton groups to nitrogen production

Since nitrogen uptake rates of each individual group (prochlorophytes, cyanobacteria, and picoeukaryotes) in the <2-µm fraction could not be measured directly, values of relative carbon biomass of each of these groups were combined with the relative uptake rates of nitrate in the <2-µm fraction (Table 2.4), to estimate the relative importance of each group for the uptake of nitrate. The numerical abundances of the three photosynthetic groups were converted to carbon biomass values using specific conversion factors, as described in Chapter 1. Average values were calculated for the oligotrophic waters and for the upwelling region, for the mixed layer and for the DCM layer. It was assumed that (1) all nitrogen uptake was accounted for by the autotrophic cells in the picoplankton fraction and not by bacteria, and that (2) all the photosynthetic groups had the same nitrogen uptake per unit biomass. Note that the first assumption may not hold, particularly in the case of regenerated production, since there is evidence of bacterial uptake of ammmonium (Wheeler and Kirchman, 1986; Laws *et al.*, 1985, Chapter 3) The second assumption was based on the following: using hourly ^{14}C uptake rates per cell measured by Li (1994) for three stations of the same cruise (located at 26 65° W on the 29.09 92, 19.27° W on 28.09.92, 10.78° W on 2.10.92), growth rates (h^{-1}) of the three picophytoplankton groups were calculated based on the same conversion factors (µgC $cell^{-1}$) used previously (Chapter 1) For two out of three stations (26 65° W, 10 78° W), the growth rates did not differ significantly from one group to another At the oligotrophic stations where ~90% of the nitrate uptake was due to picoplankton (Table 2 4), more than 80% of the picoplankton biomass was accounted for by the prochlorophyte group in the mixed layer (82%) and in the DCM layer (86%). In the upwelling region on the other hand, 53% (mixed layer) and 90% (DCM layer) of the nitrate uptake was in the <2-µm fraction, while the picoplankton biomass was mainly comprised of picoeukaryotes (~70%) These

Table 2.4 Relative contribution of prochlorophytes, cyanobacteria, and picoeukaryotes to the total picoplankton carbon biomass (see Chapter 1), and relative nitrate uptake in the <2-µm fraction (% of total nitrate uptake).

Water type	Depth	Biomass			NO_3^- uptake
		Prochlorophytes (%)	Cyanobacteria (%)	Picoeukaryotes (%)	<2 µm (%)
Oligotrophic	Mixed layer	82	12	5	87
	DCM layer	86	6	8	88
Upwelling	Mixed layer	4	21	76	53
	DCM layer	10	25	65	90

results suggest that prochlorophytes were the main contributors to measured new production in oligotrophic waters, while at the upwelling site, picoeukaryotes account for a significant fraction of the new production. The relative uptake rates of ammonium in the <2-μm fraction were similar on an average to the relative uptake rates of nitrate, suggesting that prochlorophytes also contributed to most of the regenerated production of open ocean stations, and that picoeukaryotes accounted for a substantial fraction of the regenerated production at the upwelling site. In agreement with previous studies (Olson *et al* , 1990; Veldhuis and Kraay, 1990), prochlorophytes were present throughout the water column in open-ocean areas, often outnumbering cyanobacteria (Chapter 1). In fact, in oligotrophic waters prochlorophytes dominated the picophytoplankton biomass of the water column (77%), in accordance with a significant contribution of prochlorophytes to nitrogen uptake in these areas.

2.4.4. New production and export production

Under steady-state conditions, new production is balanced by the export production of organic matter out of the photic zone (Eppley and Peterson, 1979; Platt *et al.*, 1992), which in turn can be estimated by the downward flux of particulate organic matter collected in sediment traps (Knauer *et al.*, 1984). New production is also equal, under steady-state conditions, to the input rate of nitrate to the photic zone (Lewis *et al.*, 1986). In the present study, the oligotrophic stations showed a low variability in nitrogen and carbon production rates for the entire duration of sampling, *i.e.* nearly a month (19 Sep-17 Oct, 1992), which suggests that the water column was stable during the time of sampling. A study during the following spring (May-June of 1993) in the same waters also indicated similar values of nitrogen productivity at most of the oligotrophic stations (G. Harrison, pers. comm.), thus supporting the view of a stratified and stable water column year round at the sub-tropical open-ocean stations.

New production integrated over the upper water column represented 13% (5-33%) of total production in oligotrophic waters (calculated for picoplankton and for the total fraction). These results were in agreement with previous studies carried out in tropical and subtropical areas of the Atlantic and Pacific oceans (LeBouteiller, 1993; Eppley and Peterson, 1979; Eppley *et al.*, 1977; Glibert *et al.*, 1988). Given my estimates of new production for the open-ocean stations, are they comparable with estimates of export production in ocean waters? The nitrogen flux in oligotrophic waters, estimated as the uptake of nitrate over the water column, was on average 0.77 mmole N m^{-2} d^{-1} (range of 0.28 to 1.91 mmole N m^{-2} d^{-1}) and 0.89 mmole N m^{-2} d^{-1} (range of 0.35 to 2.27 mmole N m^{-2} d^{-1}) for the <2-μm fraction and for the total fraction, respectively. These estimates are of the same order of magnitude as the estimates of nitrogen flux based on sediment trap measurements in the Sargasso Sea (Altabet, 1989, Lohrenz *et al.*, 1992) Compared with the vertical nitrate flux estimated by Lewis *et al.* (1986), which was on average 0.14 mmol NO_3^- m^{-2} d^{-1} (0.002-0.89), my estimate of nitrogen flux was not significantly different. Moreover, the nitrate uptake rates ($^{15}NO_3^-$ uptake rates) measured in the present study were similar to the ones measured by Lewis *et al.* (1986), *i.e.* 0.81 mmol NO_3^- m^{-2} d^{-1} (±0.1 /).

Thus, comparison of my results with the various estimates of export production leads me to conclude that new production by picoplankton alone can account for that exported out of the photic zone. However, this implies the existence of export mechanisms for picoplankton, and furthermore that there are no significant recycling losses associated with the transfer of picoplankton out of the photic zone. These implications are discussed in the following section.

2.4.5. Mechanisms of export for picoplankton and food web structures

As demonstrated by the Stokes Laws and by direct measurements (Smayda, 1970; Smayda and Bienfang, 1983; Takahashi and Bienfang, 1983; Bienfang, 1985; Kiørboe, 1993), small-sized cells such as picoplankton have negligible sinking rates. Yet picoplankton (cyanobacteria) have been found on the deep-sea floor of the oligotrophic North East Atlantic in the form of phytodetritus (Lochte and Turley, 1988; Pfannkuche and Lochte, 1993), suggesting that avenues other than direct sinking must be responsible for the export of picoplankton out of the photic zone.

Fecal pellets containing pigments representative of cyanobacteria and prymnesiophytes (picoeukaryotes) were actually detected in the sediment traps deployed at the upwelling site at the time of the present study (E. Head, pers. comm.). In a simple model of oceanic food webs where picoplankton were the dominant producers, Michaels and Silver (1988) showed that the probability of export of small phytoplankton varied according to the conversion efficiency of the microbial loop components, and depended on the presence of generalist grazers such as salps that can directly consume the small phytoplankton, resulting in a short food-chain. Salps were present at the time of the present study, at the upwelling site (E. Head, pers. comm.). Thus, a significant export of picoplankton production appears to be consistent with a food web model such as the one described in Michaels and Silver (1988). This food web model could then be representative of an oceanic ecosystem such as found in the subtropical North Atlantic.

Sinking aggregates, *e.g.* marine snow, have also been reported to include large numbers of picophytoplankton cells (Silver and Alldredge, 1981; Lampitt *et al.*, 1993), and could serve as efficient conveyors of organic matter out of the photic zone (Silver and Alldredge, 1981; Fowler and Knauer, 1986; Jackson and Lochmann, 1992; Alldredge *et*

al., 1993). Lampitt *et al.* (1993) suggested that the consumption by metazoans of marine snow which contain picoplankton could result in a short-cut of the food-chain (picoplankton-microzooplankton-metazooplankton).

Another avenue for the export of POM originating from picoplankton may be diapycnal mixing. I estimated the flux of PON <2 μm as the product of the PON gradient (<2 μm) at the photic depth and a diapycnal diffusivity (K_z) of 1.5×10^{-5} m^2 s^{-1} (Ledwell *et al.*, 1993). At four oligotrophic stations picked randomly (# 76, 8, 70, and 14), the resulting maximum flux was estimated to be 0.77 μmol N m^{-2} h^{-1}, which is two orders of magnitude lower than the water column integrated uptake rates of $^{15}NO_3^-$. Even if a higher value of K_z were used (*i.e.* $3.7\ 10^{-5}$ m^2 s^{-1}, Lewis *et al.*, 1986), the resulting PON flux and the uptake rates of $^{15}NO_3^-$ were still different by an order of magnitude. These results were in agreement with a previous comparison between nitrate uptake rates and PON fluxes in the oligotrophic North East Pacific (Harrison *et al.*, 1992). Note, also, that the maximum PON flux was lower than the maximum nitrate flux (computed as the product of K_z and the nitrate gradient) by two orders of magnitude. Thus, it appears that diapycnal mixing of PON was not an important mechanism for PON export.

It appears that the two most feasible routes for picoplankton export are grazing and aggregation, however, there is not enough evidence to determine which pathway is the most important. Although the mechanisms exist for exporting picoplankton production, the recycling losses associated with the export may be significant (King, 1987), and therefore short food-chains are necessary to reconcile my results (Lampitt *et al.*, 1993).

2.5. Conclusions

In the nutrient-depleted, oligotrophic environment of the sub-tropical North Atlantic, picophytoplankton cells account for most of the new production as measured by the ^{15}N tracer method, albeit a small fraction of the total. These small phytoplankton cells have higher specific rates of nitrogen uptake compared with larger cells, and are generally better adapted to growing under nutrient-poor conditions. Picophytoplankton are also important contributors to new production in the upwelling and slope waters, indicating that these small phytoplankton cells may also compete effectively under high concentrations of nutrients by virtue of their presence alone. Within the picoplankton population, prochlorophytes may be the main contributors to new production in oligotrophic waters, leading to the question of why this group would be more successful in the uptake of nutrients than cyanobacteria and picoeukaryotes.

Conventional thinking is that small cells contribute primarily to regenerated production, while larger cells account for most of the new production. Results in the present study do not support this, for example, the *f*-ratios of the <2-μm fraction were similar to the *f*-ratios of the >2-μm fraction both for discrete depths and for the upper water column. The estimated nitrogen export flux from picoplankton production is, on average for the oligotrophic waters, 0.77 mmole N m^{-2} d^{-1} (range of 0.28 to 1.91 mmole N m^{-2} d^{-1}) which is equivalent to a yearly carbon export flux of 22 g C m^{-2} $year^{-1}$ (range between 8 and 55 g C m^{-2} $year^{-1}$). This represents a significant flux of carbon compared with previous estimates by the total phytoplankton community (Martin *et al.*, 1987; Lohrenz *et al.*, 1992). Most of this new production by picoplankton must get exported out of the photic zone, presumably through mechanisms such as grazing and aggregation. However, given that there may be significant recycling losses associated with the export of picoplankton, the question remains of how picoplankton will get exported with a very high

efficiency. The most probable alternative is that of a short food-chain associated with picoplankton.

Chapter 3

Bacterial uptake of inorganic nitrogen in coastal and oceanic waters of the subtropical North Atlantic

3.1. Introduction

Our understanding of the role of marine bacteria in the nitrogen cycling of pelagic systems has expanded in recent years: it is now recognized that bacteria are not only mineralizers of organic nitrogen (Pomeroy, 1974; Williams, 1981; Azam *et al.*, 1983) but also consumers of inorganic nitrogen (Laws *et al.*, 1985; Wheeler and Kirchman, 1986). Given that bacteria represent a significant fraction of picoplankton biomass in oceanic waters (see Chapter 1) and that picoplankton in turn account for most of the new and regenerated production there (see Chapter 2), what is the contribution of bacteria to the uptake of inorganic nitrogen by picoplankton?

Organic nitrogen sources such as dissolved free amino acids (DFAA) can provide bacteria with both carbon and nitrogen, and moreover, DFAA appears to be the preferred nitrogen form over inorganic nitrogen (Kirchman *et al.*, 1989; Kirchman *et al.*, 1992). Then why do bacteria take up ammonium (Tupas and Koike, 1990; Wheeler and Kirchman, 1986; Kirchman *et al.*, 1989, 1992, 1994) and nitrate (Horrigan *et al.*, 1988; Kirchman *et al.*, 1991)? Some authors report that organic nitrogen is insufficient to meet the bacterial demand for nitrogen (Wheeler and Kirchman, 1986; Kirchman *et al.*, 1989; Tupas and Koike, 1991) and therefore bacteria turn to ammonium as a nitrogen source (Fuhrman *et al.*, 1988; Tupas and Koike, 1994). Bacteria have also been shown to take up dissolved organic nitrogen and ammonium simultaneously (Tupas and Koike, 1990; Tupas and Koike, 1991; Kroer *et al.*, 1994). However, the factors that control the bacterial uptake

of inorganic nitrogen relative to their uptake of organic nitrogen are not well known. It has been suggested that bacteria will assimilate inorganic nitrogen when the C:N ratio of the substrate ($C:N_S$) is higher than the C:N ratio of the bacterial biomass ($C:N_b$) divided by the bacterial growth efficiency (Fenchel and Blackburn, 1979; Goldman *et al.*, 1987). Goldman and Dennett (1991) showed that marine bacteria which grew on a mixture of ammonium and amino acids with a constant $C:N_b$ increased their ammonium uptake when the $C:N_S$ increased. In the field, the organic sources of carbon and nitrogen available to bacteria are not well defined and some of them are highly variable in concentration such as DFAA (Lee and Bada, 1977; Mopper and Lindroth, 1982). It is therefore difficult to characterize $C:N_S$ in the field in order to determine the role of bacteria in the uptake of inorganic nitrogen.

According to the traditional view, bacterial growth is carbon limited (Williams, 1981; Azam *et al.*, 1983) and much of this carbon originates from phytoplankton excretion of dissolved organic carbon (Larsson and Hagström, 1979; Wolter, 1982; Baines and Pace, 1991); an alternative carbon source comes from the decomposition of algal detritus (Fenchel and Blackburn, 1979). When bacteria grow on substrates poor in nitrogen (high $C:N_S$) they need to take up additional nitrogen such as ammonium. The bacterial uptake of nitrogen depends on the growth of bacteria which in turn is controlled by the availability of organic carbon. This suggests that the interaction between bacteria and phytoplankton could be important in controlling the bacterial uptake of inorganic nitrogen. In a review on bacterial-algal interactions, Cole (1982) reported, based on laboratory evidence, that bacteria and phytoplankton can interact in both stimulatory and inhibitory ways. The extracellular release of DOC by phytoplankton and its subsequent uptake by bacteria represent a stimulatory effect of phytoplankton on bacteria (Cole, 1982). Bratbak and Thingstad (1985) argued that this type of interaction may lead to bacteria and phytoplankton competing for inorganic nutrients, particularly in nutrient-depleted waters. It has been

shown that bacteria and phytoplankton can compete for inorganic nutrients such as nitrate (Parker *et al.*, 1975), ammonium (Horstmann and Hoppe, 1981), and phosphate (Currie and Kalff, 1984).

Previous measurements of bacterial uptake of inorganic nitrogen have been carried out mostly on samples where phytoplankton activity was reduced to a minimum either by size-fractionation or by dark incubation; this, however, could have eliminated or minimized potentially important interactions with the photosynthetic community *e.g.* competition (Bratbak and Thingstad, 1985) or predator-prey interactions (Stone, 1990). Other studies in the field (Laws *et al.*, 1985) in which bacterial uptake of inorganic nitrogen was estimated in unfractionated water under *in situ* light conditions are few, particularly in oligotrophic waters (Harrison *et al.*, 1992), and it is therefore difficult to generalize them; these studies estimate bacterial uptake of inorganic nitrogen indirectly, as the difference between total microbial uptake and phytoplankton uptake. Moreover, these previous estimates (Laws *et al.*, 1985; Harrison *et al* , 1992) have not been evaluated against any independent estimate of bacterial activity.

In the present study, bacterial uptake rates of inorganic nitrogen are estimated for the subtropical North Atlantic as the difference between total uptake rates of inorganic nitrogen (by both phytoplankton and bacteria) estimated using ^{15}N, and phytoplankton uptake rates of nitrogen (Laws *et al.*, 1985), both estimated using light incubations of unfractionated water; the phytoplankton uptake rates are based on ^{14}C incorporation rates into proteins according to DiTullio and Laws (1983). The estimated bacterial uptake rates of inorganic nitrogen are compared with an estimate of total bacterial nitrogen demand, based on measurements of bacterial production rates. Since the total bacterial nitrogen demand includes organic nitrogen as well as inorganic nitrogen, it is expected to be an upper bound for the uptake rate of inorganic nitrogen. The differences observed between bacterial uptake

rates of inorganic nitrogen and bacterial nitrogen demand are discussed in detail. The implications for estimating new and regenerated production are examined, and the results are analyzed in the context of possible interactions between bacteria and phytoplankton.

3.2. Materials and methods

Data were collected during a fall cruise in the North Atlantic (16 Sept-21 Oct, 1992), as described in Chapter 1. All data are from two depths: one in the mixed layer and the other in the deep chlorophyll-maximum layer (DCM). A description of sampling locations, and date and depth of sampling is given in Table 1.1 (see also Fig. 1.1).

3.2.1. Bacterial production (P_b) and bacterial nitrogen demand (D_b)

Bacterial production rates (μg C L^{-1} h^{-1}) were estimated as incorporation rates of [^{3}H] leucine and [^{3}H] thymidine (data from Dr. Li) based on the methods of Kirchman *et al.* (1985) and Fuhrman and Azam (1980), respectively, as decribed in detail in Li *et al.* (1993). Incubations lasted for three hours under simulated *in situ* conditions on deck of the ship; filtrations were made onto 0.2 μm polycarbonate Nuclepore filters. The conversion from leucine incorporation to biomass production was made using the conversion factor of 3.1 kg C mol^{-1} of leucine (Kirchman, 1993). The conversion from thymidine incorporation to biomass production was made assuming $2x10^{18}$ cells mol^{-1} of thymidine (median value of conversion factors reviewed by Ducklow and Carlson, 1992) and 20 fg C $cell^{-1}$, resulting in a conversion factor of 40 kg C mol^{-1} of thymidine.

Values of bacterial nitrogen demand (μg-at N L^{-1} h^{-1}) were calculated as the bacterial production rates (in units of μg-at C L^{-1} h^{-1}) divided by a bacterial C:N ratio (atomic) equal to 4; this C:N ratio corresponds to a minimum value (Nagata, 1986;

Goldman *et al.*, 1987; Goldman and Dennett, 1991) in order to obtain a maximum value of bacterial nitrogen demand (range of 4-5, Goldman *et al.*, 1987).

The bacterial nitrogen demand, D_b, is computed from a net bacterial production, P_b (measured by leucine and thymidine incorporation rates), and represents therefore a net demand. However, D_b is used as an estimate of the bacterial demand for substrates because losses associated with bacterial production such as respiration appear to be low: Kroer *et al.* (1994) reported low values of respiration on DFAA. Tupas *et al.* (1994) measured 23% of respiration on leucine, while Fenchel and Blackburn (1979) reported 10-20% of respiration on the same substrate. Furthermore, these values were estimated after more than 24 hours of incubation. Suttle *et al.* (1991) measured the fraction of radioactive leucine respired in the Sargasso sea and found during short incubations (30-40 minutes) values less than 10% and values as high as 20% for 24 hour incubations. Therefore, given that short-term incubations were carried out to estimate P_b in the present study, respiratory losses were likely small, and thus values of D_b were not corrected.

3.2.2. Picophytoplankton production (P_p) and picophytoplankton nitrogen demand (D_p)

Primary production rates (µg C L^{-1} h^{-1}) were measured by addition of $NaH^{14}CO_3$ to water samples (200 ml) and subsequent incubation for 3 hours on deck (simulated *in situ* conditions); 3 light bottles and 3 dark bottles were incubated for each depth. These measurements were made simultaneously with the uptake measurements of ^{13}C and ^{15}N and therefore under identical conditions (see Chapter 2 for more details). The amount of ^{14}C added was 20-30 µCi per 100 ml of sample. Samples were size-fractionated after incubation (post-screening) through 2-µm Nuclepore filters and the material was collected onto glass-fiber filters (MFS). The filters were fumed with HCl (10 min.) and ^{14}C activity

on the filters was analyzed in a scintillation counter (Beckman). The ^{14}C activity of the dark bottle was subtracted from the ^{14}C activity of the light bottle and an average value of the triplicates was computed for each depth. On average, the ^{14}C activity in the dark represented 13% of the activity in the light bottle, for the <2-µm fraction. The ^{14}C uptake rates were estimated using the equation of Strickland and Parsons (1972). Replicate filters were frozen for protein extraction (see following section).

Values of picophytoplankton nitrogen demand (µg-at N L^{-1} h^{-1}) were estimated as the <2 µm ^{14}C uptake rates divided by a C:N assimilation ratio in the Redfield proportions (6.6 by atoms).

3.2.3. Picophytoplankton uptake of inorganic nitrogen (U_p)

Uptake rates of inorganic nitrogen by picophytoplankton (µg-at N L^{-1} h^{-1}) were estimated using the equation of DiTullio and Laws (1983):

$$U_p = [\, Pe \frac{0.3}{0.85} \,] \frac{1}{14} \qquad (1)$$

where P_e (µg C L^{-1} h^{-1}) is the incorporation rate of ^{14}C into the protein fraction of picophytoplankton, 0.3 is the nitrogen to carbon ratio by weight in phytoplankton protein (assumed to be equal to the N:C assimilation ratio), the value of 0.85 is the fraction of nitrogen taken up into proteins and the value of 14 represents the molecular weight of nitrogen which converts nitrogen uptake in units of µg N L^{-1} h^{-1} into units of µg-at N L^{-1} h^{-1}. The assumptions used in this equation are (1) that 85% of the nitrogen taken up by phytoplankton is allocated to proteins, (2) that the N:C ratio by weight in proteins is constant and equal to 0.3, and (3) that the uptake of carbon and nitrogen is coupled. The first assumption holds under nitrogen-limited conditions *e.g.* at the oligotrophic stations of

the present study; however, this assumption may not hold in nutrient-rich waters (DiTullio and Laws, 1983) such as the upwelling waters of the present study (see Discussion). The assumptions are examined in section 3.4.4.

Values of P_e were measured by differential solvent extraction based on the method of Roberts *et al.* (1955) as modified by Morris *et al.* (1974). Samples were incubated with $NaH^{14}CO_3$ as previously explained. After incubation, triplicate samples were filtered onto glass-fiber filters (MFS) and immediately stored at -20 °C. In the laboratory, each filter was treated with 3 ml of boiling absolute ethanol; the extract plus the filter were poured onto a second glass-fiber filter and both filters were rinsed twice with 2 ml of cold absolute ethanol. The resulting filtrate represents the ethanol-soluble fraction. Both filters were then extracted in 3 ml of TCA (5%) at 95°C for 30 minutes; the extract plus the two filters were poured onto a third glass-fiber filter, and all three filters were then rinsed twice with 2 ml of cold TCA (5%); this filtrate represents the hot TCA-soluble fraction. The residual fraction retained on the three filters represents the protein fraction. The three different fractions (ethanol-soluble, hot TCA-soluble, and protein) were measured in a scintillation counter for the corresponding ^{14}C activity (see previous section); only the measurements of ^{14}C uptake in the protein fraction were used in the present study. The ^{14}C total recovery was 70% on average.

3.2.4. Bacterial uptake of inorganic nitrogen (U_b)

Bacterial uptake rates of inorganic nitrogen U_b (µg-at N L^{-1} h^{-1}) were estimated according to Laws *et al.* (1985) using the following equation:

$$U_b = U_t - U_p \quad (2)$$

where U_t is the total uptake (phytoplankton and bacteria) of inorganic nitrogen as measured by the uptake of [$^{15}NH_4^+$ + $^{15}NO_3^-$] in the <2-µm fraction; U_p was estimated from equation (1). The values of U_t did not include the uptake of ^{15}N-Urea for the following reasons. As a result, U_t was underestimated by ~10% in upwelling waters, and ~30% in oligotrophic waters. Although bacteria have been shown to decompose urea in coastal waters (Rajendran *et al.*, 1980; Taga, 1970), in estuaries (Savidge and Johnston, 1987; Remsen *et al.*, 1972), in lake waters (Satoh, 1980) and in cultures (Jahns, 1992), these studies were carried out in waters where urea concentrations were higher by at least an order of magnitude compared with ambient urea measured in the present study (all stations). Moreover, most of these studies are based on dark incubations which could have included a phytoplankton uptake of urea. Therefore, these studies cannot be taken as representative of waters such as in the present study. In addition, a marine study carried out by Wheeler and Kirchman (1986), which was not based on dark incubations did not find a significant uptake of urea in the <1-µm fraction which includes prokaryotes. Given these uncertainties and the fact that the urea uptake rates measured in the present study may have been associated with significant errors (see Chapter 2), U_t did not include the uptake of ^{15}N-Urea.

3.3. Results

3.3.1. Bacterial and picophytoplankton carbon production

Bacterial production (leucine and thymidine incorporation) and photosynthetic production <2 μm (^{14}C uptake) both increased from open-ocean waters to upwelling waters (Figs. 3.1 a, b). Bacterial production (Table 3.1) represented ~10% of photosynthetic production in the mixed layer (9% and 8% for leucine and thymidine incorporation, respectively), and ~25% of photosynthetic production in the DCM layer (20% and 26% for leucine and thymidine incorporation, respectively) Note that ^{14}C uptake rates varied more across the transect in the DCM layer than in the mixed layer, following the variation in chlorophyll *a* concentration (see Chapter 1)

3.3.2. Bacterial and phytoplankton nitrogen demand

The ratio of bacterial nitrogen demand (D_b) to picophytoplankton nitrogen demand (D_p), D_b:D_p, varied from 0.2 to 1.2 (Fig. 3.2) At the oligotrophic stations (50°-15°W), the ratio was close to 1 in most of the DCM samples whereas it ranged from 0.1 to 0.5 in the mixed layer. At three stations sampled in the DCM layer (#76, 70, 67) the ratio was lower compared with the remaining stations (see Table 1.1 for date, depth and station # of sampling); at these three stations the values of D_p were significantly higher than at the other oligotrophic stations, while the corresponding values of D_b were similar to those at the remaining stations. On the other hand, the two outliers (#17, 54 not shown in Fig. 3.2) corresponding to ratios much larger than 1 were located at stations where values of D_p were significantly lower than those at the remaining stations At the upwelling stations, despite a large variability, higher ratios were also observed in the DCM layer compared with the mixed layer. A similar distribution of D_b:D_p was observed for values of D_b

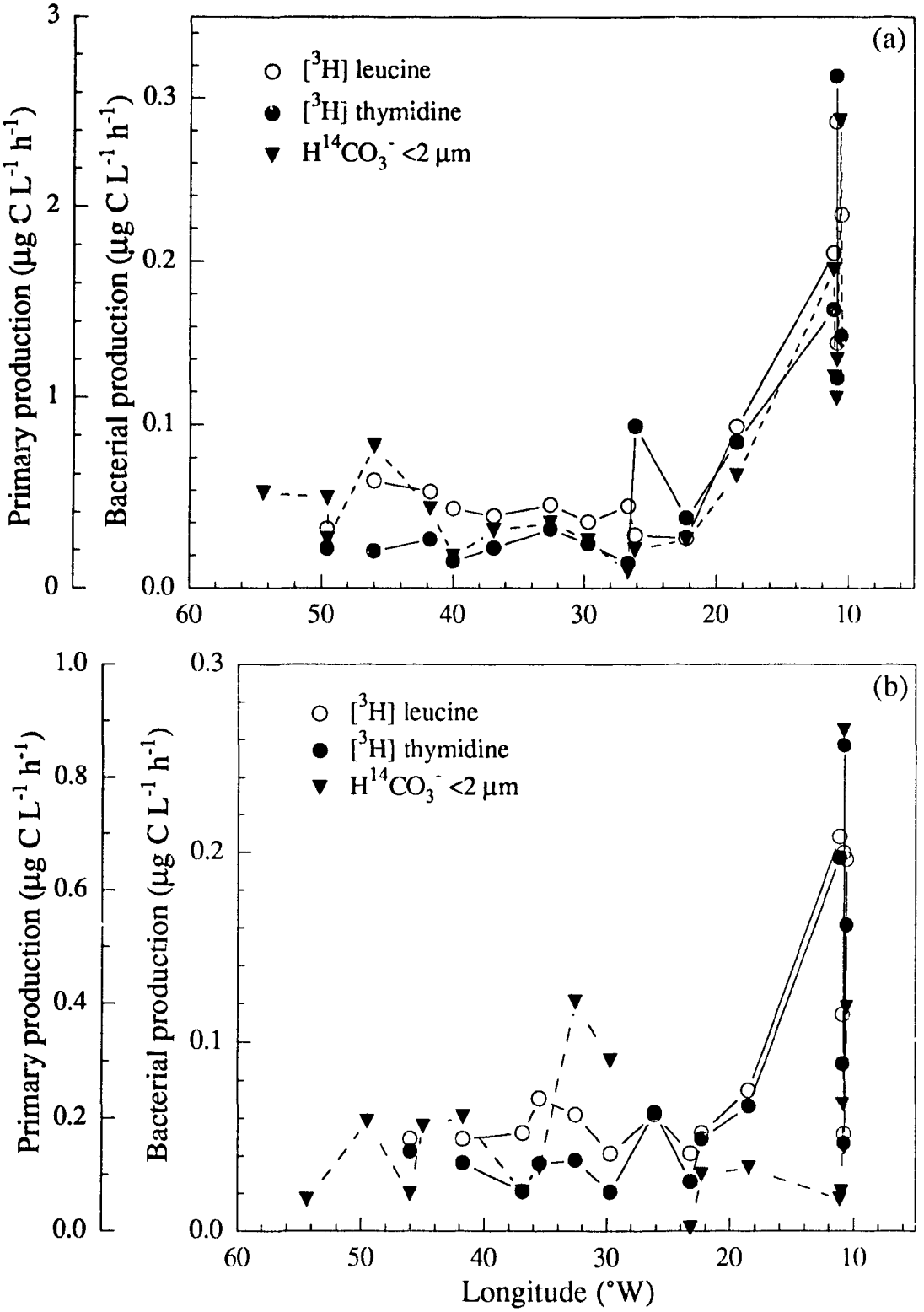


Fig. 3.1 Bacterial production rates (based on leucine incorporation rates and thymidine incorporation rates) and primary production rates (based on ^{14}C uptake rates in the <2-µm fraction), in (a) the mixed layer, and (b) the DCM layer.

Table 3.1 *Slope and coefficient* of correlation (R^2) for linear fit of bacterial production rates based on leucine (P_b^1) and thymidine (P_b^2) incorporation rates, versus picophytoplankton production rates (P_p), and for linear fit of P_b^2 versus P_b^1. Picophytoplankton production rates are based on ^{14}C uptake rates in the <2-µm fraction. Model II regression was used.

Depth	P_b^1 vs P_p		P_b^2 vs P_p		P_b^2 vs P_b^1	
	Slope	R^2	Slope	R^2	Slope	R^2
Mixed layer	0.09	0.79	0.08	0.67	0.97	0.92
DCM layer	0.20	0.52	0.26	0.64	1.14	0.96

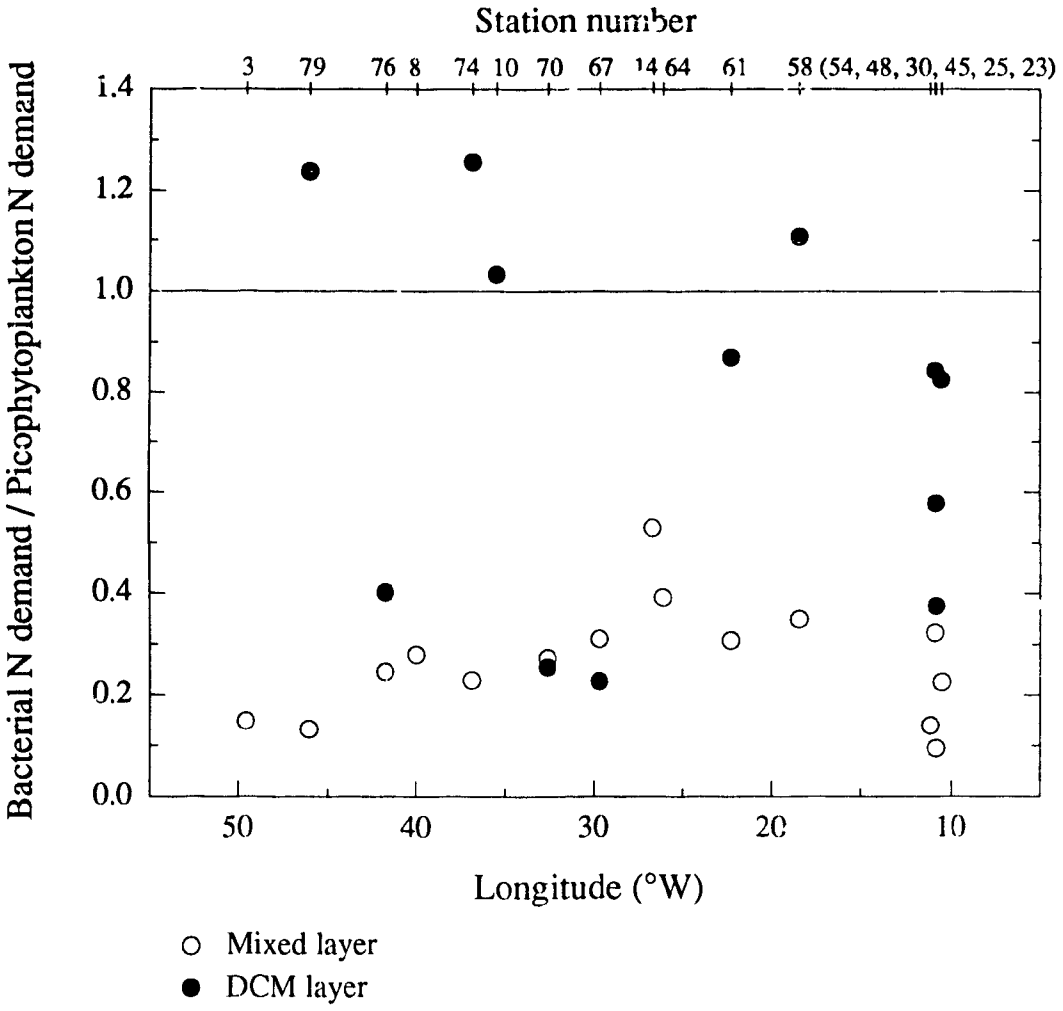


Fig. 3.2 Ratios of bacterial nitrogen demand, D_b (based on leucine incorporation rates) to picophytoplankton nitrogen demand, D_p, in the mixed layer, and in the DCM layer. DCM samples from stations 17 and 54 showed ratios >>1 (14 and 6, respectively) and were not included.

computed from thymidine incorporation (not shown here) compared with the values of D_b computed from leucine incorporation (Fig 3.2)

3.3.3. Bacterial uptake of inorganic nitrogen

Bacteria contributed on average 56% (range from 26% to 85%) and 62% (range from 18% to 93%) to the microbial uptake (bacteria+picophytoplankton) of inorganic nitrogen in oligotrophic waters for the mixed layer and for the DCM layer, respectively (Tables 3.2 a, b). In upwelling and slope waters, on the other hand, heterotrophic bacteria contributed 45% (range from 8% to 80%) and 69% (range from 14% to 98%) to the total uptake in the mixed layer and in the DCM layer, respectively, note that in the mixed layer in this region bacterial uptake was averaged over three values only (Table 3 2 a) Although bacterial uptake rates were higher on average in the DCM layer compared with the mixed layer, values (absolute and relative) were highly variable making it difficult to statistically evaluate any difference between depths or between regions

3.3.4. Comparison between bacterial uptake of inorganic nitrogen (U_b) and bacterial nitrogen demand (D_b)

Bacterial uptake rates of inorganic nitrogen were compared with an estimate of bacterial nitrogen demand (Figs. 3 3 a, b). Given that bacterial nitrogen demand includes both organic and inorganic nitrogen, it is expected that bacterial uptake rates of inorganic nitrogen would be smaller or equal to bacterial nitrogen demand In fact, at the oceanic stations (#3-17, and #58-79) D_b was lower than U_b by an order of magnitude. However, at some stations (# 76, 8 in the mixed layer) where replicate values of U_b were highly variable (see standard deviation) the corresponding D_b was situated within the range of variation in U_b. Note that the peak values of U_b observed in the mixed layer (#70, 61) and

Table 3.2a. Picophytoplankton uptake rates of inorganic nitrogen (U_p) and bacterial uptake rates of inorganic nitrogen (U_b) estimated according to equations (1) and (2) for the mixed layer. Values are given in absolute units and as contributions (%) to the total uptake (phytoplankton + bacteria) of inorganic nitrogen. na = not available, *i.e.* when U_b could not be calculated ($U_p > U_t$).

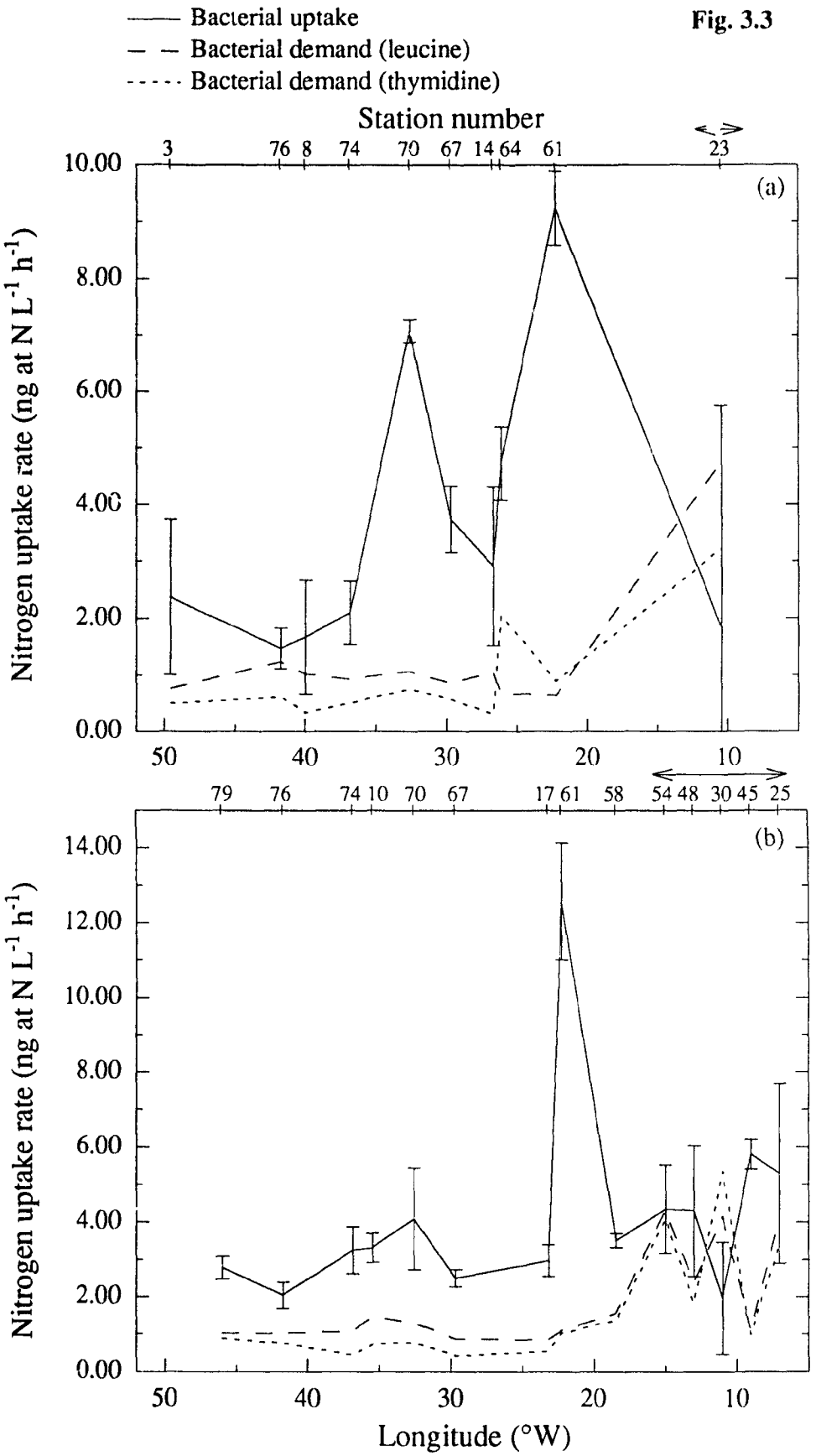
Longitude	Depth	U_p		U_b	
°W	m	ng at L^{-1} h^{-1}	%	ng at L^{-1} h^{-1}	%
54.35	10	9.35	52	8.56	48
49.55	40	2.71	53	2.37	47
49.50	1	3.14	20	12.26	80
46.02	20	9.79		na	
41.73	20	4.24	74	1.46	26
39.97	30	2.63	61	1.66	39
36.87	20	3.22	61	2.09	39
32.57	20	3.16	31	7.07	69
29.67	20	1.95	34	3.73	66
26.65	20	2.34	45	2.91	55
26.10	20	1.01	18	4.72	82
22.27	20	1.60	15	9.24	85
18.44	20	4.89		na	
11.10	20	27.87		na	
10.98	20	23.91		na	
10.86	10	22.23		na	
10.78	20	14.84		na	
10.61	1	36.07		na	
10.47	5	20.37	92	1.83	8

Table 3.2b. As in Table 3.2a, but for the DCM layer.

Longitude °W	Depth m	U_p ng at L^{-1} h^{-1}	U_p %	U_b ng at L^{-1} h^{-1}	U_b %
54.35	60	0.54	2	22.51	98
49.50	20	2.37	13	15.27	87
46.02	60	0.98	26	2.78	74
45.00	70	3.03	82	0.66	18
41.73	60	2.49	55	2.03	45
36.87	60	1.09	25	3.24	75
35.48	70	0.86	21	3.32	79
32.57	60	3.86	49	4.09	51
29.67	60	3.39	58	2.50	42
23.20	80	1.08	27	2.97	73
22.27	50	0.91	7	12.57	93
18.44	80	1.35	28	3.51	72
11.10	60	0.78	15	4.34	85
10.98	60	0.56	13	3.87	87
10.86	50	2.43	36	4.30	64
10.78	40	12.07	86	1.97	14
10.78	60	2.38	29	5.79	71
10.53	25	5.16	49	5.29	51

Fig. 3.3 Estimates of bacterial uptake rates of inorganic nitrogen ($NH_4^+ + NO_3^-$) in the <2-μm fraction, and estimates of bacterial nitrogen demand in (a) the mixed layer and (b) the DCM layer. Error bars are standard deviations. The upwelling stations are indicated by an arrow above the corresponding station numbers. In (b) the upwelling stations are equally spaced only for clarity.

Fig. 3.3


Bacterial uptake
Bacterial demand (leucine)
Bacterial demand (thymidine)
Station number
3
76 8
74
70
67 14 64
61
23
(a)
Nitrogen uptake rate (ng at N L-1 h-1)
10.00
8.00
6.00
4.00
2.00
0.00
50
40
30
20
10
79
76
74 10
70
67
17 61
58
54 48
30 45
25
(b)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Longitude (°W)

in the DCM layer (#61) corresponded to stations where $^{15}NH_4^+$ uptake rates were higher than the remaining stations by an order of magnitude. These stations also had the highest values of bacterial uptake rates relative to total microbial uptake rates (Tables 3.2 a, b): 69% and 85% in the mixed layer (#70, 61), 93% in the DCM layer (#61). At the upwelling site (stations #23-54) the comparison did not show any systematic trend (Figs. 3.3 a, b): at three stations (#23, 30, 54) D_b was higher or equal to U_b. At the other three stations (#48, 45, 25) D_b was lower than U_b. The two stations located in slope waters off the Scotian shelf (#85, 82) showed high values of U_b; however, no values of D_b were available for comparison (not shown here).

3.4. Discussion

3.4.1. Bacterial production and nitrogen demand compared with picophytoplankton production (P_b:P_p) and nitrogen demand (D_b:D_p)

Cole *et al.* (1988) reported that bacterial production represents 20% of the primary production on average over a variety of fresh and saltwater ecosystems. The values of P_b were based on the different methods currently available for measuring bacterial production. Ducklow and Carlson (1992) indicated values of P_b:P_p ranging from 2-37% in open ocean areas and 2-10% in upwelling waters. Joint and Pomroy (1983, 1987) measured primary production in the <1-μm size-fraction and found values of P_b:P_p less than 10% in coastal waters. Estimates of P_b:P_p in the present study were ~10% in the mixed layer and ~25% in the DCM layer (Table 3.1), thus comparable with previous estimates. If computed for individual depths, P_b:P_p ratios ranged from 2 to 32% in the mixed layer and from 6 to 76% in the DCM layer, whereas ratios integrated over the water column (based on profiles of leucine and thymidine incorporation rates from Dr. Li and on profiles of ^{13}C uptake rates

from Dr. Harrison) ranged from 6 to 34% (leucine) and from 4 to 30% (thymidine), with one outlier eliminated (station #48 where P_b was anomalously high) in both cases. Thus P_b:P_p ratios for discrete depths were in agreement with the corresponding P_b:P_p ratios integrated over the water column. Note that the integrated values of primary production were based on ^{13}C uptake rates whereas values of primary production for individual depths were based on ^{14}C uptake rates; however, uptake rates of ^{13}C and ^{14}C were measured under identical conditions and showed a strong correlation (Appendix 1). Moreover, in the oligotrophic waters, ^{14}C uptake rates were similar to ^{13}C uptake rates within 10%. At the upwelling and slope water stations, on the other hand, ^{14}C uptake rates were higher than ^{13}C uptake rates; note that at many of these stations, ^{14}C and ^{13}C uptake rates showed a high variability *i.e.* high coefficients of variation (up to 28% for ^{14}C uptake and up to 42% for ^{13}C uptake); no explanation could be given for the difference in the latter case.

The estimates of D_b:D_p, based on the P_b:P_p ratios (using a C:N ratio for bacteria and phytoplankton of 4 and 6.6, respectively), suggest that bacteria require a significant amount of nitrogen (Fig. 3.2; Table 3.1). Note that the D_b:D_p ratios vary considerably between the mixed layer depth and the DCM-layer depth. These ratios (Table 3.1, Fig. 3.2) depend mostly on the variation in D_p, which in turn is controlled by the light-level to which the samples were exposed. In the mixed layer, where photosynthetic production was close to light-saturation and therefore D_p was high, the D_b:D_p ratio may therefore represent a minimum estimate of bacterial activity relative to photosynthetic activity. In the DCM layer, on the other hand, where phytoplankton production was light-limited and therefore D_p at its lowest, the ratio of D_b:D_p probably represents a maximum estimate. Since measurements were carried out under light conditions, and the diel variations in P_p are greater than the diel variations in P_b (Li and Dickie, 1991), the D_b:D_p ratios presented here represent on the whole conservative estimates of the bacterial activity relative to photosynthetic activity. Traditional thinking states that D_b is satisfied for most part by organic nitrogen forms

(Williams, 1981). What then are the sources and concentrations of organic nitrogen available to bacteria?

Dissolved free amino acids (DFAA) which represent the predominant organic nitrogen form for bacterial growth (Kirchman and Hodson, 1986) are usually present at low concentrations (Fuhrman, 1987): 1-15 nM in coastal waters (Fuhrman and Ferguson, 1986) and <0.5 nM in offshore waters (Fuhrman, 1987). At these typical natural concentrations of DFAA bacterial uptake rates do not often reach saturation (Fuhrman, 1987; Jørgensen and Søndergaard, 1984; Fuhrman and Ferguson, 1986). Fuhrman (1987) estimated in the case of alanine, one of the highest concentrations of DFAA, that although turnover rates were high (<0.5 to 5 h^{-1}) this DFAA only supported 25% of the bacterial nitrogen demand. Wheeler and Kirchman (1986) reported, based on values reported in the literature, that bacterial production rates were substantially higher than bacterial uptake rates of DFAA, indicating that bacteria need inorganic nitrogen forms as well. These observations suggest that bacteria will normally require inorganic nitrogen in addition to organic nitrogen forms to fulfill their growth requirements (Wheeler and Kirchman, 1986). Studies of pure cultures of marine bacteria have shown that bacteria can indeed metabolize inorganic nitrogen, principally NH_4^+ but also NO_3^- because they have the enzymes required to assimilate both nitrogen sources (Brown *et al.*, 1972; Brown *et al.*, 1975; Hoch *et al.*, 1992). It seems that bacteria would prefer to use preexisting amino acids to synthesize proteins, due to the lower energy cost involved, rather than inorganic nitrogen which would first have to be synthesized into amino acids (Kirchman *et al.*, 1985; Kirchman and Hodson, 1986). Given the relatively high turnover rates of DFAA measured (Fuhrman, 1987; Fuhrman and Ferguson, 1986), bacteria may completely exhaust the sources of organic nitrogen before turning to inorganic nitrogen.

How important is bacterial uptake of inorganic nitrogen then? The values of P_b:P_p and D_b:D_p suggest that bacterial uptake of nitrogen is important but do not reveal the amount of inorganic nitrogen taken up by bacteria. To address this question, we consider estimates of U_b, the uptake rates of inorganic nitrogen by bacteria.

3.4.2. Bacterial uptake rates of inorganic nitrogen (U_b)

Bacterial uptake rates of inorganic nitrogen represented a substantial fraction of the total microbial uptake of inorganic nitrogen (Table 3.2) both in oligotrophic and upwelling waters. Bacterial uptake rates compiled from literature (Table 3.3) show that the range of reported values is large. To some extent, this may be due to the variety of methods used and the different experimental conditions applied *e.g.* time of incubation, depths sampled, integrated values over water column versus individual depth samples. But given the spatial and temporal variability in bacterial substrate concentration, such as DFAA, DCAA (dissolved combined amino acids), or DON (dissolved organic nitrogen), and in the type of substrate used by bacteria, the different estimates reported in Table 3.3 may in fact represent real differences in U_b. Results from the present study (~60% uncorrected), and from Harrison *et al.* (1992) and Laws *et al.* (1985) were generally higher (40-75%) than previous estimates using different methods (≤50%, Table 3.3). Note that the method used in the present study is the only one that gives estimates of U_b based on incubations of an entire community, in close to natural conditions. Interestingly, both the studies based on the method of DiTullio and Laws (1983) and Laws *et al.* (1985) yield similar results. This suggests that the bacterial contribution to the uptake of inorganic nitrogen is indeed greater than 50% in this study. However, the uncertainties associated with the various methods used cannot be disregarded.

Table 3.3 Bacterial contributions (%) to the uptake of ammonium, nitrate, and dissolved free aminoacids (DFAA) reported in the literature, including estimates from the present study.

Location	Reference	Relative contribution of bacteria to uptake of:			
		NH_4^+	NO_3^-	$NH_4^+ + NO_3^-$	NH_4^+ + DFAA
Sapelo Island, off coast of Georgia (U.S.A)	Wheeler and Kirchman, 1986	78			
Long Island Sound, New York	Fuhrman *et al.*, 1988	<30			
Subarctic Pacific and Delaware Estuary	Keil and Kirchman, 1991				>90
Subarctic Pacific	Kirchman *et al.*,1989	44-47			
Seawater cultures	Tupas and Koike, 1990	50-88			
Long Island Sound, New York	Suttle *et al.*, 1990	25-50			
Antarctic waters	Tupas *et al.*, 1994	8-25			
North Atlantic (47°N, 20°W) during spring bloom	Kirchman *et al.*, 1994	22-39	4-14		
Hawaii Islands (offshore and coastal)	Laws *et al.*, 1985	50-75*			
Oligotrophic North East Pacific	Harrison *et al.*, 1992			40*	
North Atlantic, 43°-28°N and 54°-10°W)	Present study			60* (25* after correction) in oligotrophic waters 63* (23* after correction) in upwelling and slope waters	

* Methods of DiTullio and Laws (1983) and Laws *et al.* (1985).

3.4.3. Previous studies of bacterial uptake of inorganic nitrogen

Previous studies of U_b in natural communities and in seawater cultures are based on the assumptions that (1) phytoplankton would not take up inorganic nitrogen in the dark or that (2) the small size-fractions (from <1 μm and smaller) which contain all heterotrophic bacteria would not comprise any significant fraction of photosynthetic cells (Table 3.4). The validity of the first assumption is challenged by the fact that phytoplankton have been shown to take up inorganic nitrogen (NH_4^+ and NO_3^-) under dark conditions (MacIsaac and Dugdale, 1972; Nelson and Conway, 1979; see also Martin-Jézéquel, 1992 for axenic culture study). This dark uptake by phytoplankton is not a constant and may vary with the nitrogen form taken up, with the water type, with the depth at which the phytoplankton were sampled, and with the species composition of the community (Cochlan *et al*, 1991a, b). Note that in the typical N-I experiments (nitrogen uptake rates measured at different levels of irradiance) such as carried out by Cochlan *et al.* (1991 a, b), measurements of dark uptake by phytoplankton may in fact include a bacterial uptake which can result in biased values of phytoplankton uptake under dark conditions; this renders measurements of dark uptake of nitrogen difficult to interpret

When size-fractionation is used to separate heterotrophic bacteria from phytoplankton cells (Table 3.4), it is important to estimate the fraction of photosynthetic biomass in the filtrate. Such measurements (chlorophyll *a*) have been made in many of the studies (Harrison and Wood, 1988; Fuhrman *et al.*, 1988; Kirchman *et al.*, 1989; Kirchman *et al.*, 1990; Kirchman *et al.*, 1991; Keil and Kirchman, 1991; Kirchman *et al*, 1992; Tupas *et al*, 1994; Tupas and Koike, 1991). In most cases, chlorophyll *a* represented ≤10% of the unfractionated chlorophyll *a*, except for the studies of Harrison and Wood (1988) and Kirchman *et al.* (1994) where chlorophyll *a* measurements were higher. However, in waters where picophytoplankton dominate the photosynthetic biomass

Table 3.4 Summary of the methods used for estimating non-photosynthetic uptake of inorganic nitrogen compiled from the literature.

Type of experiment			Measurement of bacterial uptake	Assumptions used to separate bacteria from phytoplankton	Potential problems	References
Field / culture	Light / dark incubation	Use of tracers, size-fractionation				
Field	Light	$^{15}NH_4^+$, ^{14}C	$^{15}NH_4^+$ uptake when ^{14}C uptake=0	N uptake by phytoplankton is light dependent	May include photosynthetic uptake of NH_4^+ in dark	Eppley *et al.* (1977)
Continuous seawater culture	Dark	Prescreened (<1 μm)	ΔNH_4^+ and ΔNO_3^- over time	N uptake by phytoplankton is light dependent Size	May include photosynthetic uptake of NH_4^+ and NO_3^- in dark	Horrigan *et al.* (1988)
Field	Light	$^{15}NH_4^+$, postincubation filtered (1μm)	$^{15}NH_4^+$ uptake when ^{14}C uptake is low	Size	*idem*	Harrison and Wood (1988)
Field	Light	$^{13}NH_4^+$, prescreened (0.2-0.6μm)	$^{13}NH_4^+$ uptake	Size	May include photosynthetic uptake of NH_4^+ Exclude any interaction with organisms >0.6μm during incubation	Fuhrman *et al.*, (1988)
Field	Light	$^{15}NH_4^+$, postfiltered (1μm)	$^{15}NH_4^+$ uptake	Size	May include photosynthetic uptake of NH_4^+	Kirchman *et al.* (1989)

Table 3.4 cont.

Field	Light	$^{13}NH_4^+$; postfiltered (1μm)	$^{13}NH_4^+$ uptake	Size	May include photosynthetic uptake of NH_4^+	Suttle *et al.* (1990)
Field	Light	$^{15}NH_4^+$, $^{15}NO_3^-$ postincubation filtered (0.8μm)	$^{15}NH_4^+$ uptake, $^{15}NO_3^-$ uptake corrected for photosynthetic uptake assuming relative N uptake <0.8μm is equal to relative chlorophyll <0.8μm; also corrected by using phytoplankton nitrogen demand (DiTullio & Laws, 1983)	Size	Chlorophyll biomass is not directly proportional to production Assigning equal importance to NO_3^- uptake and NH_4^+ uptake by phytoplankton	Kirchman *et al.* (1994)
Seawater batch cultures	Dark	$^{15}NH_4^+$; prescreened (GF/F filter)	$^{15}NH_4^+$ uptake	N uptake by phytoplankton is light dependent; Size	Exclude any interaction with organisms >0.7μm Comparable to field bacteria? May include photosynthetic uptake in dark	Tupas & Koike (1990)

Table 3.4 cont.

Seawater cultures	Dark	$^{15}NH_4^+$, $^{15}NO_3^-$; prescreened (GF/C filter)	$^{15}NH_4^+$ uptake, $^{15}NO_3^-$ uptake	N uptake by phytoplankton is light dependent; Size	Exclude any interaction with organisms >0.7-1.0µm Comparable to field bacteria? May include photosynthetic uptake in dark	Tupas and Koike (1991)
Seawater batch culture	Dark	Prescreened (1µm)	ΔNH_4^+ and ΔNO_3^- over time	N uptake by phytoplankton is light dependent; Size	Exclude any interaction with organisms >1µm May include photosynthetic uptake in dark Comparable to field bacteria?	Kroer *et al.* (1994); Jørgensen *et al.* (1994)

Table 3.4 cont.

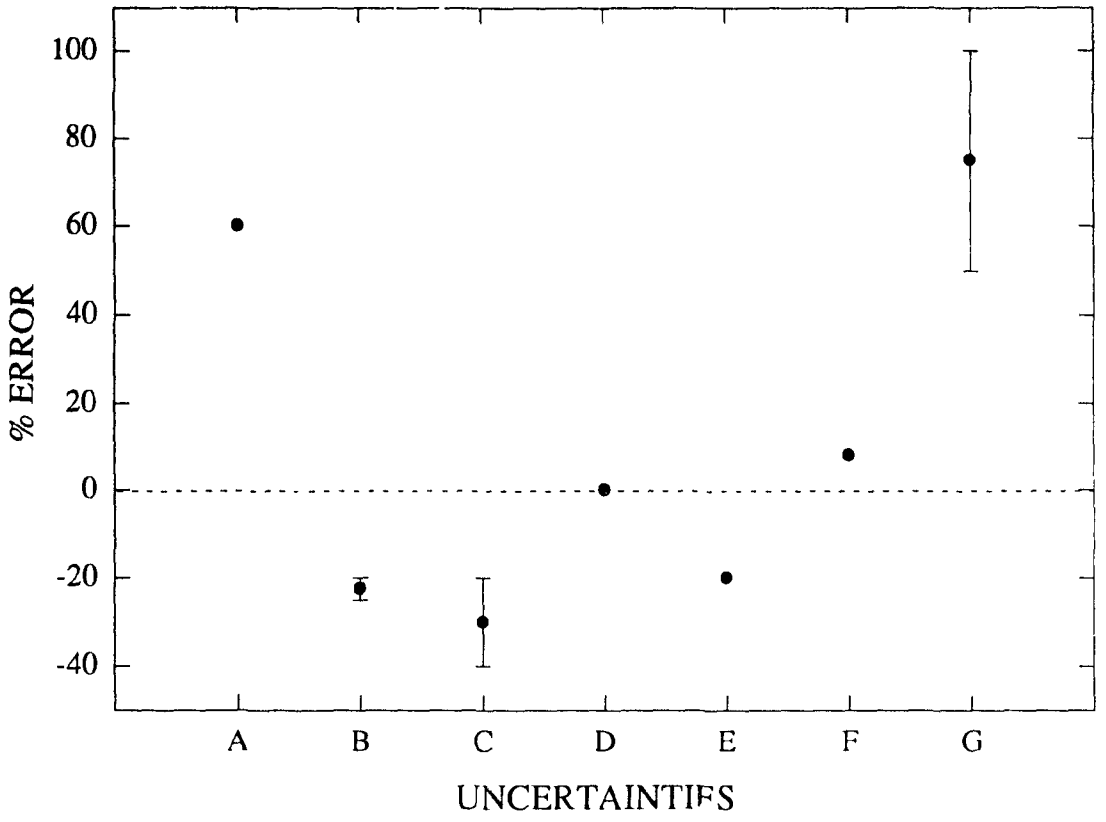
Field	Dark	Prescreened (0.8μm)	ΔNH_4^+ and ΔNO_3^- over time	N uptake by phytoplankton is light dependent; Size	May include photosynthetic uptake in dark Exclude any interaction with organisms >0.8μm	Kirchman *et al.* (1992); Kirchman *et al.* (1991)
			ΔNH_4^+ , ΔNO_3^- , ΔNO_2^- over time			Keil and Kirchman (1991)
			ΔNH_4^+ over time			Kirchman *et al.* (1989); Kirchman *et al.* (1990); Tupas *et al.* (1994)
Field	Light	$^{15}NH_4^+$, $^{15}NO_3$; prescreened (1μm) and treated with specific inhibitors of protein synthesis for prokaryotes and eukaryotes	$^{15}NH_4^+$ uptake, $^{15}NO_3^-$ uptake by prokaryotes	Prokaryote/ Eukaryote; Size	May include uptake by photosynthetic prokaryotes such as cyanobacteria and prochlorophytes	Wheeler and Kirchman (1986)

such as open-ocean waters (Li *et al.*, 1983; Harrison and Wood, 1988; the present study) or equatorial waters (Chavez, 1989), size-fractionation may be inappropriate. In these waters, phytoplankton <1 μm usually account for 70-80% of the total chlorophyll *a*, and therefore this technique may not result in an effective separation of the two communities. In addition, size-fractionation was made before incubation in many of the previous studies (Fuhrman *et al.*, 1988; Kirchman *et al.*, 1992; Keil and Kirchman, 1991; Kirchman *et al.*, 1989; Kirchman *et al.*, 1990; Tupas *et al.*, 1994; Kirchman *et al.*, 1991; Wheeler and Kirchman, 1986). This prescreening results in measuring uptake only on a portion of the initial community, thus eliminating important interactions within the natural community (Glibert *et al.*, 1992; Cole *et al.*, 1982; Bratbak and Thingstad, 1986; Stone, 1990).

3.4.4. Evaluation of the present method used to estimate U_b

The method used in the present study (DiTullio and Laws, 1983; Laws *et al.*, 1985) enables one to differentiate between bacterial and photosynthetic uptake of nitrogen without using size-fractionation or dark incubations as in most previous studies. The uncertainties associated with this method (Fig. 3.4) are discussed in detail in Appendix 2 and comprise uncertainties in the values of U_p (see equation (1)) and U_t (see equation (2)). The errors in U_p are associated with (a) diel periodicity of protein synthesis, (b) uncoupling of carbon and nitrogen uptake by phytoplankton, and (c) deviation from the assumption that most of the nitrogen taken up by phytoplankton goes into proteins.

By not taking into account the diel periodicity in protein synthesis, U_p may be overestimated by as much as 60% (Fig. 3.4). This appears to be the main error in the estimation of U_p. Uncertainties in the estimates of U_b, on the other hand, are mainly associated with uncertainties in the measurements of U_t (see following section and Appendix 3); these values can be overestimated by as much as 90% leading to values of U_b that are overestimated by 80% on average.



Relative error in U_P (*) and in U_b (**) due to:

A. Diel periodicity of protein synthesis (*)
B. Diel periodicity of protein synthesis (**) in oligotrophic waters
C. Diel periodicity of protein synthesis (**) in upwelling waters
D. Uncoupling of C and N uptake by phytoplankton (* and **)
E. < 85% of N uptake goes into proteins (*)
F. < 85% of N uptake goes into proteins (**)
G. Uncertainties in U_t (**)

Fig. 3.4 Relative errors in the estimates of U_p and U_b due to uncertainties associated with diel periodicity in protein synthesis, uncoupling of C and N uptake, the case in which <85% of the N uptake goes into proteins, and the measurement of U_t. The estimation of the relative errors in U_p and U_b is detailed in Appendix 2 and 3 (see also Discussion). Error bars indicate the range of uncertainty.

3.4.5. Comparison between estimates of U_b and D_b

In order to evaluate the method of DiTullio and Laws (1983) and Laws *et al.* (1985), the values of U_b obtained using this method were compared with estimates of D_b. Estimates of D_b comprise both organic and inorganic nitrogen whereas estimates of U_b include inorganic nitrogen only ($NH_4^+ + NO_3^-$); therefore D_b should be equal or greater than U_b. At the majority of the stations sampled, however, values of U_b were significantly higher than D_b (Figs. 3.3 a, b). This discrepancy may be explained by (1) an underestimation in D_b , or (2) an overestimation in U_b, or both.

(1) Measurements of bacterial production (based on leucine and thymidine incorporation rates) were within the lower range of previous measurements reported for open-ocean areas (see review of Ducklow and Carlson, 1992). Given that the conversion factors used (kgC $mole^{-1}$ of leucine or thymidine) can vary by almost an order of magnitude from one study to another (Ducklow and Carlson, 1992; Robarts and Zohary, 1993), they can have a significant effect on computed bacterial production rates. However, this variation is not sufficient to explain all the discrepancy observed between D_b and U_b in the present study because the maximum estimates of D_b (using the higher conversion factors available in the literature) were still lower than the corresponding values of U_b. If values of D_b are corrected (increased) so that 50% of the bacterial nitrogen demand is satisfied by inorganic nitrogen (range of 26-53% reported by Kirchman *et al.*, 1994) then the resulting values of bacterial production rates would range from 0.1 to 1.6 $\mu g\ C\ L^{-1}\ h^{-1}$, and the P_b:P_p ratios would range from 0.2 to over 1, which are too high compared with previous studies (Cole *et al.*, 1988). This suggests that a potential bias in D_b towards lower values would not have been sufficient to account for the discrepancy observed between U_b and D_b.

(2) A bias in U_b towards higher values could have resulted from an underestimation in U_p and/or an overestimation in U_t. Only at the upwelling stations could U_p have been underestimated (Fig. 3.4) resulting in values of U_b that were overestimated by <1% up to 20%. Regarding uncertainties in the values of U_t, these include uncertainties in (i) the measurements of ambient NH_4^+, (ii) the measurements of PON and atom enrichment, (iii) the isotope enrichment, (iv) the isotope dilution, and are estimated in Appendix 3.

In summary, the uncertainties associated with measurements of ambient ammonium and with isotope enrichment result in a significant overestimation of U_t which in turn can account for the discrepancy observed between U_b and D_b. The bacterial contribution to uptake of inorganic nitrogen is, on average, after correction, 25% (Appendix 3) in oligotrophic waters (15±5% for the mixed layer and 36±25% for the DCM layer) and 23% in upwelling waters (10±1% for the mixed layer and 34±24% for the DCM layer). As in the case of the uncorrected estimates, no significant difference was observed between the mixed layer and the DCM layer for the corrected estimates due to the high standard deviation. Although initial values of U_t were decreased significantly, the resulting C:N assimilation ratios were not unreasonably high: 6.5 for the mixed layer and 5.4 for the DCM layer. Note that a number of stations were eliminated for the mixed layer depth (# 28, 51, 82, 85) and for the DCM layer depth (#6, 28, 51, 82, 85); these stations corresponded to samples where U_b could not be computed, and are all upwelling and slope water stations except one (#6). The corresponding C:N ratios for uncorrected values of U_t were 8.8 in the mixed layer and 5.9 in the DCM layer (excluding station #61 as an outlier). In the study of Kirchman *et al.* (1994) and in the present study, where an independent estimate was used for comparison and a correction was applied, the estimates are similar, even though the methods used are different. Corrected values of U_b were also lower than previous estimates based on the same method (Laws *et al.*, 1985; Harrison *et al.*, 1992). This suggests that previous estimates which have not been corrected are probably too high.

In the previous studies based on ^{15}N uptake measurements (Tables 3.3, 3.4) and in the present study, microbial cells (heterotrophic bacteria or picophytoplankton) may have been lost through the glass-fiber filters resulting in an underestimate of the ^{15}N uptake rates. These losses have been estimated to be at least 30% for bacteria (Lee and Fuhrman, 1987; Lee *et al.*, 1995), and up to 35% (Taguchi and Laws, 1988) or insignificant for phytoplankton cells (Chavez *et al.*, 1995). In the present study, the microbial fraction which passed through the glass-fiber filters used was estimated by counting picophytoplankton (by flow cytometry) and bacteria (by DAPI technique) collected in the filtrate (Chapter 1). On average, only 2% and 6% of the picophytoplankton cells were lost through glass-fiber filters in oligotrophic and upwelling waters, respectively. On the other hand, bacterial losses averaged 30% (±13%) over a number of transect stations including those located in slope waters (stations # 58-85). These results suggest that values of U_b could have been underestimated by at most 30% assuming that all bacterial cells counted were taking up inorganic nitrogen. This error is low in comparison with the bias in U_b due to uncertainties in U_t (Fig. 3.4, Appendix 3).

3.4.6. Implications for phytoplankton-bacteria interactions

Heterotrophic bacteria are able to grow efficiently in low-substrate environments (Wiebe, 1984; Azam and Fuhrman, 1984), and have also been reported to have a higher substrate affinity for phosphorus than phytoplankton (Currie and Kalff, 1984). As a result, bacteria have the capability to out-compete phytoplankton for the uptake of inorganic phosphate (Brown *et al.*, 1981; Currie and Kalff, 1984), nitrate (Parker *et al.*, 1975; Parsons *et al.*, 1981) and ammonium (Horstmann and Hoppe, 1981). Because inorganic nitrogen forms such as ammonium and nitrate are usually present at nanomolar levels in oligotrophic waters (Garside, 1985; Brzezinski, 1988; Eppley *et al.*, 1990), bacteria should out-compete phytoplankton for inorganic nitrogen in nutrient-depleted waters. In the

present study, however, bacteria only contribute ~25% of the total uptake of inorganic nitrogen and values of bacterial biomass and production are relatively low compared with phytoplankton. This may suggest that the limiting element for bacterial growth is not the nitrogen source, as for phytoplankton, but rather the available organic carbon (Williams, 1981; Azam *et al.*, 1983). Also, previous studies which report a competitive advantage for bacteria are based on microbial communities where phytoplankton species are substantially larger in size than bacteria (Parker *et al.*, 1975; Horstmann and Hoppe, 1981) or based on culture studies (Currie and Kalff, 1984; Parsons *et al.*, 1981). In the present study, however, the photosynthetic community of oligotrophic waters was dominated by picophytoplankton, which are close in size to bacterial cells and therefore have a similarly high surface-to-volume ratio (S/V ratio). Given that the influx of nutrients by cell surface increases with an increase in the S/V ratio (Raven, 1986), bacteria and picophytoplankton are both at an advantage in the uptake of nutrients compared with larger cells. My estimates of bacterial contribution to inorganic nitrogen uptake therefore suggest that bacteria may not necessarily out-compete the phytoplankton for inorganic nitrogen. This was supported by a kinetic study carried out during the same cruise (Harrison *et al.*, 1996): values of the half-saturation constant (K_S) for nitrate and ammonium uptake were in the same range as ambient concentrations of nitrate and ammonium. This suggests that phytoplankton could adapt to low concentrations of inorganic nitrogen, assuming that mainly phytoplankton are taking up nitrogen during incubation. Koike *et al.* (1983) reported K_S values of 3 nM for a marine microflagellate in the oligotrophic Pacific. This implies that picophytoplankton cells may possess similar competitive advantages to bacteria for the uptake of nutrients. Moreover, picophytoplankton cells albeit light-limited at deeper depths, *e.g.* in the DCM layer, can still grow by fixing inorganic carbon efficiently at low-light levels (Li *et al.*, 1983; Glover *et al.*, 1987; Moore *et al.*, 1995). Bacteria, on the other hand, remain limited by the supply of organic carbon (Williams, 1981; Azam *et al.*, 1983) and therefore ultimately dependent on phytoplankton production.

3.4.7. Implications for new and regenerated production

Given that new and regenerated production are most often estimated by the uptake of $^{15}NO_3^-$ and $^{15}NH_4^+$, respectively (Dugdale and Goering, 1967; Eppley and Peterson, 1979; Platt *et al.*, 1992), how will these estimates be affected by a bacterial uptake of nitrate and ammonium? Dugdale and Goering (1967) originally defined new and regenerated production as an autotrophic process, assuming that bacteria take up no inorganic nitrogen. However, as shown in the present study, bacteria can contribute significantly to the uptake of inorganic nitrogen and therefore, new and regenerated production correspond to the total microbial uptake of $^{15}NO_3^-$ and $^{15}NH_4^+$, respectively (Fig. 3.5). New and regenerated production also imply the synthesis of organic carbon from CO_2 in photosynthesis, the sum of new and regenerated production representing total primary production. Since heterotrophic bacteria require presynthesized organic carbon, bacterial uptake of nitrate and ammonium is not associated with any synthesis of organic carbon from dissolved inorganic carbon. This implies that the fraction of nitrate which is consumed by bacteria does not contribute to carbon-based new production. If the bacterial u ake of NO_3^- was not accounted for, calculation of carbon-based new production may lead to overestimates, under steady-state conditions, since the Redfield ratio (6.6) routinely used to convert nitrate uptake to carbon uptake is higher than the bacterial C:N ratio (4-5). Therefore, new production as measured by $^{15}NO_3^-$ uptake should be corrected for bacterial uptake of $^{15}NO_3^-$ before conversion into carbon production. Moreover, the C:N ratio to be used should also take into account any variation in C:N ratio within the phytoplankton community. For example, when phytoplankton species are richer in nitrogen, as shown for cultured *Synechococcus* (Kana and Glibert, 1987), the C:N ratio is likely to be lower than the Redfield proportions; this may also apply for prochlorophytes, prokaryotic cells which are similar to *Synechococcus*, and thus may have lower C:N ratios as well.

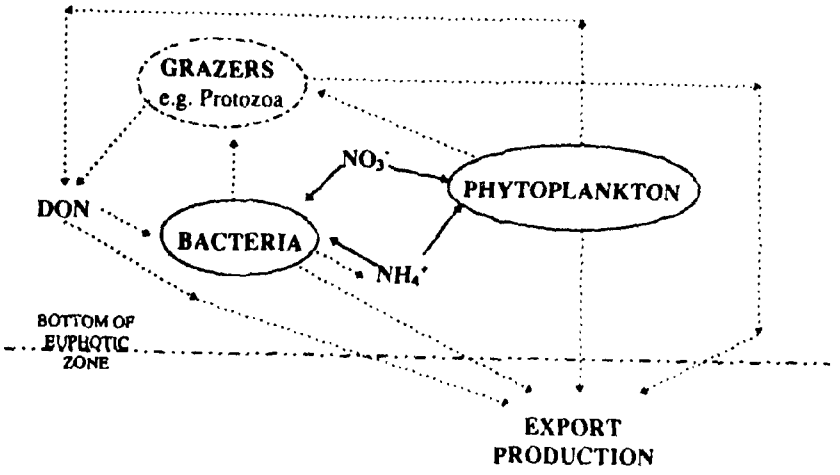


Fig. 3.5 Major pathways of N in the upper water column showing the production that is exported out of the euphotic layer. Nitrogen uptake, which is measured by the uptake of $^{15}NO_3^-$ and $^{15}NH_4^+$, is represented by solid lines and includes both phytoplankton and bacterial uptake. Pathways not estimated in the present study are represented by dashed lines. New production is the total microbial uptake of nitrate under the assumptions that 1) NO_3^- represents new inputs only, *i.e.*, does not include NO_3^- produced by nitrification within the euphotic zone, and 2) other external sources of nitrogen such as nitrogen fixation are not significant. Likewise, regenerated production is the total microbial uptake of NH_4^+.

Since bacterial contribution to the uptake of inorganic nitrogen is estimated to be ~25% in the present study, what is the resulting error in new production? Based on an error analysis of f, and assuming a partitioning of U_b into 90% of ammonium and 10% of nitrate (Appendix 4, Wheeler and Kirchman, 1986; Kirchman *et al.*, 1992, 1994), the error in f, E_f/f, was ~10%. This error is smaller than the spatial variation observed in f across the transect. Moreover, in comparison to the range of f-ratios observed across different open-ocean waters (Dugdale and Wilkerson, 1992) and across different eutrophic areas, E_f/f is low. It remains that an error of 10% in f can make a significant change in the absolute value of f, and in turn for the calculation of carbon-based new production.

Assuming, as for the analysis of errors, that U_b was partitioned into 90% of ammonium and 10% of nitrate, the uncorrected values of new production ($^{15}NO_3^-$ uptake rates) were overestimated on average by 27% in oligotrophic waters and by 9% in upwelling waters compared with values corrected for bacterial uptake. Initial values of regenerated production ($^{15}NH_4^+$ uptake rates), on the other hand, were overestimated on average by 28% in oligotrophic and upwelling waters. These uncertainties are lower than some of the methodological uncertainties associated with the ^{15}N-technique (Appendix 3), for example measurement of ambient ammonium and isotope enrichment. This suggests that we cannot determine the extent of the bias due to $U_{b(a)}$ and $U_{b(n)}$. However, with recent improvements in the ^{15}N methodology (McCarthy *et al.*, in press), particularly the measurement of low level nitrate and ammonium, these uncertainties are significantly decreased, and we would therefore be able to determine the extent of this bias.

In comparing f-ratios of different size-fractions (Chapter 2), the relative error in f is higher for f-ratios of the <2-μm fraction compared with f-ratios of the >2-μm fraction since the bulk of bacterial biomass is contained in the <2-μm fraction. This may introduce a bias when comparing the importance of small versus large cells in new production because the

contribution of small cells to new production would be overestimated. However, given that in the present study, Ef/f was small, the comparison between the <2-μm and >2-μm fractions would not have been significantly biased (Chapter 2).

3.5. Conclusion

Bacterial production (P_b) and nitrogen demand (D_b) represented a significant fraction of primary production (10-25%) and picophytoplankton nitrogen demand (20-100%), respectively, suggesting that bacteria may require a substantial fraction of the available inorganic nitrogen.

Bacterial uptake rates of inorganic nitrogen ($NO_3^- + NH_4^+$), U_b, were estimated as the difference between total microbial uptake rates of inorganic nitrogen, U_t, and picophytoplankton uptake rates of inorganic nitrogen, U_p, and represented ~60% of the total microbial uptake of inorganic nitrogen. Comparison with D_b, however, indicated that U_b was overestimated. It was concluded that an overestimation in U_t (by as much as 90%) would have contributed most to this error; this could in turn be explained by an overestimation of the ambient ammonium concentration and by the effect of uptake enhancement due to isotope enrichment. The estimates of U_b were corrected and resulted on average in 25% of the total microbial uptake of inorganic nitrogen.

In order to improve the estimation of U_b, we need to decrease the uncertainties associated with the measurements of U_t, by improving the estimates of ammonium concentration and the effect of isotope enrichment. This can be accomplished by the use of low-level techniques for measuring ambient ammonium (Brzezinski, 1988), and by applying a kinetic correction for isotope enrichment (based on nutrient kinetic experiments, Harrison *et al.*, 1996). The methods of DiTullio and Laws (1983) and Laws *et al.* (1985)

remain the only approach available so far in which the entire community is taken into account in the estimation of U_b; moreover, this estimate does not require any correction for potential phytoplankton contamination, as in the case of previous methods that are based on size-fractionation and/or dark incubations. Thus, if improvements in the measurements of ^{15}N uptake rates can be met, this approach would yield the more accurate results.

The high variability observed in U_b suggests, beyond the methodological uncertainties, real differences due to factors such as the varying concentrations of available nitrogen which in turn are controlled by phytoplankton-bacteria interactions. The fact that U_b (~25%) does not dominate the microbial uptake of inorganic nitrogen would suggest, moreover, that the phytoplankton community could compete efficiently with bacteria for inorganic nitrogen.

New and regenerated production as defined originally by Dugdale and Goering (1967) implied no bacterial uptake of nitrate and ammonium. Thus, any bacterial uptake of nitrate or ammonium should be subtracted from the total microbial uptake to compute autotrophic new and regenerated production. Bacteria take up carbon and nitrogen in different proportions from phytoplankton, implying that a bacterial uptake of nitrate could lead to the C:N ratio deviating from the Redfield ratio. This would in turn result in the overestimation of carbon new production and, over longer time scales, in the overestimation of carbon export production, when computed as nitrate uptake times the Redfield ratio. Based on the estimates of U_b in the present study and assuming a partitioning of 1:9 into nitrate and ammonium, new and regenerated production were overestimated by ~30%, and the relative error in f was ~10%. These errors are lower than some of the methodological uncertainties associated with the measurements of ^{15}N uptake. However, as the methods improve to estimate new and regenerated production using ^{15}N uptake experiments (McCarthy in press, 1995), the error due to a bacterial uptake of

inorganic nitrogen will represent one of the major uncertainties associated with the measurement of new and regenerated production.

General Discussion and Conclusions

In this thesis, I examined the biomass composition and productivity of an oligotrophic ecosystem in the subtropical North Atlantic ocean. Most of the stations sampled were located in open-ocean waters which, on a global scale, represent >80% of the world's oceans. The pelagic community consisted mainly of picoplankton (69% of total chlorophyll *a* <2 μm), and moreover, prochlorophytes and bacteria were the main contributors to the picoplankton carbon biomass (Chapter 1). A coastal upwelling site off North West Africa was also studied for comparison; coastal areas represent, on a global scale, ~10% of the world's oceans. Here, picoplankton only contributed 41% to the total chlorophyll *a* (Chapter 1). The dominance by picoplankton, both in number and biomass, of the pelagic community in oceanic waters raises the question of what the role of picoplankton is in the productivity and transfer of energy and material to the higher trophic levels.

In the last fifteen years, numerous studies have determined the contribution of picophytoplankton to primary production (Bienfang and Takahashi, 1983; Joint and Pomroy, 1983; Li *et al.*, 1983; Platt *et al.*, 1983; Takahashi and Bienfang, 1983). Concomitantly, other studies have examined the role of heterotrophic bacteria in the transformation of organic matter produced (Williams, 1981; see review of Fuhrman, 1992), leading to the important concept of the microbial loop in which bacteria utilize the dissolved organic matter produced by phytoplankton, and can compete with phytoplankton for mineral nutrients (Azam *et al.*, 1983). More recently, studies have examined the link between the microbial food web and higher trophic levels, focussing on the predators of picophytoplankton and bacteria (Fenchel, 1982; Caron, 1991). For example, Caron and coworkers (Caron *et al.*, 1988; Caron, 1991; Caron *et al.*, 1991) found that the microbial loop can transfer part of the primary production to higher trophic levels through an effective grazing by protozoa. Under the steady-state assumption and

over the proper time scales, the fraction of primary production which is available for the higher trophic levels, *i.e.* export production, is equal to new production. Regenerated production, on the other hand, represent the fraction of primary production which is recycled within the photic zone. In this thesis, the main objective was to assess the contribution of picoplankton to new and regenerated production in the <2-μm fraction, taking into account the photosynthetic picoplankton and the heterotrophic bacteria.

Picoplankton are usually the main contributors to primary production in open-ocean oligotrophic waters (Li *et al.*, 1983; Joint and Pomroy, 1983; Platt *et al.*, 1983; Iturriaga and Mitchell, 1986). Given the extension of these areas over the world's oceans (>80%), picoplankton must therefore account for a significant fraction of the global primary production. New production represents a small fraction of total primary production in oligotrophic waters; however, because these waters cover such a large area of the world's oceans, picoplankton are also likely to be significant contributors to new production on a global scale. In support of this hypothesis, I showed that picoplankton were the main contributors to new and regenerated production at the oligotrophic stations, and that they accounted for a significant fraction at the upwelling stations. Previous studies, however, have argued that small cells (*e.g.* nanoplankton, ultraplankton, or picoplankton) contribute mainly to regenerated production (Malone, 1980; Probyn, 1985; Probyn and Painting, 1985; Legendre and Lefèvre, 1989), whereas larger cells account for most of the new production, *i.e.* new and regenerated production would be size-dependent. In this thesis, I clearly showed that picoplankton can contribute equally, compared with the larger cells, to new and regenerated production (Chapter 2).

The data set in the present study covers a large area and could be extrapolated to an entire basin in the subtropical North Atlantic, also including an upwelling area. Therefore, I extrapolated my results of size-fractionated (<2 μm) primary production and

f-ratios to make a first order estimate of global values of primary production and new production, using the most recent global estimates of primary production from remote sensing of ocean colour (Longhurst *et al.*, 1995). These estimates are shown below, together with other previous global estimates of primary production and new production. Picoplankton production represented a significant fraction of the global

Global primary production (Gt C y^{-1})	Global new production (Gt C y^{-1})	Method of estimation	Reference
50.1	5.0*	Remote sensing; partitioning by biogeochemical domains	Longhurst *et al.* (1995)
50.3	5.0*	Remote sensing; partitioning by oceans	Longhurst *et al.* (1995)
51.0	7.4	^{14}C assimilation; sediment traps	Martin *et al.* (1987)
19.1	3.4	^{14}C and $^{15}NO_3^-$ assimilation (polar oceans not included)	Eppley and Peterson (1979)
27.5	4.9	Size-fractionated C uptake and *f* applied to estimates of Longhurst *et al.* (1995) by biogeochemical domains	Present study
28.4	3.7	Size-fractionated C uptake and *f* applied to estimates of Longhurst *et al.* (1995) by oceans	Present study

* Assuming new production is 10% of primary production.

primary production and picoplankton accounted for most of the global new production. Given that new production estimates in the present study were comparable with previous estimates of export production (Chapter 2), this suggests that picoplankton could account for a significant fraction of the export production. Although mechanisms for exporting these small cells have been reported in the literature, recycling losses associated with the export of picoplankton may be significant. Therefore, the question remains of how these small cells get exported out of the photic zone with high efficiencies (Chapter 2).

Picoplankton were shown to take up nitrogen at higher rates (biomass-normalised and specific) than the larger cells (Chapter 2), suggesting that they are more efficient in the uptake of nitrogen (in terms of μg-at N L^{-1} h^{-1}) than the larger size-fraction. This can

be explained by their higher surface-to-volume ratio, and their capacity to adapt to nutrient-depleted conditions (*e.g.* by having a lower half-saturation constant, K_S), as well as to nutrient-rich areas (*e.g.* upwelling site). Within the picoplankton community, it was suggested that prochlorophytes would account for most of the new and regenerated production at the oligotrophic stations (Chapter 2), raising the question of whether prochlorophytes are better adapted for nutrient uptake in these waters than cyanobacteria or picoeukaryotes. Because nutrient uptake by phytoplankton cells is usually controlled by molecular diffusion processes (Raven, 1986), which in turn depend on the relative surface area of the cell, the diffusion of nutrients through the cell membrane may be facilitated for prochlorophytes due to their higher relative surface area, compared with the two larger picophytoplankton groups.

Over a large area of the subtropical North Atlantic, I showed that heterotrophic bacteria take up a significant fraction of inorganic nitrogen (~25%) relative to phytoplankton, and furthermore that their relative contribution did not vary significantly between oligotrophic waters and upwelling waters. Based on these open-ocean measurements, I therefore concluded that the ^{15}N uptake measurement is not exclusively an autotrophic measurement, as was assumed implicitly by Dugdale and Goering (1967). Therefore, it is important to estimate the contribution of bacteria to the total uptake of inorganic nitrogen, when measuring the uptake of ^{15}N.

Routine estimates of bacterial activity (using tracers) to be carried out were those of bacterial production, *i.e.* bacterial consumption of organic carbon (Fuhrman and Azam, 1980; Kirchman *et al.*, 1985), where it was shown to represent a significant fraction of phytoplankton production (see review by Cole *et al.*, 1988). It was later argued that, to sustain this level of bacterial production, bacteria needed inorganic nitrogen sources in addition to organic nitrogen (Wheeler and Kirchman, 1986; Kirchman *et al.*, 1989). In

this thesis, I estimated bacterial uptake rates of inorganic nitrogen as the difference between ^{15}N uptake rates ($^{15}NH_4^+ + {}^{15}NO_3^-$) and autotrophic uptake rates of nitrogen (DiTullio and Laws, 1983; Laws *et al.*, 1985). The estimates were made on unfractionated water under light conditions, thus taking into account potential interactions within the community. Moreover, these estimates were compared with bacterial production rates which had been converted into values of bacterial nitrogen demand (assuming balanced growth). Based on the discrepancy found, I concluded that the estimates of bacterial uptake rates had been overestimated. The corrected bacterial uptake rates were lower than most of the previous uncorrected estimates, however, they were similar to recent estimates from Kirchman *et al.* (1994) which had also been corrected for, based on a comparison with an independent estimate. This suggests that many of the previous estimates had probably been overestimated.

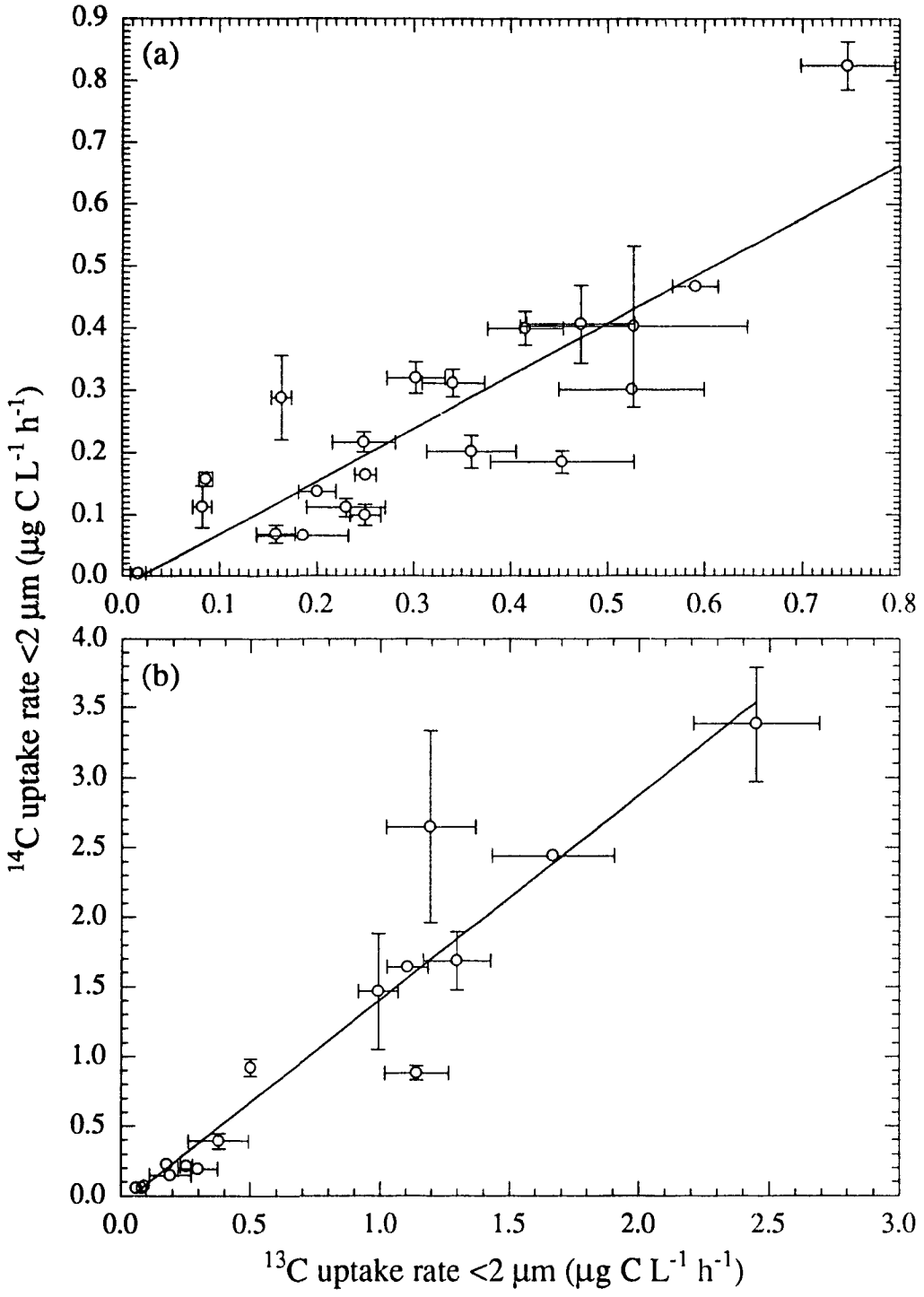
Because the ^{15}N uptake measurement includes a bacterial uptake, and because the bulk of bacteria is in the <2-μm fraction, the contribution of picoplankton to new and regenerated production may be systematically overestimated. In the present study, I estimate that $^{15}NO_3^-$ uptake rates and $^{15}NH_4^+$ uptake rates in the <2-μm fraction were overestimated by as much as 30%. This error is smaller than some of the current uncertainties associated with the ^{15}N measurement. Moreover, after correction, the contribution of picoplankton to new and regenerated production is still ~60% at the oligotrophic stations. Thus, the main conclusions of Chapter 2, that picoplankton are the major contributors to new and regenerated production, remain unchanged when the bacterial uptake of inorganic nitrogen is taken into account.

A bacterial uptake of inorganic nitrogen also has important implications for the carbon flux studies. The operational definition of new and regenerated production (Dugdale and Goering, 1967) is based on the assumption that only phytoplankton take up

inorganic nitrogen. By not correcting the new production estimates (defined as the autotrophic uptake of NO_3^-) for a bacterial uptake, this could lead to the C:N assimilation ratio deviating from Redfield ratio towards lower values. Consequently, carbon-based new production would be overestimated when calculated as the product of nitrate uptake and the Redfield ratio. In order to assess this error, an analysis of error was performed on the *f*-ratio (Chapter 3). Based on the estimates of bacterial uptake rates and assuming a partitioning into 90% of ammonium and 10% of nitrate, the error on *f* was ~10%. This error is small in comparison with some of the methodological uncertainties of the ^{15}N-technique. However, with the recent improvements in the ^{15}Nmeasurement, the error associated with a bacterial uptake of inorganic nitrogen is likely to remain one of the major hurdles in the estimation of new and regenerated production.

Appendices

Appendix 1. ^{14}C uptake rates versus ^{13}C uptake rates in the <2-μm fraction for oligotrophic waters (a), and for upwelling and slope waters (b). R^2=0.86, slope=0.92 and R^2=0.95, slope=1.50, respectively (Model II regression).



Appendix 2: Potential errors in U_p

The estimates of U_p are associated with potential errors due to (a) diel periodicity of protein synthesis, (b) uncoupling between carbon and nitrogen uptake, and (c) to deviation from the assumption that 85% of the nitrogen taken up goes into proteins.

(a) Protein synthesis can show diel periodicity *i.e.* non-linearity of the uptake of ^{14}C into the protein fraction (Morris & Skea, 1978) and could therefore result in higher hourly values of ^{14}C uptake when measured over 3 hours compared with measurements over 24 hours (DiTullio & Laws, 1983). In the present study, this implies higher values (see equation 1) of U_p and consequently lower values (see equation 2) of U_b. Previous studies carried out in cultures and in nutrient-rich waters have shown a continuous incorporation of ^{14}C into proteins at night (Morris & Skea, 1978; Cuhel *et al.*, 1984) or a higher incorporation at low light levels (Morris *et al.*, 1974; Li & Platt, 1982; Lignell & Lindqvist, 1992); this suggests that protein synthesis is maintained during the dark period, sometimes by utilization of carbon that is stored in the polysaccharide fraction (Barlow, 1984). Smith & D'Souza (1993), on the other hand, showed for the northeastern Sargasso sea that rates of protein synthesis were significantly reduced during night. Assuming that protein synthesis during the night-time represents 23% of the day-time rate (Smith & D'Souza, 1993) in the present study, the values of P_e (measured over 3 hours) if extrapolated over 24 hours would be 60% higher compared with values of P_e corrected for this dark uptake; this supports similar observations by Morris *et al.* (1981) for an 8 hours' incubation and a 20 hours' incubation in the Caribbean sea (overestimation by 57%). Values of U_p would in turn be overestimated by 60% whereas values of U_b would be underestimated by 20-25% in oligotrophic waters and 20-40% in upwelling and slope waters. Given that most studies other than Smith & D'Souza (1993) have shown a

continuous protein synthesis through night the estimated error probably represents an upper limit.

(b) Carbon incorporation into the protein fraction of phytoplankton is correlated with carbon uptake in the total fraction (DiTullio & Laws, 1986). Since carbon uptake is directly dependent on light whereas nitrogen uptake is not (uncoupling of C and N uptake) the estimated values of U_p (see equation 1) may be biased (DiTullio & Laws, 1986). It is noteworthy that most studies of the light effect on nitrogen uptake are based on ^{15}N experiments in which bacterial uptake of inorganic nitrogen cannot be distinguished from the photosynthetic uptake; this may further complicate the problem of investigating the uncoupling between carbon and nitrogen uptake by phytoplankton.

In the mixed layer where ^{14}C uptake is close to light saturation level (20-60%Io) the corresponding values of U_p may be overestimated (see equation 1) and the resulting values of U_b therefore underestimated (see equation 2). In the DCM layer, on the other hand, ^{14}C uptake is light-limited (0.8-5% I_0) and therefore the values of U_p may be underestimated (see equation 1) and the values of U_b overestimated (see equation 2).

Values of $U_p$were corrected for this bias in the following way. For the samples located in the mixed layer, values of U_p were compared with the corresponding values of U_p measured in the DCM layer corrected so as to represent an upper limit of U_p at light-saturation level: assuming that the DCM values were light-limited, these values were corrected by a ratio which is nitrogen uptake ($NH_4^+ + NO_3^-$) at the 30% light-level divided by nitrogen uptake at the 1% light-level (Cochlan *et al.*, 1991a). At 6 out of 7 oligotrophic stations, the "corrected" values of U_p were higher than the initial values of U_p in the mixed layer suggesting that the initial estimates could not have been significantly

overestimated. At the upwelling stations, on the other hand, initial estimates of U_p were overestimated by up to an order of magnitude using the same ratio for correction.

For the samples located in the DCM layer, values of U_p may have been underestimated. To determine this bias, values of U_p were compared with the corresponding values of U_p measured in the mixed layer corrected so as to represent a lower limit of U_p under light-limited conditions. The correction was made in the following way: assuming that nitrogen uptake under dark conditions equals 50% of the day-time rate (central tendency among previous studies), the mixed layer-values of U_p were multiplied by 0.5. At 4 out of 6 oligotrophic stations, the "corrected" values of U_p were lower than the initial value in the DCM layer suggesting that the initial estimates had not been significantly underestimated. At the upwelling stations, on the other hand, estimates of U_p in the DCM layer were underestimated by 70-95%.

Based on this crude correction of U_p, I conclude that the estimates of U_p were not significantly biased at the oligotrophic stations. At the upwelling stations, on the other hand, where the gradients in carbon and nitrogen uptake are higher, values of U_p were significantly biased at both depths.

(c) One of the assumptions used in equation (1) is that a large fraction of nitrogen taken up is incorporated into proteins (85%). This assumption may not hold for measurements carried out in upwelling and slope waters *i.e.* waters that are not nutrient-depleted (DiTullio & Laws, 1983). Wheeler *et al.* (1982) reported for Chesapeake Bay NH_4^+ uptake rates in the protein fraction that represented 85% (±7%) of the total uptake. Glibert & McCarthy (1984), on the other hand, reported for Chesapeake Bay and for the Caribbean sea NH_4^+ uptake rates in the protein fraction ranging from 70% to 100% and from 70% to 90%, respectively, of the total uptake. In order to estimate a maximum error,

therefore, it was assumed that nitrogen uptake ($NH_4^+ + NO_3^-$) into the protein fraction represented 70% of the total uptake. In this case, the resulting values of U_p in upwelling and slope waters were underestimated by 17% and as a result values of U_b were overestimated by 8% on average (0.5-27%).

Appendix 3: Potential errors in U_t

Uncertainties in U_t are associated with uncertainties in (i) measurements of ambient NH_4^+, (ii) measurements of PON and atom enrichment, (iii) isotope enrichment, and (iv) isotope dilution.

(i) Measurements of ambient NH_4^+ may have been overestimated at the oligotrophic stations since it was assumed that NH_4^+ concentrations equalled 0.03 µg-at L^{-1} (*i.e.* the detection limit) when NH_4^+ could not be detected (see chapter 2). This could lead to an overestimation in the values of U_t and U_b (see equation (2)). Assuming that the true values of U_b should be 50% of D_b (Kirchman *et al.*, 1994) and that all other values used to compute U_t and U_p remain unchanged, the NH_4^+ concentrations necessary to yield $U_b = 0.5*D_b$ were computed. These revised NH_4^+ concentrations were significantly lower (by as much as an order of magnitude), than initial measurements and often were < 0.03 µg-at L^{-1} (*e.g.* 0 025, 0.002, 0.001 µg-at L^{-1}). These revised NH_4^+ concentrations were similar to typical NH_4^+ concentrations measured in oligotrophic waters using a low level technique (Brzezinski, 1988) As a result also, the revised values of U_b were lower by at least an order of magnitude than initial values of U_b . Thus, uncertainties in the measurement of ambient NH_4^+ can account for a significant overestimation in ^{15}N uptake rates (from 20% to an order of magnitude) which in turn could have contributed to the discrepancy observed between U_b and D_b.

(ii) Filtration of different volumes of water at the end of an ^{15}N experiment can result in different values of PON and atom enrichment (L. Harris, pers. comm.): higher values of PON and concomitantly lower values of atom enrichment (APE) have been observed in smaller volumes filtered (*e.g.* 450 ml) compared with larger volumes filtered (*e.g.* 900 ml). In the present study, measurements of PON in unfractionated water

(13.1±2.9 μg L^{-1} for oligotrophic waters) were consistently higher (85% on average) than measurements of PON carried out during the same cruise based on larger volumes filtered (7.2±1.5 μg L^{-1} for oligotrophic waters). A possible explanation of this difference is the trapping of colloidal material onto the filters when smaller volumes are filtered (Gordon and Sutcliffe, 1974; Sharp, 1974; Johnson and Wangersky, 1985). In the present study, although measurements of PON may be overestimated and measurements of atom enrichment of APE underestimated, they essentially compensate one another and therefore the resulting uptake rates of ^{15}N, based on the product of APE*PON, are unaffected.

(iii) The substrate enrichment produced by the addition of $^{15}NH_4^+$ and $^{15}NO_3^-$ can lead to increased uptake rates particularly in nutrient-depleted waters where the ambient nutrient concentrations are low and close to the constant of half-saturation (see review of Harrison, 1983). At the oligotrophic stations, the addition of $^{15}NH_4^+$ and $^{15}NO_3^-$ resulted in enrichments of up to 60% and 100%, respectively. A kinetic correction was applied to the uptake rates of $^{15}NH_4^+$ and $^{15}NO_3^-$ using the kinetic parameters of K_S and p_{max} estimated during the same cruise (Harrison *et al.*, 1996). The corrected values of $^{15}NH_4^+$ uptake and $^{15}NO3^-$ uptake were then compared with the initial uptake rates. The initial values of U_t (NH_4^+ + NO_3^-) were overestimated by ~20-30% in oligotrophic waters (range of 4-88%) and by ~20% in upwelling and slope waters (range of 1-33%) when compared with the values of U_t corrected kinetically. As a result, in oligotrophic waters the initial values of U_b were in most cases overestimated by up to an order of magnitude compared with the corrected values and occasionally were negative in the case of $U_t < U_p$ (U_t had been decreased below the values of U_p). In upwelling and slope waters, initial values of U_b were overestimated by 60% compared with the corrected values. It is noteworthy, however, that the corrected values of U_b were still higher than the values of D_b. This suggests that the error associated with isotope enrichment can account for only part of the discrepancy observed between U_b and D_b.

(iv) The effect of isotope dilution on the $^{15}NH_4^+$ uptake rates can be corrected for by assuming that uptake rates and regeneration rates are constant and of equal magnitude over the incubation period (Kanda *et al.*, 1987). These calculations suggest that the initial values of $^{15}NH_4^+$ uptake were underestimated by 7% in oligotrophic waters and by 6% in upwelling and slope waters compared with the corrected values; this results in values of U_b being underestimated by 12% in oligotrophic waters and by 9% in upwelling and slope waters. This indicates that isotope dilution would not affect significantly the uptake rates of $^{15}NH_4^+$, and therefore the values of U_b, unless regeneration rates are >> uptake rates.

Appendix 4: Error analysis of the *f*-ratio

To estimate the error in *f* due to a bacterial uptake of nitrate and ammonium, an analysis of errors (Topping, 1972) was performed. Based on previous studies which report a bacterial preference of NH_4^+ over NO_3^- (Kirchman *et al.*, 1992) and a small (10% according tc Kirchman *et al.*, 1994) to insignificant contribution (Wheeler and Kirchman, 1986) of bacteria to nitrate uptake it is assumed that the estimated values of U_b (corrected) represent 90% of ammonium and 10% of nitrate. Defining *f* as:

$$f = \frac{pNO_3^-}{pNH_4^+ + pNO_3^-} \quad (3),$$

the relative error in *f*, E_f/f (where E_f is the absolute error in *f*), due to the absolute errors in pNH_4^+ ($U_{b(a)}$) and pNO_3^- ($U_{b(n)}$) can be computed as:

$$\frac{E_f}{f} = \frac{1}{(pNO_3^- + pNH_4^+)} \left\{ \frac{pNH_4^+}{pNO_3^-} U_n - U_a \right\} \quad (4)$$

($U_{b(a)}$) and ($U_{b(n)}$) represent the bacterial uptake of NH_4^+ and NO_3^-, respectively, and were calculated for each station. At the oligotrophic stations, nearly half of the *f*-ratios were underestimated by 8% (1-17%) on average while the other half of *f*-ratios were overestimated by 9% (2-26%) on average. At the upwelling stations, on the other hand, *f*-ratios were systematically underestimated by 6% on average (1-23%).

References

Alldredge A. L., Passow U., and Logan B. E. (1993). The abundance and significance of a class of large, transparent organic particles in the ocean. Deep-Sea Res. I 40(6): 1131-1140

Altabet M. A. (1989). Particulate new nitrogen fluxes in the Sargasso sea. J. Geoph. Res. 94(C9): 12,771-12,779

Azam F., Fenchel T., Field J. G., Gray J. S., Meyer-Reil L. A., Thingstad F. (1983). The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10: 257-263

Azam F., Fuhrman J. A. (1984). Measurement of bacterioplankton growth in the sea and its regulation by environmental conditions. *In*: Hobbie J. E., Williams P. J. leB. (eds.) Heterotrophic activity in the sea. NATO Conference Series Vol. 15, p.179-196

Baines S. B., Pace M. L. (1991). The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. Limnol. Oceanogr. 36(6): 1078-1090

Barlow R. G. (1984). Time-series uptake of carbon into photosynthetic products of Benguela phytoplankton populations. J. Plankton Res. 6(3): 435-442

Berger W. H., Smetacek V. S., Wefer G. (1989). Ocean productivity and paleoproductivity - An overview. *In:* Berger, W. H., Smetacek V. S., Wefer G. (eds.) Productivity of the ocean: present and past. J. Wiley & Sons Ltd, pp: 1-34

Bienfang P. K. (1985). Size structure and sinking rates of various microparticulate constituents in oligotrophic Hawaiian waters. Mar. Ecol. Prog. Ser. 23: 143-151

Bienfang P. K., Morales L., Klein K., Takahashi M. (1984). Picoplankton growth rates in subtropical Hawaiian embayments. Pacific Science 38(2): 134-140

Bienfang P. K., Takahashi M. (1983). Ultraplankton growth rates in a subtropical ecosystem. Mar. Biol. 76: 213-218

Bienfang P. K., Ziemann D. A. (1992). The role of coastal high latitude ecosystems in global export production. *In*: Falkowski P. G., Woodhead A. D. (eds.) Primary productivity and biogeochemical cycles in the sea. Plenum Press, pp: 285-316

Bird D. F., Kalff J. (1984). Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters. Can. J. Fish. Aquat. Sci. 41: 1015-1023

Booth B. C. (1988). Size classes and major taxonomic groups of phytoplankton at two locations in the subarctic Pacific ocean in May and August, 1984. Mar. Biol. 97: 275-286

Bratbak G., Thingstad T. F. (1985). Phytoplankton-bacteria interactions: an apparent paradox? Analysis of a model system with both competition and commensalism. Mar. Ecol. Prog. Ser. 25: 23-30

Brown C. M., Macdonald-Brown D. S., Stanley S. O. (1972). Inorganic nitrogen metabolism in marine bacteria: nitrogen assimilation in some marine pseudomonads J. Mar. Biol. Ass. U. K. 52: 793-804

Brown C. M., Macdonald-Brown D. S., Stanley S. O. (1975). Inorganic nitrogen metabolism in marine bacteria: nitrate uptake and reduction in a marine pseudomonad. Mar. Biol. 31: 7-13

Brown E. J., Button D. K., Lang D. S. (1981). Competition between heterotrophic and autotrophic microplankton for dissolved nutrients. Microb. Ecol. 7: 199-206

Brzezinski M. A. (1988). Vertical distribution of ammonium in stratified oligotrophic waters. Limnol. Oceanogr. 33(5): 1176-1182

Campbell L., Vaulot D. (1993). Photosynthetic picoplankton community structure in the subtropical North pacific ocean near Hawaii (station ALOHA). Deep-Sea Res. I, 40(10): 2043-2060

Campbell L., Nolla H. A., Vaulot D. (1994). The importance of *Prochlorococcus* to community structure in the central North Pacific ocean. Limnol. Oceanogr. 39(4): 954-961

Caron D. A., Goldman J. C., Dennett M. R. (1988). Experimental demonstration of the roles of bacteria and bacterivorous protozoa in plankton nutrient cycles. Hydrobiologia 159: 27-40

Caron D. A., Lim E. L., Miceli G Waterbury J. B., Valois F. W. (1991). Grazing and utilization of chroococcoid cyanobacteria and heterotrophic bacteria by protozoa in laboratory cultures and a coastal plankton community. Mar. Ecol. Prog. Ser. 76: 205-217

Caron D. A. (1991). Evolving role of protozoa in aquatic nutrient cycles. *In:* Reid P.C., Turley C. M., Burkill P. H. (eds.) Protozoa and their role in marine processes. NATO ASI Series, Vol. 25, pp: 387-415

Carpenter E. J., Guillard R. R. L. (1971). Intraspecific differences in nitrate half-saturation constants for three species of marine phytoplankton. Ecology 52(1): 183-185

Chang F. H., Vincent W. F. and Woods P. H. (1989). Nitrogen assimilation by three size fractions in the winter phytoplankton off Westland, New Zealand. New Zealand Journal of marine and freshwater research 23: 491-505

Chavez F. P. (1989). Size distribution of phytoplankton in the central and Eastern tropical Pacific. Global Biogeochemical cycles 3(1): 27-35

Chavez F. P., Buck K. R., Barber R. T. (1990). Phytoplankton taxa in relation to primary production in the equatorial Pacific. Deep-Sea Res. 37(11): 1733-1752

Chavez F. P., Buck K. R., Bidigare R. R , Karl D. M., Hebel D., Latasa M., Campbell L. (1995). On the chlorophyll *a* retention properties of glass-fiber GF/F filters. Limnol. Oceanogr. 40(2): 428-433

Chisholm S. W., Olson R. J., Zettler E. R., Goericke R., Waterbury J. B., Welschmeyer N. A. (1988). A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature 334: 340-343

Chisholm S. W. (1992). Phytoplankton size. *In:* Falkowski P. G., Woodhead A. D. (eds.) Primary productivity and biogeochemical cycles in the sea. Plenum Press, pp: 213-237

Cho B. C., Azam F. (1990). Biogeochemical significance of bacterial biomass in the ocean's euphotic zone. Mar. Ecol. Prog. Ser. 63: 253-259

Christian J. R., Karl D. M. (1994). Microbial community structure at the U.S.-Joint Global Ocean Flux Study stations ALOHA: inverse methods for estimating biochemical indicator ratios. J. Geophys. Res. 99: 14269-14276

Cochlan W. P., Harrison P. J., Denman K. L. (1991a). Diel periodicity of nitrogen uptake by marine phytoplankton in nitrate-rich environments. Limnol. Oceanogr. 36(8): 1689-1700

Cochlan W. P., Price N. M., Harrison P. J. (1991b). Effects of irradiance on nitrogen uptake by phytoplankton: comparison of frontal and stratified communities. Mar. Ecol. Prog. Ser. 69: 103-116

Cole J. J., Likens G. E., Strayer D. L. (1982). Photosynthetically produced dissolved organic carbon: an important carbon source for planktonic bacteria. Limnol. Oceanogr. 27(6): 1080-1090

Cole J. J. (1982). Interactions between bacteria and algae in aquatic ecosystems. Ann. Rev. Ecol. Syst. 13: 291-314

Cole J. J., Findlay S., and Pace M. L. (1988). Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Mar. Ecol. Prog. Ser. 43: 1-10

Collos Y. (1986). Time-lag algal growth dynamics: biological constraints on primary production in aquatic environments. Mar. Ecol. Prog. Ser. 33: 193-206

Cuhel R. L., Ortner P. B., Lean D. R. S. (1984). Night synthesis of protein by algae. Limnol. Oceanogr. 29(4): 731-744

Cuhel R. L., Waterbury J. B. (1984). Biochemical composition and short term nutrient incorporation patterns in a unicellular marine cyanobacterium, *Synechococcus* (WH7803). Limnol. Oceanogr. 29(2): 370-374

Currie D. J., Kalff J. (1984). Can bacteria outcompete phytoplankton for phosphorus? A chemostat test. Microb. Ecol. 10: 205-216

Davis P. G., Caron D. A., Johnson P. W., Sieburth J. McN. (1985). Phototrophic and apochlorotic components of picoplankton and nanoplankton in the North Atlantic: geographic, vertical, seasonal and diel distributions. Mar. Ecol. Prog. Ser. 21: 15-26

DiTullio G. R., Laws E. A. (1983). Estimates of phytoplankton N uptake based on $^{14}CO_2$ incorporation into protein. Limnol Oceanogr. 28: 177-185

DiTullio G. R., Laws E. A. (1986). Diel periodicity of nitrogen and carbon assimilation in five species of marine phytoplankton: accuracy of methodology for predicting N-assimilation rates and N/C composition ratios. Mar. Ecol. Prog. Ser. 32: 123-132

Dortch Q., Packard T. T. (1989). Differences in biomass structure between oligotrophic and eutrophic marine ecosystems. Deep-Sea Res. 36(2): 223-240

Ducklow H.W., Carlson C.A. (1992). Oceanic bacterial production. Adv. Microb. Ecol. 12(3): 113-181

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

Eikrem W., Throndsen J. (1990). The ultrastructure of *Bathycoccus* gen. nov. and *B. prasinos* sp. nov., a non-motile picoplanktonic alga (Chlorophyta, Prasinophyceae) from the Mediterranean and Atlantic. Phycologia 29(3): 344-350

El Hag A. G. D., Fogg G. E. (1986). The distribution of coccoid blue-green algae (Cyanobacteria) in the Menai straits and the Irish sea. Br. Phycol. J. 21 (1): 45-54

Eppley R. W., Rogers J. N., McCarthy J. J. (1969). Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. 14: 912-920

Eppley R. W., Renger E. H., Venrick E. L., Mullin M. M. (1973). A study of plankton dynamics and nutrient cycling in the central gyre of the North Pacific ocean. Limnol. Oceanogr. 18(4): 534-551

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

Eppley R. W., Peterson B. J. (1979). Particulate organic matter flux and planktonic new production in the deep ocean. Nature 282: 677-680

Eppley R. W., Garside C., Renger E. H., Orellana E. (1990). Variability of nitrate concentration in nitrogen-depleted subtropical surface waters. Mar. Biol. 107: 53-60

Estep K. W., Davis P. G., Hargraves P. E., Sieburth J. McN. (1984). Chloroplast containing microflagellates in natural populations of North Atlantic nanoplankton, their identification and distribution; including a description of five new species of *Chrysochromulina* (Prymnesiophyceae). Protistologica XX, fasc. 4, pp. 613-634

Fenchel T. (1982). Ecology of heterotrophic flagellates. IV. Quantitative occurrence and importance of bacterial consumers. Mar. Ecol. Prog. Ser. 9: 35-42

Fenchel T., Blackburn T. H. (1979). Bacteria and mineral cycling. Academic press, 225pp.

Fogg G. E. (1995). Some comments on picoplankton and its importance in the pelagic ecosystem. Aquat. Microb. Ecol. 9: 33-39

Fowler S. W., Knauer G. N. (1986). Role of large particles in the transport of elements and organic compounds through the oceanic water column. Prog. Oceanogr. 16: 147-194

Fuhrman J. A. (1987). Close coupling between release and uptake of dissolved free amino acids in seawater studied by an isotope dilution approach. Mar. Ecol. Prog. Ser. 37: 45-52

Fuhrman J. A., Azam F. (1980). Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. Environ. Microbiol. 39(6): 1085-1095

Fuhrman J. A., Ferguson R.L. (1986). Nanomolar concentrations and rapid turnover of dissolved free amino acids in seawater: agreement between chemical and microbiological measurements. Mar. Ecol. Prog. Ser. 33: 237-242

Fuhrman J. A., Horrigan S. G., Capone D. G. (1988). Use of ^{13}N as trace. for bacterial and algal uptake of ammonium from seawater. Mar. Ecol. Prog. Ser. 45: 271-278

Fuhrman J. A., Sleeter T. D., Carlson C. A., Proctor L. M. (1989). Dominance of bacterial biomass in the Sargasso sea and its ecological implications. Mar. Ecol. Prog. Ser. 57: 207-217

Fuhrman J. (1992). Bacterioplankton roles in cycling of organic matter: the microbial food web. *In:* Falkowski P. G., Woodhead A. D. (eds.) Primary productivity and biogeochemical cycles in the sea. Plenum Press, pp: 361-383

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

Garside C. (1982). A chemiluminescent technique for the determination of nanomolar concentrations of nitrate and nitrite in seawater. Mar. Chem. 11: 159-167

Garside C. (1985). The vertical distribution of nitrate in open ocean surface water. Deep-Sea Res. 32: 723-732

Garside C., Glover H. E. (1991). Chemiluminescent measurements of nitrate kinetics; I. *Thalassiosira pseudonana* (clone 3H) and neritic assemblages. J. Plankton Res. 13, Suppl.: 5-19

Glibert P. M., McCarthy J. J. (1984). Uptake and asimilation of ammonium and nitrate by phytoplankton: indices of nutritional status for natural assemblages. J. Plankton Res. 6(4): 677-697

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

Glibert P. M., Kana T. M., Olson R. J., Kirchman D. L., Alberte R.S. (1986). Clonal comparisons of growth and photosynthetic responses to nitrogen availability in marine *Synechococcus* spp. J. Exp. Mar. Biol. Ecol. 101: 199-208

Glibert P. M., Ray R. T. (1990). Different patterns of growth and nitrogen uptake in two clones of marine *Synechococcus* spp. Mar. Biol. 107: 273-280

Glibert P. M., Miller C. A., Garside C., Roman M. R., McManus G. B. (1992). NH_4^+ regeneration and grazing: interdependent processes in size-fractionated $^{15}NH_4^+$ experiments. Mar. Ecol. Prog. Ser. 82: 65-74

Glover H. E., Keller M. D., Spinrad R. W. (1987). The effects of light quality and intensity on photosynthesis and growth of marine eukaryotic and prokaryotic phytoplankton clones. J. Exp. Mar. Biol. Ecol. 105(2-3): 137-159

Glover H. E., Prézelin B B., Campbell L., Wyman M., Garside C. (1988a). A nitrate-dependent *Synechococcus* bloom in surface Sargasso sea water. Nature 331: 161-163

Glover H. E., Prézelin B. B., Campbell L., Wyman M. (1988b). Pico- and ultraplankton Sargasso sea communities: variability and comparative distributions of *Synechococcus* spp. and algae. Mar. Ecol. Prog. Ser. 49: 127-139

Glover H. E. (1991). Oceanic phytoplankton communities: our changing perception. Reviews in Aquatic Sciences 5(3-4): 307-331

Goericke R., Repeta D. J. (1993). Chlorophylls *a* and *b* and divinyl chlorophylls *a* and *b* in the open subtropical North Atlantic ocean. Mar. Ecol. Prog. Ser. 101: 307-313

Goericke R., Welschmeyer N. A. (1993). The marine prochlorophyte *Prochlorococcus* contributes significantly to phytoplankton biomass and primary production in the Sargasso Sea. Deep-Sea Res. 40(11/12): 2283-2294

Goldman J. C., Caron D. A., Dennett M. R. (1987). Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. Limnol. Oceanogr. 32(6): 1239-1252

Goldman J. C. (1988). Spatial and temporal discontinuities of biological processes in pelagic surface waters. *In*:: Rotschild B. J. (ed.) Toward a theory on biological-physical interactions in the world ocean, Kluwer Academic Publ., pp: 273-296

Goldman J. C., Dennett M. R. (1991). Ammonium regeneration and carbon utilization by marine bacteria grown on mixed substrates. Mar. Biol. 109: 369-378

Goldman J. C. (1993). Potential role of large oceanic diatoms in new primary production. Deep-Sea Res. I 40(1): 159-168

Gordon D. D. Jr., Sutcliffe W. H. Jr. (1974). Filtration of seawater using silver filters for particulate nitrogen and carbon analysis. Limnol. Oceanogr. 19: 989-993

Guillard R. R. L., Keller M., O'Kelly C. J., Floyd G. L. (1991). *Pycnococcus provasolii* gen. et sp. nov., a coccoid prasinoxanthin-containing phytoplankter from the Western North Atlantic and Gulf of Mexico. J. Phycol. 27: 39-47

Hall J. A., Vincent W. F. (1990). Vertical and horizontal structure in the picoplankton communities of a coastal upwelling system. Mar. Biol. 106: 465-471

Hama T., Miyazaki T., Ogawa Y., Iwakuma T., Takahashi M., Otsuki A., Ichimura S. (1983). Measurement of photosynthetic production of a marine phytoplankton population using a stable ^{13}C isotope. Mar. Biol. 73: 31-36

Harrison W. G., Wood L. J. E. (1988). Inorganic nitrogen uptake by marine picoplankton; evidence for size-partitioning. Limnol. Oceanogr. 33(3): 468-475

Harrison W. G., Harris L. R., Karl D. M., Knauer G. A., Redalje D. G. (1992). Nitrogen dynamics at the VERTEX time-series site. Deep-Sea Res. 39(9): 1535-1552

Harrison W. G., Harris L., Irwin B. D. (1996). The kinetics of nitrogen utilization in the oceanic mixed layer: nitrate and ammonium interactions at nanomolar concentrations. Limnol. Oceanogr.

Herbland A., LeBouteiller A., Raimbault P. (1985). Size structure of phytoplankton biomass in the equatorial Atlantic ocean. Deep-Sea Res. 32(7): 819-836

Herman A. W., Mitchell M. R., Young S. W. (1984). A continuous pump sampler for profiling copepods and chlorophyll in the upper oceanic layers. Deep-Sea Res. 31(4): 439-450

Hobbie J. E., Daley R., Jasper S. (1977). Use of nucleopore filters for counting bacteria by flurescence microscopy. Appl. Environ. Microbiol. 33: 1225-1228

Hoch M. P., Fogel M. L., Kirchman D. L. (1992). Isotope fractionation associated with ammonium uptake by a marine bacterium. Limnol. Oceanogr. 37(7): 1447-1459

Hoepffner N., Haas L. W. (1990). Electron microscopy of nanoplankton from the North Pacific Central gyre. J. Phycol. 26: 421-439

Holm-Hansen O., Lorenzen C. J., Holmes R. W., Strickland J. D. (1965). Fluorometric determination of chlorophyll. J. Cons. Int. Expl. Mer 30: 3-15

Horrigan S. G., Hagström Å., Koike I., Azam F. (1988). Inorganic nitrogen utilization by assemblages of marine bacteria in seawater culture. Mar. Ecol. Prog. Ser. 50: 147-150

Horstmann U., Hoppe H. G. (1981). Competition in the uptake of methylamine/ammonium by phytoplankton and bacteria. Kieler Meeresforsch., Sonderh. 5: 110-116

Iturriaga R., Mitchell B. G. (1986). Chrococcoid cyanobacteria: a significant component in the food web dynamics of the open ocean. Mar. Ecol. Prog. Ser. 28: 291-297

Jackson G. A., Lochmann S. E. (1992). Effect of coagulation on nutrient and light limitation of an algal bloom. Limnol. Oceanogr. 37(1): 77-89

Jahns T. (1992). Urea uptake by the marine bacterium *Deleya venusta* HG1. Journal of General Microbiology 138: 1815-1820

Johnson B. D., Wangersky P. J. (1985). Seawater filtration: particle flow and impaction considerations. Limnol. Oceanogr. 30(5): 966-971

Johnson P. W., Sieburth J. McN. (1979). Choococcoid cyanobacteria in the sea: a ubiquitous and diverse phototrophic biomass. Limnol. Oceanogr. 24(5): 928-935

Johnson P. W., Sieburth J. McN. (1982). In-situ morphology and occurrence of eukaryotic phototrophs of bacterial size in the picoplankton of estuarine and oceanic waters. J. Phycol. 18: 318-327

Joint I. R., Pomroy A. J. (1987). Activity of heterotrophic bacteria in the euphotic zone of the Celtic Sea. Mar. Ecol. Prog. Ser. 41: 155-165

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

Jørgensen N. O. G., Søndergaard M. (1984). Are dissolved free amino acids free? Microb. Ecol. 10: 301-316

Kana T. M., Glibert P. M. (1987). Effect of irradiances up to 2000 μE m^{-2} s^{-1} on marine *Synechoccus* WH7803-I. Growth, pigmentation, and cell composition. Deep-Sea Res. 34(4): 479-495

Kanda J., Laws E. A., Saino T., Hattori A. (1987). An evaluation of isotope dilution effect from conventional data sets of ^{15}N uptake experiments. J. Plankton Res. 9(1): 79-90

Keil R. G., Kirchman D. L. (1991). Contribution of dissolved free amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton. Mar. Ecol. Prog. Ser. 73:1-10

King DF. D. (1987). Nitrogen recycling efficiency in steady-state oceanic environments. Deep-Sea Res. 34(5/6): 843-856

Kirchman D. L. (1993). Leucine incorporation as a measure of biomass production by heterotrophic bacteria. *In:* Handbook of methods in aquatic microbial ecology. Kemp, Sherr (Eds.), Lewis Publ., pp: 509-512

Kirchman D. L., Ducklow H. W., McCarthy J. J., Garside C. (1994). Biomass and nitrogen uptake by heterotrophic bacteria during the spring phytoplankton bloom in the North Atlantic ocean. Deep-Sea Res. 41(5/6): 879-895

Kirchman D. L., Hodson R. E. (1986). Metabolic regulation of amino acid uptake in marine waters. Limnol. Oceanogr. 31(2): 339-350

Kirchman D. L., Keil R. G., Wheeler P. A. (1989). The effect of amino acids on ammonium utilization and regeneration by heterotrophic bacteria in the subarctic Pacific. Deep-Sea Res. 36(11): 1763-1776

Kirchman D. L., Keil R. G., Wheeler P. A. (1990). Carbon limitation of ammonium uptake by heterotrophic bacteria in the subarctic Pacific. Limnol. Oceanogr. 35(6): 1258-1266

Kirchman D. L., K'nees E., Hodson R. (1985). Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49(3): 599-607

Kirchman D. L., Moss J., Keil R. G. (1992). Nitrate uptake by heterotrophic bacteria: does it change the *f*-ratio ? Arch. Hydrobiol. Beih. 37:129-138

Kirchman D. L., Suzuki Y., Garside C., Ducklow H. W. (1991). High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. Nature 352: 612-614

Kiørboe T. (1993). Turbulence, phytoplankton cell size, and the structure of pelagic food webs. Adv. Mar. Biol. 29: 1-72

Knauer G. A., Martin J. H., Karl D. H. (1984). Further implications of a two-layered euphotic zone in oceanic waters. EOS Trans. Am. Geophys. Soc. 65: 923 (abstr.)

Knauer G. A., Redalje D. G., Harrison W. G. W., Karl D. M. (1990). New production at the VERTEX time-series site. Deep-Sea Res. 37(7): 1121-1134

Koike I., Redalje D. G., Ammerman J. W., Holm-Hansen O. (1983). High-affinity uptake of an ammonium analogue by two marine microflagellates from the oligotrophic Pacific. Mar. Biol. 74: 161-168

Kroer N., Jørgensen N. O. G., Coffin R. B. (1994). Utilization of dissolved nitrogen by heterotrophic bacterioplankton: a comparison of three ecosystems. Appl. Environ. Microbiol. 60(11): 4116-4123

Lampitt R. S., Wishner K. F., Turley C. H., Angel M. V. (1993). Marine snow studies in the Northeast Atlantic ocean: distribution, composition and role as a food source for migrating plankton. Mar. Biol. 116: 689-702

Landry M. R., Kihrstein J., Constantinou J. (in press). Abundances and distributions of picoplankton populations in the central equatorial Pacific from 12° N to 12° S, 140° W. Deep-Sea Res. II

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

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

LeBouteiller A. (1986). Environmental control of nitrate and ammonium uptake by phytoplankton in the Equatorial Atlantic ocean. Mar. Ecol. Prog. Ser. 30: 167-179

LeBouteiller A., Blanchot J., Rodier M. (1992). Size distribution patterns of phytoplankton in the western Pacific: towards a generalization for the tropical open ocean. Deep-Sea Res. 39(5): 805-823

LeBouteiller A. (1993). Comparison of in-bottle measurements using ^{15}N and ^{14}C ICES Mar. Sci. Symp 197: 121-131

Ledwell J. R , Watson A. J., Law C. S. (1993). Evidence for slow mixing across the pycnocline from an open-ocean tracer-release experiment. Nature 364: 701-703

Lee C., Bada J. L. (1977). Dissolved amino acids in the equatorial Pacific, the Sargasso sea, and Biscayne Bay. Limnol. Oceanogr. 22(3): 502-510

Lee C., Fuhrman J. A. (1987). Relationships between biovolume and biomass of naturally received marine bacterioplankton. Appl. Environ Microbiol. 53: 1298-1303

Lee C., Kang Y., Fuhrman J. A. (1995). Imperfect retention of natural bacterioplankton cells by glass fiber filters. Mar. Ecol. Prog. Ser. 119: 285-290

Legendre L., Le Fèvre J. (1989). Hydrodynamical singularities as controls of recycled versus export production in oceans. *In:* Berger, W. H., Smetacek V. S., Wefer G. (eds.) Productivity of the ocean: present and past. J. Wiley & Sons Ltd, pp: 49-63

Letelier R. M., Bidigare R. R., Hebel D. V., Ondrusek M., Winn C. D., Karl D. M. (1993). Temporal variability of phytoplankton community structure based on pigment analysis. Limnol. Oceanogr. 38(7): 1420-1437

Lewis M. R., Harrison W. G. W., Oakey N. S., Herbert D., Platt T. (1986). Vertical nitrate fluxes in the oligotrophic ocean. Science 234: 870-873

Li W. K. W., Platt T. (1982). Distribution of carbon among photosynthetic end-products in phytoplankton of the Eastern Canadian Arctic. J. Phycol. 18: 466-471

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

Li W. K. W., Platt T. (1987). Photosynthetic picoplankton in the ocean. Sci. Prog., Oxf. 71: 117-132

Li W. K. W., Wood A. M. (1988). Vertical distribution of North Atlantic ultraphytoplankton: analysis by flow cytometry and epifluorescence microscopy. Deep-Sea Res. 35(9): 1615-1638

Li W. K. W. (1989). Shipboard analytical flow cytometry of oceanic ultraplankton. Cytometry 10: 564-579

Li W. K. W., Dickie P. M. (1991). Light and dark ^{14}C uptake in dimly-lit oligotrophic waters: relation to bacterial activity. J. Plankton Res. 13: 29-44

Li W. K. W., Dickie P. M., Irwin B. D., Wood A. M. (1992). Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso sea. Deep-Sea Res. 39(3/4): 501-519

Li W. K. W., Dickie P. M., Harrison W. G., Irwin B. D. (1993). Biomass and production of bacteria and phytoplankton during the spring bloom in the western North Atlantic ocean. Deep-Sea Res. II 40(1/2): 307-327

Li W. K. W. (1994). Primary production of prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton: measurements from flow cytometric sorting. Limnol. Oceanogr. 39(1): 169-175

Li W. K. W. (1995). Composition of ultraphytoplankton in the central North Atlantic. Mar. Ecol. Prog. Ser. 22: 1-8

Lignell R., Lindqvist K. (1992). Effect of nutrient enrichment and temperature on intracellular partitioning of $^{14}CO_2$ in a summer phytoplankton community in the Northern Baltic. Mar. Ecol. Prog. Ser. 86: 273-281

Lochte K., Turley C. M. (1988). Bacteria and cyanobacteria associated with phytodetritus in the deep sea. Nature 335:67-69

Lohrenz S. E., Knauer G. A., Asper V. L., Tuel M., Michaels A. F., Knap A. M. (1992). Seasonal variability in primary production and particle flux in the northwestern Sargasso sea: U. S. JGOFS Bermuda Atlantic time-series study. Deep-Sea Res. 39(718): 1373-1391

Longhurst A. R., Sathyendranath S., Platt T., Caverhill C. (1995). An estimate of global primary production in the ocean from satellite radiometer data. J. Plankton Res. 17(6): 1245-1271

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

Malone T. C. (1980). Size-fractionated primary productivity of marine phytoplankton. *In*: Falkowski P. G. (ed.) Primary productivity in the sea. Brookhaven Symp. Biol., Plenum Press, pp: 301-319

Martin J. H., Knauer G. A., Karl D. M., Broenkow W. W. (1987). VERTEX: carbon cycling in the northeast Pacific. Deep-Sea Res. 34(2): 267-285

Martin-Jézéquel (1992). Effect of Si-status on diel variation of intracellular free amino acids in *Thalassiosira weissflogii* under low-light intensity. Hydrobiologia 238. 159-167

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

McCarthy J. J., Taylor R. W., Taft J. L. (1977). Nitrogenous nutrition of the plankton in the Cheasapeak Bay. I. Nutrient availability and phytoplankton preferences. Limnol. Oceanogr. 22(6): 996-1011

McCarthy J. J., Garside C., Nevins J. L. (1992). Nitrate supply and phytoplankton kinetics in the euphotic layer of a Gulf stream warm-core ring. Deep-Sea Res. 39, Suppl. 1: 393-403

McCarthy J. J., Garside C., Nevins J. L. (in press). "New" production along 140° W in the equatorial Pacific during and following the 1992 El Niño event. Deep-Sea Res.

Michaels A. F., Silver M. W. (1988). Primary production, sinking fluxes and the microbial food web. Deep-Sea Res. 35(4): 473-490

Monger B. C., Landry M. R. (1993). Flow cytometric analysis of marine bacteria with Hoechst 33342. Appl. Environ Microbiol. 95: 905-911

Moore L. R., Goericke R., Chisholm S. W. (1995). Comparative physiology of *Synechococcus* and *Prochlorococcus* isolated from the subtropical open ocean: growth regulation by light and temperature. Mar. Ecol. Prog. Ser. 116: 259-275

Mopper K., Lindroth P. (1982). Diel and depth variations in dissolved free amino acids and ammonium in the Baltic sea determined by shipboard HPLC analysis. Limnol. Oceanogr. 27(2): 336-347

Morris I., Glover H. E., Yentsch C. S. (1974). Products of photosynthesis by marine phytoplankton: the effect of environmental factors on the relative rates of protein synthesis. Mar. Biol. 27: 1-9

Morris I., Skea W. (1978). Products of photosynthesis in natural populations of marine phytoplankton from the Gulf of Maine. Mar. Biol. 47: 303-312

Morris I., Smith A. E., Glover H. E. (1981). Products of photosynthesis in phytoplankton off the Orinoco River and in the Caribbean sea. Limnol. Oceanogr. 26(6): 1034-1044

Nagata T. (1986). Carbon and nitrogen content of natural planktonic bacteria. Appl. Environ. Microbiol. 52(1): 28-32

Nalewajko C., Garside C. (1983). Methodological problems in the simultaneous assessment of photosynthesis and nutrient uptake in phytoplankton as functions of light intensity and cell size. Limnol. Oceanogr. 28: 591-597

Nelson D. M., Conway H. L. (1979). Effects of the light regime on nutrient assimilation by phytoplankton in the Baja California and northwest Africa upwelling systems. J. Mar. Res. 37(1): 301-318

Olson R. J., Chisholm S. W., Zettler E. R., Altabet M. A., Dusenberry J. A. (1990a). Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic ocean. Deep-Sea Res. 37(6): 1033-1051

Olson R. J., Chisholm S. W., Zettler E., R., Armbrust E. V. (1990b). Pigments, size, and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans. Limnol. Oceanogr. 35(1): 45-58

Olson R. J., Zettler E., R., Durand M. D. (1993). Phytoplankton analysis using flow cytometry. *In:* Handbook of methods in aquatic microbial ecology. Kemp P. F., Sherr B. F., Sherr E. B., Cole J. J. (eds.). Lewis Publ., pp: 175-186

Parker R. R., Sibert J., Brown T. J. (1975). Inhibition of primary productivity through heterotrophic competition for nitrate in a stratified estuary. J. Fish. Res. Board Can. 32(1): 72-77

Parsons T. R., Albright L. J., Whitney F., Wong C. S., Williams P. J. LeB. (1981). The effect of glucose on the productivity of seawater: an experimental approach using controlled aquatic ecosystems. Mar. Environ. Res. 4: 229-242

Peña M. A., Lewis M. R., Harrison W. G. (1990). Primary productivity and size-structure of phytoplankton biomass on a transect of the equator at 135°N in the Pacific ocean. Deep-Sea Res. 37(2): 295-315

Pfannkuche O., Lochte K. (1993). Open ocean pelago-benthic coupling: cyanobacteria as tracers of sedimenting salp faeces. Deep-Sea Res. I 40(4): 727-737

Platt T., Subba Rao D. V., Irwin B. (1983). Photosynthesis of picoplankton in the oligotrophic ocean. Nature 301: 702-704

Platt T., Jauhari P., Sathyendranath S. (1992). The importance and measurement of new production. *In:* Falkowski P. G., Woodhead A. D. (eds.) Primary productivity and biogeochemical cycles in the sea. Plenum Press, pp: 273-284

Pomeroy L. R. (1974). The oceans's food web, a changing paradigm. BioScience 24(9): 499-504

Porter K. G., Feig Y. S. (1980). The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25: 943-948

Price N. M., Harrison P. J. (1988). Urea uptake by Sargasso sea phytoplankton: saturated and *in situ* uptake rates. Deep-Sea Res. 35(9): 1579-1593

Probyn T. A., Waldron H. N., James A. G. (1990). Size-fractionated measurements of nitrogen uptake in aged upwelled waters: implications for pelagic food webs. Limnol. Oceanogr. 35(1): 202-210

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

Probyn T. A., Painting S. J. (1985). Nitrogen uptake by size-fractionated phytoplankton populations in Antarctic surface waters. Limnol. Oceanogr. 30(6): 1327-1332

Probyn T. A. (1992). The inorganic nitrogen nutrition of phytoplankton in the southern Benguela: new production, phytoplankton size and implications for pelagic foodwebs. S. Afr. J. Mar. Sci. 12: 411-420

Raimbault P., Gentilhomme V. (1990). Short- and long-term responses of the marine diatom *Phaeodactylum tricornutum* to spike additions of nitrate at nanomolar levels. J. Exp. Mar. Biol. Ecol. 135: 161-176

Rajendran A., Joseph T., Reddy C. V. G. (1980). Experimental studies on urea degradation in seawater. Mahasagar Bulletin of the National Institute of Oceanography 13(4): 303-313

Rassoulzadegan F., Laval-Peuto M., and Sheldon R. W. (1988). Partitioning of the food ration of marine ciliates between pico- and nanoplankton. Hydrobiologia 159: 75-88

Raven J. A. (1986). Physiological consequences of extremely small size for autotrophic organisms in the sea. Can. Bull. Fish. Aquat. Sci. 214: 1-70

Raven J. A. (1994). Why are there no picoplanktonic O_2 evolvers with volumes less than 10^{-19} m^3? J. Plankton Res. 16(5): 565-580

Remsen C. C., Carpenter E. J., Schroeder B. W. (1972). Competition for urea among estuarine microorganisms. Ecology 53: 921-926

Robarts R. D., Zohary T. (1993). Fact or fiction - Bacterial growth rates and production as determined by [*methyl*-^{3}H]-thymidine? Adv. Microb. Ecol. 13: 371-425

Roberts R. B., Abelson P. H., Cowie D. B., Bolton E. T., Britten R. J. (1955). *In:* Studies of biosynthesis in *Escherichia coli*. Carnegie Institution of Washington publication 607, pp: 13-30

Ryther J. H., Dunstan W. M. (1971). Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science 171: 1008-1013

Satoh Y. (1980). Distribution of urea-decomposing bacteria in waters of lake Suwa. Hydrobiologia 71: 233-237

Savidge G., Johnston J. P. (1987). Urea degradation rates by size-fractionated plankton populations in a temperate estuary. Estuarine, coastal and shelf science 24: 433-447

Selmer J., Ferrier-Pages C., Cellario C., Rassoulzadegan F. (1993). New and regenerated production in relation to the microbial loop in the NW Mediterranean sea. Mar. Ecol. Prog. Ser. 100: 71-83

Sharp J. H. (1974). Improved analysis for "particulate" organic carbon and nitrogen from seawater. Limnol. Oceanogr. 19: 984-989

Sherr B. F., Sherr E. B. (1984). Role of heterotrophic protozoa in carbon and energy flow in aquatic ecosystems. *In:* Klug M. J., Keddy C. A. (eds.) Current perspectives in microbial ecology. Proceedings of the Third International Symposium on Microbial Ecology, pp: 412-423

Sieburth J. McN., Smetacek V., Lenz J. (1978). Pelagic ecosystem structure: heterotrophic components of the plankton and their relationship to plankton size fractions. Limnol. Oceanogr. 23(6): 1256-1263

Silver M. W., Alldredge A. L. (1981). Bathypelagic marine snow: deep-sea algal and detrital community. J. Mar. Res. 39: 501-530

Smayda T. J. (1970). The suspension and sinking of phytoplankton in the sea. Oceanogr. Mar. Biol. Ann. Rev. 8: 353-414

Smayda T. J., Bienfang P. K. (1983). Suspension properties of various phyletic groups of phytoplankton and tintinnids in an oligotrophic, subtropical system. Marine Ecology 4(4): 289-300

Smith R. E. H., D'Souza F. M. L. (1993). Macromolecular labelling patterns and inorganic nutrient limitation of a North Atlantic spring bloom. Mar. Ecol. Prog. Ser. 92: 111-118

Solorzano L. (1969). Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14:799-801

Stockner J. G., Antia N. J. (1986). Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. Can. J. Fish. Aquat. Sci. 43: 2472-2503

Stockner J. G. (1988). Phototrophic picoplankton: an overview from marine and freshwater ecosystems. Limnol. Oceanogr. 33(4, part 2): 765-775

Stone L. (1990). Phytoplankton-bacteria-protozoa interactions: a qualitative model portraying indirect effects. Mar. Ecol. Prog. Ser. 64: 137-145

Strathmann R. R. (1967). Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol. Oceanogr. 12: 411-418

Strickland J. D. H., Parsons T. R. (1972). A practical handbook of seawater analysis. Bulletin 167. Fisheries Research Board of Canada, 311pp.

Suttle C. A., Fuhrman J. A., Capone D. G. (1990). Rapid ammonium cycling and concentration-dependent partitioning of ammonium and phosphate: implications for carbon transfer in planktonic communities. Limnol. Oceanogr. 35(2): 424-433

Suttle C. A., Chan A. M., Fuhrman J. A. (1991). Dissolved free amino acids in the Sargasso Sea: uptake and respiration rates, trunover times, and concentrations. Mar. Ecol. Prog. Ser. 70: 189-199

Suzuki K., Handa N., Kiyosawa H., Ishizaka J. (1995). Distribution of the prochlorophyte *Prochlorococcus* in the central Pacific Ocean as measured by HPLC. Limnol. Oceanogr. 40(5): 983-989

Søndergaard M., Jensen L. M., and Ærtebjerg G. (1991). Picoalgae in Danish coastal waters during summer stratification. Mar. Ecol. Prog. Ser. 79: 139-149

Taga N. (1970). Occurrence of urea-decomposing bacteria in the neritic and oceanic waters of the northwest Pacific ocean. Proc. of the 2nd CSK Symposium, Tokyo, Saikon Publishing Company Limited, pp: 341-351

Taguchi S., Laws E. A. (1988). On the microparticles which pass through glass fiber filter type GF/F in coastal and open waters. J. Plankton Res. 10(5): 99-1008

Takahashi M., Bienfang P. K. (1983). Size structure of phytoplankton biomass and photosynthesis in subtropical Hawaiian waters. Mar. Biol. 76: 203-211

Topping J. (1972). Errors of observation and their treatment. Science paperbacks, Chapman and Hall, London, New York

Troussellier M., Courties C., Zettelmaier S. (1995). Flow cytometric analysis of coastal lagoon bacterioplankton and picophytoplankton: fixation and storage effects. Estuarine, Coastal and Shelf Science 40: 621-633

Tupas L. M., Koike I. (1990). Amino acid and ammonium utilization by heterotrophic marine bacteria grown in enriched seawater. Limnol. Oceanogr. 35(5): 1145-1155

Tupas L. M., Koike I. (1991). Simultaneous uptake and regeneration of ammonium by mixed assemblages of heterotrophic marine bacteria. Mar. Ecol. Prog. Ser. 70: 273-282

Tupas L. M., Koike I., Karl D. M., Holm-Hansen O. (1994). Nitrogen metabolism by heterotrophic bacterial assemblages in Antarctic coastal waters. Polar Biol. 14: 195-204

Turley C. M., Hughes D. J. (1992). Effects of storage on direct estimates of bacterial numbers of preserved seawater samples. Deep-Sea Res. 39(3/4): 375-394

Vaulot D., Marie D., Olson R. J., Chisholm S. W. (1995). Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the Equatorial Pacific ocean. Science 268: 1480-1482

Veldhuis M. J. W., Kraay G. W. (1990). Vertical distribution and pigment composition of a picoplanktonic prochlorophyte in the subtropical North Atlantic: a combined study of HPLC-analysis of pigments and flow cytometry. Mar. Ecol. Prog. Ser. 68: 121-127

Veldhuis M. J. W., Kraay G. W., Gieskes W. W. (1993). Growth and fluorescence characteristics of ultraplankton on a north-south transect in the eastern North Atlantic. Deep-Sea Res. II 40(1/2): 609-626

Verity P. G., Robertson C. Y., Tronzo C. R., Andrews M. G., Nelson J. R., Sieracki M. E. (1992). Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. Limnol. Oceanogr. 37(7): 1434-1446

Waterbury J. B., Watson S. W., Guillard R. R. L., Brand L. E. (1979). Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. Nature 277: 293-294

Waterbury J. B., Watson S. W., Valois F. W., Franks D. G. (1986). Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. *In*: Can. Bull. Fish. Aquat. Sci. 214: 71-120

Weber L. H., El-Sayed S. Z. (1987). Contributions of the net, nano- and picoplankton to the phytoplankton standing crop and primary productivity in the Southern ocean. J. Plankton Res. 9(5): 973-994

Wheeler P. A., Glibert P. M., McCarthy J. J. (1982). Ammonium uptake and incorporation by Chesapeake Bay phytoplankton: short term uptake kinetics. Limnol. Oceanogr. 27(6): 1113-1128

Wheeler P. A., Kirchman D. L. (1986). Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnol. Oceanc [illegible]): 998-1009

Wiebe W. J. (1984). Physiological and biochemical aspects of marine bacteria. *In*: Hobbie J. E., Williams P. J. leB. (eds.) Heterotrophic activity in the sea. NATO Conference Series Vol. 15, p.55-82

Williams LeB. P. J. (1981). Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. Kieler Meeresforsch., Sonderh. 5: 1-28

Wolter K. (1982). Bacterial incorporation of organic substances released by natural phytoplankton populations. Mar. Ecol. Prog. Ser. 7: 287-295

Yentsch C. M. (1990). Environmental health: flow cytometric methods to assess our water world. *In:* Darzynkiewicz Z., Crissman H. A. (eds.) Methods in cell biology. Academic Press, Vol. 33, pp: 575-561