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# THE UPTAKE AND FLUX OF DISSOLVED NITROGEN IN MARINE WATERS

REES, ANDREW PAUL

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# THE UPTAKE AND FLUX OF DISSOLVED NITROGEN IN MARINE WATERS

By

**ANDREW PAUL REES**

A thesis submitted to the University of Plymouth
in part fulfillment for the degree of

**DOCTOR OF PHILOSOPHY**

**November 2001**

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**For Two Little Boys**
**& their Mother**

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**Acknowledgements**

A lot of water has flowed past the tide gauge since the first of the fieldwork included in this thesis began in January 1991. In recent years people have often observed how the 1980's I.M.E.R. "wild" boys are now a bunch of bald(ing) married men who trek home each night to put their kids to bed. I would fail miserably if I attempted to name and shame everyone who has played a part in this change, but there are a number of people whose contribution to both work and play really deserve more than the fleeting recognition given here.

It has been my privilege to have had Nick Owens and Ian Joint as my managers during my time at the Plymouth Marine Laboratory. To them both I am indebted for their inspiration and support, and for the sharing of their great wealth of knowledge and experience.

I would particularly like to thank Malcolm Nimmo for responding to my request to supervise the submission of this thesis and for the enthusiasm and thoroughness with which he has done so, and also to Geoff Millward who has with Malcolm rigorously reviewed and advised on the preparation and submission.

Nick Owens was my original mentor, and provided my introduction to nitrogen cycling and all that goes with the daily grind of repeated pre-dawn CTD casts and post-midnight "preparation". From my early days at I.M.E.R. to now, Malcolm Woodward and Cliff Law have been close friends and colleagues. There were a number of years when I spent more time sleeping in the same room as Malcolm than with Sharon (it certainly felt that way anyway!), and the sight of Cliff descending from the top bunk is certainly one that will stay with me for too many years, no matter how hard I try to lose it.

Axel Miller has played a large part in my life since 1987 and despite a stubbornness beyond belief, for me, he really is one of life's good guys. Ashley Rowden, Duncan Plummer, Stuart Gibb, Elaine Fileman, Angela Hatton, Thomas Raabe, Sue Turner, Carol Robinson, Claire Widdicombe and John Stephens have made varied but in all cases significant contributions to my work and/or sanity. To spend time at sea with people on a regular basis is to open to them the best and the worst of your character. For them to be still speaking to you after suffering your mood swings, sweaty armpits and taste in music for a month on some rusting research vessel certainly reflects in the strength of their

character, for those who fall into this category and are not named – I apologise on all counts and salute you, cheers.

In September 1984, I discovered in Faddy my alter ego. Since then we've shared most of life's experiences, and as with Cliff and the top bunk, some are best long forgotten, thanks for your friendship and good luck for what comes next. I am generally a content and happy soul, this is due in no small part to the love I have always received from my family. I will take this opportunity to thank my Mum, Dad, Steve and Karen for the support that they have constantly given. Sharon must have known what she was letting herself in for when she married me – she'd known me long enough; for everybody else, that balding family man that slopes off from work every evening actually then does his best to sneak off to go fishing or play rugby whenever possible. Thanks Curly for everything, but especially for those two little boys Dan and Tommy.

And finally, to the PML fishing boys, thanks for the offer, but leave me alone - I'm in enough trouble as it is!

## Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award. The work here was carried out as part of the author's duties at the Plymouth Marine Laboratory (PML) as an employee of the Natural Environment Research Council (NERC) between 1991 and 2001.

During 1991 an intensive multidisciplinary study of Loch Linnhe on the West coast of Scotland was performed. 12 monthly research cruises were undertaken onboard the Irish Research Vessel (RV) Lough Foyle. Sample analysis was performed between February 1991 and December 1992. The results of primary production and nitrogen assimilation experimental work is presented in Paper I: *Rees et al., 1995a. Seasonal nitrogen assimilation and carbon fixation in a fjordic sea loch. Journal of Plankton Research. 17(6), 1307-1324.*

EROS-2000 was an European Commission (EC) funded project primarily focused on the impact of the River Rhone on the Mediterranean coastal zone. In July 1993 the Royal Research Ship (RRS) Discovery cruise 203 involved working a transect of stations along a gradient from coastal to oceanographic conditions. Sample analysis took place at PML between September and December 1993 and form the basis of Paper II: *Rees et al., 1995b. Phytoplankton nitrogen assimilation at low nutrient concentrations in the NW – Mediterranean Sea (July 1993). In: Martin J-M and Barth H., editors. Proceedings of the fifth EROS-2000 workshop. Water Pollution Reports No. 32. European Commission, p.141-148.*

The U.K. contribution to the JGOFS Southern Ocean study was the STERNA expedition which involved a two ship study (RRS James Clark Ross and RRS Discovery) to the South East Pacific sector of Southern Ocean during October to December 1992. The concentration and air-sea exchange of nitrous oxide for a number of transects and stations at the marginal ice-edge are presented in Paper III: *Rees et al., 1997. Nitrous Oxide in the Bellingshausen Sea and Drake Passage. Journal of Geophysical Research. 102(C2), 3383-3391.*

Fieldwork during RRS Charles Darwin cruise 85 in April/May 1994 formed a part of the contribution to another EC funded project OMEX. The aims of the Ocean Margin EXchange experiment were to quantify and characterise processes occurring at the continental shelf break of the western European margin and specifically for this study the Goban Spur in the Celtic Sea. Laboratory analysis was performed between August 1994 and April 1995. This study coincided with the onset of the spring bloom as described in Paper IV: *Rees et al., 1999. Early spring bloom phytoplankton-nutrient dynamics at the Celtic Sea shelf edge. Deep-Sea Research I. 46, 483-510.*

Experimental work performed during RRS Discovery cruise 211 during June/July 1996 as a contribution to the NERC PRIME thematic project followed by laboratory analysis which took place between September 1996 and April 1997 resulted in the publication of papers V and VI: *Rees et al., 1999. Measurement of nitrate and ammonium uptake at ambient concentrations in oligotrophic waters of the north-east Atlantic Ocean. Marine Ecology Progress Series, 187, 295-300;* and, *Rees et al., 2001. Carbon, nitrogen and phosphorous budgets within a mesoscale eddy: comparison of mass balance with in vitro determination. Deep-Sea Research II, 48, 859-872.*

Signed: Date: 23rd November 2001

## Abstract

The biological uptake and transformation of inorganic nitrogen species is described for contrasting marine environments which include a sea loch, riverine plume, polar marginal ice-zone, continental shelf break, oligotrophic ocean and a mesoscale eddy. Uptake of nitrate and ammonium by phytoplankton has been determined using the stable isotope $^{15}N$ as a tracer and continuous flow stable isotope-ratio mass spectrometry. The analysis of dissolved and atmospheric nitrous oxide was made using electron capture detector gas chromatography in a novel configuration which also allowed for the simultaneous analysis of methane from a single sample.

Significant advances in the study of the marine nitrogen cycle have been made and presented to the scientific community via publication in high quality research journals and by the placement of data into the British Oceanographic Data Centre. These are important from local and global perspectives; information on the trophic status of seawaters is presented with respect to seasonal and hydrographical variability, as is the contribution made to knowledge regarding the role of nitrogen in climate change.

Nitrogen availability is limiting in many oceans and attempts to constrain the global atmosphere - ocean fluxes of $CO_2$ are based on basin scale nitrogen balances. The development of novel analytical procedures and their subsequent use during a number of field programs and modeling exercises has increased the available knowledge regarding the role of nitrogen in the removal of carbon to the deep ocean. In particular new insights into new production and ultimately carbon export have been made, these include: (i) The accepted threshold limit for phytoplankton uptake of nitrate in the oligotrophic ocean has been reduced from 0.016 to 0.005 mmol $m^{-3}$, (ii) a relationship has been described relating the size structure of the phytoplankton productivity to new production which can be used as a direct estimate of carbon export, (iii) a series of algorithms have been produced to allow the basin scale estimation of new production from satellite and ship derived data, and (iv) whilst nitrous oxide plays a significant role in radiative forcing and stratospheric ozone depletion, an area in the Southern Ocean was found to provide a seasonal sink to atmospheric $N_2O$.

## List of published work forming the basis of this thesis

**Paper I** Rees A.P., Owens N.J.P., Heath M.R., Plummer D.H. & Bellerby R.S. (1995). Seasonal nitrogen assimilation and carbon fixation in a fjordic sea loch. *Journal of Plankton Research.* 17(6), 1307-1324.

**Paper II** Rees A.P., Owens N.J.P. & Woodward E.M.S. (1995). Phytoplankton nitrogen assimilation at low nutrient concentrations in the NW-Mediterranean Sea (July 1993). In: Martin J-M. and Barth H., editors. *Proceedings of the fifth EROS-2000 workshop. Water Pollution Research Reports No. 32.* European Commission, p.141-148, 1995.

**Paper III** Rees A.P., Owens N.J.P. & Upstill-Goddard R.C. (1997). Nitrous oxide in the Bellingshausen Sea and Drake Passage. *Journal of Geophysical Research: Oceans.* 102, 3383-3391.

**Paper IV** Rees A.P., Joint I. & Donald K.M. (1999). Early spring bloom phytoplankton-nutrient dynamics at the Celtic Sea shelf edge. *Deep-Sea Research I.* 46, 483-510.

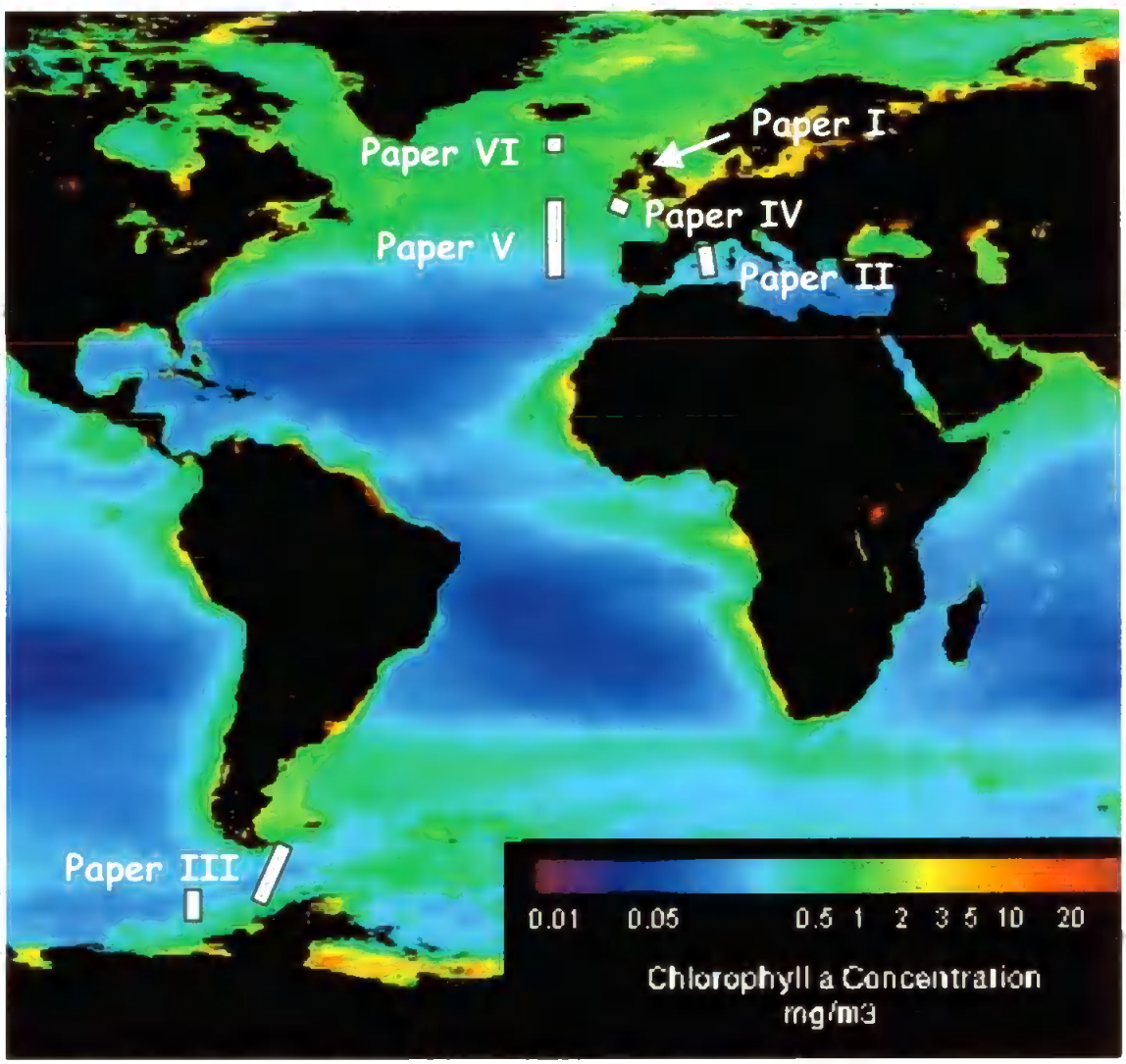
**Paper V** Rees A., Woodward M. & Joint I. (1999). Measurement of nitrate and ammonium uptake at ambient concentrations in oligotrophic waters of the north-east Atlantic Ocean. *Marine Ecology Progress Series.* 187, 285-300.

**Paper VI** Rees A.P., Joint I., Woodward E.M.S. & Donald K.M. (2001). Carbon, nitrogen and phosphorous budgets within a mesoscale eddy: Comparison of mass balance with in vitro determination. *Deep-Sea Research II.* 48(4/5), 859-872.

A complete publication record for A.P. Rees can be found in the Appendix.

# CRITICAL APPRAISAL

## The Uptake and Flux of Dissolved Nitrogen in Marine Waters




Approximate positions of cruise tracks presented in Papers I - VI. (*Image generated by the SeaWifs project, NASA/Goddard space flight centre and ORBIMAGE*).

## 1. *Introduction*

Nitrogen is thought to limit primary productivity throughout much of the global ocean. In recent decades interest in the role of nitrogen in the marine environment has been focussed on anthropogenic rather than natural perturbations, at regional and global scales. Such perturbations are in part due to the increase in fertilizer production and usage. At the regional scale, increased inputs of nitrogen to coastal waters may enhance both the frequency and intensity of eutrophication events. More recently however, research has largely been focussed on the role of the nitrogen cycle in global climate change. Nitrous oxide ($N_2O$) can react with and destroy stratospheric ozone and is a potent greenhouse gas, but more importantly, the availability of nitrogen in the ocean may govern the capacity of the biosphere to fix and sequester atmospheric $CO_2$ (Capone, 2000). The marine nitrogen cycle is a complex system owing to the existence of many forms that are not readily converted from one to another. The current research is dedicated to the study of nitrate ($NO_3^-$) and ammonium ($NH_4^+$) which account for the majority of the phytoplankton nitrogen requirement, and fluxes between the two forms, which may result in the production of $N_2O$.

The concept of new and regenerated production was introduced by Dugdale and Goering (1967), the basic premise being that primary production is supported either by new nitrogen that exists as $NO_3^-$ or fixed $N_2$ and regenerated nitrogen as $NH_4^+$ or dissolved organic nitrogen (DON). Large reservoirs of $NO_3^-$ (677 Tg N) exist below the permanent thermocline in the deep ocean (Capone, 1991), whilst the major supply of $NO_3^-$ to the ocean is from freshwater via estuaries, with groundwater and atmospheric inputs being increasingly recognized as other important sources (Owens, 1993). Regenerated nitrogen is that produced within the upper layers of the ocean following excretion by zooplankton or by bacterial transformation. $N_2O$ in the oceans is generally assumed to be biologically produced (Capone, 1991) as either an intermediate or end product of denitrification (bacterial degradation of $NO_3^-$ to $N_2$) or as a by-product of the nitrification process (microbial oxidation of $NH_4^+$ to $NO_2^-$ and $NO_3^-$). Nitrification is considered to be the dominant source of $N_2O$ (Nevison et al., 1995) and in the euphotic zone may provide an important source of regenerated $NO_3^-$ (Dore and Karl 1996).

At the cellular level, $NO_3^-$ taken up must first be reduced to $NH_4^+$ by assimilatory $NO_3^-$ reductase before being assimilated into the cell biomass (Falkowski 1983). This impinges a higher energy requirement on the cell assimilating $NO_3^-$ compared to $NH_4^+$, such energetics account in part for the difference in uptake rates relative to abundance and also on the ability of some species to take up $NH_4^+$ at a greater rate compared to $NO_3^-$ in the dark. Measurement of the uptake of nitrogen during these studies has utilized the stable isotope $^{15}N$ as a tracer and continuous flow stable isotope-ratio mass spectrometry techniques for sample analysis. The fundamental principles introduced by Dugdale and Goering (1967) following Nees et al. (1962) still apply today. The main advances since then have been the improved precision and sample throughput of the instrumentation, providing greater flexibility in experimental design (Preston and Owens, 1983; Owens and **Rees** 1989) and confidence at much lower nutrient concentrations following advancements in nutrient detection systems (Garside, 1982; Jones, 1991). $N_2O$ determinations were performed using a novel gas chromatography system developed and are described by Upstill-Goddard, **Rees** and Owens (1996).

## *2. Objectives*

The underlying objective of the presented work was to relate biological activity to transformations taking place in the marine nitrogen cycle relative to the prevailing physical conditions:

- By, improving current methodology, through the development of novel analytical procedures and the refinement of existing protocols.

In order to:

- Investigate the uptake of $NO_3^-$ and $NH_4^+$ at ambient concentrations in marine waters.
- Determine the relative rates of new to total production.
- Asses the microbial contribution to $N_2O$ flux in Antarctic waters.
- Investigate the effect of water column stability on phytoplankton productivity and gaseous exchange with the atmosphere.

Table 1. Summary of the experimental approach taken in each of the published works presented within this thesis.

| Paper | Environment | Study Mode | Concentration (mmol $m^{-3}$) $NO_3^-$ | Concentration (mmol $m^{-3}$) $NH_4^+$ | Incubation Period | $^{15}N$ addition | Bottle Size | Incubation Method | Comment |
|---|---|---|---|---|---|---|---|---|---|
| I | Sea Loch | Seasonal Survey – Fixed Stations | 0.2 - 8.8 | <0.1 - 3.5 | Dawn - Dusk | 0.1µM | 2.4 l | On-deck | Restricted tidal exchange, receiving external inputs from riverine and anthropogenic sources |
| II | River Plume | Nutrient Gradient | 0.05 - 8.53 | 0.02 - 0.12 | 24 hour | 10% ambient | 2.4 l | In-situ | Coastal zone study along decreasing nutrient gradient in surface waters |
| III | Southern Ocean | Latitudinal Transect & Marginal ice-zone | >25 | 0.01 - 2.5 | n/a $N_2O$ | - | - | - | Concentration of dissolved and atmospheric $N_2O$ |
| IV | Continental Shelf-Break | Depth Gradient | 6.1 - 8.6 | <0.1 - 1.5 | 24 hour | 0.1µM | 2.4 l | On-deck /In-situ | Two week study during onset of the spring bloom |
| V | Oligotrophic | Meridional Transect 50° – 36°N | 0.004 – 0.65 | 0.044 – 0.11 | 4 hour | 10% (- 3 nM minimum) | 2.4 l | On-deck | Determination of uptake characteristics during summertime oligotrophy |
| VI | Mesoscale Eddy | Lagrangian | 5.0 - 7.2 | 0.07 - 0.10 | 24 hour | 10% | 0.62 l | On-deck | Comparison of in-vitro and in-situ nitrogen uptake |

## *3. Discussion*

The papers presented in this thesis are intimately linked through the key, fundamental processes under investigation. Diverse environments were studied, requiring a varied experimental approach (Table 1). This diversity is a reflection of the progressive nature of the research and the specific aims of the individual projects, which were in part dictated by the requirements of the funding bodies. The emphasis is on the presentation of the key findings of these papers, by discussing in a logical sequence the improvement and advancements made in technological and methodological design followed by a description and critical assessment of the impact made to our knowledge of the marine nitrogen cycle.

### *3.1 Methodology and Developments*

Prior to these studies Owens and **Rees** (1989) presented a modification to the $^{15}N$-nitrogen analysis technique which was paramount in enabling a large part of this work to take place. $^{15}N$-nitrogen analysis of gaseous $N_2$ is made using a triple collector isotope-ratio mass spectrometer. Solid samples are converted by high temperature catalytic oxidation in the prescence of oxygen to $NO_x$, this is subsequently reduced to $N_2$ over copper wire at 500°C. The oxygen pulse carries with it a contaminant nitrogen signal. A modification to the process separates in time the sample from the oxygen and whilst maintaining optimal oxidation conditions allows the simultaneous measurement of nitrogen and $^{15}N$ at levels as low as 0.010 mmol $m^{-3}$ of total nitrogen. Where nitrogen levels had previously proved insufficient to allow precise analysis this development has proved invaluable. This protocol also allows smaller volumes of seawater (~0.5 l) to be used in experimental work enabling greater replication and improving statistical viability. Parallel to this development was the introduction of more sensitive methods of $NO_3^-$ and $NH_4^+$ analysis (Garside, 1982; Jones, 1991) which were developed and used in Papers II, III, V and VI. Paper II reported the first deployment of the nanomolar $NH_4^+$ system in European waters (Woodward, 1995), the significance of detecting $NH_4^+$ <0.1 mmol $m^{-3}$ (previous limit of detection) is evident from Fig. 1. For the first time $NH_4^+$ uptake was determined at the actual ambient concentration (0.02 – 0.12 mmol $m^{-3}$) and additions of

$^{15}N$-$NH_4^+$ tracer were made at concentrations not likely to stimulate phytoplankton uptake.

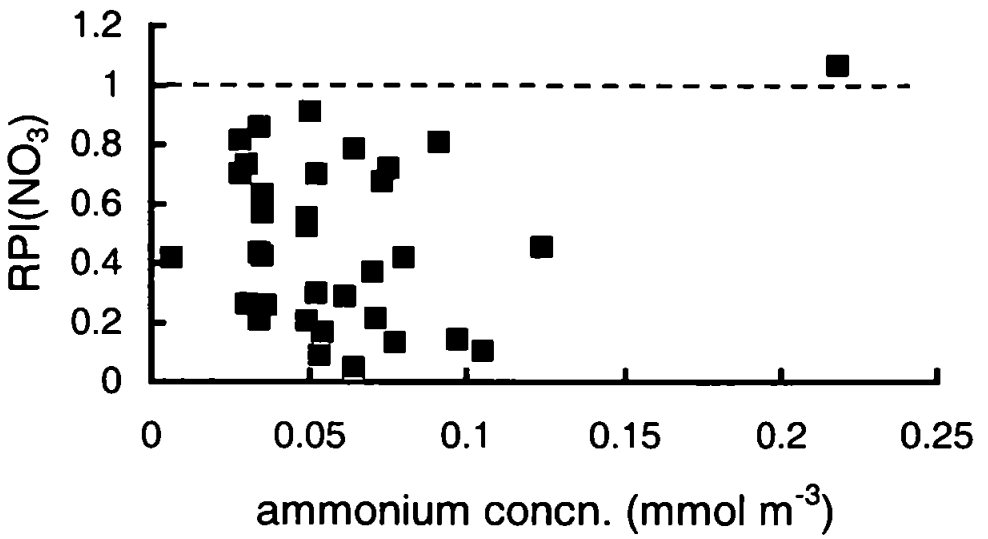



Fig. 1. Relationship between $NH_4^+$ concentration and RPI($NO_3$) in the NW Mediterranean during July 1993. (From: Paper II; Rees et al., 1995b).

The EU funded Ocean Margin Exchange (OMEX) project (Paper IV) involved three cruises to the Celtic Sea shelf break. In collaboration with the Vrije Universiteit Brussel and University of Hamburg a number of data sets were amalgamated which resulted in a novel approach to estimating new production in surface waters using satellite derived data. The hyperbolic relationship between the f-ratio (defined as the rate of $NO_3^-$ uptake as a function of total nitrogen uptake; Eppley & Peterson, 1979) and $NO_3^-$ concentration is used in association with an algorithm linking $NO_3^-$ concentration with satellite (AVHRR) derived sea-surface temperature data as described in Joint et al., (2001). This data set was further used to improve the estimation of f-ratio (Elskens, Goeyens, Dehairs, **Rees**, Joint, and Baeyens, 1999) by including the effect of $NH_4^+$ and expressing f-ratio as a function of both $NO_3^-$ concentration and $NH_4^+$ inhibition.

Paper V describes experiments which combined measurements of $NO_3^-$ and $NH_4^+$ at nanomolar concentrations with $^{15}N$-nitrogen tracer experiments. This was the first reported simultaneous deployment of these techniques and successfully recorded $NO_3^-$ uptake at ambient concentrations of 0.004 mmol $m^{-3}$ (Fig. 2). This enabled the

quantification of the relationship between $NO_3^-$ and $NH_4^+$ at concentrations typical of summer conditions in the North East Atlantic. The most significant development was the refinement of the lower limit of phytoplankton $NO_3^-$ uptake to 0.005 mmol $m^{-3}$ from 0.016 mmol $m^{-3}$ (McCarthy et al., 1992) hence defining the upper limit for any threshold $NO_3^-$ uptake.

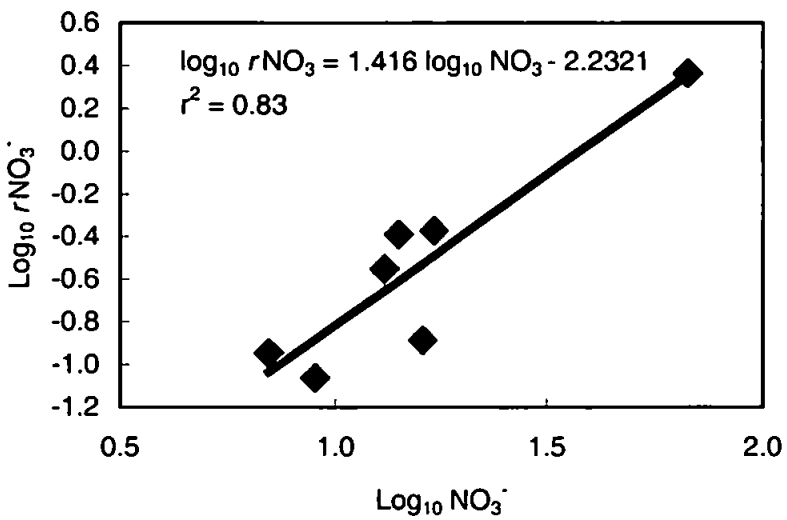


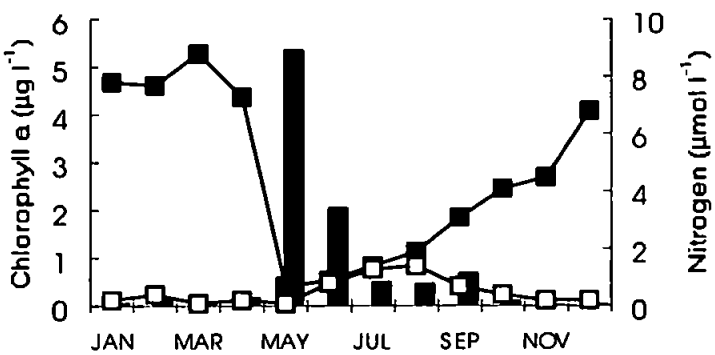

Fig. 2. Log transformation of nitrate uptake ($rNO_3^-$) versus $NO_3^-$, at concentrations <70 nM for data obtained between 50°and 36°N along the 20°W meridian during July 1996. (From Paper V; Rees et al., 1999b).

Upstill-Goddard, **Rees** and Owens (1996) present a novel system for the simultaneous measurement of $N_2O$ and methane ($CH_4$), which was subsequently deployed in the Southern Ocean (Paper III). This was the first reported case of an analytical system capable of determining $N_2O$ and $CH_4$ in a single seawater sample. The procedure involved a single-stage phase equilibration followed by gas chromatography using an electron capture detector for $N_2O$ and flame ionisation detector for $CH_4$. Significant advantages over previous methods include the avoidance of complicated sample purging or cold trapping procedures and the requirement for the minimum of sample manipulation, thus eliminating a number of potential sources of error. Consequently, the method is a substantial improvement on established procedures, and the analytical results are inherently more precise than those obtained previously.

### *3.2 Biological uptake and transformation of nitrogen*

#### *3.2.1 Investigation of processes*

The overriding control on phytoplankton production and therefore the uptake and utilization of nitrogen is the distinct seasonal pattern experienced by the majority of temperate waters. Butler et al. (1979) describe the change in the status and relative concentrations of dissolved nutrients for the English Channel. Typically $NO_3^-$ concentrations are maximal during the winter and become depleted during the spring bloom to be succeeded during the summertime by increases in $NH_4^+$ and DON following the onset of seasonal stratification and the establishment of regenerative processes in




surface waters.

Fig. 3. Seasonal trend of surface nitrogenous nutrient and chlorophyll a for station IB, Loch Linnhe 1991. The histogram represents the chlorophyll a concentration, $NO_3^-$ is shown by filled squares and $NH_4^+$ by open squares. (From Paper I; Rees et al 1995a).

The findings of Paper I are implicit in characterizing a full annual cycle (Fig. 3) and provide a sound platform for the introduction of a number of hypotheses on which subsequent papers are founded. During the winter whilst algal activity was at a minimum and chlorophyll concentration was of the order of 0.1 mg $m^{-3}$; $NO_3^-$ in surface waters increased to the maximum level (~8 mmol $m^{-3}$) whilst $NH_4^+$ was typically at or below the detection limit (~0.1mmol $m^{-3}$) of the analytical system used. Following the spring bloom during April and May, when chlorophyll biomass increased up to ~8 mg $m^{-3}$, $NO_3^-$ was

reduced to mid-summer values of 0.2 mmol $m^{-3}$ whilst between June and October $NH_4^+$ concentrations were generally >0.5 mmol $m^{-3}$ with infrequent peaks up to 3.5 mmol $m^{-3}$.

Inherent with changes in the nutrient status of seawaters are changing patterns in the relative rates of uptake of $NO_3^-$ and $NH_4^+$. The f-ratio provides valuable information on the prevailing trophic conditions. In Paper I Fig. 6, f-ratios are seen to increase from approximately 0.5 during the early part of the year to 0.8 – 0.9 during the peak of the bloom in May reflecting the dominance of $NO_3^-$ uptake. It is generally considered that $NH_4^+$ is the preferred nitrogen substrate for phytoplankton productivity (e.g. Goldman and Glibert 1983) though because of the manner of it's supply was found only to be quantitatively important during the summer and for Loch Linnhe $NO_3^-$ provided >50 % of the annual nitrogen demand (Paper I).

During the Celtic Sea shelf break spring bloom (Paper IV) the transition from winter to springtime conditions was monitored over a period of 16 days, changes in nutrient concentrations and phytoplankton productivity were followed and discussed relative to the phytoplankton assemblage size structure. Phytoplankton succession in the North Atlantic is now fairly well understood and the summary provided by Donald, Joint, **Rees,** Woodward and Savidge (2001) agreed well with that of Paper IV. The bloom was characterized by large (>2 $\mu$m) phytoplankton which were dominated by a number of species of diatoms responsible for the rapid uptake of $NO_3^-$ and resultant high f-ratios (>0.8). $NH_4^+$ uptake remained fairly constant whilst the production of the picoplankton (<2 $\mu$m) which originally represented 42% of the population declined as the bloom progressed.

Seasonal changes in $NO_3^-$ and $NH_4^+$ distributions were effectively mirrored in their spatial variability as described in Paper V, for a transect along the 20°W meridian. Sampling took place between 50°N and 36°N over 11 days in waters which changed from relatively nutrient replete conditions (0.65 mmol $m^{-3}$ $NO_3^-$) to severe oligotrophy (0.004 mmol $m^{-3}$ $NO_3^-$). Therefore quantifying changes, which may have otherwise only been experienced by prolonged temporal sampling at a fixed position, as the post bloom condition progressed into mid-summer. The general relationship between large phytoplankton associated with high f-ratios noted above was extended with an overall increase in the proportion of phytoplankton smaller than 5 $\mu$m from north to south (30 –

75% of the total community) as f-ratios decreased from 0.39 – 0.02. and the rate of $NO_3^-$ uptake ($rNO_3^-$) decreased with increasing values of the $NH_4^+$:$NO_3^-$ concentration ratio according to:

$$rNO_3^- = 3.19e^{-0.45(NH4:NO3)}$$

The importance of nitrification in the supply of $NO_3^-$ to oligotrophic waters is increasingly recognised as supporting productivity in surface waters (Dore and Karl, 1996; Diaz and Raimbault, 2000; **Rees** et al., in press). Nitrate regeneration through nitrification is reflected by a positive correlation between $NO_3^-$ and $\Delta N_2O$ (apparent nitrous oxide consumption/production). In Paper III nitrification was considered responsible for $N_2O$ production at oceanic stations (J & K) of the South East Pacific sector of the Southern Ocean whilst supply to the continental shelf waters (F to I) of the Bellingshausen Sea was from upwelling of supersaturated Continental Deep Water (Table 2).

Table 2. Correlation coefficients ($r$) of $\Delta N_2O$ on $NO_3^-$ for Stations F-K. * Correlation significant at $P = 0.001$; ** Correlation significant at $P < 0.0001$. (From Paper III; Rees et al., 1997).

| Station | Depth Range, (m) | $\Delta N_2O/NO_3^-$ | $n$ |
|---|---|---|---|
| F | 2-51 | 0.337 | 6 |
| G | 50-600 | -0.324 | 10 |
| I | 2-1380 | 0.344 | 14 |
| J | 10-3775 | 0.776* | 14 |
| K | 2-3000 | 0.943** | 12 |

### *3.2.2 Derived indices of productivity*

Throughout the published papers included in this thesis, reference is made to the f-ratio, the relative preference index – RPI (McCarthy et al., 1977) and the C:N uptake ratio (e.g. Sambrotto et al., 1993). Any one of these three concepts may be used

individually or in combination with either or both of the others with confidence to provide a descriptive tool of the ecosystem function with respect to nitrogen uptake and productivity providing the limitations of each approach are recognized.

The f-ratio indicates the uptake of $NO_3^-$ as a function of total nitrogen uptake and may in specific conditions be used in the estimate of new production and exportable production. More often than not it's use is restricted to describing the nutrient status of a sea area (Papers I, II, IV, V, VI). New production (the product of primary production and the f-ratio) is considered to approximate the sinking flux of particulate matter to the deep ocean and in the Celtic Sea was found to be significantly correlated to carbon fixation in the >2 $\mu$m phytoplankton size fraction (Fig. 4). This not only confirms but also quantifies the hypothesis that during the spring bloom the dominance of large phytoplankton provides the potential for significant vertical export. A significant correlation is often found between the f-ratio and $NO_3^-$, which may be represented by a simple hyperbolic function. Data presented in Paper IV have been used by Joint et al. (2001) in order to present this relationship for the Celtic Sea Shelf break:

$$\text{f-ratio} = 0.1177 \log_e NO_3^- + 0.5591 \quad (r^2 = 0.725)$$

The RPI is often used to assess whether or not there is preference for a particular nitrogen source (e.g. Papers I, II and V). Paper I (Fig. 6) and Paper II (Fig. 3) confirm that $NH_4^+$ is preferentially assimilated over $NO_3^-$ in the majority of occasions. Stolte and Riegman (1996) however, warn that the RPI should not be used to indicate a physiological preference and that as nutrient uptake is a non-linear function of the concentration of that particular nutrient then the RPI is really an indicator of uptake relative to the ambient concentration. Paper V supports this finding such that the $RPI_{NO3}$ is positively correlated ($r^2$= 0.70) with the $NH_4^+$:$NO_3^-$ concentration ratio;

$$RPI_{NO3} = -0.049 NH_4^+ : NO_3^- + 0.532$$

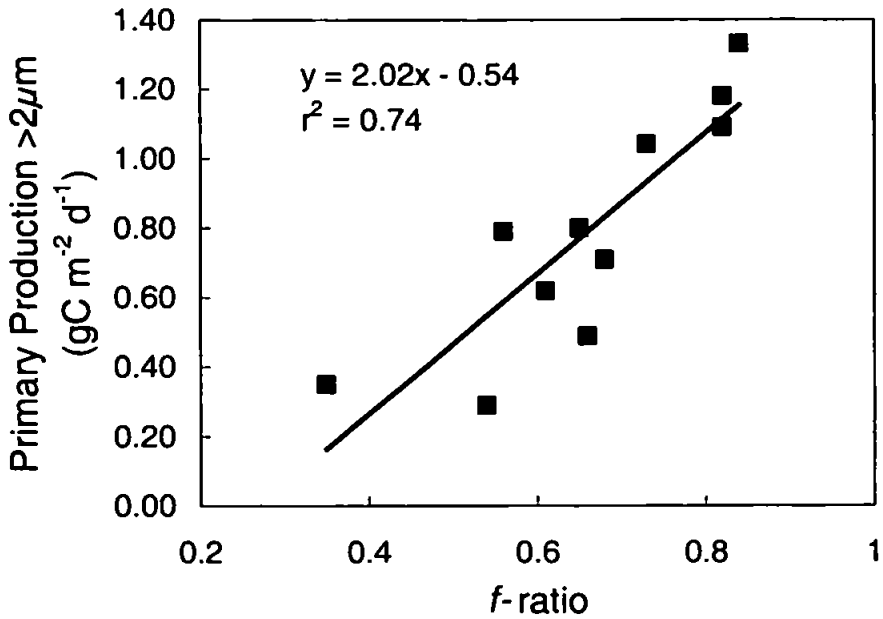



Fig. 4. Relationship between derived estimates of potential particulate vertical flux. Carbon fixed by phytoplankton larger than 2.0 μm against f-ratio, during April-May 1994 at the Celtic Sea shelf break. (From Paper IV; Rees et al., 1999a).

and the main cause in reduction of $RPI_{NO3}$ from 0.5 to 0.1 (where values equal to or greater than unity indicate preference) was a decrease in $NO_3^-$ concentration of two orders of magnitude.

The Redfield ratio (Redfield et al., 1963) is a useful concept in nutrient assimilation studies to link carbon and nitrogen uptake (Sambrotto et al., 1993) and in the case of Papers IV and VI, phosphorous uptake. If the elemental composition of a phytoplankton cell corresponds to the Redfield ratio of 106C:16N:1P or 6.6C:1N then nutrient assimilation ratios should also be close to this ratio when measured over the generation time of the cell. Though the C:N(:P) assimilation ratio will depend on a number of factors including nutrient availability and phytoplankton physiology. A number of processes are likely to result in deviations from the Redfield ratio, including; (i) nutrient uptake and photosynthesis may be de-coupled due to $NH_4^+$ and phosphate uptake in the dark, (ii) photosynthesis continuing after nutrients are exhausted, and (iii) changes occurring in the phytoplankton assemblage composition during nutrient-limited conditions. Papers I, IV and VI present a range of C:N uptake ratios. The majority of data show only limited deviation from the Redfield ratio, with significantly low values (<2,

Paper I and <3, Paper IV) presented at the onset of the spring bloom which may be due to luxury uptake of $NO_3^-$ as environmental conditions optimize. At the peak of the bloom in May nitrogen becomes limiting which results in the maximum uptake ratio of 23 (Paper I, Fig. 5). In paper VI concentrations of $NO_3^-$ were always >5 mmol $m^{-3}$ and could not be considered to be limiting production, however values for the C:N (and C:P) uptake ratios elevated above the Redfield ratio indicated that primary production was potentially being limited by nutrient supply. Further investigation revealed the presence of a significant population of the coccolithophore *Coccolithus pelagicus* a prymnesiophyte alga which fixes inorganic carbon as calcite plates on it's cell walls and it is proposed that the elevated values of fixed carbon are a result of calcite production.

### *3.3 Water column stability and physical control*

Evidence of the effects of water column stability on phytoplankton productivity and microbial activity can be seen in all 6 papers. Whether a particular sea area becomes thermally stratified or not is dependent on a balance between surface heating and vertical mixing (Owens, 1993). The onset of seasonal thermal stratification results in a surface layer becoming isolated from deeper waters. Phytoplankton cells are maintained in the surface layer where light is not usually limiting and whilst the supply of nutrients from deep waters is severely restricted, the boundary between the two layers provides a site for active regeneration of nitrogenous nutrients (Woodward and **Rees**, 2001; **Rees** et al., in press). In Paper III the interface between surface and intermediate waters was seen to be supersaturated in $N_2O$ (Paper III, Figs. 3 & 4) indicating the prescence of microbial activity in the form of nitrification; denitrification the alternative biological source of $N_2O$ is an anaerobic process and is unlikely to be significant in these relatively well oxygenated waters.

Paper I reported a marked difference between the four stations occupied due to the relative rates of tidal and riverine flushing. During the peak of the spring bloom the nitrogen uptake at three stratified stations was 4-6 times greater than the one station which was completely mixed, whilst carbon fixation was enhanced by up to an order of magnitude. A similar pattern is described in Paper IV where transient stratification early

in the cruise was disturbed by a storm resulting in the mixing of nutrients back into the surface but also in the loss of phytoplankton cells to deep waters. The difference in $NO_3^-$ uptake between the mixed water column and partially stratified conditions two weeks later was 2 – 3 times.

Often the limit of light penetration through the water column – the euphotic depth is coincident with or approximates to the depth of the surface mixed layer, for Loch Linnhe this was restricted by material of a terrigenous origin (Paper I, Fig. 3). In truly oligotrophic (low $NO_3^-$, low chlorophyll) conditions, light attenuance through the water column is also low. In the oligotrophic Mediterranean and North East Atlantic (Paper II; Donald et al., 2001) the euphotic depth was often found to be greater than the mixed layer depth, making $NO_3^-$ from deep water available to phytoplankton at the base of the euphotic zone. f-ratios at depths greater than 50m were between 0.5 and 0.96. In surface waters $NH_4^+$ (and urea) appeared to be the most important nitrogen source, but on integrating over the euphotic zone, $NO_3^-$ was found to supply up to 68% of the total nitrogen.

The oceans are generally considered as a source of atmospheric $N_2O$, though little information on $N_2O$ distribution is available for the Southern Ocean. Because of it's extraordinary hydrography, localised regions of temporally and spatially variable source-sink characteristics to atmospheric $N_2O$ are displayed. Major deviations from equilibrium between the sea surface and the atmosphere were found in Paper III to be largely associated with the presence of physical discontinuities (Fig. 5) including the retreating ice edge and oceanographic fronts. The surface waters of this sector of the Southern Ocean were found to be close to equilibrium with the atmosphere. In contrast to Bouwman et al. (1995) who hypothesised that the Antarctic Ocean is a major source of atmospheric $N_2O$, Paper III concludes that; following release of $N_2O$ accumulated under the seasonal sea-ice during winter, the Bellingshausen Sea at 85°W represents a net sink to atmospheric $N_2O$. Surface waters in the region of the ice retreat were found to be undersaturated in $N_2O$ following mixing of waters with meltwaters so that overall a small negative flux of –0.06 to -0.09 $\mu$mol $N_2O$ $m^{-2}$ $d^{-1}$ was recorded.

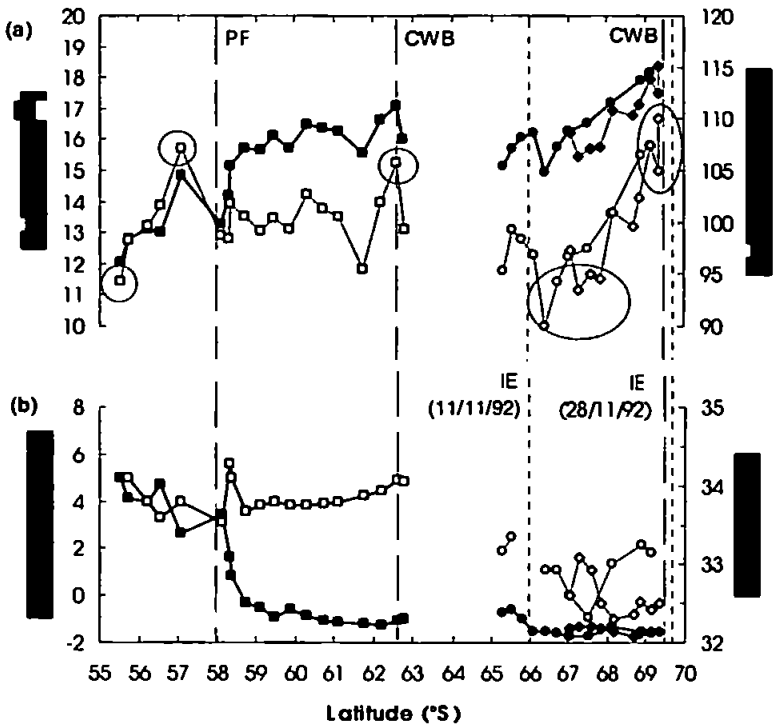



Fig. 5. Surface transects across the Drake Passage (squares) and at 85°W (circles and diamonds). (a) Change in nitrous oxide concentration (solid symbols) and percent saturation (open symbols). The circled points are those deviating from 100% saturation by ± 1 standard deviation (b) Surface temperature (solid symbols) and salinity (open symbols) profile. The dashed lines are approximate positions for the Polar Front (PF) and Continental Water Boundary (CWB). The position of the ice edge (IE) at 85°W is located by the dotted lines. (From Paper III; Rees et al., 1997).

## *4. Summary*

In Owens & **Rees** (1989) a refinement to the analysis of $^{15}$N-nitrogen procedure resulted in a method with greater precision at low levels of nitrogen typical of the oligotrophic ocean. This was the focus of ongoing research so that Paper V presented the first reported uptake of nitrogen at ambient concentrations of $NO_3^-$ and $NH_4^+$ in the oligotrophic ocean and refined the upper limit for a threshold nitrate concentration to 0.005 mmol $m^{-3}$ (from 0.016 mmol $m^{-3}$). This paper also described a significant relationship between $NO_3^-$ uptake rate and the ratio of $NH_4^+$:$NO_3^-$ concentration both of which have implications for ecosystem models of the North Atlantic. Data produced

during the OMEX I project (Paper IV) has been used to improve the f-ratio model allowing for the inhibitory effect of ammonium on nitrate uptake (Elskens et al., 1999). The same data set was used by Joint et al. (2001) to describe a series of algorithms relating the f-ratio to nitrate concentration and ultimately sea surface temperature derived from satellite (AVHRR) to estimate new production. A novel analysis system for $N_2O$ was developed (Upstill-Goddard et al., 1996) and used to identify localised areas of production and consumption of atmospheric $N_2O$ in the Southern Ocean (Paper III).

### *4.1 The significant outcomes of work presented in this thesis are:*

- The first determination of $NH_4^+$ uptake in European waters at concentrations $<0.1 mmol\ m^{-3}$.
- Rapid $NO_3^-$ uptake during the spring bloom at the European continental margin of the North East Atlantic accounted for >60 % of the annual new production.
- A significant relationship between phytoplankton size and new production was described for the Celtic Sea, which may be used in estimating the loss of organic material to the deep ocean.
- First published account of the simultaneous use of $^{15}N$ tracer techniques and analysis of $NO_3^-$ and $NH_4^+$ at low nanomolar concentrations.
- Confidence in uptake rates at low levels due to nanomolar detection limits of analytical system
- Measurement of $NO_3^-$ uptake at ambient concentration of 0.004 mmol $m^{-3}$, lowering known threshold limit of uptake to ~0.005 mmol $m^{-3}$. Critical to refinement of ecological models.
- Published algorithms to estimate satellite remotely sensed new production
- Atmospheric $N_2O$ concentration is increasing at 0.2-0.3% per year and the role of the oceans as a source or sink is uncertain and requires further quantification. The Bellingshausen Sea was found to represent a seasonal sink to atmospheric $N_2O$.

## *5. Future work*

The cycling of nitrogen in marine waters has been the object of innumerable studies over the last half century on a world-wide basis. However whilst analytical and molecular biology capabilities continue to improve there remains a vast degree of uncertainty regarding a number of the processes involved and over the numerous links and interrelationships involved. A major consequence of climate change is the need to extrapolate to ocean-basin or global scales and to refine ecosystem models and satellite based algorithms. Modelling exercises will be unable to successfully predict or replicate real-time scenarios while many processes remain unknown or poorly understood. Work at the Iberian Peninsular and in the northern North Sea (Joint, **Rees** and Woodward, 2001; **Rees** et al., in press) has highlighted the importance of urea to phytoplankton. Urea together with other components of the DON pool form an important source of nitrogen to the phytoplankton (Antia et al., 1991) particularly under oligotrophic conditions, although the source and rates of production of the DON remain largely unquantified. Paper V identified the need for further investigation of nitrogen uptake at nanomolar concentration as large areas of the ocean surfaces are characterized by concentrations of $NO_3^-$ significantly less than the detection limit for conventional analysis (Sharp, 1983). Processes such as nitrification and nitrogen fixation are often critical to the function of an ecosystem but remain largely unquantified. Nitrification occurs at the base of the mixed layer, but has recently been measured throughout the euphotic zone (Diaz and Raimbault, 2000; Lipschultz, 2001) a possible consequence of which is the underestimation of $NO_3^-$ uptake rate by approximately 2.5 times (**Rees** et al., in press). Biological fixation of atmospheric dinitrogen ($N_2$) is the dominant mechanism for the introduction of N into the biosphere. In the oligotrophic ocean gyres N fixation may fuel up to half of the new production and in light of the central role of N fixation in the ongoing debate concerning the ultimate limiting nutrient it is essential that quantitative data are obtained to address the balance of oceanic nutrient budgets. Reconciliation of these unknowns creates an ongoing requirement to unravel further the intricacies of the marine nitrogen cycle.

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**Paper I**
Seasonal nitrogen assimilation and carbon fixation in a fjordic sea loch.
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**Rees A.P.,** Owens N.J.P., Heath M.R., Plummer D.H. & Bellerby R.S.

Contribution in terms of total effort by A.P. Rees on this work was: 80%

Signed: N.J.P. Owens Date: 10th May 2001.

Signed: M.R. Heath Date: 17 April 2001

Signed: D.H. Plummer Date: 19 April 2001

Signed: R.S. Bellerby Date: 3rd May 2001

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# Seasonal nitrogen assimilation and carbon fixation in a fjordic sea loch

A.P.Rees[1], N.J.P.Owens[1,3], M.R.Heath[2], D.H.Plummer[1] and R.S.Bellerby[1]

[1]*Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH and* [2]*Scottish Office Agriculture and Fisheries Department, Marine Laboratory, Aberdeen, AB9 8DP, UK*

[3]*Present address: Department of Marine Sciences and Coastal Management, University of Newcastle, Newcastle upon Tyne, NE1 7RU, UK*

**Abstract.** Carbon (C) fixation and nitrogen (N) assimilation rates have been estimated from $^{14}C$ and $^{15}N$ techniques for a 12 month period in a Scottish sea loch. The maximum rate of nitrogen assimilated (29.92 mmol N $m^{-2}$ $day^{-1}$) was in April at the most seaward station; similar high rates were experienced during May at the other stations. Carbon fixation rates were maximal (488–4047 mg C $m^{-2}$ $day^{-1}$) at the time of high phytoplankton biomass (maximum 8.3 mg $m^{-3}$ chlorophyll *a*) during May, whilst nitrate concentrations remained >0.7 μmol $l^{-1}$. C:N assimilation ratios suggest nitrogen limitation only during the peak of the spring bloom, although at times nitrogen (nitrate and ammonium) concentration fell to 0.2 μmol $l^{-1}$ in the following months. The vertical stability of the water column, influenced by tidal and riverine flushing, varied along the axis of the loch, resulting in marked differences between sampling stations. Although ammonium was preferentially assimilated by phytoplankton, >50% of production was supported by nitrate uptake and only during the summer months was the assimilation of ammonium quantitatively important.

## Introduction

Concern over inorganic nitrogen loading to coastal waters is a problem faced by most nations. Anthropogenic inputs from various sources, including sewage and fish farming, are typical of nutrient loadings which, in combination with natural features such as water column stratification and light field structure, may ultimately result in occurrences of algal blooms and eutrophication (Richardson, 1990; Zevenboom *et al.*, 1991). In recent years, many investigations have taken place into both the incidence and causal effects of such phenomena (Lancelot and Mathot, 1987; Owens *et al.*, 1990; Joint and Pomroy, 1993). However, despite this intense study, there would appear to be no simple common relationship, and prediction of any such occurrence requires detailed field knowledge coupled with a model specific to a particular ecosystem. Such a study has been carried out jointly by the Aberdeen and Plymouth Marine Laboratories between January and December 1991. The site chosen was Loch Linnhe on the west coast of Scotland. The programme was underpinned by the development of a fully dynamic simulation model of fjord ecosystems at the University of Strathclyde (Ross *et al.*, 1993a,b, 1994).

The physical properties of most Scottish sea lochs are dominated by tidal exchange, vertical entrainment and freshwater input. The high tidal range over most of the west of Scotland ensures that, despite being virtually landlocked, the waters of the sea lochs are strongly linked to the open sea and well flushed throughout the year compared to most river-dominated estuaries. However, high

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freshwater input from mountainous terrain of much of the region (typical annual rainfall >2000 mm) results in strong haline stratification which greatly influences the biological and chemical activity (Grantham, 1981; Tett, 1986). The modelling studies (Ross *et al.*, 1993a) indicated that the gross behaviour of sea loch systems with respect to nutrients can be compared to a laboratory chemostat, in that concentrations reflect a balance between tidal exchanges of bound and dissolved nutrient with the open sea and assimilation within the loch. The overall aim of the programme was to test the relationship between the flux of dissolved nitrogen across the open-sea boundary of the sea loch and the biological dynamics within the loch. This paper describes measurements of the annual cycle in nitrate and ammonium assimilation and carbon fixation at four sampling locations, and tests the hypothesis that inorganic nitrogen supply exceeds demand throughout the year on a whole-loch scale, except at the peak of the spring bloom. This is the first reported use of $^{15}N$ techniques in the determination of nitrogen uptake in this area. The derivation of annually integrated assimilation rates for nitrate and ammonium is also the first we are aware of that has been resolved over a complete 12 month sampling regime. Irradiance, rather than nutrient concentration, was predicted by the model to be the main factor limiting primary production, whilst algal biomass and the resultant nutrient demand were predicted to be controlled for most of the year by zooplankton grazing on the phytoplankton (Ross *et al.*, 1993a,b). In addition, this paper examines the interactions between phytoplankton production and the relative contributions of nitrate and ammonium to the total nitrogen assimilation over an annual cycle, an aspect of coastal nutrient dynamics which is still little understood (Dortch, 1990).

## Method

Loch Linnhe is a fjordic sea loch which (at its southernmost end) opens into the Firth of Lorne (Figure 1). It comprises two deep basins (maximum depth >100 m) separated by a shallow (<10 m) sill at Corran Narrows. The uppermost part of the loch is separated from Loch Eil by another sill just north of the town of Fort William, the major population centre on the shores of the loch. A more detailed description can be found in Connor (1990).

Data were obtained during 12 cruises on board the RV 'Lough Foyle' between January and December 1991, at three stations positioned along the axis of the loch [the Inner Basin (IB), the Outer Basin (OB) and Lismore (Lis)] and a fourth in the Firth of Lorne (FoL) (Figure 1). Owing to inclement weather conditions, stations were not occupied at FoL and Lis during November, and at FoL during December. In addition to data described below, measurements were also made of zooplankton biomass, grazing and excretion rates, macroplankton biomass and sediment nutrient flux. Environmental variables were measured at high frequency by the deployment of ARIES, a towed undulating vehicle (Dunn *et al.*, 1993), and maintenance of moored instruments (current meters, thermistor chains, fluorometers, transmissometers, nitrate sensors, sediment traps and tide gauges) at four locations throughout the year. Incident radiation was logged by a

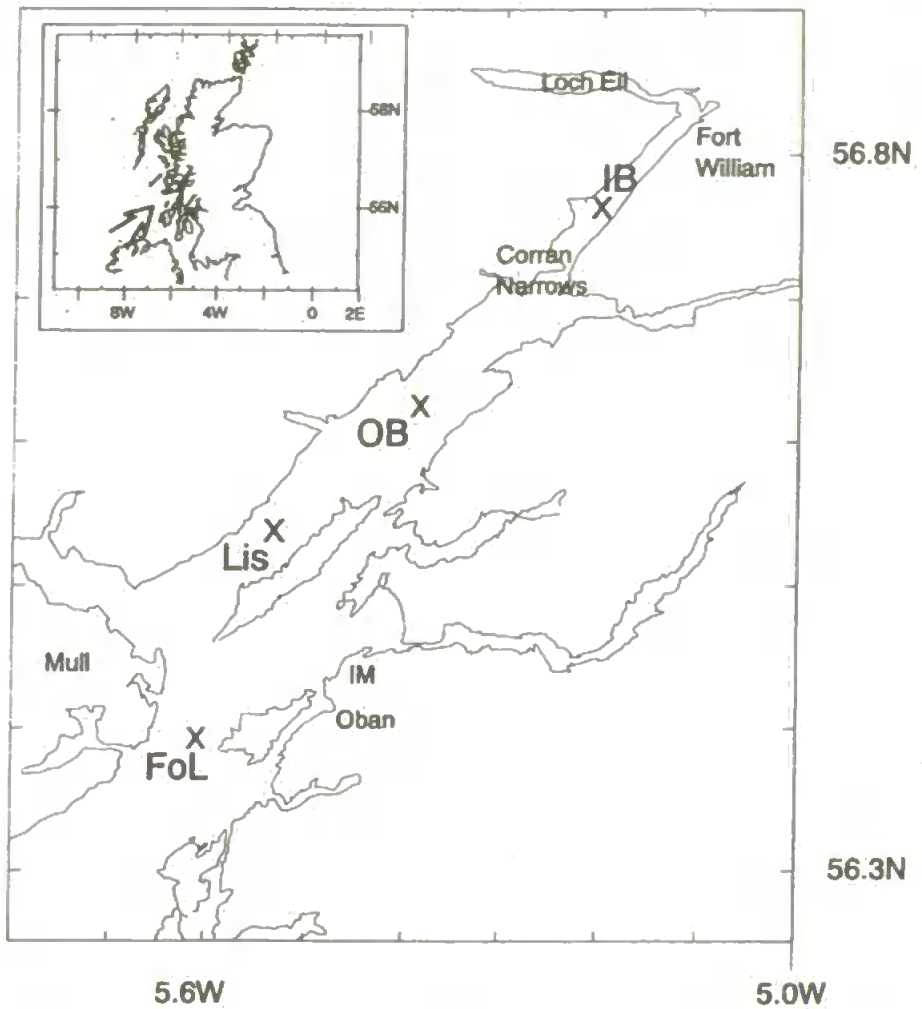


**Fig. 1.** Location of the study area, showing station positions (X) and site of irradiance meter (IM).

shore-based irradiance meter as hourly mean quantum flux (μmol quanta $m^{-2}$ $s^{-1}$) for the whole of the study period.

*Nitrogen assimilation*

The assimilation rates for both nitrate and ammonium were measured following inoculation with the stable isotope $^{15}N$. Water samples were collected pre-dawn from six depths corresponding as far as possible to the simulated light profile of an on-deck incubator. This consisted of a series of six plastic boxes with neutral-density filters to give 100, 60, 30, 16, 3 and 0.3% of ambient irradiance. Vertical light attenuation coefficients used in the calculation of simulated depths for the onboard incubator were collected by an irradiance sensor (Chelsea Instruments) interfaced to either a profiling CTD system or the towed undulating ARIES system. Surface seawater was pumped through the system to maintain the temperature at ambient levels. This method for estimation of carbon fixation gives comparable data to the method of *in situ* free-floating rigs (Joint and

Pomroy, 1993; Joint *et al.*, 1993) and this in combination with the operational convenience gives confidence in its use for this particular study. Water was obtained from a deck-mounted pump and a 40 m length of 1.5 inch bore PVC/terylene hosing. To avoid light shock to the phytoplankton, the water was kept in the dark prior to incubation. Samples from each depth were distributed into four 2.4 l clear polycarbonate bottles and 0.1 μmol $l^{-1}$ of $[^{15}N]NO_3$ and $[^{15}N]NH_4$ was added to duplicate pairs. These bottles were transferred to the on-deck incubator before dawn. Incubations were terminated at dusk by filtration (<40 cm Hg vacuum) onto ashed Whatman GF/F filters, which were then washed with filtered seawater and stored frozen until return to the laboratory, where they were oven dried at 50°C before analysis. Atom% $^{15}N$ was measured by continuous flow nitrogen analysis–mass spectrometry using the techniques described in Barrie *et al.* (1989) and Owens and Rees (1989), and rates of assimilation calculated from the equations of Dugdale and Goering (1967). Nitrate and ammonium concentrations were determined on subsamples of the water by standard analytical methods (Solorzano, 1969; Strickland and Parsons, 1972).

Long incubations (>6 h) may result in inaccuracies in the rate estimation due to substrate depletion or isotope dilution as a result of ammonium regeneration (Fisher *et al.*, 1982; Glibert *et al.*, 1982; Ward *et al.*, 1984; Kokkinakis and Wheeler, 1987). To check for this source of error in the deck incubations, surface waters inoculated as above were also incubated in a laboratory incubator at ambient temperature and normalized for light at 100 μmol quanta $m^{-2}$ $s^{-1}$ for generally <6 h. Over this time period, nitrogen uptake is accepted to be linear (La Roche, 1983), as substrate consumption is insignificant (Harrison *et al.*, 1983) and isotope dilution is minimal. Nitrate and ammonium uptake was computed to be less than one-third of the original concentration in 92% and 72%, and greater than two-thirds in 3% and 10% of experiments, respectively. Rates determined in this manner were then extrapolated to give maximum potential daily uptake rates, assuming 100% uptake rate over 24 h; and expressed as a ratio of the rates obtained from the deck incubator, in order to estimate the error introduced through the non-linearity of long incubations.

*Carbon fixation*

The rate of carbon fixation was estimated from the incorporation of $^{14}C$-labelled bicarbonate. Aliquots of the water used in the nitrogen assimilation experiments were transferred into triplicate 125 ml polycarbonate bottles and inoculated with 5.0 μCi $NaH^{14}CO_3$. Incubations were made in the on-deck incubator as described above, and were terminated after dusk by filtration (<40 cm Hg) onto 0.2 μm pore size polycarbonate filters, which were then rinsed with filtered seawater and frozen until return to the laboratory. $^{14}C$ was measured in a liquid scintillation counter, the efficiency of which was determined with an external standard, channels ratio method.

*Chlorophyll a concentration*

The data presented here are from two sources. Estimates of surface values (mg

$m^{-3}$) were made by *in vitro* fluorescence analysis, the method used was adapted from that described by Holm-Hansen *et al.* (1965). Single or duplicate aliquots from water used in the above experiments were filtered through 0.2 μm polycarbonate filters and frozen until return to the laboratory. Pigments were extracted by addition of 90% acetone and stored in the dark at 4°C for ~12 h. Depth-integrated concentrations (mg $m^{-2}$) have been determined from vertical profiles of the water column made using a calibrated Sea Tech fluorometer interfaced to a profiling CTD, deployed directly after water sample collection.

## Results

The seasonal distribution of nitrate, ammonium and chlorophyll *a* concentrations (Figure 2), and the resultant production rates, show a similar pattern at all four stations. The monthly sampling scheme did not resolve any succession of changes along the length of the loch. However, each station displayed individual characteristics with marked differences between the two basins.

### *Euphotic zone nutrients and phytoplankton biomass*

The maximum recorded nitrate concentration (8.8 μmol $l^{-1}$) was at IB in March; the mean concentration for all stations during the first 3 months of the year was 7.9 μmol $l^{-1}$ (minimum 6.2 μmol $l^{-1}$), with little evidence of vertical variation. A rapid decrease from winter nitrate levels occurred with the onset of the spring bloom between April and May; surface nitrate concentrations were reduced to 0.7 μmol $l^{-1}$ at IB, and eventually 0.2 μmol $l^{-1}$ at Lis during June and August. Summer conditions were characterized by low, but never completely deplete nitrate concentrations, with levels generally increasing with depth. Following the minima at IB, surface concentrations showed an increasing trend back towards winter levels over the rest of the year (Figure 2). Stations OB and Lis showed increases in nitrate concentration in August and July over their mean summer conditions, and displayed a sharp increase in October, by which time there was little vertical variation. A minimum surface nitrate concentration in June at FoL was followed by a gradual increase throughout the summer, again followed by a dramatic increase during October.

In general, concentrations of ammonium varied little throughout the year, being close to or below the detection limit of the analysis method (0.1 μmol $l^{-1}$) for the majority of the time. Only between the months of June and October did these levels increase above 0.5 μmol $l^{-1}$. During this time vertical variation was most apparent, with concentrations in the photic zone tending to increase with depth. However, anomalous maximum surface concentrations of 2.8 and 2.4 μmol $l^{-1}$ occurred in July at OB and at Lis, respectively, ~20 times higher than at 5 m. At both stations concentrations had returned to <0.5 μmol $l^{-1}$ by August. Elevated concentrations were experienced between June and September at IB (maximum 3.5 μmol $l^{-1}$, at 15 m in June) and between July and October at FoL (maximum 1.3 μmol $l^{-1}$, at 5 m in September).

The beginning of the spring bloom was first indicated at FoL in April, when phytoplankton biomass (measured as chlorophyll *a* concentration) showed a

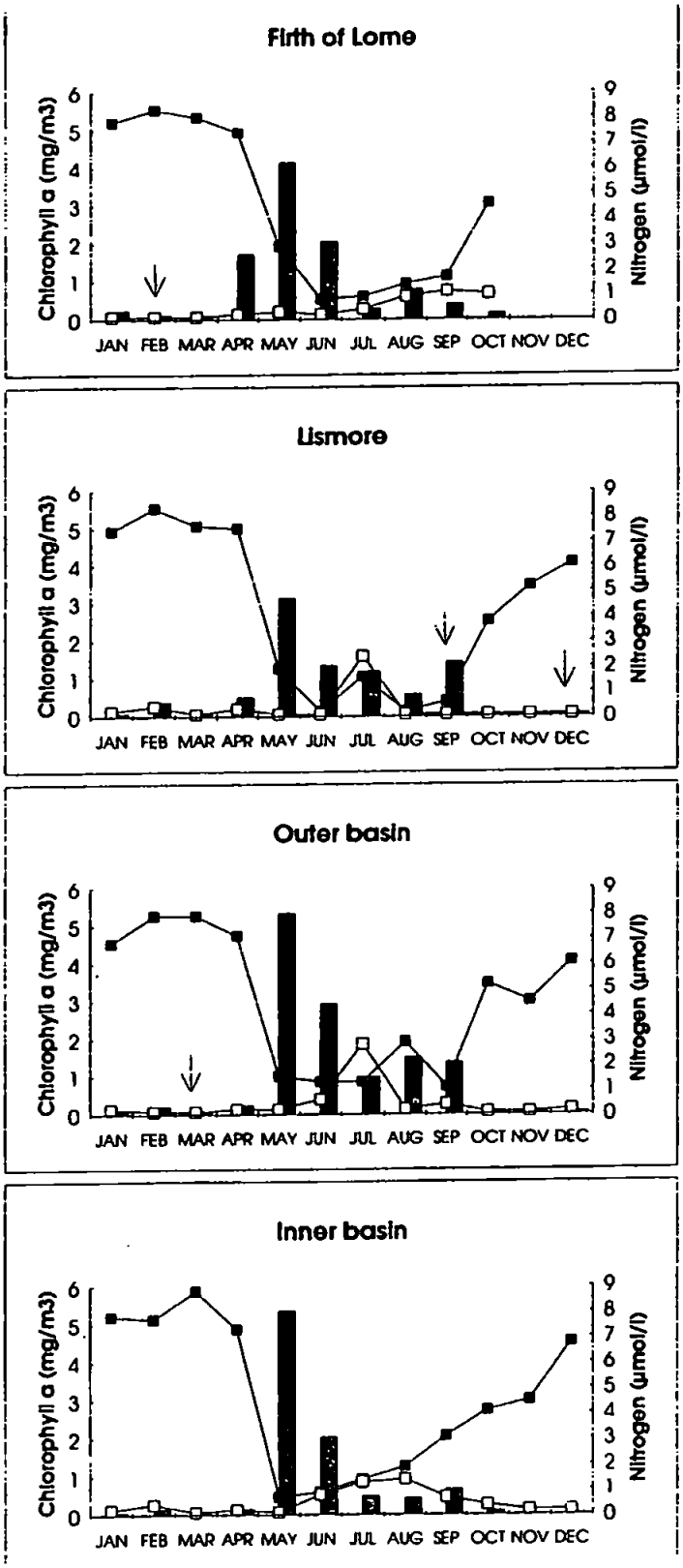


**Fig. 2.** Seasonal trend of surface nitrogenous nutrient and chlorophyll *a* for stations FoL, Lis, OB and IB, Loch Linnhe 1991. The histogram represents the chlorophyll *a* concentration, nitrate is shown by filled squares and ammonium by open squares. Arrows indicate ammonium concentration <0.1 $\mu mol^{-1}$.

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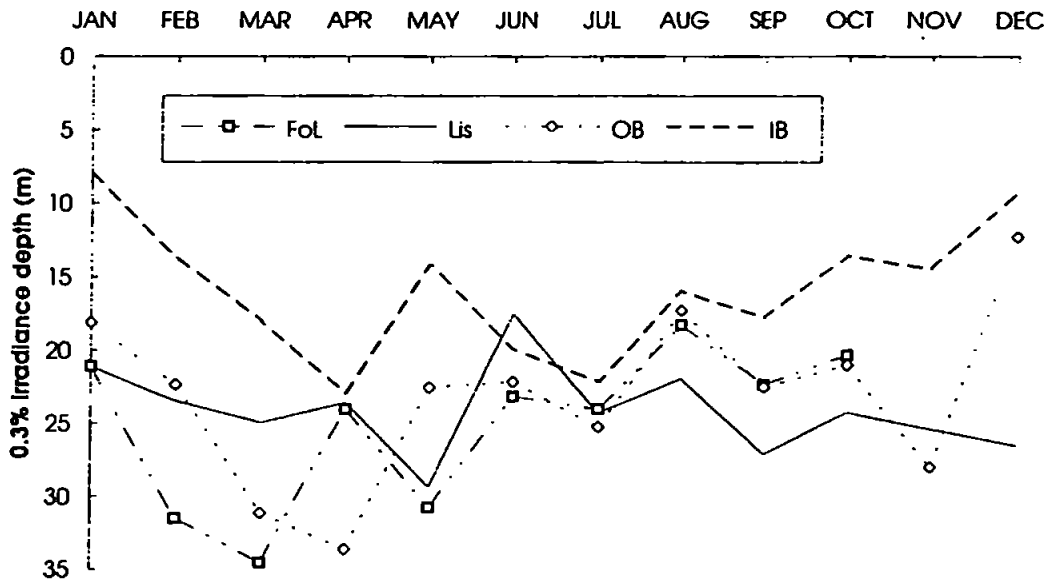


**Fig. 3.** Monthly change in the 0.3% irradiance depth for stations FoL, Lis, OB and IB, Loch Linnhe 1991.

17-fold increase from 0.1 mg $m^{-3}$ in the top 5 m. This was accompanied by increases at the other stations of 3–6 times from 0.08, 0.09 and 0.03 mg $m^{-3}$ for Lis, OB and IB, respectively, throughout the euphotic zone, and below 5 m at FoL. Maximum surface concentrations were recorded at all stations during May, and ranged from 3.1 mg $m^{-3}$ at station Lis to 5.3 mg $m^{-3}$ at stations OB and IB. Sub-surface peaks of 8.3 and 8.0 mg $m^{-3}$ were found at OB and IB, respectively, at 8 m. Although relatively high levels were maintained until September, there were summertime minima during either July or August at all stations, the lowest value being 0.3 mg $m^{-3}$ at FoL. In parallel with changes described above for nitrate, the chlorophyll concentrations returned to typical winter values ($\leq$0.1 mg $m^{-3}$) between September and October.

The euphotic zone thickness, defined by the depth of the 0.3% irradiance level, did not show a strong seasonal variation (Figure 3), suggesting that suspended sediment rather than shading by phytoplankton was probably the main contributor to vertical attenuation. This conclusion was supported by transmissometer data (not shown) and a progressive deepening of the 0.3% irradiance depth with distance from the head of the loch (shallowest at IB and deepest at FoL). Other authors have chosen to use different irradiance levels in the range 0.1–1.0% to define the base of the euphotic zone [e.g. Owens *et al.* (1991) and McCarthy *et al.* (1977)]. A value of 0.3% was chosen here since this was the lowest light level in the incubator used to measure carbon fixation and nitrogen assimilation rates. Chlorophyll concentrations integrated to the 0.3% irradiance depth were correlated with concentrations in the surface layer so that $r^2 = 0.621$ and $P < 0.0001$.

*Carbon fixation and nitrogen assimilation*

Measured rates of photosynthetic carbon assimilation integrated to the 0.3%

**Table I.** Depth-integrated values for nitrogen (nitrate and ammonium) assimilation (assim.) and carbon fixation rates for stations FoL, Lis, OB and IB in Loch Linnhe, 1991

| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Firth of Lome | | | | | | | | | | | | |
| $NO_3$ assim. (mmol N $m^{-2}$ $day^{-1}$) | ND | 2.02 | 3.41 | 27.20 | 1.86 | 6.37 | 0.65 | 1.22 | 0.59 | 1.04 | NS | NS |
| $NH_4$ assim. (mmol N $m^{-2}$ $day^{-1}$) | 0.63 | 1.02 | 1.36 | 2.72 | 1.89 | 4.06 | 5.91 | 4.03 | 5.05 | 2.33 | NS | NS |
| Total N assim. (mmol N $m^{-2}$ $day^{-1}$) | 0.63 | 3.04 | 4.76 | 29.92 | 3.75 | 10.42 | 6.56 | 5.25 | 5.64 | 3.37 | NS | NS |
| C assim. (mg C $m^{-2}$ $day^{-1}$) | 44.04 | 87.68 | 260.44 | 103.54 | 487.53 | 406.16 | 385.39 | 287.22 | 183.08 | 230.67 | NS | NS |
| C/N assim. ratio | 5.82 | 2.40 | 4.56 | 0.29 | 10.84 | 3.25 | 4.90 | 4.56 | 2.70 | 5.70 | NS | NS |
| Lismore | | | | | | | | | | | | |
| $NO_3$ assim. (mmol N $m^{-2}$ $day^{-1}$) | ND | 0.54 | 8.20 | 4.79 | 20.22 | 2.71 | 4.97 | 3.98 | 8.75 | 1.02 | NS | 3.94 |
| $NH_4$ assim. (mmol N $m^{-2}$ $day^{-1}$) | 0.98 | – | 1.36 | 1.59 | 3.27 | 3.08 | 4.45 | 8.35 | 3.87 | 5.07 | NS | 1.09 |
| Total N assim. (mmol N $m^{-2}$ $day^{-1}$) | 0.98 | – | 9.56 | 6.38 | 23.49 | 5.79 | 9.42 | 12.33 | 12.62 | 6.08 | NS | 5.03 |
| C assim. (mg C $m^{-2}$ $day^{-1}$) | 35.08 | 48.58 | 178.20 | 57.66 | 1154.38 | 348.78 | 495.19 | 609.21 | 436.00 | 175.75 | NS | 198.01 |
| C/N assim. ratio | 2.98 | – | 1.55 | 0.75 | 4.09 | 5.02 | 4.38 | 4.12 | 2.88 | 2.41 | NS | 3.28 |
| Outer basin | | | | | | | | | | | | |
| $NO_3$ assim. (mmol N $m^{-2}$ $day^{-1}$) | ND | – | 1.60 | 6.57 | 12.51 | 7.50 | 6.25 | 3.47 | 9.23 | 0.64 | 0.74 | 1.01 |
| $NH_4$ assim. (mmol N $m^{-2}$ $day^{-1}$) | 2.49 | – | 1.28 | 4.95 | 1.95 | 6.25 | 6.52 | 2.50 | 4.53 | 2.81 | 1.13 | 0.42 |
| Total N assim. (mmol N $m^{-2}$ $day^{-1}$) | 2.49 | – | 2.87 | 11.52 | 14.46 | 13.75 | 12.76 | 5.97 | 13.75 | 3.45 | 1.87 | 1.43 |
| C assim. (mg C $m^{-2}$ $day^{-1}$) | 58.34 | 47.97 | 257.14 | 65.00 | 4046.69 | 331.79 | 730.22 | 438.72 | 601.71 | 187.41 | 57.08 | 83.55 |
| C/N assim. ratio | 1.95 | – | 7.45 | 0.47 | 23.32 | 2.01 | 4.77 | 6.13 | 3.65 | 4.52 | 2.54 | 4.88 |
| Inner basin | | | | | | | | | | | | |
| $NO_3$ assim. (mmol N $m^{-2}$ $day^{-1}$) | ND | 0.49 | 0.66 | 0.70 | 22.66 | 5.31 | 0.52 | 1.37 | 2.03 | 1.14 | 0.47 | 1.19 |
| $NH_4$ assim. (mmol N $m^{-2}$ $day^{-1}$) | 0.56 | 0.93 | 0.58 | 1.26 | 0.89 | 8.42 | 8.38 | 4.43 | 5.69 | 2.11 | 1.29 | 0.83 |
| Total N assim. (mmol N $m^{-2}$ $day^{-1}$) | 0.56 | 1.42 | 1.24 | 1.95 | 23.55 | 13.73 | 8.90 | 5.80 | 7.72 | 3.26 | 1.76 | 2.02 |
| C assim. (mg C $m^{-2}$ $day^{-1}$) | 12.53 | 32.95 | 112.66 | 28.82 | 2805.90 | 723.33 | 533.74 | 415.41 | 353.15 | 157.87 | 18.85 | 40.18 |
| C/N assim. ratio | 1.87 | 1.93 | 7.59 | 1.23 | 9.93 | 4.39 | 5.00 | 5.97 | 3.81 | 4.04 | 0.89 | 1.65 |

ND, not detected; NS, no station; –, missing data.

irradiance depth (mg C $m^{-2}$ $day^{-1}$) spanned two orders of magnitude (Table I). In comparison, depth-integrated nitrogen assimilation rates (mmol N $m^{-2}$ $day^{-1}$) only varied by a factor of 50. The maximum carbon fixation rate of >4 g C $m^{-2}$ $day^{-1}$ was recorded at OB in May with the range of seasonal variation comparable to data recorded for other areas [see, for example, Reid *et al.* (1990) for the North Sea, Minas and Minas (1991) for the northwestern Mediterranean Sea, and Li *et al.* (1993) for the western North Atlantic Ocean]. The other stations also exhibited peak rates during May with, however, an order of magnitude difference in carbon fixation between OB and FoL (Figure 4b). The high rate measured at OB relative to the other stations at this time is attributed to the stability of the water column, which exhibited a well-defined pycnocline with the surface mixed layer established to 10 m. This was in contrast to the other stations which varied from fully mixed at the FoL to IB where a layer of warm freshwater was clearly evident (Figure 4a).

Nitrogen assimilation also showed maximum rates during May (14.5–23.6 mmol N $m^{-2}$ $day^{-1}$) at the three northernmost stations, but the maximum rate at

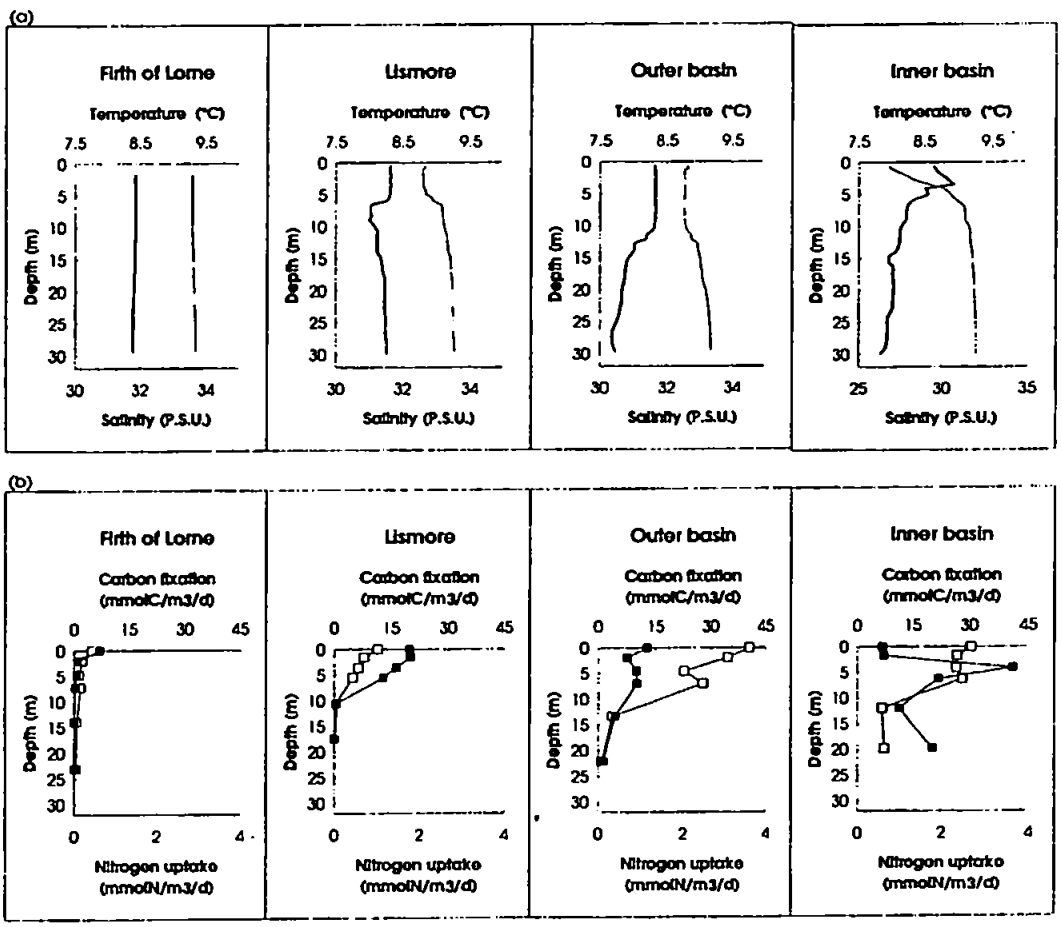


Fig. 4. Variation between the four stations during May 1991, for the upper 30 m. (a) Thermal (bold line) and haline (dashed line) structure. (b) Carbon fixation rate (open squares) and total nitrogen (nitrate and ammonium) uptake (filled squares). Note that in each case the deepest point is equivalent to the 0.3% irradiance level.

**Table II.** Pearson correlation matrix of environmental variables and depth-integrated (d.i.) carbon fixation and nitrogen assimilation (assim.) rates for Loch Linnhe, 1991

| | $NO_3$ | $NH_4$ assim. | Total N assim. | C assim. | C/N assim. | *f* ratio | RPI–$NH_4$ | RPI–$NO_3$ | Chloro–d.i. | ($NO_3$)–d.i. | ($NH_4$)–d.i. | 0.3% LL | Irradiance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $NO_3$ assim. | 1 | | | | | | | | | | | | |
| $NH_4$ assim. | −0.057 | 1 | | | | | | | | | | | |
| Total N assim. | 0.937*** | 0.296 | 1 | | | | | | | | | | |
| C assim. | 0.492** | −0.01 | 0.468** | 1 | | | | | | | | | |
| C/N assim. | 0.105 | −0.142 | 0.05 | 0.828*** | 1 | | | | | | | | |
| *f* ratio | 0.701*** | −0.531** | 0.485** | 0.361* | 0.155 | 1 | | | | | | | |
| RPI–$NH_4$ | −0.351* | −0.613*** | −0.551*** | −0.356* | −0.129 | 0.058 | 1 | | | | | | |
| RPI–$NO_3$ | 0.305 | −0.339* | 0.172 | −0.004 | −0.018 | 0.56*** | 0.186 | 1 | | | | | |
| Chloro–d.i. | 0.542*** | 0.118 | 0.56*** | 0.729*** | 0.515** | 0.361* | −0.532** | 0.081 | 1 | | | | |
| ($NO_3$)–d.i. | 0.005 | −0.566*** | −0.193 | −0.305 | −0.206 | 0.371* | 0.7*** | 0.345* | −0.45 | 1 | | | |
| ($NH_4$)–d.i. | −0.185 | 0.766*** | 0.092 | −0.013 | 0.014 | −0.598*** | −0.516*** | −0.366* | −0.094 | −0.425** | 1 | | |
| 0.3%LL | 0.063 | −0.045 | 0.044 | −0.142 | −0.123 | 0.221 | 0.207 | 0.186 | −0.19 | 0.635*** | 0.005 | 1 | |
| Irradiance | 0.112 | 0.405* | 0.249 | 0.041 | −0.082 | −0.21 | −0.347* | −0.167 | 0.291 | −0.234 | 0.344* | −0.201 | 1 |

Correlations significant at: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (LL, light level).

FoL was during April (29.9 mmol N $m^{-2}$ $day^{-1}$). The onset of the spring bloom is assumed to have begun at this time. It is interesting to note a decrease in carbon fixation at all stations in April, resulting in the molar carbon:nitrogen (C:N) assimilation ratio falling below 1.2, a phenomenon exhibited throughout the depth profile. Uptake of nitrate was not detected during January at any station. Similar situations have been experienced in the northern North Sea during January and the southern North Sea in February (A.P.Rees and N.J.P.Owens, unpublished data). Production rates showed a pattern analogous to the chlorophyll *a* distribution, and indeed both carbon fixation and nitrogen assimilation rates were significantly correlated with chlorophyll concentrations ($r^2$ = 0.531 and $P < 0.001$; and $r^2$ = 0.314 and $P < 0.001$, respectively). This relationship, and that between other variables, is summarized in Table II.

## Discussion

Recorded values of chlorophyll *a* and nutrient concentrations are comparable to those of Grantham (1981) for Loch Eil and the Lynn of Lorne, and to those of Gowen *et al.* (1983) for Loch Ardbhair and Loch na Droighniche. Most of the Scottish sea lochs, including Loch Linnhe, are characterized by high rates of tidal flushing, whilst freshwater input leads to haline stratification with consequences for vertical distributions of phytoplankton and nutrients, and primary production (Solorzano and Grantham, 1975). The effects of freshwater input were most dramatic at station IB (Figure 4a), being the station closest to the head of the loch and the outflows from the Lochy and Nevis rivers. In contrast, little evidence of haline stratification was found at FoL where the water column was vertically well mixed throughout the year. The effect of such vertical stability of the water column is evident by comparing rates of carbon fixation for OB (thermocline and halocline to 10 m) and FoL (fully mixed) in May. An 8-fold increase in production at OB over FoL is attributed to the benefits associated with phytoplankton remaining in the photic zone (Figure 4b).

The measured C:N assimilation ratios (Table I) showed a variation of approximately one order of magnitude from 0.3 to 23.3. The mean ratios of nitrate and ammonium uptake rates (1.2, nitrate; 1.8, ammonium) derived from short-term versus long-term incubations suggest that nitrogen uptake rates described above may have been underestimated due to substrate depletion and/or isotope dilution, and that C:N assimilation ratios adjusted accordingly would generally be lower. Variations of the molar C:N ratio in marine particulate and dissolved material of between 0.1 and 750 have been reported (Fisher *et al.*, 1982); in this study, the C:N assimilation ratio uncorrected for substrate depletion and isotope dilution artefacts increased above the Redfield ratio of 6.6 (Redfield *et al.*, 1963) on only five occasions (Table I). Two of these occurrences showed a deviation of less than two Redfield units, inferring slight nitrogen limitation only in May at the peak of the spring bloom (Figure 5), at three of the four sites (FoL, OB and IB), conforming closely with the model simulations of Ross *et al.* (1993a). Low values of C:N assimilation have been previously reported (Fisher *et al.*, 1982; Harrison *et al.*, 1983; Owens *et al.*, 1986, 1993).

Owens *et al.* (1986) proposed that there is no reason why the C:N assimilation ratio should be constant, or reflect a perceived or predicted cellular composition. Banse (1994) has described the results of several experiments which have suggested that the ratios of removal (of carbon and nitrogen) from the water may not reflect the plankton composition. Owens *et al.* (1993) attribute the deviation of their measured values from the Redfield ratio to stimulation of nitrogen uptake rates, owing to the enrichment of ambient nitrogen concentrations by the addition of high concentrations (2.5 $\mu mol^{-1}$) of substrate. This is a limitation of the $^{15}N$ technique which is generally experienced at times of low ambient concentrations (Harrison *et al.*, 1983). The introduction of novel techniques with greater sensitivity for the determination of nanomolar ammonium concentrations (Brezinski, 1987; Jones, 1991) will in future make it possible to make additions at concentrations which are unlikely to perturb ambient nutrient levels. During this study, stimulation of uptake was a potential problem during the winter months, as an addition of 0.1 $\mu mol\ l^{-1}$ [$^{15}N$]ammonium was at times effectively doubling the available nitrogen. There was, however, no evidence of C:N uptake ratios being significantly lower in the winter than during the post-bloom period when ammonium additions were <10% of ambient. The large anomaly in April (Figure 5) can probably be better explained by a physiological response of the phytoplankton to changing environmental conditions associated with the onset of summer, resulting in a proportionally large uptake of nitrate. Comparisons between C:N assimilation and particulate matter C:N ratios are of limited interest to this particular investigation because of the dominance of terrestrially derived material in the suspended material (M.R.Heath *et al.*, in preparation) for the most of the year. Kokkinakis and Wheeler (1987) used a

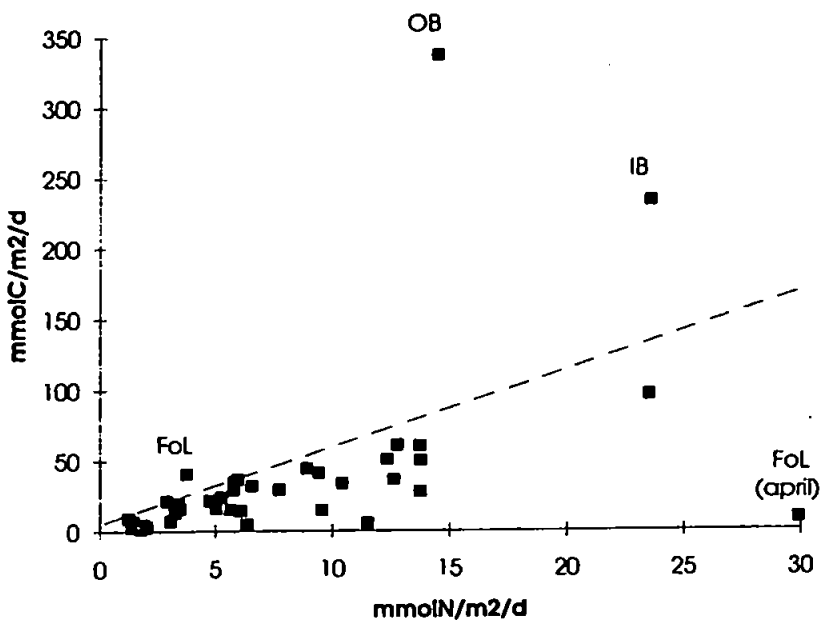


**Fig. 5.** Relationship between depth-integrated carbon fixation and nitrogen assimilation, Loch Linnhe 1991. The dashed line is equivalent to the Redfield ratio for C/N = 6.6. Points above the line indicate nitrogen limitation during May.

figure of 2.25 (μg Chl *a*:μmol cell N) in order to estimate the proportion of the particulate nitrogen present as phytoplankton nitrogen. Using the same number, we found that during the winter months the algal contribution was <3% of the total particulate nitrogen, this increased to 16% in April, and ranged from 9 to 75% during May at the peak of the spring bloom.

Although nitrate uptake accounted for >50% of the total nitrogen assimilation, nitrate concentrations in the water did not reach a seasonal minimum until June, when C:N assimilation ratios were significantly <6.6. Water column concentrations of the other nutrient elements phosphorus and silicon decreased during the spring bloom (data not shown here), but never even approached detection limits, and recovered rapidly after the peak of the bloom. It is very unlikely, therefore, that either phosphate or silicate were limiting at any time. Thus, the model predictions of Ross *et al.* (1993a), that under normal circumstances light rather than nutrients is limiting primary productivity in sea lochs, appear to be borne out in Loch Linnhe. Wassmann and Aadnesen (1984), in their study of a shallow Norwegian fjord system, agree that this is also the case in polls (land-locked fjords) and the innermost parts of fjords. Paasche and Erga (1988) in their study of the inner Oslofjord, however, found evidence of nutrient limitation (nitrogen, phosphorus and silicate) at times between April and October, following the spring bloom at the beginning of April.

The data indicate that following the spring bloom, ammonium rather than nitrate became the more important source of inorganic nitrogen for phytoplankton. Utilization of nitrate and ammonium was examined by the calculation of two indices: the *f* ratio and relative preference index (RPI). The *f* ratio, the conventional method used for describing the relative proportions of nitrogen assimilated, is defined as the nitrate assimilation rate as a function of the total nitrogen assimilation rate (Eppley and Peterson, 1979). Although more generally used in open-ocean or 'steady-state' situations in the derivation of estimates of new production, the concept is less useful here because of the influence of terrestrially derived nutrient species; however, it is still a useful model to describe phytoplankton–nutrient relationships for coastal ecosystems. The RPI is used as an indication of the preference of the phytoplankton for a particular nitrogen species compared with its concentration (McCarthy *et al.*, 1977), where preference is implied by a value greater than unity. The contribution of bacteria to the measured ammonium uptake has not been evaluated during this study; however, it has previously been shown to be significant (Eppley *et al.*, 1977; Laws *et al.*, 1985; Wheeler and Kirchman, 1986). Flynn and Butler (1986) report that bacteria, for a given mass, actually assimilate more nitrogen than do phytoplankton. Although the retention of bacteria by Whatman GF/F filters is only 50–66% efficient (Lee, 1993), the lack of correlation between ammonium assimilation and both carbon fixation and chlorophyll *a* (Table II) would infer that, at times, the heterotrophic ammonium uptake is significant in Loch Linnhe. In assuming that nitrate uptake is confined to autotrophs, Harrison *et al.* (1987) proposed that the elevation of the *f* ratio resultant from considering only autotrophic ammonium uptake is of the same order as the reduction envisaged for the effects of isotope dilution and uptake of urea. The use of both indices,

although not providing a completely accurate assessment of phytoplankton nitrogen preference, is considered to be sufficiently close to allow the comparison of nitrate and ammonium behaviour in this loch ecosystem.

On only one occasion during the year does the RPI-$NH_4$ fall below one or the RPI-$NO_3$ increase above one (see Figure 6); both occurred at OB in May, coincident with the maximum recorded carbon fixation rate. At times of low ammonium, the RPI-$NH_4$ reached values of up to 41. In contrast with this, during times when nitrate concentration was reduced, the RPI-$NO_3$ remained below one and, in particular at FoL and IB, appeared to be depressed further during the summer months. Co-variance analysis of the relationship between RPI ($-NO_3$ and $-NH_4$) and nutrient concentration revealed the independence of RPI-$NO_3$ from both nitrate and ammonium concentrations, values of 0.3 and −0.6, respectively. RPI–$NH_4$ showed positive co-variance with nitrate concentration (value 559.0) and negative co-variance with ammonium concentration (value −61.9), correlation coefficients of $r^2 = 0.49$, $P < 0.001$ and $r^2 = 0.266$, $P < 0.001$, respectively. These values of RPI suggest a preference for ammonium over nitrate for the whole of the loch and for the majority of time. The loch is thus similar to other sea areas; the algae demonstrated a preference for the reduced nitrogen species as has been previously well documented, e.g. in Goldman and Glibert (1983) and Paasche (1988). Although the evidence indicates a preference for ammonium relative to its availability, it can be seen that the most of the production is supported by nitrate assimilation ($f$ ratio > 0.5), and it is mainly in the post-bloom period that ammonium becomes quantitatively important as a nitrogen source. An overall trend in the $f$ ratio would appear to be: high values (>0.5) inferring nitrate assimilation proportionally greater than ammonium assimilation experienced during periods of high nitrate concentration in winter and early spring; lower values of the $f$ ratio are then associated with elevated ammonium concentrations (Figure 6), so that there is a negative relationship between $f$ ratio and ammonium concentration ($r^2 = 0.358$; $P < 0.001$).

Annually integrated values of nitrogen assimilation and carbon fixation at the four sites (Table III) show the same pattern along the length of the loch, as described above. Values for carbon fixation are within the range described for other sea areas [Reid *et al.* (1990) for the North Sea, Joint *et al.* (1986) for the Celtic Sea] and within the low to mid range for a number of Norwegian fjords as reviewed by Wassmann and Aadnesen (1984). We are unaware of other directly measured estimates of annually integrated nitrogen assimilation to allow a comparison of rates, although several other studies would potentially allow the

**Table III.** Annually integrated values of carbon fixation and nitrogen assimilation, Loch Linnhe, 1991

| Station | (g C $m^{-2}$ $year^{-1}$) | $NO_3$ (mol N $m^{-2}$ $year^{-1}$) | $NH_4$ (mol N $m^{-2}$ $year^{-1}$) | $NO_3$ as per cent of total N assimilated |
|---|---|---|---|---|
| FoL | 78.5 | 1.28 | 0.91 | 58 |
| Lis | 112.6 | 1.73 | 1.11 | 61 |
| OB | 198.6 | 1.43 | 1.09 | 57 |
| IB | 147.2 | 1.03 | 1.00 | 51 |

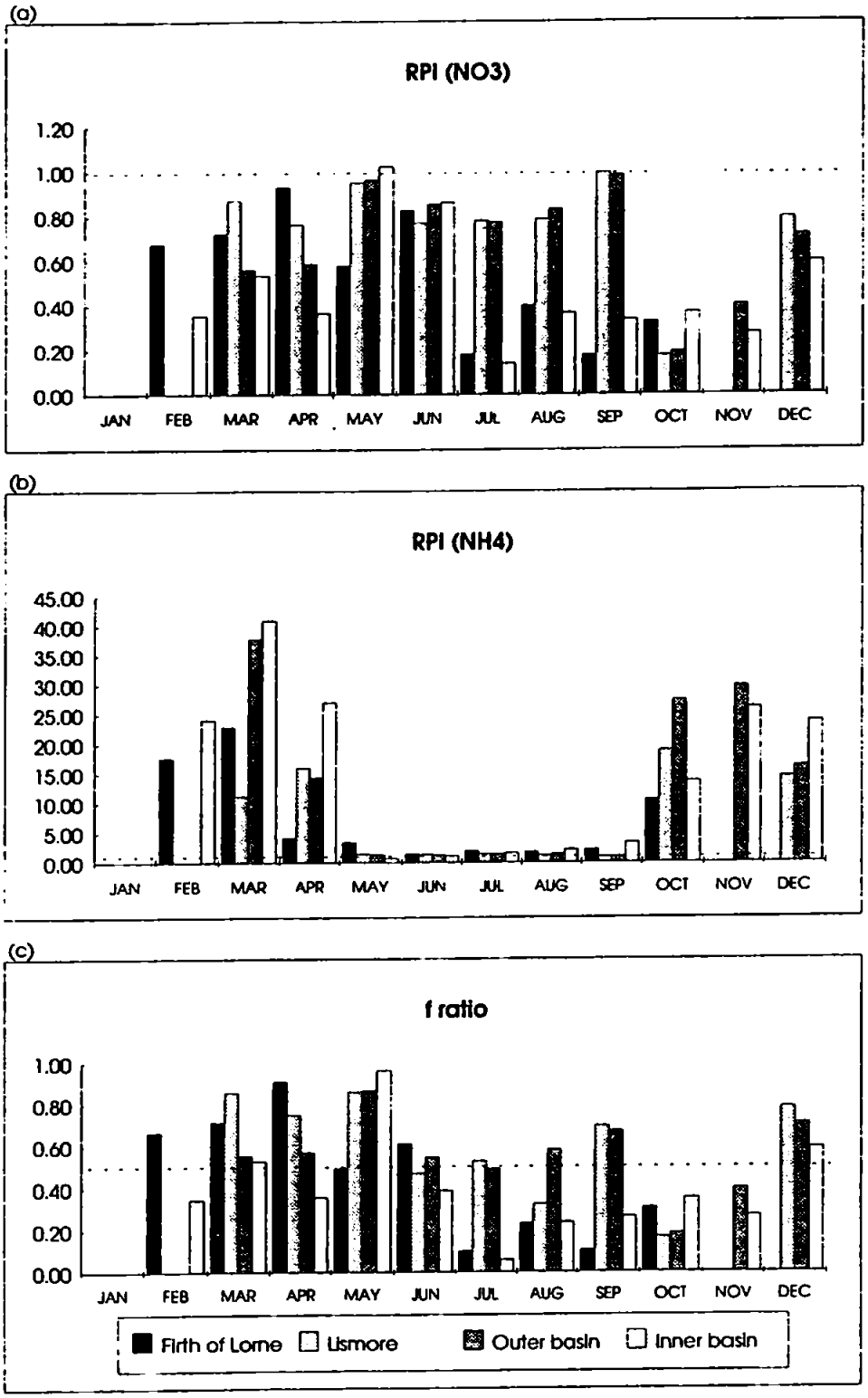


**Fig. 6.** Seasonal trend of (a) RPI($NO_3$), (b) RPI($NH_4$) and (c) *f* ratio. In (a) and (b), the dotted line is equivalent to one, a value greater than this indicates preference by phytoplankton. In (c), the dotted line is equivalent to 0.5, values greater than this indicate times when nitrate assimilation exceeded 50%.

derivation of such a figure (Fisher *et al.*, 1982; Furnas *et al.*, 1986). We recognize that methodological problems in the $^{15}N$ technique may have resulted in underestimates of nitrate and ammonium uptake by factors of 1.2 and 1.8, respectively.

Paasche and Kristiansen (1982) and McCarthy *et al.* (1977), following similar studies to this one in the Oslofjord and Chesapeake Bay, respectively, report that on an annual basis ammonium is more important than nitrate as a nitrogen source for phytoplankton growth and for the Oslofjord that nitrate is only important during the spring. Although rates of nitrogen uptake are similar in the latter studies to this one, hydrodynamic and nutrient conditions vary enormously. The Oslofjord and Chesapeake Bay both receive substantial amounts of sewage effluent and experience elevated ammonium concentrations, so that nitrate uptake is suppressed by ammonium concentrations >~1.0 μM. The addition of sewage not only affects the proportions in which nitrogen species are assimilated, but shifts natural systems toward nitrogen limitation (Sakshaug and Olsen, 1986). According to our data, ammonium within Loch Linnhe is generally a result of internal cycling, occurring during the post-bloom period, and not from any external input; so that in contrast to these other studies, between 39 and 49% of annual production in Loch Linnhe is supported by ammonium uptake and nitrogen limitation is minimal.

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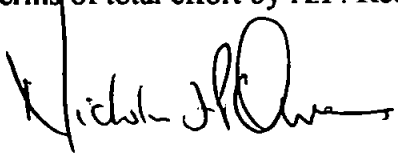
**Rees A.P.,** Owens N.J.P., & Woodward E.M.S.

Contribution in terms of total effort by A.P. Rees on this work was: 70%

Signed: 
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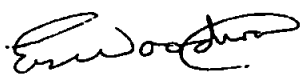
Signed: 
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# PHYTOPLANKTON NITROGEN ASSIMILATION AT LOW NUTRIENT CONCENTRATIONS IN THE NW - MEDITERRANEAN SEA (JULY 1993).

A.P. REES[1], N.J.P. OWENS[2] AND E.M.S. WOODWARD[1].
[1]*Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth, PL1 3DH, U.K.*
[2]*Department of Marine Sciences and Coastal Management, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, U.K.*

## INTRODUCTION

The influence of riverine inputs to coastal waters and the resultant nutrient enrichment and eutrophication effects, have been the focus of numerous recent investigations. The river Rhone is a major source of nutrients to the North West Mediterranean, although offshore areas; particularly in summer become severely nutrient limited. Previous studies have shown that coastal areas may show production rates elevated four to ten times relative to those offshore. This paper describes variations in nitrogen assimilation along a transect, covering the gradient between offshore waters and those in direct influence of the river plume. The preference of ammonium (over nitrate) as a nitrogen species by phytoplankton (Owens *et al.*, 1991) has been determined in all of the previous investigations during this programme. Although, the last in this series of cruises, this was the first time that the assimilation of urea was determined. Which in addition to rates of nitrate and ammonium gives a more complete view of the phytoplankton-nitrogen relationship. A further refining of this study, was the introduction of a novel analysis of low level ammonium concentrations, allowing addition of the $^{15}N$ substrate at trace amounts.

## METHODS

Assimilation rates for nitrate, ammonium and urea were determined along an offshore-onshore transect (MA), during RRS Discovery cruise 203. Incorporation of $^{15}N$ labelled substrates by phytoplankton throughout the photic zone was estimated during *in-situ* incubations. Water samples were collected pre-dawn from six depths from the upper 100m, which were selected for each incubation depending on the specific conditions encountered. To avoid light shock to the phytoplankton, the water was kept in the dark prior to incubation. Samples from each depth were distributed into 2.4 litre clear polycarbonate bottles and amended with $^{15}N$ substrates. Nitrogen was added as salts of nitrate,

ammonium and urea at approximately 10% of ambient concentrations. This was made possible through the onboard capability to detect nanomolar concentrations of ammonium and nitrate. Urea was added at the same concentration as ammonium. Incubations were commenced at dawn with the deployment of a free-floating incubation rig. At five of the six stations, the rig was recovered at dusk and the samples maintained during the dark period at surface sea-water temperatures. At the sixth station (MA-1a), incubations were made *in situ* for the full twenty four hours. Incubations were terminated by gentle filtration onto ashed Whatman GF/F filters. Samples were size fractionated into two size classes using 5.0μm polycarbonate screens. Filters were washed with filtered sea-water and stored frozen until return to the laboratory, where they were oven dried at 50°C before analysis. Atom% $^{15}N$ was measured by continuous flow nitrogen analysis-mass spectrometry using the techniques described in Barrie *et al.* (1989) and Owens and Rees (1989), and rates of assimilation calculated from the equations of Dugdale and Goering (1967). Nitrate, ammonium and urea concentrations were determined onboard on subsamples of the water, by the methods described by Woodward *(this volume)*.

## RESULTS AND DISCUSSION

Nutrient concentrations were low for the most of the study area. Nitrate concentrations for the four most southerly stations were all below 0.25μmol $l^{-1}$ in the surface 50m. With the depth of the photic zone extending down to 80-90m, and with nitrate concentrations increasing to 8.5μmol $l^{-1}$ (MA5), sub-surface chlorophyll and nitrogen uptake maxima were experienced in the offshore stations. Stations MA-7 and MA-9 the two under greatest influence of the river Rhone showed elevated nitrate concentrations (4.94μmol $l^{-1}$) in the upper 10m. The introduction of a novel technique for the analysis of ammonium on this cruise allowed inspection of low level variability which had previously been undetected. The detection limit of conventional analysis methods were in the order of 0.1μmol $l^{-1}$. In this study only three of the samples analysed for ammonium concentration were greater than 0.1μmol $l^{-1}$. Urea concentrations remained below the detection limit (0.05μmol $l^{-1}$) for much of the time. Maximum concentrations were detected at station MA-9 (0.23μmol $l^{-1}$, surface; 0.28μmol $l^{-1}$, at 40m).

Size-fractionated data for the particulate nitrogen concentration suggested that in most instances the community was dominated by phytoplanton in the <5μm fraction (data not shown). Barlow (*pers comm.*) confirmed this conclusion to be true, from analysis of pigment biomarkers. An f test on the two data sets (<5.0 μm and total community) confirmed that, at the 5 per cent level of significance, there was no difference between the the total particulate nitrogen concentration and that of the <5μm fraction. Differences between the total

Table 1. Nitrogen assimilation rates. NW-Mediterranean, July1993.

| | depth (m) | Nitrate µmolN/l/day | Ammonium µmolN/l/day | Urea µmolN/l/day | total N µmolN/l/day |
|---|---|---|---|---|---|
| MA-1b (20/7/93) | | | | | |
| | 5 | 0.0004 | 0.0049 | 0.0004* | 0.0057 |
| | 10 | 0.0008 | 0.0042 | 0.0005* | 0.0055 |
| | 20 | 0.0015 | 0.0028 | 0.0007 | 0.0050 |
| | 50 | 0.0008 | 0.0019 | 0.0006 | 0.0032 |
| | 75 | - | 0.0017 | 0.0013 | - |
| | 90 | 0.0084 | 0.0030 | 0.0003 | 0.0117 |
| MA-1a (18/7/93) | | | | | |
| | 5 | 0.0005 | 0.0022 | 0.0010* | 0.0037 |
| | 10 | 0.0003 | 0.0006 | 0.0003 | 0.0011 |
| | 20 | 0.0006 | 0.0014 | 0.0006 | 0.0026 |
| | 46 | 0.0001 | 0.0006 | 0.0007 | 0.0015 |
| | 60 | 0.0042 | 0.0016 | 0.0008* | 0.0067 |
| | 91 | 0.0278 | 0.0004 | 0.0008 | 0.0290 |
| MA-3 (24/7/93) | | | | | |
| | 5 | 0.0003 | 0.0012 | 0.0012* | 0.0027 |
| | 20 | 0.0012 | 0.0016 | 0.0014* | 0.0042 |
| | 40 | - | 0.0020 | - | - |
| | 50 | 0.0010 | 0.0013 | 0.0003* | 0.0026 |
| | 57 | 0.0032 | 0.0011 | 0.0008* | 0.0051 |
| | 80 | - | 0.0018 | 0.0006* | - |
| MA-5 (26/7/93) | | | | | |
| | 5 | 0.0018 | 0.0011 | 0.0013 | 0.0042 |
| | 15 | 0.0015 | 0.0010 | 0.0014 | 0.0039 |
| | 20 | 0.0006 | 0.0014 | 0.0008 | 0.0029 |
| | 30 | 0.0004 | 0.0012 | 0.0015 | 0.0031 |
| | 46 | 0.0013 | 0.0015 | 0.0022 | 0.0050 |
| | 80 | 0.0547 | 0.0010 | 0.0013 | 0.0570 |
| MA-7 (28/7/93) | | | | | |
| | 0 | 0.0003 | 0.0018 | 0.0012* | 0.0033 |
| | 10 | 0.0003 | 0.0019 | 0.0011* | 0.0033 |
| | 20 | 0.0002 | 0.0013 | 0.0011* | 0.0026 |
| | 40 | 0.0022 | 0.0010 | 0.0013* | 0.0045 |
| | 60 | 0.0127 | 0.0013 | 0.0012* | 0.0152 |
| | 80 | 0.0119 | 0.0018 | 0.0020* | 0.0157 |
| MA-9 (22/7/93) | | | | | |
| | 0 | 0.5888 | 0.0090 | 0.0040 | 0.6019 |
| | 5 | 0.0023 | 0.0024 | 0.0027 | 0.0074 |
| | 10 | 0.0011 | 0.0027 | 0.0035 | 0.0073 |
| | 15 | 0.0010 | 0.0022 | 0.0040 | 0.0072 |
| | 20 | 0.0002 | 0.0020 | 0.0036 | 0.0059 |
| | 40 | 0.0002 | 0.0018 | 0.0034 | 0.0054 |

- , missing data; * , concentration below detection limit (0.05µmol/l).

nitrogen uptake and that by the <5um fraction were also minimal, and so the data described are for the total community. The importance of the nanoplankton (phytoplankton <5μm) is well documented. Joint (1987) showed from a survey of the literature that 25% to 80% of the production is attributable to phytoplankton in this size class. Owens *et al.* (1989) showed that for this region during winter the minimum contribution by nanoplankton was more than 50%.

Calculation of the nitrogen assimilation rate is dependent on the ambient nutrient concentration. At times when urea concentration was below the detection limit (Table 1.), a value of 0.05μmol $l^{-1}$ was substituted into the equation. This results in an overestimate of the rate, such that a 50% increase in urea concentration resulted in a change of rate approximately two-fold. This situation has been previously experienced during similar experiments in oligotrophic conditions for both nitrate and ammonium uptake, and is recognised as a limitation of the technique.

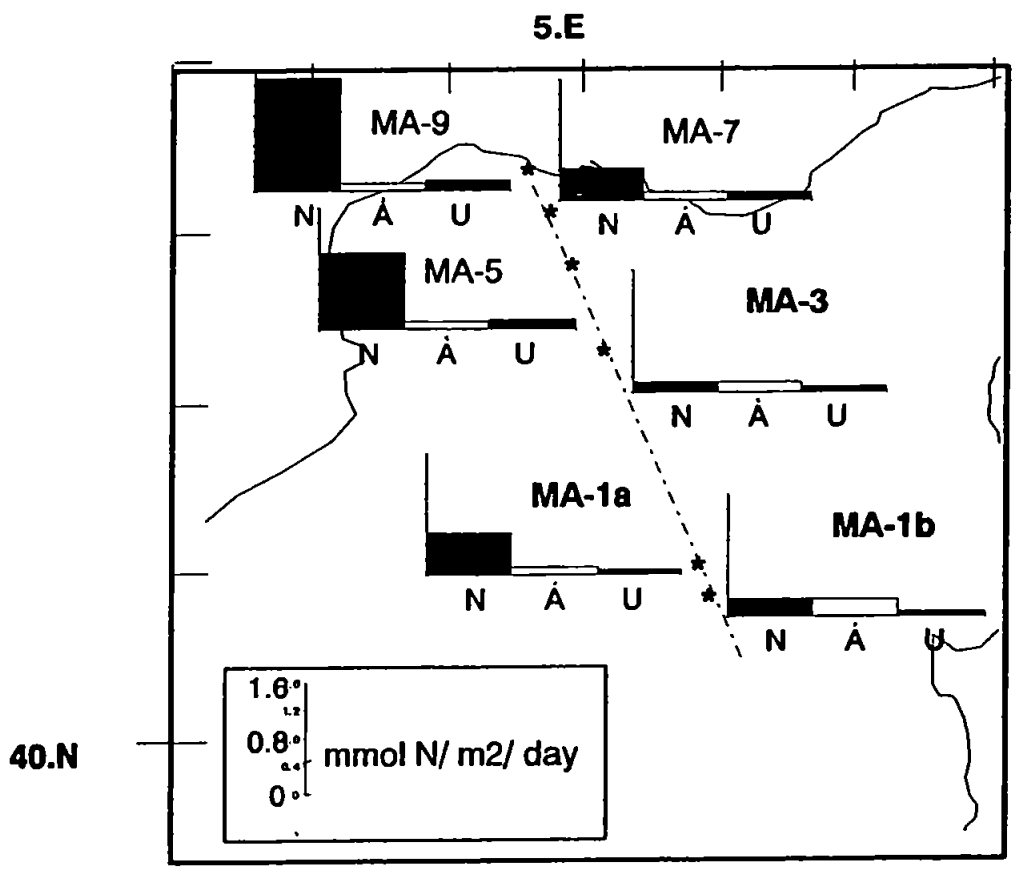


Figure 1. Depth integrated nitrogen assimilation rates at stations on transect MA, during July1993. * indicates station position; N, nitrate; A, ammonium; U, urea.

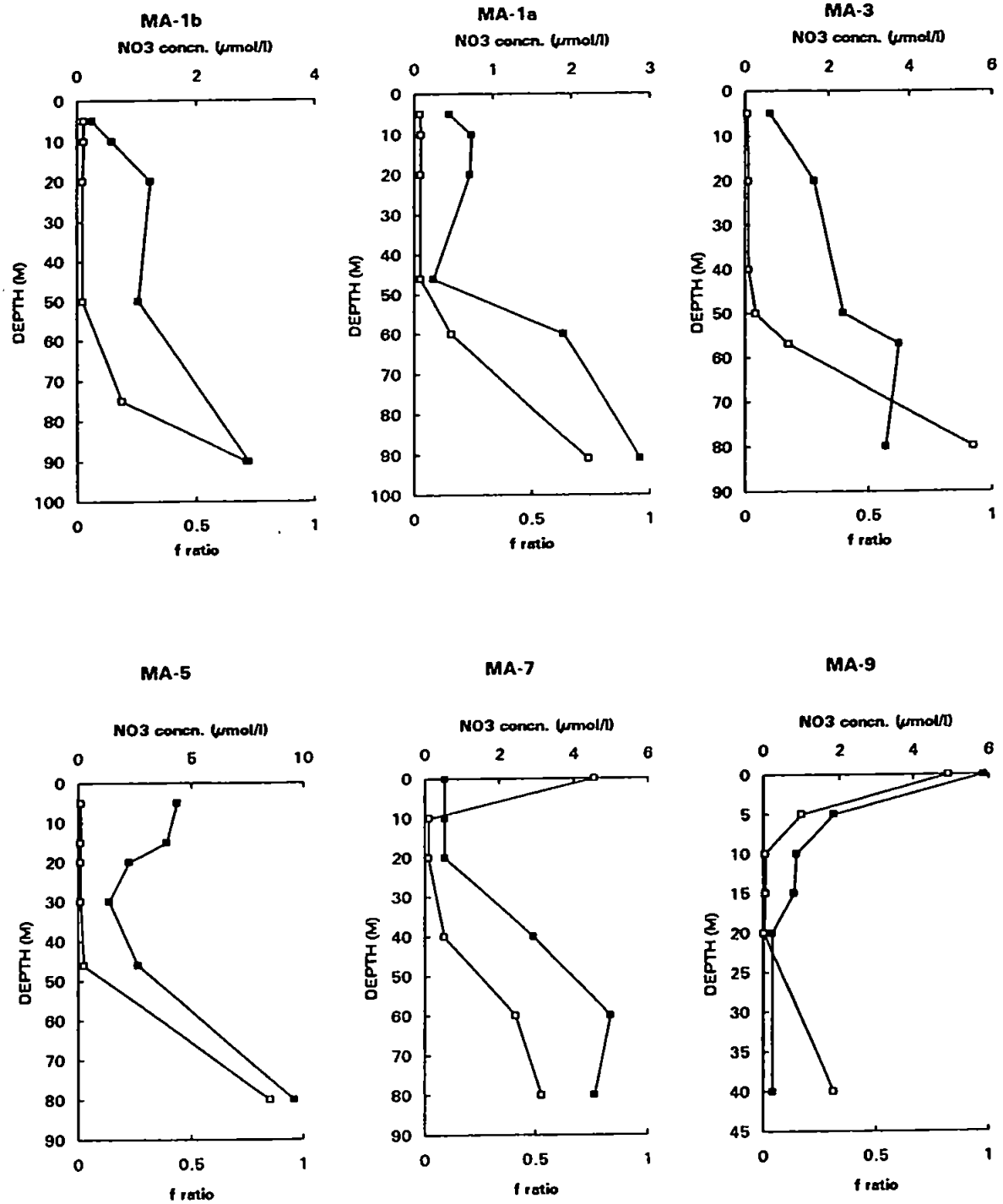


Fig. 2 Depth profiles of nitrate concentration (µmol/l) - open squares and f ratio - solid squares, along transect MA during July 1993.

Williams and Robinson, (1991) have previously identified the "*post bloom*" period during the summer months as exhibiting low rates of production. Depth integrated values for the rates of nitrate, ammonium and urea assimilation (Figure 1.) were the lowest experienced during the EROS 2000 project. The maximum rate of total nitrogen uptake (1.742 mmol N $m^{-3}$ $day^{-1}$) was experienced at the most inshore station; where the depth integrated value was heavily biased by the uptake of nitrate at the surface (~98% of the total). Unexpectedly maybe, the minimum value of 0.326 mmol N $m^{-3}$ $day^{-1}$ was at station MA-3 and not at stations MA-1a or b. And although surface chlorophyll *a* concentrations were low (~0.1µg $l^{-1}$), there was a sub-surface maximum (1.2µg $l^{-1}$), greater than that at the other stations (Barlow *et al.*, *this volume*).

Use of the f ratio, (Eppley and Peterson, 1979) is the concept generally used in the estimation of new and regenerated production (Dugdale and Goering, 1967). F describes the relative proportions of nitrogen assimilated, and is defined as: the rate of nitrate assimilated as a function of the total nitrogen assimilation rate. Although not particularly applicable as such in areas inflenced by riverine inputs, it is still a useful model in the comparison of stations along the transect (Figure 2.). Depth profiles of nitrate concentration and f ratio show similar structure at all stations, indeed the two are significantly correlated ($r = 0.611$; $p = 0.001$). At the five offshore stations, in the upper 40 - 50m, the f ratio is consistently less than 0.5. Indicating that more than 50% of nitrogen uptake was of the reduced nitrogen species (ammonium and urea). At depths greater than this nitrate becomes quantitatively more important, and values of f between 0.5 and 0.96 were found. The influence of the river at station MA-9 produced profiles of nitrate concentration and f ratio much different to the other stations, with maximum values at the surface, decreasing with depth.

For the greater part of the study area f ratios were less than 0.5, suggesting that the reduced species of nitrogen were proportionally more important than was nitrate. McCarthy *et al.* (1977) described a relative preference index (RPI), as a means of investigating the nitrogen species preferred by phytoplankton. Calculation of the RPI(NO3) confirmed that ammonium and urea were preferred in all cases but one (Figure 3.). The results shown in figure 3 clearly demonstrate the importance of the detection of ammonium concentrations less than 0.1µmol $l^{-1}$. Nitrate was the preferred nitrogen species at the surface, station MA-9 only. As a proportion of the study area ammonium and urea appear to be the dominant nitrogen species. And as shown through use of the RPI, are preferred over nitrate by phytoplankton. It is interesting to note, that taking the study area as a whole, 75% of nitrogen assimilation is met by nitrate; and even ignoring station MA-9 this figure is still 68%.

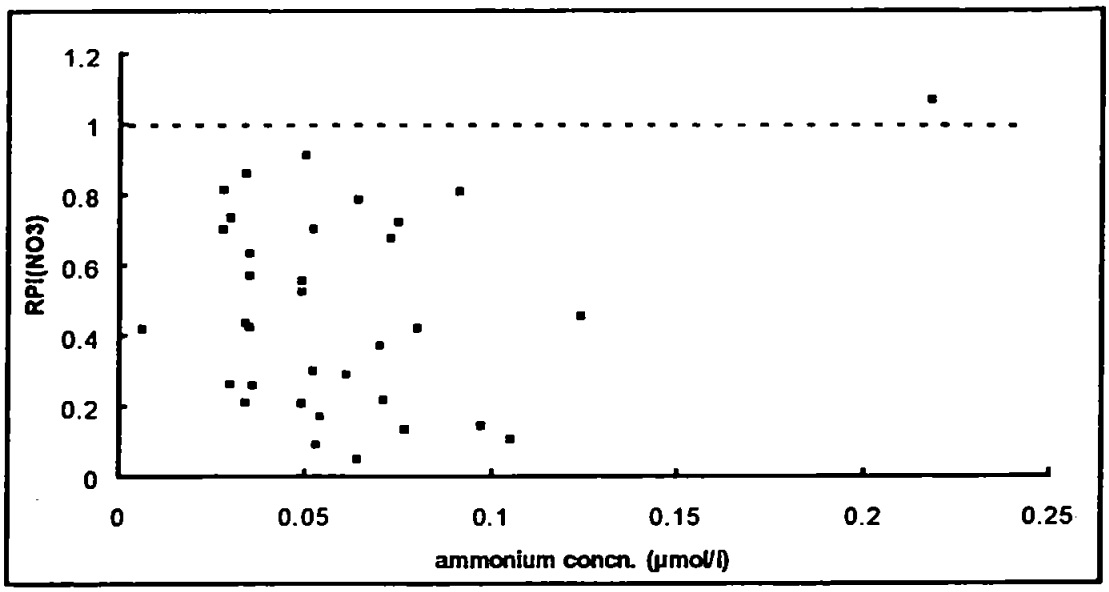


Figure 3. Relationship between ammonium concentration and RPI(NO3). Values less than 1 (dotted line) indicate preference for the reduced nitrogen species.

## Conclusion

Summer production was dominated by phytoplankton smaller than 5.0µm. Except at station MA-9 ammonium was the major nitrogen source throughout the upper 50m, although quantitatively nitrate was the dominant form over the whole of the photic zone due to light penetration to the nitracline. A major success of this cruise was the coupling of the information on levels of ammonium less than 0.1µmol $l^{-1}$ with the nitrogen uptake technique.

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**Paper III**
Nitrous Oxide in the Bellingshausen Sea and Drake Passage
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**Rees A.P.,** Owens N.J.P. & Upstill-Goddard R.C.

Contribution in terms of total effort by A.P. Rees on this work was: 75%

Signed: N.J.P. Owens Date: 28th June 2001

Signed: R.C. Upstill-Goddard Date: 25/05/01

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# Nitrous oxide in the Bellingshausen Sea and Drake Passage

A.P. Rees, N.J.P. Owens,[1] and R.C. Upstill-Goddard[1]

Plymouth Marine Laboratory, Plymouth, England, United Kingdom

**Abstract.** Concentrations of dissolved and atmospheric nitrous oxide, $N_2O$, were measured in the austral spring of 1992 in the Drake Passage and Bellingshausen Sea as part of the United Kingdom Joint Global Ocean Flux Study expedition to the Southern Ocean. The measured atmospheric mixing ratio was 313 ± 5 parts per billion by volume, in agreement with the hemispherically corrected global mean. In the Drake Passage, surface $N_2O$ saturations were generally very close to atmospheric equilibrium, 99.7 ± 3%, although several anomalous points were associated with the presence of frontal and eddy-like features within the Antarctic Polar Frontal Zone and at the Continental Water Boundary. Further to the south, a series of oceanographic stations and two surface transects along the 85°W meridian between 65.28°S and 70.32°S revealed a transition from undersaturated conditions in open water to oversaturated conditions in the marginal ice zone, in the upper mixed layer (75-100 m). These observations reflect upwelling of Circumpolar Deep Water at approximately 70°S, resulting in the accumulation of $N_2O$ under the winter sea ice and its subsequent release to the atmosphere following the ice retreat. Sea-air $N_2O$ fluxes were estimated from the product of the surface $N_2O$ anomaly and the modeled gas transfer coefficients of *Liss and Merlivat* [1986] and *Wanninkhof* [1992] to find a maximum rate of +3.1 μmol $N_2O$ $m^{-2}$ $d^{-1}$. North of the upwelling region, Antarctic Surface Water formed from the mixing of surface waters and ice melt was moderately depleted in $N_2O$ with respect to the atmosphere, a minimum 90% of saturation. This sink area was estimated to extend between 65.28°S and 69.57°S with a mean sea-air flux of between -0.6 ± 0.4 and -0.9 ± 0.7 μmol $N_2O$ $m^{-2}$ $d^{-1}$. The region studied at 85°W (65.28°S to 70.32°S) revealed source and sink areas which were largely determined by the changing physical hydrography, so that overall there was a small net negative flux of between -0.06 ± 0.9 and -0.09 ± 1.4 μmol $N_2O$ $m^{-2}$ $d^{-1}$.

## Introduction

Nitrous oxide ($N_2O$) is an important atmospheric trace gas with significant natural and anthropogenic sources [*Badr and Probert*, 1992]. It is the source gas for stratospheric nitric oxide radicals ($NO^{\cdot}$), whose contribution to ozone depletion is well documented [*Crutzen*, 1971; *Crutzen and Howard*, 1978; *Hahn and Crutzen*, 1982], and it is an efficient greenhouse gas [*Wang et al.*, 1976; *Ramanthan et al.*, 1985]. Because of the imbalance between known sources and sinks, the atmospheric $N_2O$ burden is currently increasing at a rate of 0.2-0.3% $yr^{-1}$ [*Butler et al.*, 1989; *Prinn et al.*, 1990]. The oceans are generally considered to represent a net source of atmospheric $N_2O$, and source-sink characteristics have been examined by numerous authors in order to evaluate the ocean-atmosphere exchange [e.g., *Elkins et al.*, 1978; *Butler et al.*, 1989; *Law and Owens*, 1990a; *Oudot et al.*, 1990; *Naqvi and Noronha*, 1991; *Nevison et al.*, 1995; *Bange et al.*, 1996]. These studies have revealed large regional anomalies in the oceanic distribution of $N_2O$. For example, *Law and Owens* [1990a] detail the Arabian Sea as a significant source, reporting supersaturation values of up to 246% in surface waters, equivalent to approximately 5-18% of the total marine flux from an area equivalent to 0.43% of the world ocean. Elevated $N_2O$ concentrations have also been reported for surface waters in the Peruvian upwelling [*Codispoti et al.*, 1992] and tropical upwelling regions of the Pacific Ocean [*Elkins et al.*, 1978; *Weiss et al.*, 1992]. In contrast, *Elkins et al.* [1978] present data from southeast tropical regions of the Pacific Ocean and Chesapeake Bay which demonstrate consumption of dissolved $N_2O$ in conditions of low oxygen at depths between 150 and 250 m and in surface waters, respectively.

For the Southern Ocean, physical and biological characteristics suggest the potential for both production and removal of $N_2O$ in different areas. However, little information on dissolved $N_2O$ distributions is currently available. Increased gas solubility in low-temperature Antarctic waters, combined with downwelling associated with deep water formation over the continent and convergences within the Antarctic Polar Frontal Zone, may provide substantial sink conditions, whereas the upwelling of deep and intermediate waters may result in this region providing a source of atmospheric $N_2O$. Significant $N_2O$ production in areas of high biological production, as observed in other ocean regions [e.g., *Codispoti and Christensen*, 1985; *Law and Owens*, 1990a], is generally coincident with a pronounced subsurface oxygen minimum. In contrast, Southern Ocean waters are well oxygenated and high productivity is generally associated with elevated nutrient concentrations and water column stability.

[1] Now at Department of Marine Sciences and Coastal Management, University of Newcastle, Newcastle upon Tyne, England, United Kingdom.

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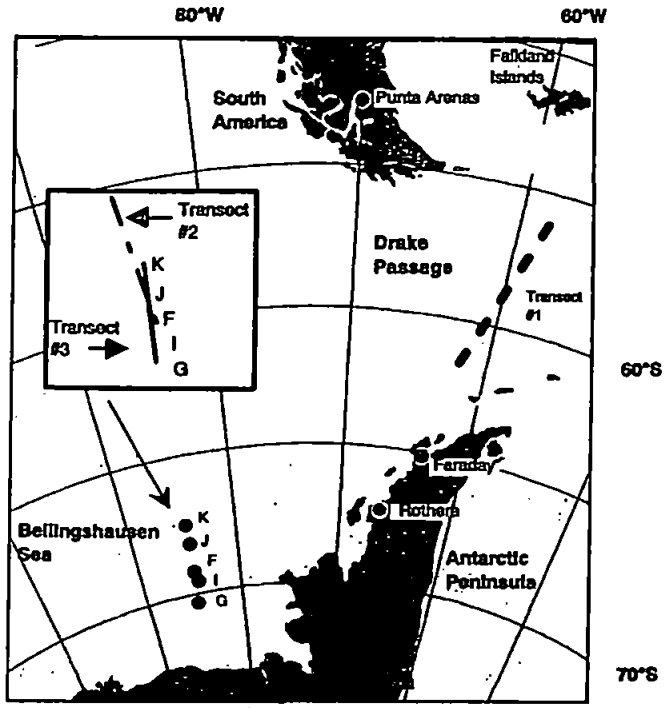


**Figure 1.** Location of transect 1 in the Drake Passage; transects 2 and 3; and oceanographic stations G, I, F, J, and K in the Bellingshausen Sea during November-December 1992.

This paper describes measurements of the concentrations of $N_2O$ and related oceanographic variables made onboard the Royal Research Ship *James Clark Ross* in the Bellingshausen Sea and Drake Passage (Figure 1) during November/December 1992. These were determined as part of a two-ship multidisciplinary study to investigate biogeochemical fluxes in the region primarily within and adjacent to the marginal ice zone of the SE Pacific sector of the Southern Ocean. *Turner and Owens* [1995] provide a detailed background and overview of the expedition, including summaries of the hydrographical and ice-cover conditions experienced.

## Methods

### Sample Sites

As part of the overall aims of the cruise program, investigations were made to (1) determine ocean-atmosphere exchanges of $N_2O$ (and other radiatively active gases) and the factors influencing such fluxes, over a wide latitudinal range, and (2) to assess the impact of seasonal sea ice on these fluxes. Three surface transects were made (Figure 1), the first of which crossed the Drake Passage (55.35°S, 59.15°W to 62.80°S, 62.12°W), while subsequent transects made the transition from open water to the marginal ice zone at approximately 85°W. In addition to surface transects, a series of vertical profiles through the water column were made at stations F-K, which were chosen to give a surface range from ten tenths first-year pack ice (stations F and G) to open water (K) along the 85°W meridian. Station positions, dates, and ice cover characteristics are summarized in Table 1. Station K was coincident with an area of elevated phytoplankton biomass observed on previous transects by both ships and was associated with the presence of a frontal feature between 66° and 67°S, identified by *Read et al.* [1995] as the Southern Polar Front. Station F (69.43°S) was sampled to 50 m, primarily as an exercise in testing sampling protocols in full ice cover, prior to the more extensive sampling at stations G-K. Because of the rapid ice retreat at this time, station F is considered apart from the G-K transect to allow a spatially and temporally coherent description of the effects of the ice-cover gradient. The retreat of the seasonal ice was such that a well-defined ice edge was located at approximately 66°S on November 11, 1992, and at 69.3°S on November 28, with conditions in the study area changing significantly over a relatively short timescale.

### Collection of Seawater Samples

Seawaters were collected in two sampling modes. For surface transects, samples were taken from the ships nontoxic seawater supply (intake at 8 m). Samples for the vertical profiles were collected from a conductivity-temperature-depth (CTD) rosette sampler, using 10-L Go-Flo bottles. Sample collection was in 1-L volumetric flasks, overfilled at least three times to aid exclusion of air bubbles. Following the addition of 200-μL saturated $HgCl_2$ solution to arrest microbial action, flasks were sealed with ground glass stoppers, inverted to disperse the $HgCl_2$ and temperature equilibrated at 25.0 ± 0.05°C prior to $N_2O$ analysis. In preliminary work, water samples treated in this way showed no significant changes in $N_2O$ concentration during several days storage. Using a similar sampling protocol, *Elkins* [1980] reports dissolved $N_2O$ stabilities of at least 1.5 months.

### $N_2O$ Analysis

Analyses of $N_2O$ in air and seawater were by gas chromatography with electron-capture detection (Shimadzu GC-8AIE, 10mCi $^{63}Ni$). For seawaters, analysis was always within 48 hours of collection using a single-phase equilibration technique. The analytical system, which also provides simultaneous analyses of methane by flame ionization detection, is described in detail by R.C. Upstill-Goddard et al. (Simultaneous high-precision measurements of

**Table 1.** Station Positions and Characteristics

| Station | Position | Duration | Ice Conditions |
|---|---|---|---|
| F | 69.43°S 85.06°W | Nov. 13-14 | 100% ice cover; ~ 0.5 m thick; evidence of melting |
| G | 70.32°S 85.27°W | Nov. 14-18 | 100% ice cover; 1-2 m first-year ice |
| I | 69.57°S 84.98°W | Nov. 30 to Dec. 3 | at distinct ice edge |
| J | 68.25°S 85.05°W | Dec. 3-6 | open water |
| K | 67.50°S 85.00°W | Dec. 6-12 | open water |

methane and nitrous oxide in water and seawater by single phase equilibration gas chromatography, submitted to *Deep Sea Research, Part I,* 1996), where full details of method calibration are also presented. Corresponding methane data will be reported elsewhere (N.J.P. Owens et al., manuscript in preparation, 1996). In brief, chromatographic separation of $N_2O$ was at 65°C on Porapak-Q® (80-100 mesh, 2 m x 2 mm stainless steel column), with prior removal of $H_2O$ vapor and $CO_2$ on precolumns of magnesium perchlorate and carbosorb, respectively. The detector temperature was 320°C. Carrier gas was ultrahigh purity (UHP) nitrogen at a flow rate of 15 mL $min^{-1}$. Primary method calibration was through a commercially prepared, certified $N_2O/CH_4$ mixture in an UHP $N_2$ matrix (Air Products) with a certified accuracy of ±1% or better. For routine analytical calibrations a series of four secondary standards (in the linear range of the detector) were used, prepared from the primary mixtures by pressure dilution [*Upstill-Goddard et al.*, 1990]. Consequently, the best estimate for overall accuracy of the $N_2O$ secondary standards was ±2%. The variability of the method during this study was generally better than 2-3% (mean = 0.97%, $n$=44), based on replicate analyses of standards, atmospheric air, and compressed air. In support of this calibration, the measured atmospheric mixing ratio of 313 ± 5 parts per billion by volume (ppbv) shows less than 1% deviation from the interhemispheric corrected global mean and at worst provides an overestimate of 1.3% relative to 1992 data for the South Pole [*Swanson et al.*, 1992].

The method returns gas partial pressures in the samples at the equilibrium temperature. Dissolved $N_2O$ concentrations in situ were calculated from the solubility tables of *Weiss and Price* [1980], for the equilibrium temperature (~25°C) and in situ salinity. Percent saturation of seawater with $N_2O$ was determined by ratioing the $N_2O$ partial pressures corrected to in situ temperature to the values determined in atmospheric air samples (mean 313 ± 5 ppbv).

### Other Hydrographical Variables

Temperature, salinity, and oxygen and nitrate concentrations are described in a summary by *Turner and Owens* [1995]. Nitrate was determined by segmented flow autoanalysis using an adaptation of the method of *Brewer and Riley* [1965] [*Whitehouse et al.*, 1995]. Oxygen concentrations were determined both with a calibrated sensor interfaced to the CTD package and with bottle samples determined by a modified Winkler titration using the method of *Williams and Jenkinson* [1982]. Temperature and salinity were calibrated using reversing thermometers and salinometer, respectively.

## Results

### Surface Transects

Figure 2a shows concentrations and percent saturations of $N_2O$ in surface transects, deviations from equilibrium saturation by ± 1 standard deviation are highlighted. These data are also summarized in Table 2. The most striking observation is a general overall increase in dissolved $N_2O$ with increasing southerly latitude. Despite a relatively large temperature decrease of Δ 6.67°C (Figure 2b) over the transects, percent $N_2O$ saturation data show that these observations cannot be attributed to temperature-solubility

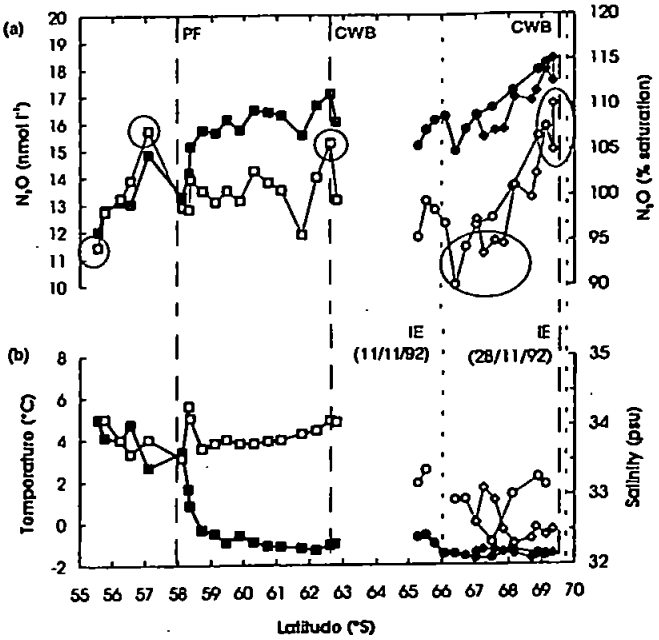


**Figure 2.** Surface transects 1 (squares) across the Drake Passage and 2 (circles) and 3 (diamonds) at 85°W. (a) Change in nitrous oxide concentration (solid symbols) and percent saturation relative to the mean atmospheric mixing ratio of 313 ppbv (open symbols) with latitude. The circled points are those deviating from 100% saturation by ± 1 standard deviation (b) Surface temperature (solid symbols) and salinity (open symbols) profile. The dashed lines are approximate positions for the Polar Front (PF) and Continental Water Boundary (CWB). The position of the ice edge (IE) at 85°W is located by the dotted lines.

effects alone and reveal important features of the dissolved $N_2O$ distribution; (1) a region of moderate $N_2O$ undersaturation north of 57°S in which saturations increase steadily with increasing southerly latitude, becoming oversaturated by about 57°S, most probably in association with the Subantarctic Front; the physical hydrography of this region was difficult to interpret, and though the position of the Subantarctic Front was defined at 57.4°S, an eddy-like feature to the north confused this somewhat (J. Read, personal communication, 1995); (2) a region between the Polar Front at 58°S and the Continental Water Boundary at 63°S, in which dissolved $N_2O$ is in approximate atmospheric equilibrium with the exception of a peak at the Continental Water Boundary; and (3) a region at 85°W where $N_2O$ is undersaturated at approximately 65°S with saturations (>90%) steadily increasing towards the south from 66.40°S, a position just north of the seasonal ice edge on November 11, 1992. South of 67.85°S, surface waters most recently subject to ice edge retreat and within the marginal ice zone were always supersaturated in $N_2O$, increasing steadily to a maximum of 110% at 69.35°S. These features indicate the presence of $N_2O$ removal and production processes within clearly defined regions of the study area.

### Station F (69.43°S)

At the time of sampling this station had similar ice cover characteristics to station G, though occupying a geographical position north of station I (Figure 1). So although considered

Table 2. Latitudinal Position of Sampling Points (Depth ~ 8 m), Observed Nitrous Oxide Concentration, Relative Saturation, and Flux Rate During November 1992 in the Southern Ocean

| Latitude, °S | Concentration, nmol $L^{-1}$ | Saturation, % | Flux (LM), $\mu mol\ m^{-2}\ d^{-1}$ | Flux (WK), $\mu mol\ m^{-2}\ d^{-1}$ |
|---|---|---|---|---|
| *Transect 1, Nov. 5, 1992, Drake Passage* | | | | |
| 55.55 | 12.0 | 94 | -0.8 | -1.2 |
| 55.75 | 12.8 | 98 | -0.2 | -0.4 |
| 56.23 | 13.1 | 100 | 0.0 | -0.1 |
| 56.54 | 13.0 | 102 | 0.2 | 0.4 |
| 57.09 | 14.8 | 107 | 1.0 | 1.6 |
| 58.12 | 13.3 | 99 | -0.2 | -0.3 |
| 58.33 | 14.2 | 98 | -0.2 | -0.3 |
| 58.37 | 15.1 | 102 | 0.2 | 0.4 |
| 58.73 | 15.7 | 101 | 0.1 | 0.1 |
| 59.11 | 15.6 | 99 | -0.1 | -0.2 |
| 59.47 | 16.1 | 100 | 0.1 | 0.1 |
| 59.88 | 15.7 | 99 | -0.1 | -0.1 |
| 60.30 | 16.5 | 103 | 0.4 | 0.6 |
| 60.73 | 16.4 | 101 | 0.2 | 0.3 |
| 61.13 | 16.3 | 100 | 0.1 | 0.1 |
| 61.76 | 15.5 | 96 | -0.6 | -1.0 |
| 62.20 | 16.6 | 102 | 0.3 | 0.4 |
| 62.61 | 17.1 | 106 | 0.1 | 1.3 |
| 62.77 | 16.0 | 99 | -1.0 | -0.1 |
| *Transect 2, Nov. 11, 1992, 85°W* | | | | |
| 65.28 | 15.1 | 95 | -0.7 | -1.0 |
| 65.53 | 15.7 | 99 | -0.1 | -0.1 |
| 65.79 | 16.1 | 98 | -0.2 | -0.4 |
| 66.10 | 16.2 | 97 | -0.5 | -0.7 |
| 66.40 | 14.9 | 90 | -1.4 | -2.3 |
| 66.71 | 15.8 | 94 | -0.8 | -1.3 |
| 67.03 | 16.3 | 97 | -0.5 | -0.7 |
| 67.51 | 16.5 | 98 | -0.4 | -0.6 |
| 68.13 | 17.2 | 101 | 0.1 | 0.2 |
| 68.90 | 17.9 | 106 | 0.9 | 1.5 |
| 69.14 | 18.2 | 107 | 1.1 | 1.7 |
| *Transect 3, Nov. 28, 1992, 85°W* | | | | |
| 67.59 | 16.2 | 97 | -0.4 | -0.6 |
| 67.04 | 15.5 | 94 | -0.9 | -1.5 |
| 67.25 | 15.7 | 95 | -0.7 | -1.1 |
| 67.85 | 15.8 | 95 | -0.8 | -1.2 |
| 68.18 | 16.9 | 101 | 0.2 | 0.2 |
| 68.70 | 16.8 | 100 | 0.0 | -0.1 |
| 68.84 | 17.1 | 102 | 0.3 | 0.5 |
| 69.14 | 17.9 | 108 | 1.1 | 1.7 |
| 69.35 | 17.5 | 105 | 0.7 | 1.1 |
| 69.35 | 18.4 | 110 | 1.4 | 2.3 |

Flux rates derived from *Liss and Merlivat* [1986] (LM) and *Wanninkhof* [1992] (WK).

apart from the other stations along the 85°W meridian because of the difference in time and ice cover, data from this station give an initial hint as to a positive sea-air flux of $N_2O$ as the ice retreats. The vertical distribution of $N_2O$ was fairly uniform in the upper 50 m of the water column (Table 3), with saturations of 113-117%, with the exception of a distinct maximum at 30 m (125%), coincident with a peak in $O_2$ distribution (77.8% and 293.1 $\mu mol\ L^{-1}$).

### Vertical Section G to K

Figure 3 shows percent $N_2O$ saturations as a function of depth and latitude for stations G, I, J, and K. Figure 4 shows profiles of $N_2O$ concentration and percent saturation, temperature, salinity, $NO_3$, and $O_2$ for the upper 200 m at the same stations. Overall vertical features are summarized as the following; (1) moderate undersaturations to moderate supersaturations are found above 200 m, with undersaturations confined to the upper mixed layer north of station G (Figure 4) with a general increase in saturation toward the south as reflected in the surface data; (2) a band of high $N_2O$ water (saturations of 130-142%) at the interface between surface and intermediate water approximates to the base of the oxycline, at 200 m in the south, shallowing to 150 m at station K; (3) a tongue of Warm Deep Water (>1°C) with $N_2O$ saturations of 115-130% from 200 to 500 m on the continental shelf descends and broadens in depth toward the north consistent with the bathymetry, so that its base is about 1800 m at 68°S; and (4) deep moderately $N_2O$ saturated waters (115%) progressively decrease in saturation with depth to <100% close to the bottom.

Stations F and I were separated laterally by approximately 16 km, but were sampled 17 days apart. During this time the ice retreated south by over 2° latitude [*Turner and Owens*, 1995]. Interestingly, the $N_2O$ characteristics of the upper 50 m of the water column appear to have changed during this retreat (Figure 5) from 113% saturation at 10 m on November 13, 1992, to 90% saturation at 10 m on December 1,1992; this is coincident with a decrease in salinity from 33.99 to 33.92, which suggests that the decrease in $N_2O$ saturation may be attributed at least in part to dilution by ice meltwater. The area of undersaturation between stations I and K corresponds to the upper mixed layer (Figures 3 and 4) as identified by the density section of *Turner and Owens* [1995] and described in detail by *Read et al.* [1995]. The maxima recorded at 200 m for stations G-J and at 150 m for station K (Figure 4) are at the interface between the surface water and Circumpolar Deep Water. Circumpolar Deep Water is identified by *Keir et al.* [1992] as a southward directed tongue of Warm Deep Water, which is characterized by mid depth temperature and salinity maxima that slope upward toward the Antarctic continent. This water, which originates from a combination of North Atlantic Deep Water, Mediterranean water and central waters formed in the sub tropical gyres, is clearly evident from the isotherms in Figure 3.

We have shown above for the Drake Passage transect that the physical hydrography of the region accounts for a substantial fraction but not all of the dissolved $N_2O$

Table 3. Station F (69.43°S 85.06°W)

| Depth, m | $N_2O$ Concentration, nmol $L^{-1}$ | $N_2O$ Saturation, % | $O_2$ Concentration, $\mu mol\ L^{-1}$ |
|---|---|---|---|
| 2 | 19.1 | 113 | 282.2 |
| 10 | 19.1 | 113 | 286.2 |
| 20 | 19.1 | 113 | 291.8 |
| 30 | 21.0 | 125 | 293.1 |
| 40 | 19.3 | 114 | 292.4 |
| 51 | 19.7 | 117 | 288.1 |

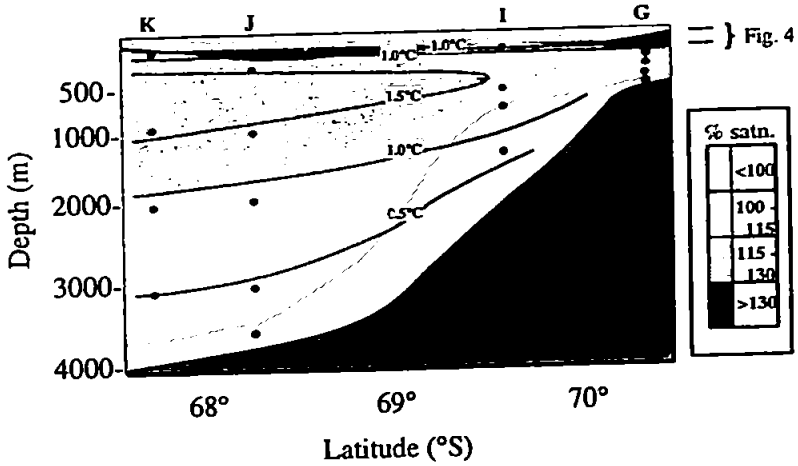


Figure 3. Vertical section of the percentage of nitrous oxide saturation relative to an atmospheric mixing ratio of 313 ppbv for stations G, I, J, and K at 85°W in the Bellingshausen Sea. Solid lines are isotherms, labeled as degrees Celsius.

distribution. Investigation of the relationships between $N_2O$ and hydrographical variables along transect G - K is confused by the complex series of events associated with the retreating ice edge. Positive and negative correlations between $N_2O$ and nitrate and oxygen respectively, as observed previously [*Badr and Probert*, 1992; *Naqvi et al.*, 1994], were evident particularly at station K ($r^2$= 0.889 and 0.604). However, these relationships broke down and were insignificant at station G ($r^2$= 0.042 and 0.057).

### Air ↔ Sea Fluxes

Exchange of $N_2O$ across the sea-air interface may be estimated from the measured surface $N_2O$ anomaly and modeled gas transfer coefficients, so that

$$F = K_W L \Delta p\, N_2O \qquad (1)$$

where $F$ is the $N_2O$ flux (micromoles of $N_2O$ per square meter per day) and $K_W$ is the gas transfer velocity, based on a monthly mean wind speed for November and December of 6 m $s^{-1}$ [*Launiainen and Vihma*, 1994; *Tilbrook and Karl*, 1994], with adjustment for $N_2O$ at ambient temperature using the relevant Schmidt numbers [*Wanninkhof*, 1992]. $L$ is the solubility and $\Delta pN_2O$ is the partial pressure gradient across the interface. The relationship between wind speed and transfer velocity is not a simple linear one; discrepancies between published transfer velocities [*Nevison et al.*, 1995; *Bange et al.*, 1996] highlight the uncertainties introduced by the different models, and the use of averaged wind speeds in the estimation of $K_W$ may lead to some further uncertainty. Following the work of *Nevison et al.* [1995], in which the derivation and use of three such models are discussed in detail, gas transfer velocities have been calculated for this study using the relationships of *Liss and Merlivat* [1986] and *Wanninkhof* [1992], which provide lower and upper limits of $N_2O$ flux, respectively.

Sea-air $N_2O$ fluxes reflect the surface pattern of percent saturation; in all cases, minimum fluxes are those produced by *Liss and Merlivat* [1986] and maximum fluxes are those produced by *Wanninkhof* [1992]. For the Drake Passage transect 1 (Table 2), there is a net ocean release in the range +0.05 ± 0.4 to +0.08 ± 0.7 μmol $N_2O$ $m^{-2}$ $d^{-1}$. Along the 85°W meridian, there is great variability in flux estimates from ocean uptake (maximum -1.4 to -2.3 μmol $N_2O$ $m^{-2}$ $d^{-1}$) to release to the atmosphere (maximum +1.9 to +3.1 μmol $N_2O$ $m^{-2}$ $d^{-1}$). These data give an overall mean flux of between -0.06 ± 0.9 and -0.09 ± 1.4 μmol $N_2O$ $m^{-2}$ $d^{-1}$ for the area between 65.28°S and 70.32°S. Results for transects 2 and 3 indicate a sink for $N_2O$ between 65.28°S and 68.70°S; stations G to K show that this area may extend as far south as the ice edge at 69.57°S (station I) with a mean flux of between -0.6 ± 0.40 and -0.9 ± 0.7 μmol $N_2O$ $m^{-2}$ $d^{-1}$. The two stations with full ice cover, F and G both display potential positive fluxes of 1.9-3.1 and 0.8-1.3 μmol $N_2O$ $m^{-2}$ $d^{-1}$, respectively. The maximum rate of 1.9-3.1 μmol $N_2O$ $m^{-2}$ $d^{-1}$ was recorded at station F only ~16 km south of station I, separated by 17 days and following retreat of the ice edge by 2° south. Positive fluxes south of 68.13° recorded toward the ends of transects 2 and 3 are associated with upwelling of Circumpolar Deep Water coincident with the position of the Continental Water Boundary as is the point highlighted at the southern end of transect 1 (Figure 2). For this area (68.13°-70.32°S) we calculate a mean flux of between +0.9 ± 0.5 and +1.5 ± 0.9 μmol $N_2O$ $m^{-2}d^{-1}$.

## Discussion

Figure 6 shows the relationship between apparent nitrous oxide production/consumption ($\Delta N_2O$) and the apparent oxygen utilization (AOU), where

$$\Delta N_2O = [N_2O]_{obs} - [N_2O]_{eq} \qquad (2)$$

$$AOU = [O_2]_{eq} - [O_2]_{obs} \qquad (3)$$

$[X]_{obs}$ is the measured concentration and $[X]_{eq}$ is the expected equilibrium concentration calculated from the solubility at in situ temperature and salinity, from the equations of *Weiss and Price* [1980] for $N_2O$ and *Garcia and Gordon* [1992] for $O_2$. A positive correlation between $\Delta N_2O$ and AOU for waters below the mixed layer is accepted to be indicative of $N_2O$ production associated with nitrification [*Law and Owens*, 1990a; *Nevison et al.*, 1995]. Exceptions to this have previously been associated with oxygen deficient

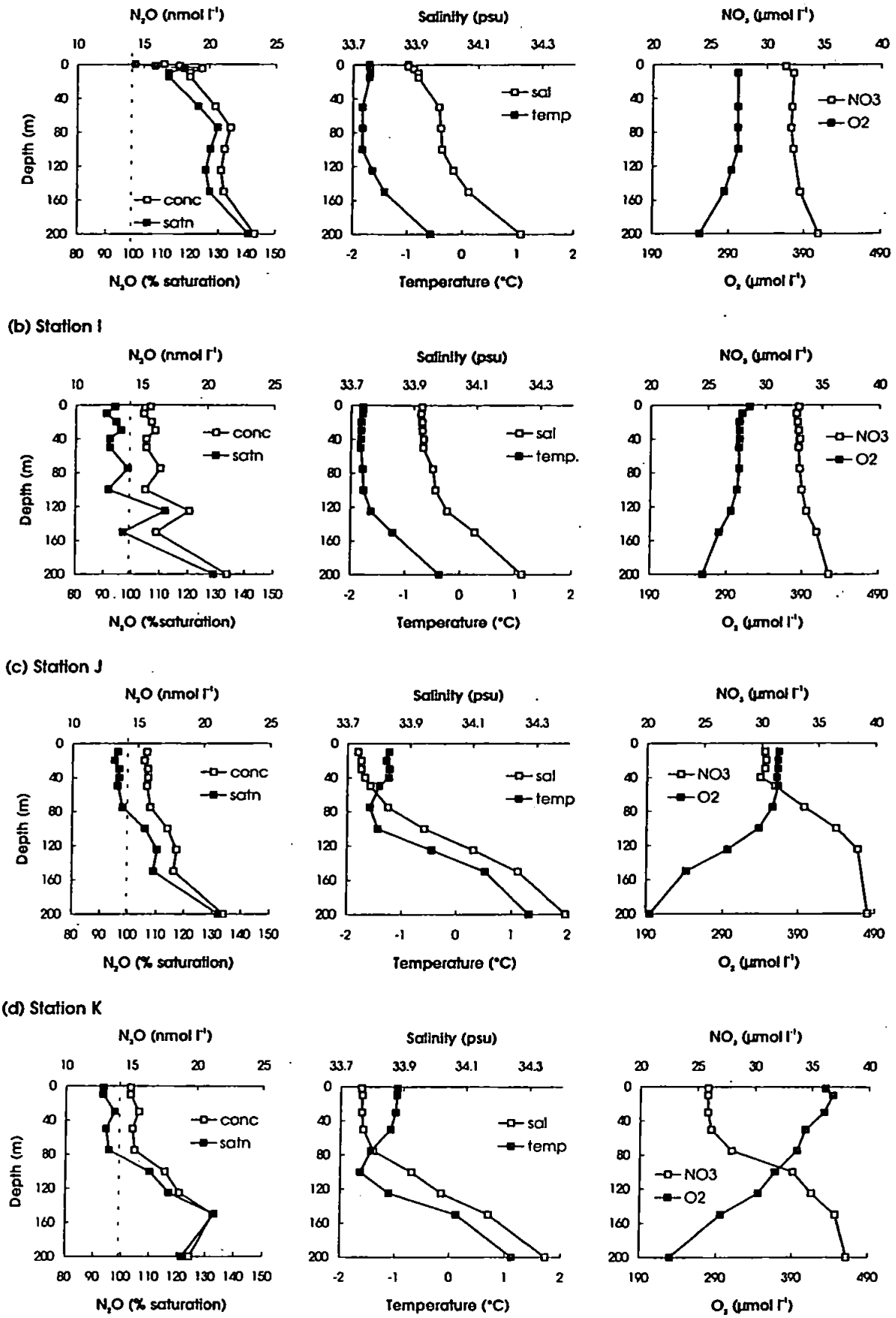


Figure 4. Zero to 200 m profiles of nitrous oxide concentration and saturation (the dashed line indicates 100% saturation), salinity and temperature, and nitrate and oxygen concentrations for stations (a) G, (b) I, (c) J, and (d) K at 85°W in the Bellingshausen Sea.

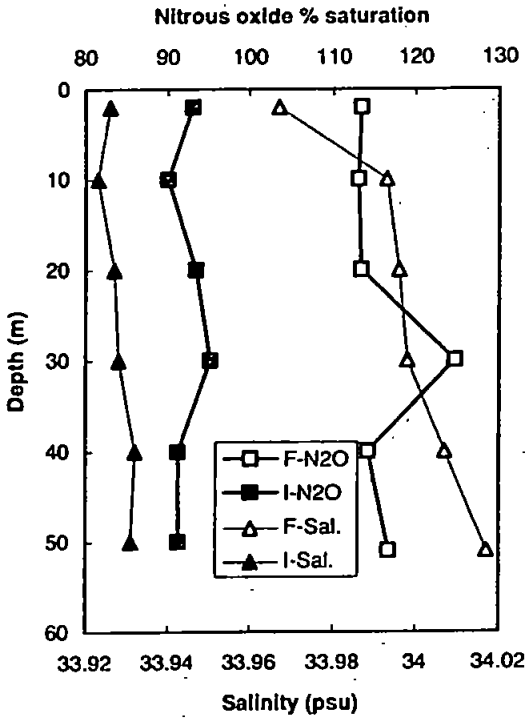


Figure 5. Change in nitrous oxide percentage of saturation and salinity between stations F (69.43°S) and I (69.57°S) following retreat of the ice edge. F was under full ice cover, while I was at a distinct ice edge.

environments [*Cohen and Gordon*, 1979] and within the surface mixed layer [*Levitus*, 1982]; where nitrification is believed to be inhibited by light, oxygen concentrations vary due to respiration and photosynthesis, and the relative concentrations of the two gases are subject to modification by differential fluxes across the sea-air interface. The wide scatter of data in Figure 6 may be attributed to the wide depth range displayed, and though there is a significant positive correlation ($r^2 = 0.185$, $p = 0.001$), this is biased by station K; removal of these data reduces the correlation to $r^2 = 0.126$, $p > 0.1$. The linear relationships described for other oceanic areas [*Elkins et al.*, 1978; *Cohen and Gordon*, 1979; *Oudot et al.*, 1990; *Law and Owens*, 1990a; *Naqvi and Noronha*, 1991] are evident for open water stations J and K but not for those

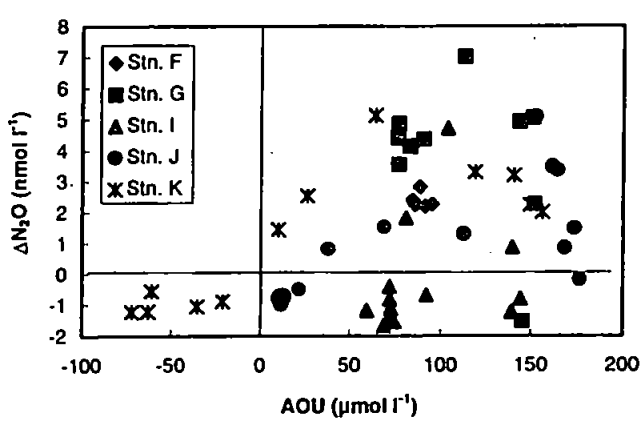


Figure 6. Relationship between $\Delta N_2O$ and AOU for stations F, G, I, J, and K at 85°W in the Bellingshausen Sea.

Table 4. Correlation Coefficients ($r$) of $\Delta N_2O$ on AOU for Stations F-K

| Station | Depth Range, m | $\Delta N_2O$/AOU | $n$ |
|---|---|---|---|
| F | 2 - 51 | -0.474 | 6 |
| G | 50 - 600 | -0.323 | 10 |
| I | 2 - 1380 | 0.261 | 14 |
| J | 10 - 3775 | 0.703* | 15 |
| K | 2 - 3000 | 0.780† | 12 |

$\Delta N_2O$ is given in nanomoles per liter; AOU is given in micromoles per liter. Here $n$ is the number of paired data points.
*Correlation significant at $P = 0.003$.
†Correlation significant at $P = 0.002$.

directly influenced by ice (Table 4). The relationship between oxygen consumption (AOU) and nitrate regeneration through nitrification is also reflected by a positive correlation between $\Delta N_2O$ and $NO_3$ concentration [*Oudot et al.*, 1990]. Data in Table 5 provide further evidence for the occurrence of nitrification as a source of $N_2O$ at the northerly two stations (K and J) at 85°W and for an alternative supply at stations F, G, and I. It is unlikely that denitrification within the water column was responsible for $N_2O$ production, as oxygen concentrations were significantly higher (> 4.0 mL $L^{-1}$) than those required for denitrification to occur (< 0.1 mL $L^{-1}$), [*Codispoti and Christensen*, 1985]. Supply of $N_2O$ to shelf waters in the Bellingshausen Sea would appear to be from the upwelling of Continental Deep Water, whose source waters display supersaturated concentrations [*Cohen and Gordon*, 1979; *Law et al.*, 1990; *Oudot et al.*, 1990; *Badr and Probert*, 1992; *Weiss et al.*, 1992].

Although sea ice permits the slow exchange of gases through it [*Gosink*, 1980; *Broecker et al.*, 1986], this is considered to be negligible by *Weiss et al.* [1979]. The ventilation of shelf waters is a dynamic process which is dependent on the relative rates of Warm Deep Water input and gas transfer across the air-sea interface [*Keir et al.*, 1992]. Our data support these observations, in that an accumulation of $N_2O$ under the ice during the winter is available for release, so that with the onset of spring a pulse of $N_2O$ is released to the atmosphere as the ice retreats, equivalent to a maximum rate recorded of +1.9 to +3.1 μmol $N_2O$ $m^{-2}$ $d^{-1}$. Following the ice retreat, significant $N_2O$ undersaturation develops as surface waters mix with sea ice meltwaters which typically show subsaturations in the range 4 - 33% relative to air [*Gosink*, 1980]. Whereas one would expect a state of

Table 5. Correlation Coefficients ($r$) of $\Delta N_2O$ on $NO_3$ for Stations F-K

| Station | Depth Range, (m) | $\Delta N_2O/NO_3$ | $n$ |
|---|---|---|---|
| F | 2 - 51 | 0.337 | 6 |
| G | 50 - 600 | -0.324 | 10 |
| I | 2 - 1380 | 0.344 | 14 |
| J | 10 - 3775 | 0.776* | 14 |
| K | 2 - 3000 | 0.943† | 12 |

$\Delta N_2O$ is given in nanomoles per liter; $NO_3$ is given in micromoles per liter. Here $n$ is the number of paired data points.
*Correlation significant at $P = 0.001$.
†Correlation significant at $P < 0.0001$.

equilibrium between surface waters and the atmosphere to be reached, $N_2O$ saturations decline to significant values. Such undersaturations have been previously reported in Antarctic waters [*Gosink*, 1980; *Priscu et al.*, 1990; *Watson et al.*, 1990; *Badr and Probert*, 1992], and a similar decline in saturations from 110 to 105% was reported between winter and summer by *Weiss et al.* [1992].

$N_2O$ undersaturations and sink conditions previously reported for the Antarctic [*Gosink*, 1980; *Priscu et al.*, 1990; *Badr and Probert*, 1992] do not include estimates of gas exchange; however, the saturations reported here are within the range of previous measurements (68-333%). Maximum ocean uptakes of $N_2O$ comparable to our own of -1.18 and -1.37 μmol $m^{-2}$ $d^{-1}$ were reported by *Cohen and Gordon* [1978] and *Pierotti and Rasmussen* [1980] for the east tropical Pacific and of -0.10 μmol $m^{-2}$ $d^{-1}$ in the Bay of Bengal by *Naqvi et al.* [1994]. The rates of sea-air $N_2O$ flux from this study are at the lower end of the published range, for example, 0.19 μmol $m^{-2}$ $d^{-1}$ for upwelling in the west Pacific [*Butler et al.*, 1989], 0.49 μmol $m^{-2}$ $d^{-1}$ for the northern North Sea [*Law and Owens*, 1990b], 2.6-5.2 μmol $m^{-2}$ $d^{-1}$ for the eastern equatorial Indian Ocean [*Butler et al.*, 1989], and 8.64 μmol $m^{-2}$ $d^{-1}$ for the Arabian Sea upwelling [*Law and Owens*, 1990a].

Our rate estimate for the air-sea exchange of $N_2O$ at 85°W suggests that the area of the Southern Ocean affected by annual sea ice represented a small sink at the time of sampling. Significant variations in the range of our estimates indicate that small changes in the physical environment could potentially lead to significant changes in the size and direction of $N_2O$ flux.

*Bouwman et al.* [1995] hypothesize that the Antarctic Ocean is a major source region for atmospheric $N_2O$, due to the supersaturations associated with upwellings reported by *Weiss et al.* [1992]. Because our data show that net fluxes may be particularly sensitive to the seasonal retreat of sea ice, Bouwman et al.'s estimate may require revision. It is important to emphasize that our data only describe conditions for the austral spring/summer, north of the continental shelf, and that it is conceivable that continued sea ice retreat throughout the summer may facilitate the release to the atmosphere of upwelled $N_2O$ over an area greater than we are able to account for.

## Conclusion

The Antarctic Ocean, because of its extraordinary hydrography, displays localized regions of spatially and temporally variable source-sink characteristics to atmospheric $N_2O$. Our data suggest that upwelling water supersaturated in $N_2O$ accumulates under annual sea ice during the winter, to be released to the atmosphere on retreat of the ice during the spring months. The flux to the atmosphere is mediated by dilution with low $N_2O$ sea ice meltwater; this gives rise to a surface layer (75-100 m) of Antarctic Surface Water, representing a seasonal sink to atmospheric $N_2O$. The Drake Passage was found to be in equilibrium with the atmosphere, though areas of subsaturated and supersaturated water were found in association with frontal and eddy features. Overall, the surface waters in this area are close to equilibrium with the atmosphere, although significant reduction in seasonal sea ice could accentuate the release of $N_2O$ to the atmosphere.

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N.J.P. Owens and R.C. Upstill-Goddard, Department of Marine Sciences and Coastal Management, University of Newcastle, Newcastle upon Tyne, NE1 7RU, England, United Kingdom. (e-mail: n.owens@ncl.ac.uk; rob.goddard@ncl.ac.uk)

A. P. Rees, Plymouth Marine Laboratory, Prospect Place, Plymouth, PL1 3DH, England, United Kingdom. (e-mail: a.rees@pml.ac.uk)

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Signed:
I.R. Joint

Date: 22/11/01

Signed:
K.M. Donald

Date: 12-4-01

PERGAMON

Deep-Sea Research I 46 (1999) 483–510

DEEP-SEA RESEARCH
PART I

# Early spring bloom phytoplankton-nutrient dynamics at the Celtic Sea Shelf Edge

Andrew P. Rees, Ian Joint*, Kirsten M. Donald

*NERC Centre for Coastal and Marine Sciences, Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH, UK*

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**Abstract**

Phytoplankton production was measured at the shelf edge region of the Celtic Sea in April/May 1994 at the beginning of the spring bloom. Size fractionated $^{14}C$ uptake experiments showed that phytoplankton $>2$ μm dominated the bloom although, in the period immediately before the increase in phytoplankton biomass, picophytoplankton ($<2$ μm) was responsible for up to 42% of the production; in these late winter conditions, chlorophyll concentrations were generally $<0.7\ \mu g\,l^{-1}$ and primary production was ca. 70 mmol $C\,m^{-2}\,d^{-1}$. As the spring bloom developed, phytoplankton production rates of 120 mmol $C\,m^{-2}\,d^{-1}$ were measured. Chlorophyll concentration increased to $>2\ \mu g\,l^{-1}$ as a result of growth of larger phytoplankton, including diatoms, with large numbers *of Nitzschia, Thalassionema* and *Chaetoceros* dominating the assemblage. Picophytoplankton production declined as the spring bloom progressed. Nutrient concentrations were not depleted during the sampling period, and $NO_3^-$ concentrations were $>6\ \mu mol\,l^{-1}$. Nutrient assimilation rates were measured at the same time as primary production was estimated. Before the development of any substantial phytoplankton biomass, the uptake rates for ammonium and nitrate were very similar, with *f*-ratios ranging from 0.5 to 0.6. Assimilation of ammonium remained relatively constant after the onset of stratification and bloom development, but nitrate uptake increased by a factor of 2 or more, resulting in *f*-ratios $>0.8$. There was significant phosphate uptake in the dark, which was generally ca. 50% of the rate in the light. The C:N:P assimilation ratios changed as the bloom developed; in the pre-bloom situation, when small phytoplankton cells dominated the assemblage, the C:N assimilation ratio was variable, with some stations having ratios less than (ca 2.5), and some higher than (ca. 9), the Redfield ratio. The most actively growing assemblages had N:P ratios close to the Redfield ratio, but the C:N ratios were consistently lower. New production was found to be closely correlated with the size of the species making up the phytoplankton assemblage, and high *f* ratios were measured when larger phytoplankton dominated the assemblage. © 1999 Elsevier Science Ltd. All rights reserved.

*Corresponding author. Fax: 01752 633101; *E-mail address*: i.joint@pml.ac.uk.

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## 1. Introduction

Coastal oceans and the deep sea have distinct physical and biological properties, but the region where they meet, at the shelf edge, is complex, with special processes that result from the combination of stratification with steep bathymetry (Huthnance, 1995). The shelf edge is generally poorly understood, yet the processes taking place there have profound implications for the interchange of materials between the coastal and open oceans. For example, the exchange of water masses at the shelf edge, with their associated nutrient and particle loads, is a poorly quantified process with important consequences for shelf sea productivity; it has been suggested that the high productivity of shelf seas is sustained by nutrient fluxes from the ocean (Wollast, 1993). The boundary between the shelf and the deep ocean is also a complex physical environment, with the processes of shelf-edge circulation, exchange and mixing having important consequences for phytoplankton production. Huthnance (1995) has provided a comprehensive review of physical processes controlling exchange at the shelf edge and highlights the problems in measuring and modelling exchange at the shelf edge.

The shelf edge has been the focus for a number of biogeochemical studies since Walsh et al. (1981) suggested that the importance of exchange between the shelf and the continental slope had been underestimated and that a large proportion of the primary production on the continental shelf was exported. A major study to test the hypothesis in the Mid-Atlantic Bight, the SEEP I experiment, provided equivocal results. Estimates of export from the shelf varied from $< 10\%$ for carbon flux calculations (Biscaye et al., 1988) to a maximum of 38% in a modelling study (Walsh et al., 1988). Within SEEP I, there was also evidence that the hypothesis was not sustainable. Falkowski et al. (1988) reported that much of the carbon fixed in the spring bloom was utilised on the shelf and that, at most, 10–20% of the phytoplankton production during the spring bloom might be available for export. A second experiment in the Mid-Atlantic Bight, SEEP II, showed unequivocally that the carbon export hypothesis was not valid in this region (Biscaye et al., 1994) and that export of biogenic organic matter from the shelf to the slope was not a significant process; much less than 5% of the organic carbon produced by biological activity on the US continental shelf was exported to the adjacent slope, with the majority of biogenic material undergoing heterotrophic recycling on the shelf.

Although evidence for the depocentre hypothesis at the shelf edge is equivocal, shelf edge processes are, nevertheless, extremely important for the fluxes of nutrients and contaminants from and to the coastal oceans. One important region is the wide shelf sea of NW Europe, which is bordered by major industrialised nations making extensive use of their coastal seas. The research described in this paper was done at the shelf edge of the Celtic Sea and is part of the Ocean Margin Exchange (OMEX) Project, funded by the European Commission. The overall aims of the project are to investigate the importance of the ocean margin for exchange between the ocean and shelf seas, to determine if there is enhanced production in this region and to estimate the fraction of primary production that sediments into intermediate and deep water; this latter topic is the subject of a separate paper (Joint et al., 1999).

A number of studies of the Celtic Sea area have investigated primary production (Joint and Pomroy, 1983; Joint and Pomroy 1986; Joint et al., 1986; Martin-Jezequel

and Videau, 1992 and Platt et al., 1994), though few have specifically studied the shelf edge. Of particular relevance for phytoplankton production are processes that enhance water column stability or nutrient supply. A major part of phytoplankton production in this region occurs in April, when the seasonal suppression of deep winter mixing results in a well defined spring bloom. However, at other times of the year, variability in vertical mixing is important (Pingree et al., 1982; New 1988) since these processes introduce nutrient-rich water into the surface mixed layer. Satellite images of the Celtic Sea in the summer months frequently show a band of cold water at the shelf edge (Dickson et al., 1980; Pingree and New, 1995). Pingree et al. (1986a, b) showed that the cool surface water was a consequence of an internal tide, generated at spring tides at the 200 m contour, which resulted in the mixing of cooler, nutrient-rich waters to the sea surface, increasing chlorophyll concentrations and presumably enhancing phytoplankton production.

This paper describes measurements made at the time of the development of the spring bloom. The assimilation of nitrate, ammonium and phosphorus was measured in relation to photosynthetic carbon fixation prior to and during the initial period of the spring bloom in April and May 1994. That is, this paper quantifies the simultaneous assimilation of the major elements required by phytoplankton– carbon, nitrogen and phosphorus. The relative rates of nitrate and ammonium assimilation are compared to provide an estimate of new production; i.e. that proportion of primary production that is supported by nitrate (Dugdale and Goering, 1967) and that is considered to be equivalent to the production that is exportable to the deep ocean (Eppley and Peterson, 1979).

The study investigated the relative importance of phytoplankton size classes as a means of estimating the proportion of material likely to be sedimented rather than recycled; since larger phytoplankton cells have faster sinking velocities and are grazed by copepods, which produce faecal pellets with rapid sinking velocities, there is a greater potential for vertical flux. Copepods are the major mesozooplankton grazers in the Celtic Sea (Joint and Williams, 1985), but a species such as *Calanus helgolandicus* cannot filter efficiently any particle that is smaller than 10 μm in diameter. Therefore, the picophytoplankton ( $<2$ μm), which is present mostly as single cells, is unlikely to be grazed directly by copepods and must be grazed by heterotrophic flagellates (microzooplankton) as part of the microbial loop. Microzooplankton does not produce discrete faecal pellets, and most mineralisation by these small grazers occurs within the surface mixed layer. Therefore, it appears likely that large phytoplankton cells have a greater potential to form the organic detritus that sediments to the sea bed, but picoplankton is more likely to be mineralised within the surface water. A study of size fractionated phytoplankton activity should be invaluable in relating primary production to sedimentation and vertical flux.

## 2. Methods

A transect across the Celtic Sea shelf edge, along the Goban Spur (Fig. 1), was sampled during April–May 1994. This was a period of rapidly changing conditions:

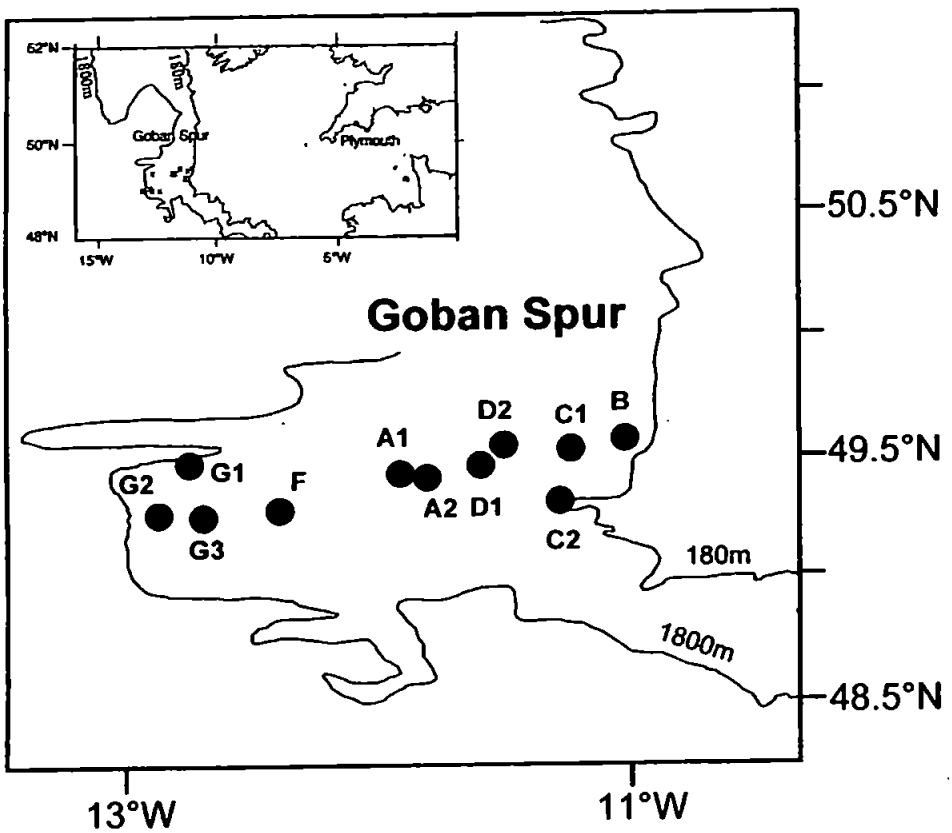


Fig. 1. Chart of the Shelf Edge region of the Celtic Sea showing the stations sampled.

increasing stratification, the development of a spring phytoplankton bloom towards the end of the cruise and declining nutrient concentrations. The transect was sampled several times during the cruise (Table 1).

The aim of the study was to estimate phytoplankton production; therefore, nitrogen, carbon and phosphorus assimilation rates were determined using radio- and stable isotope tracers. Analyses were also made of the concentrations of chlorophyll and the dissolved inorganic nutrient species nitrate, nitrite, ammonium, phosphate and silicate. The physical structure of the water column was determined using a calibrated CTD system. Water samples were collected before dawn from 6 to 9 depths in the surface mixed layer using pre-cleaned 30l "Go-Flo" bottles on a Kevlar cable; to avoid light shock to the phytoplankton, water samples were kept in the dark. Following addition of relevant tracers, samples were incubated either in-situ, using a free-floating buoyed rig, which allowed incubation at the depth from which samples were taken, or in an on-deck incubator with neutral density filters corresponding approximately to 100, 60, 30, 16, 3 and 1% of surface irradiance to give a simulated ambient light profile. Samples incubated on deck were maintained at surface temperature by pumping sea water from a depth of ca. 2.5 m through the system. Previous studies in the North Atlantic (Joint et al., 1993) and North Sea (Joint and Pomroy, 1993) have found no significant differences in the estimates of primary production by

Table 1
Position and date of stations occupied on Goban Spur transect in April and May 1994

| Station | G1 | G2 | G3 | F | D1 | A1 | A2 | B | C1 | D2 | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | 16 April | 18 April | 19 April | 21 April | 23 April | 25 April | 26 April | 27 April | 28 April | 1 May | 2 May |
| Julian Day | 106 | 108 | 109 | 111 | 113 | 115 | 116 | 117 | 118 | 121 | 122 |
| Latitude °N | 49.40 | 49.14 | 49.13 | 49.18 | 49.42 | 49.34 | 49.34 | 49.51 | 49.43 | 49.51 | 49.33 |
| Longitude °W | 12.80 | 12.78 | 12.82 | 12.34 | 11.59 | 11.65 | 11.63 | 11.00 | 11.25 | 11.48 | 11.40 |
| 1% light depth[a] (m) | 42 | 47 | 42 | 41 | 36 | 40 | 35 | 37 | 27 | 26 | 30 |
| Water column depth (m) | 1383 | 1450 | 1630 | 1091 | 765 | 798 | 767 | 192 | 242 | 620 | 349 |

[a]Depth at which the value of PAR is 1% of that immediately below the sea surface.

these two methods. On this cruise, the majority of estimates were obtained by on-deck incubations, since this gave the maximum flexibility for associated sampling of the mid-water and benthos.

### 2.1. Nutrient concentrations

Analyses of dissolved nutrient concentrations were done as soon as practicable after sampling and were always completed within 12 h of collection; prior to analysis, all samples were stored at 4°C in the dark. Nutrient concentrations were determined by colorimetric autoanalysis using the methods of Brewer and Riley (1965) for nitrate, Grasshoff (1976) for nitrite, Mantoura and Woodward (1983) for ammonium, and Kirkwood (1989) for silicate and phosphate.

### 2.2. Chlorophyll concentration

Chlorophyll concentrations were measured by spectrophotometric (Parsons et al., 1984) and fluorometric (Holm-Hansen et al., 1965) analysis of extracted pigments. 1–2 l aliquots of water from the above experiments were filtered through Whatman GF/F filters for spectrophotometric analysis, or 100 ml samples were filtered through polycarbonate filters for fluorometric determination of chlorophyll concentration in two size fractions ($<2$ and $>2$ μm); all filters were frozen until return to the laboratory. Pigments were extracted by addition of 90% acetone and stored in the dark at 4°C for ca. 12 h prior to analysis. Underway surface chlorophyll concentrations were determined by fluorometry on the ship's non-toxic sea water supply and calibrated with water samples analysed for chlorophyll content by spectrophotometry (Parsons et al., 1984).

### 2.3. Phytoplankton identification

The phytoplankton assemblage was characterised by light microscopy on samples preserved with 1% Lugol's solution and stored in the dark until analysis. Phytoplankton counts and species identification were by the procedures described by Joint and Pomroy (1988). Species identification was largely confined to phytoplankton cells $>5$ μm in diameter, since the taxonomy of many of the smaller species is difficult because of the lack of distinct morphological features.

### 2.4. Estimation of euphotic depth

On deck incubations require information on light transmission profiles to establish the shape of the depth-integrated production curve. A functional light meter was not available during the cruise and it was necessary to employ an alternative method to estimate the depth of the 1% light level ($Z_e$). The method of Berthon and Morel (1992), was used; $Z_e$ is related to the total pigment content ($C_{tot}$) within the euphotic layer (0 to $Z_e$) and modelled using the iterative process:

$$Z_e = 568.2\,(C_{tot})^{-0.746}$$

when $Z_e$. $<102$ m. This parameter was determined for each of the stations occupied and used to relate sampling depth to euphotic depth and hence to calculate production rates integrated to the depth equivalent to 1% surface light (Table 1). The depths equivalent to the light attenuation obtained by each of the screens of the on-deck incubator were calculated from Beer's Law.

### 2.5. Carbon fixation

The rate of carbon fixation was estimated from the incorporation of $^{14}$C-bicarbonate. Aliquots of water samples from each depth were transferred into three 60 ml clear polycarbonate bottles and a single black polycarbonate bottle; all bottles were cleaned following JGOFS protocols (IOC, 1994) to reduce trace metal contamination. Each bottle was inoculated with 370 kBq (10 μCi) $NaH^{14}CO_3$ and transferred to the incubation system before dawn and incubated until dusk. Experiments were by either in situ (stations G2, F and D2) or on-deck incubations (all other stations). Samples incubated in situ were brought on board at dusk and maintained in the dark overnight at surface sea water temperature. On-deck incubations had their graded light screens replaced with dark screens to ensure that they were not affected by the ship's lights. Incubations were terminated after 24 h by filtration; each sample was filtered sequentially through 2.0 and 0.2 μm polycarbonate filters, and the filters were dried and stored in a desiccator until return to the laboratory. $^{14}$C was measured in a liquid scintillation counter, the efficiency of which was determined with an external standard, channels ratio method.

### 2.6. Nitrogen assimilation

Assimilation rates for nitrate and ammonium were determined following inoculation of water samples with the stable isotope $^{15}$N. Replicate samples from each depth were distributed into 2.4l clear polycarbonate bottles, and $^{15}N$–$NO_3^-$ and $^{15}N$–$NH_4^+$ were added at 0.1 μmol $l^{-1}$. Incubations were for 24 h, as described above, and were terminated by filtration ($<40$ cmHg) onto ashed Whatman GF/F filters, which were then washed with filtered sea water and stored frozen until return to the laboratory. The filters were oven dried at 50°C before analysis, and atom% $^{15}$N was measured by continuous flow nitrogen analysis-mass spectrometry (Europa Scientific Ltd., UK) using the techniques described in Barrie et al. (1989) and Owens and Rees (1989). Rates of assimilation were calculated from the equations of Dugdale and Goering (1967).

Several limitations are inherent in the $^{15}$N method, though probably the greatest is caused by lack of information on ambient nutrient concentration when concentrations are at or below the detection limit of the analysis system (Harrison et al., 1996). Dugdale and Goering (1967) originally proposed addition of $^{15}$N at ~10% of ambient concentration; for nutrient depleted waters, this has recently been refined so that addition is made at the level of detection for the nutrient analysis system used (McCarthy et al., 1992; IOC, 1994). During this study, concentrations of $NO_3^-$ were always $>1$ μmol $l^{-1}$ so that 0.1 μmol $l^{-1}$ additions were always less than 10% of

ambient concentration. However, ambient $NH_4^+$ concentrations were in the main below the detection limit for the analyser (0.1 μmol $l^{-1}$), and tracer additions at these levels could potentially enhance uptake rates. Nitrogen uptake kinetic parameters described by Harrison et al. (1996) allowed us to account for this rate enhancement and to estimate a range of $NH_4^+$ uptake rates according to the equation:

$$rN_H = \frac{rN_o}{N_{sp}/(K + N_{sp}) \times (K + N_A)/N_A},$$

where N is $NO_3^-$ or $NH_4^+$, $rN_0$ is the original uptake rate (μmol $l^{-1}$ $d^{-1}$), $rN_H$ is the uptake rate adjusted for stimulation by tracer (μmol $l^{-1}$ $d^{-1}$), $N_{SP}$ dissolved nitrogen concentration ambient + tracer (μmol $l^{-1}$), $N_A$ is ambient dissolved nitrogen concentration, K is the half saturation parameter (0.025 μmol $l^{-1}$). Determination of both $rN_0$ and $rN_H$ requires a value for $N_A$. When ammonium concentrations were at or below detection limit, then rates were estimated assuming 0.1 and 0.05 μmol $l^{-1}$ as maximum and minimum boundaries. Information on ammonium concentrations is relatively sparse, though it often falls to 0.005–0.01 μmol $l^{-1}$ in oceanic waters (Brzezinski, 1988). We have chosen 0.05 μmol $l^{-1}$ as representative of minimum $NH_4^+$ concentration for this area; similar values were reported by both the CJGOFS-93 springtime cruise in the Atlantic (Harrison et al., 1996) and a June 1996 cruise between 60° and 37°N along the 20°W meridian (E.M.S. Woodward, Plymouth Marine Laboratory, pers. comm.). Ammonium regeneration within the incubation bottles could cause an underestimation of ammonium uptake rates. Elskens et al. (1999) carried out a series of experiments in the same area between 1993 and 1995 and suggest that the maximum effect of ammonium regeneration would be a doubling of the measured rates. Similar estimates were obtained for a fjordic system (Rees et al., 1995) and in an upwelling off Portugal (Slawyk et al., 1997). Therefore, combining these factors gives a range of estimates for ammonium uptake that account for stimulation of uptake by added tracer, ammonium regeneration and an assumed minimum $NH_4^+$ concentration.

### 2.7. *Phosphate assimilation*

Incorporation of $^{33}P$ labelled orthophosphate was determined following procedures similar to those adopted for $^{14}C$ uptake measurements. Water samples from each depth were distributed into one dark and three 60 ml clear polycarbonate bottles, each inoculated with 37 kBq $^{33}P$ orthophosphate; incubations were for 24 h at ambient light or in the dark. At the end of the experiment, samples were size-fractionated by filtration through 2.0 and 0.2 μm polycarbonate filters that had previously been boiled in 0.5 mol $l^{-1}$ lithium chloride made up in 1 mmol $l^{-1}$ phosphate-buffer (pH9.0). Since phosphate is readily adsorbed onto surfaces and particles, filters were rinsed twice with 10 ml lithium chloride/phosphate buffer, following the method of Grillo and Gibson (1979). This method has been extensively tested with laboratory cultures (Donald, 1996) and gives a true estimate of phosphate incorporated into cellular material, with no adsorption onto the cell surface, the filter or other detritus. The

filters were dried and stored in a desiccator until return to the laboratory, where $^{33}P$ activity on the filters was measured with a liquid scintillation counter, the efficiency of which was determined with an external standard, channels ratio method.

## 3. Results

### 3.1. Environmental conditions

This cruise sampled a large geographic area (Fig. 1) at a time of rapidly changing phytoplankton biomass. Though constrained by the sampling frequency, the observed bloom development is best described as a chronological change, since the physical data, temperature, salinity and density plots, suggest that only one water mass was sampled. Fig. 2 shows a series of temperature-depth profiles (data are not available for G3). At the first four stations, the water column was generally well mixed, at least to 80 m, with a surface temperature of 11.0–11.1°C; there was some evidence of thermal stratification at station F, with a small thermocline at 50 m. After station D1 on Day 113, a storm caused all sampling activities to be curtailed for 48 h. The four stations sampled immediately after the storm (A1, A2, B and C1) all showed lower surface temperatures (10.7–10.8°C), suggesting that the storm had resulted in deep mixing and destruction of the developing thermocline; analysis of temperature and salinity profiles for the upper 300 m confirmed that deep mixing had occurred. There was, therefore, the potential for reintroduction of nutrients from deep water into the surface mixed layer to replace any utilised by phytoplankton in the transient stratified conditions; but the enhanced mixing would also have dispersed any developing phytoplankton assemblage and mixed some of the population below the euphotic zone. Stations D2 and C2, sampled on Days 121 and 122, eight days after the storm, showed that surface temperatures had returned almost to pre-storm values of 10.98 and 11.12°C, respectively. At station D2, a distinct thermocline was developing at the base of the surface mixed layer at 30 m.

Silicate concentrations give a clue to the level of biological activity occurring during these periods of transient stratification (Fig. 3a). Stations occupied prior to the storm G1, F and D1 (no data are available for silicate concentration at stations G2 and G3) had mean concentrations of 2.3, 1.8 and 2.4 $\mu mol\,l^{-1}$ for the upper 50 m. Following the storm, silicate concentrations at the next four stations (A1, A2, B and C1) sampled between 25 and 28 April (Days 115–118) were higher at 2.8–3.4 $\mu mol\,l^{-1}$. This suggests that diatom growth before 23 April (Day 113) had removed silicate in the surface 50 m, which was then replaced as a consequence of the storm-induced mixing event during Days 114 and 115. At stations D2 and C2 on Days 121 and 122, there was further silicate removal with concentrations declining to 1.1 $\mu mol\,l^{-1}$ at 2.5 m and to 1.2 and 1.6 $\mu mol\,l^{-1}$ at 30 m. The profiles of silicate concentration at stations D2 and C2 generally correspond with the temperature profiles shown in Fig. 2, suggesting that the phytoplankton assemblages that developed in the stabilised surface mixed layer included diatoms.

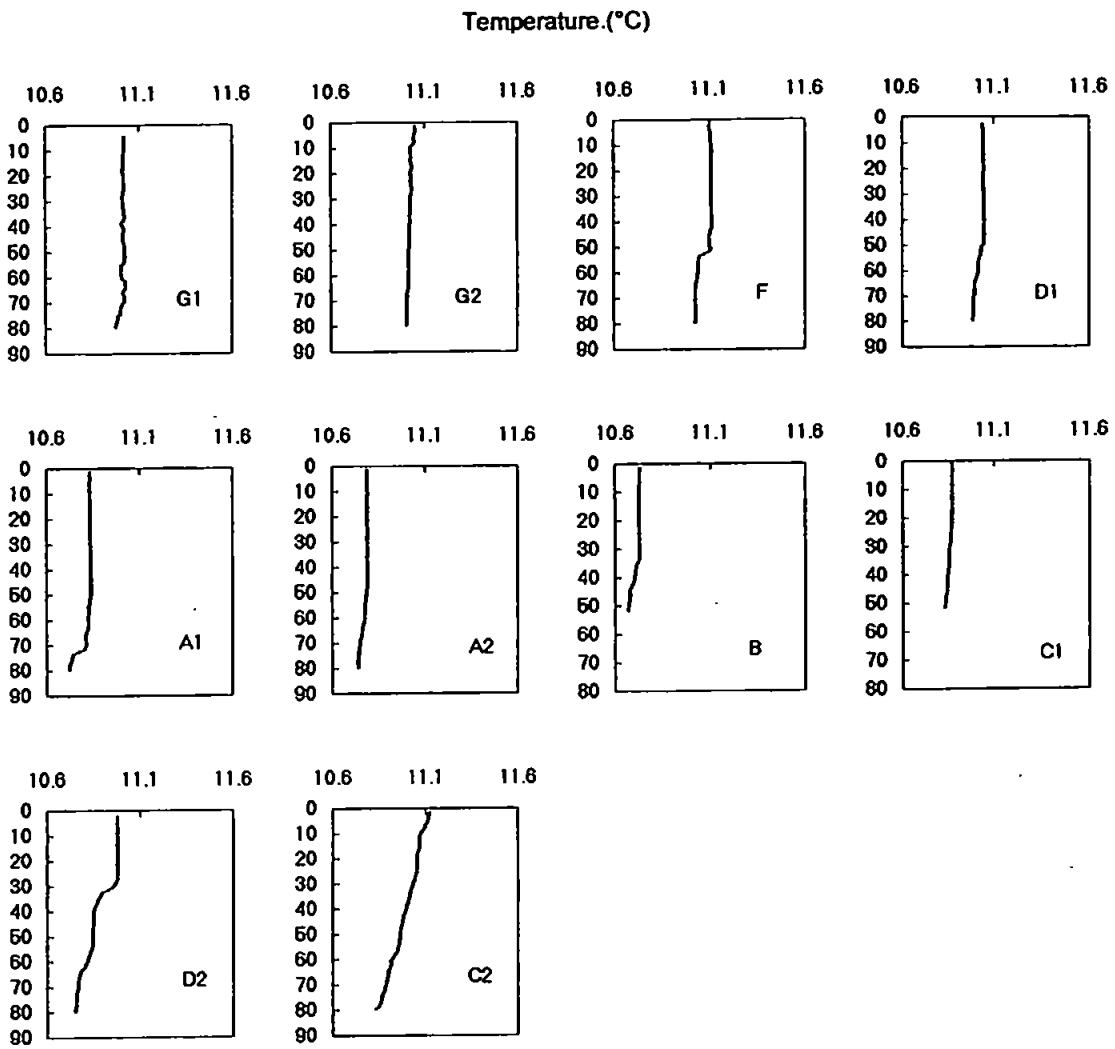


Fig. 2. Temperature profiles (°C) obtained during the cruise.

The vertical distributions of nitrate and phosphate at each station are shown in Fig. 3b and c, respectively. Evidence for an effect of the storm on the concentration of these nutrients in surface waters is not as clear cut as for silicate. However both showed maximal values immediately following the storm of 8.6 $\mu mol\,l^{-1}$ for nitrate and 0.6 $\mu mol\,l^{-1}$ for phosphate at 10 m at station A1 (Day 115) and minima of 6.1 and 0.3 $\mu mol\,l^{-1}$ at the same depth at station D2 (Day 121). Nitrate concentrations were typically $> 6.8\ \mu mol\,l^{-1}$ for the majority of stations in the upper 50 m. Only at station D2 on 1 May (Day 121), towards the end of the cruise, was there evidence of significant $NO_3^-$ removal, with a mean concentration of 6.1 $\mu mol\,l^{-1}$ in the surface 30 m. Ammonium concentrations were generally at or below the detection limit of the analytical method (0.1 $\mu mol\,l^{-1}$), though, at station G2 (Day 108), concentrations of approximately 0.2 $\mu mol\,l^{-1}$ were measured throughout the euphotic zone, and maximum values of 0.3 and 1.5 $\mu mol\,l^{-1}$ were recorded at 40 and 50 m, respectively, at

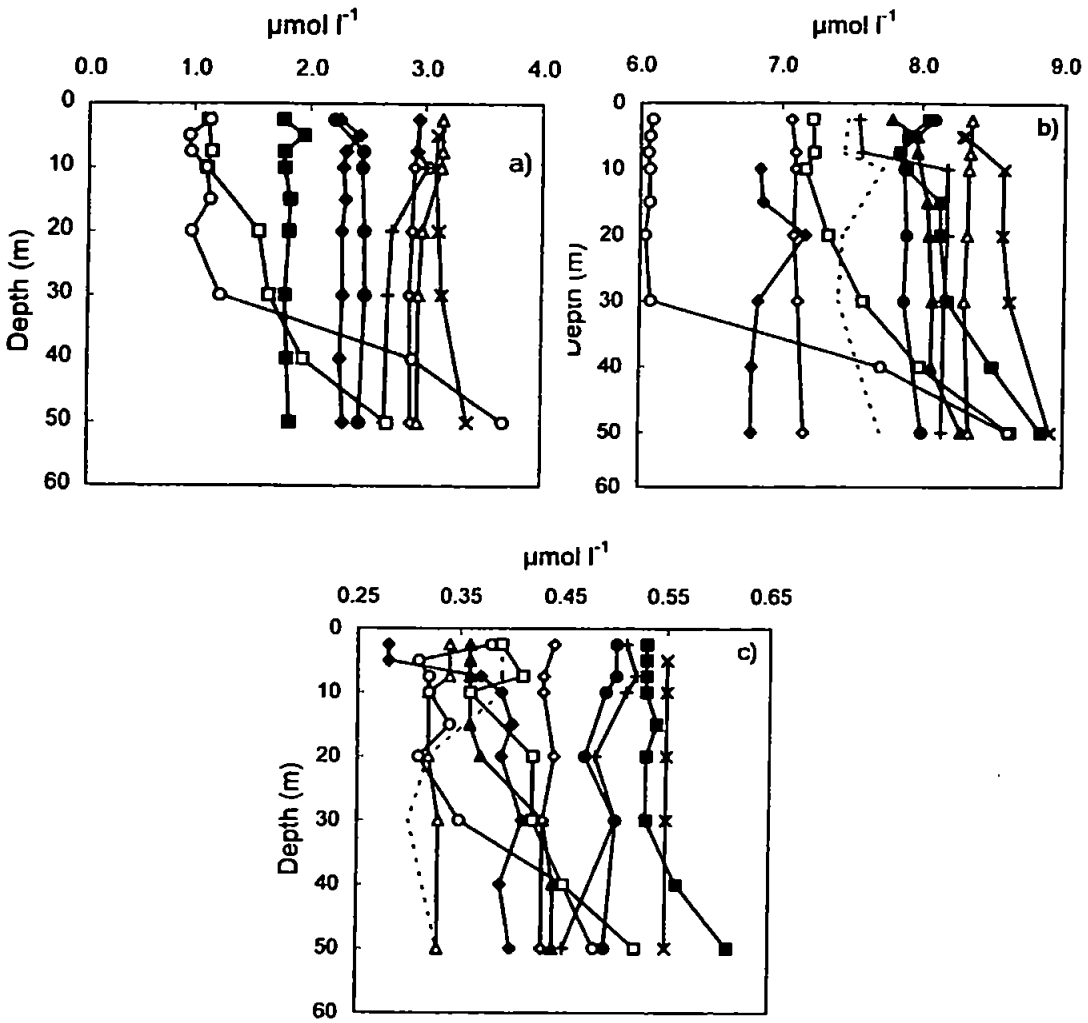


Fig. 3. Vertical profiles of: (a) silicate, (b) nitrate, and (c) phosphate concentrations at Station G1 (◆), G2 (▲), G3 (dashed line), F (■), D1 (●), A1 (×), A2 (◇), B (Δ), C1 (+), D2 (○), C2 (□). Stations G1, G2, G3, F and D1 were sampled before the storm on Day 113. Silicate data are not available for station G2 and G3.

station D2 (Day 121). Phosphate concentrations (Fig. 3c) ranged between 0.3 and 0.6 µmol $l^{-1}$, with relatively homogeneous distribution throughout the upper 50 m for the majority of stations. Again, there was evidence of phosphate removal from the upper 30 m at station D2 on Day 121.

### 3.2. *Changes in phytoplankton biomass.*

Phytoplankton biomass was initially low, with surface chlorophyll concentrations of ca. 0.5 µg $l^{-1}$ (Fig. 4a). There was little change over the first twelve days of the study, with only a slight increase in chlorophyll concentration at the surface, but slightly higher values below 20 m (Fig. 4b). The storm on Day 113 had little effect on

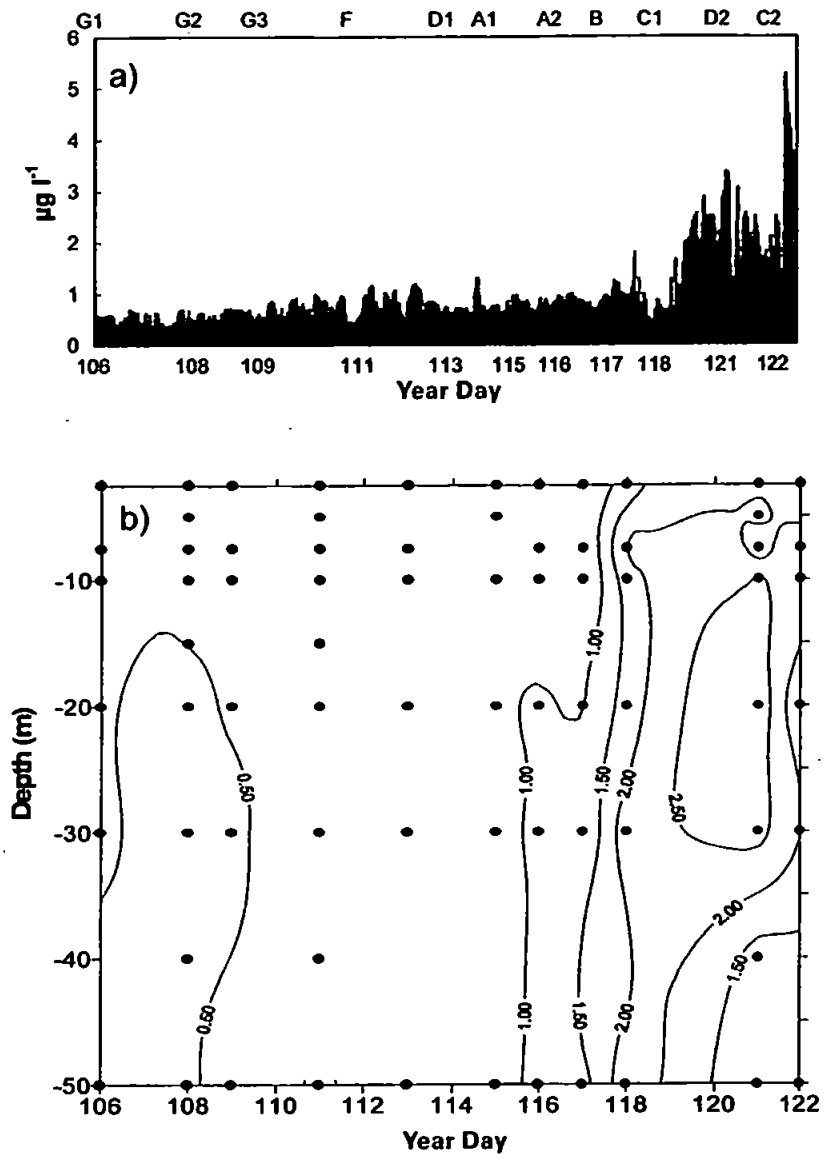


Fig. 4. (a) Chlorophyll concentration, determined by continuous flow fluorometry of water sampled from 2.5 m depth ($n = 721$), plotted against sample date. (b) Depth profiles of chlorophyll concentration ($\mu g l^{-1}$), contoured against sample date. Sample position is indicated by ●.

chlorophyll concentrations, other than the disappearance of the slightly elevated values below 20 m. After Day 118 at station C1, concentrations increased consistently to $>1.0\,\mu g l^{-1}$ throughout the surface 50 m and by 2 May (Day 122), the phytoplankton assemblage was growing vigorously. The maximum concentration measured was $5.3\,\mu g l^{-1}$ (Fig. 4a) at station B (49.45°N 11.10°W), in the vicinity of the shelf edge.

Chlorophyll measurements of size-fractionated samples showed that almost 50% of the phytoplankton consisted of picoplankton ($<2\,\mu m$) at all stations at the beginning of the cruise. At those stations where chlorophyll concentration increased as the

spring bloom developed, there was little change in concentration of the small fraction, and the increase in chlorophyll was the result of growth of phytoplankton cells larger than 2 μm. At the end of April, when chlorophyll concentrations were $>1.5\,\mu g\,l^{-1}$ throughout the water column, the $>2$ μm fraction accounted for more than 90% of the chlorophyll. The chlorophyll increase was largely the result of diatom growth. This is clear from both an analysis of the species present (Table 2) and the decline in silicate concentration (Fig. 3a). The assemblage was typical of the spring bloom in this region and was dominated by diatoms, with large numbers of *Nitzschia*, *Thalassionema* and *Chaetoceros*. However, the assemblage was not exclusively diatoms; dinoflagellates were present, and small unidentified flagellates were also abundant.

### 3.3. Carbon assimilation

Early in the cruise, when chlorophyll concentrations and carbon fixation rates were low, up to 42% of the primary production was by the picophytoplankton (Table 3). At this time, there was considerable day-to-day variation in primary production rates. Over a 4 day period at station G from 16–19 April, depth-integrated primary production varied between 41.5 and 91.2 mmol C fixed $m^{-2}d^{-1}$ (0.50–1.10 g C $m^{-2}$ $d^{-1}$), although there was little change in chlorophyll concentration. Fig. 5 shows the change in vertical distribution of carbon fixation rates with time. After the storm on Day 113, there was an increase in primary production, which was generally 75 mmol C $m^{-2}d^{-1}$ or greater at every station, rising to a maximum measured rate of 121.5 mmolC $m^{-2}d^{-1}$ at station C2 on Day 122.

There was little change in picophytoplankton production over the period; from Day 106 to 117 carbon fixation by the $<2$ μm fraction was between 17 and 26 mmol C $m^{-2}d^{-1}$. At the stations sampled after Day 117, picoplankton production declined to ca 10 mmol C $m^{-2}d^{-1}$. The increase in total carbon fixation was due to $>2$ μm cells. There was a three fold change in primary production between the station with least activity (41.5 mmol C $m^{-2}d^{-1}$), station G1 – sampled in the middle of April – and the highest rate (121.5 mmol C $m^{-2}d^{-1}$) in early May at station C2 (Table 3); 91% of the production at Station C2 was attributed to the non-picoplankton fraction.

### 3.4. Nitrate and ammonium assimilation

Nitrate and ammonium uptake rates prior to the bloom development were generally low and roughly equivalent in magnitude (Table 4). Nitrate assimilation was ca. 8–9 mmol N $m^{-2}d^{-1}$, although at Station F a higher rate of 13.1 mmol N $m^{-2}$ $d^{-1}$ was measured. This may be a result of light since the total photosynthetically available radiance on Day 111 (station F) was twice that on Days 108 and 109. Ammonium assimilation rates showed little change over the period of the cruise and were generally between 3 and 6 mmol N $m^{-2}d^{-1}$, although the highest rate (13.1 mmol N $m^{-2}d^{-1}$) was measured at Station G2 on 18 April. A range of values for ammonia assimilation are given (Table 4); these attempt to account for ammonia regeneration and possible enrichment through isotope addition (see Methods section). Therefore, there is some uncertainty about the absolute ammonium assimilation rates,

Table 2
Species composition of >5 μm phytoplankton in April/May 1994 (cells $l^{-1}$ – counted in 50 ml sample)

| Station<br>Date | G1<br>16 April | G2<br>18 April | F<br>21 April | D1<br>23 April | A1<br>25 April | A2<br>26 April | B<br>27 April | C1<br>28 April | C2<br>05 May |
|---|---|---|---|---|---|---|---|---|---|
| Dinoflagellates | | | | | | | | | |
| *Ceratium furca* | | | | | | | | | 100 |
| *C. fusus* | | | | 100 | | | | | |
| *C. lineatum* | | | 200 | | 100 | | | 100 | 400 |
| *D. punctata* | | | | | | | 100 | | 200 |
| *G. polygramma* | 200 | | | | | | | | 100 |
| *Gyrodinium aureolum* | | | | | | | | | |
| *Heterocapsa minima* | 200 | | | 200 | | | 5900 | | |
| *Mesoporus perforatus* | 100 | | | | 100 | | 100 | | 100 |
| *Oxytoxum caudatum* | | | | | | 100 | | | |
| *O. scolopax* | 200 | | 200 | | 200 | 100 | 100 | | 100 |
| *O. sphaeroideum* | | | | | | | | | 100 |
| *Peridinium faeroense* | | | | | 200 | 100 | | | |
| *Prorocentrum balticum* | | | 100 | | | | 2900 | | |
| *P. compressum* | | 200 | 200 | | 200 | | | | 200 |
| *P. dentatum* | | 100 | 200 | 300 | 300 | | 200 | | 100 |
| Diatoms | | | | | | | | | |
| *Bacteriastrum* delicatulum | | | | | | 100 | 100 | 2700 | 100 |
| *Cerataulina pelagica* | | | | | | | | | 200 |
| *Chaetoceros spp.* (*hyalochaete*) | | | | 800 | 900 | 1400 | 400 | 146700 | 37000 |
| *Chaetoceros spp.* (*phaeoceros*) | | | 100 | 100 | 100 | | | | 1500 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Lauderia borealis* | | | | 2100 | 700 | 500 | | 1300 | 2100 |
| *Leptocylindricus danicus* | | | | 200 | | | | | |
| *Navicula sp.* | | | | | | | | | 100 |
| *Nitzschia delicatissima* | 700 | 200 | 600 | 5500 | 7000 | 35400 | 44200 | 357500 | 292300 |
| *N. longissima* | 100 | | | 100 | | 100 | | | |
| *N. seriata* | 1500 | 900 | 3500 | 13400 | 10600 | 37000 | 23500 | 354800 | 188700 |
| *Rhizosolenia alata* | | | | 400 | | 500 | 100 | 200 | 900 |
| *R. fragilissima* | | | | 300 | | 2000 | 300 | 34700 | 1800 |
| *R. styliformis* | | 100 | | | 300 | 300 | | | 100 |
| *Thalassionema nitzschioides* | 1900 | 900 | 600 | 1200 | 1600 | 7700 | 7400 | 72000 | 181500 |
| *Thalassiosira spp.* | 100 | 200 | 300 | 600 | 100 | 700 | 600 | 13300 | 600 |
| *Thalassiothrix longissima* | | | 100 | | | | | | |
| Others | | | | | | | | | |
| *Phaeocystis spp.* | | | | | 1000 | | | | |
| *Distephanus speculum* | | 100 | | | | | 100 | | |

Table 3
Size fractionated carbon fixation measured at each station by the incorporation of $^{14}C$; the depth-integrated carbon fixation by > 2 μm and picophytoplankton is shown with the percentage production

| Station | Latitude °N | Longitude °W | Date 1994 | Day | Total C fixation (mmol C $m^{-2} d^{-1}$) | > 2.0 μm (mmol C $m^{-2} d^{-1}$) | > 2.0 μm (%) | < 2– > 0.2 μm (mmol C $m^{-2} d^{-1}$) | <2–>0.2 μm (%) |
|---|---|---|---|---|---|---|---|---|---|
| G1 | 49.40 | 12.80 | 16 April | 106 | 41.5 | 23.8 | 57 | 17.7 | 43 |
| G2 | 49.14 | 12.78 | 18 April | 108 | 50.1 | 28.7 | 58 | 21.4 | 42 |
| G3 | 49.13 | 12.82 | 19 April | 109 | 91.2 | 65.7 | 72 | 25.5 | 28 |
| F | 49.18 | 12.34 | 21 April | 111 | 63.8 | 40.9 | 64 | 22.9 | 36 |
| D1 | 49.42 | 11.59 | 23 April | 113 | 78.6 | 59.6 | 76 | 19.0 | 24 |
| A1 | 49.34 | 11.65 | 25 April | 115 | 75.0 | 51.5 | 69 | 23.5 | 31 |
| A2 | 49.34 | 11.63 | 26 April | 116 | 107.4 | 86.9 | 81 | 20.5 | 19 |
| B | 48.51 | 11.00 | 27 April | 117 | 92.9 | 66.0 | 71 | 26.9 | 29 |
| C1 | 49.43 | 11.25 | 28 April | 118 | 113.2 | 98.3 | 87 | 14.9 | 13 |
| D2 | 49.51 | 11.48 | 1 May | 121 | 100.1 | 91.5 | 91 | 8.6 | 9 |
| C2 | 49.33 | 11.40 | 2 May | 122 | 121.5 | 110.6 | 91 | 10.9 | 9 |

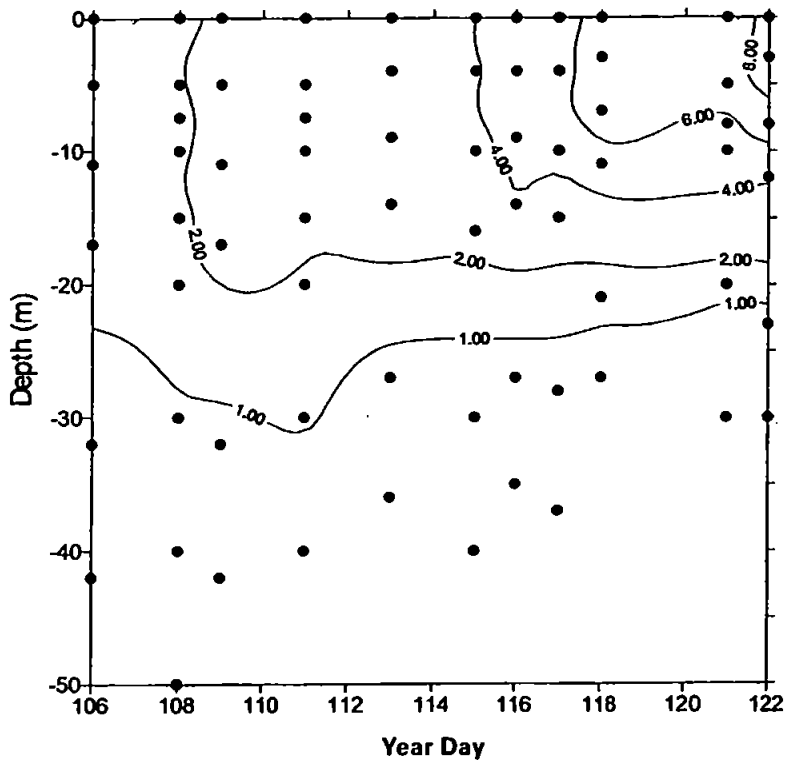


Fig. 5. Depth profile of total carbon fixation rates (mmol C $m^{-3} d^{-1}$), contoured against Year Day. The symbol ● indicates the depth of the in situ incubation, or the depth equivalent to the PAR experienced during on-deck incubations. The deepest point is the depth at which PAR is 1% of the surface value.

Table 4
Depth-integrated assimilation rates of nitrate and ammonium measured at each station by the incorporation of $^{15}N$ and the calculated *F*-ratio. See text for explanation of range in ammonium uptake values

| Station | Date | Day | Nitrate uptake (mmolN $m^{-2} d^{-1}$) | Ammonium uptake (mmolN $m^{-2} d^{-1}$) | *f*-ratio |
|---|---|---|---|---|---|
| G1 | 16 April | 106 | 8.1 | 6.8 (5.5–12.4) | 0.54 (0.40–0.60) |
| G2 | 18 April | 108 | 7.0 | 13.1 (12.7–25.4) | 0.35 (0.22–0.36) |
| G3 | 19 April | 109 | 8.0 | 6.2 (4.6–11.2) | 0.56 (0.42–0.63) |
| F | 21 April | 111 | 13.1 | 6.7 (3.8–12.2) | 0.66 (0.52–0.78) |
| D1 | 23 April | 113 | 9.4 | 4.4 (2.5–7.8) | 0.68 (0.55–0.79) |
| A1 | 25 April | 115 | 5.5 | 3.5 (2.0–6.3) | 0.61 (0.47– 0.73) |
| A2 | 26 April | 116 | 8.8 | 3.2 (1.8–5.8) | 0.73 (0.60–0.83) |
| B | 27 April | 117 | 7.9 | 4.2 (2.4–7.8) | 0.65 (0.50–0.77) |
| C1 | 28 April | 118 | 16.9 | 3.6 (2.4–6.6) | 0.82 (0.72–0.88) |
| D2 | 1 May | 121 | 21.0 | 4.6 (2.6–8.4) | 0.82 (0.71–0.89) |
| C2 | 2 May | 122 | 15.4 | 3.0 (1.7–5.5) | 0.84 (0.74–0.90) |

hence the range of *f*-ratio estimates in Table 4. However, a comparison between stations is valid. The exceptionally high value for ammonium assimilation at Station G2 results from high ammonium concentrations (0.2 – 0.25 μmol $l^{-1}$) measured at this station and the deeper euphotic depth; the 1% PAR value was measured at 47 m. At those stations that showed increased primary production rates after Day 117, it is clear that ammonium assimilation remained low, but nitrate assimilation increased (Stations C1, D2 and C2, Table 4), with depth-integrated uptake rates doubling to $>15$ mmol N $m^{-2}$ $d^{-1}$.

The vertical profile of nitrate uptake was similar to that of carbon fixation, with low rates ($<0.2$ mmol N $m^{-3}$ $d^{-1}$) below 20 m; highest uptake rates were measured at the surface. After Day 117, the maximum measured rate was 1.57 mmol N $m^{-3}$ $d^{-1}$ at station D2. In contrast ammonium uptake was measurable below the euphotic depth, with comparable uptake rates measured at some stations at the surface and at 50 m; since $NH_4^+$ uptake occurred below the euphotic zone, it must have been the result of heterotrophic activity, presumably due to bacteria. Determination of the *f*-ratio shows high rates of new production at all stations (Table 4). That is, nitrate was the dominant source of nitrogen, which increased from approximately 50% (minimum 35% at G2) to a maximum of 84% of total nitrogen assimilation.

### 3.5. *Phosphate assimilation*

Phosphate uptake rates, measured by in situ or on-deck incubations in ambient light, are shown in Fig. 6. As with $^{14}C$ assimilation, a significant proportion (ca. 40%) of the $^{33}P$ uptake at the beginning of the cruise was by the smallest cells (Table 5). Throughout the cruise, assimilation by the $<2$ μm fraction showed little variation. The mean assimilation rate was 0.25 ($\pm$ 0.09) mmol P $m^{-2}$ $d^{-1}$, but lower rates were measured at the first 2 stations and the highest at Station C1. There was a significant increase in $^{33}P$ uptake rates by the $>2$ μm fraction at stations C1, C2 and D2 when the spring bloom was underway. Phosphate uptake by the non-picoplankton fraction ranged from 0.17 mmol P $m^{-2}$ $d^{-1}$ at station G2 (Day 108) to 1.20 mmol P $m^{-2}$ $d^{-1}$ at station C2 (Day 122).

Phosphate assimilation was also measured in the dark. In contrast to carbon fixation, which is largely photosynthetic, phosphate uptake is more complex. Heterotrophic microbes, and particularly bacteria, will readily assimilate orthophosphate from sea water and are not restricted to organic forms of phosphate. So it is difficult to distinguish uptake by phytoplankton from that by heterotrophic bacteria. Therefore, the exact proportion of the $^{33}P$ taken up by the heterotrophs is not known, but we assume that it is unlikely to be the major fraction of the $^{33}P$ assimilation by the $>2$ μm assemblage.

## 4. Discussion

The spring bloom is important for the productivity of the Celtic Sea and is estimated to account for almost half of the annual primary production (Joint et al.,

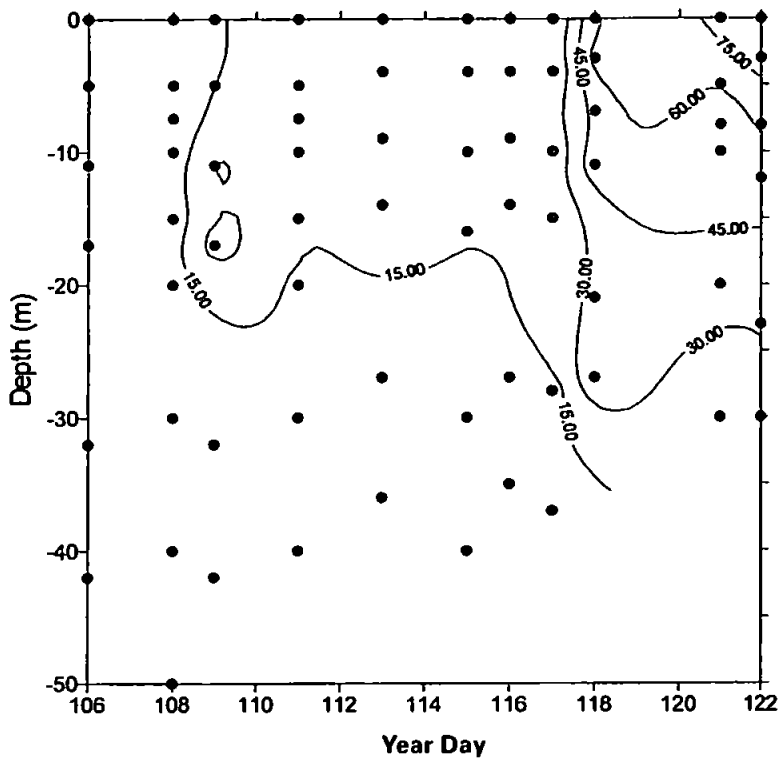


Fig. 6. Depth profile of total phosphate uptake rate (μmol P $m^{-3} d^{-1}$) contoured against Year Day. The Symbol ● indicates the depth of the in situ incubation, or the depth equivalent to the PAR experienced during on-deck incubations. The deepest point is the depth at which PAR is 1% of the surface value.

1999). The duration of the bloom varies from year to year but generally lasts for about 2 months. The data presented in this paper describe the changes occurring at the beginning of the bloom. However, a chronological description of the bloom development is complicated because water column stability was not well established and was vulnerable to wind mixing events, which break down incipient stratification. The storm that occurred between Days 113 and 114 is a good example of how a period of phytoplankton growth can be interrupted by deep mixing of the surface water column. In an area such as the Celtic Sea, there is inevitably spatial variability in phytoplankton biomass and production that cannot be resolved from the spatial and temporal scales of sampling on this cruise. Given the dynamic changes occurring, it would have required truly synoptic sampling to be able to distinguish any specific features, such as an effect of the shelf edge on production rates, within this overall region of increasing phytoplankton production. Therefore, these data represent average changes over the region sampled.

At the beginning of the cruise, sampling was largely confined to the western end of the transect of the Goban Spur. There was some evidence of stratification, with indications of a weak thermocline at stations G, F and D1 (Fig. 2); however, diurnal mixing is likely to have been intense. There is little evidence for an increase in phytoplankton biomass, and chlorophyll concentrations remained low. Changes in

Table 5
Depth-integrated phosphate assimilation by $> 2\ \mu m$ and $< 2\ \mu m$ size fractions, measured in the light at each station by the incorporation of $^{33}P$; the percentage assimilated in each size fraction is also shown

| Station | Date | Day (JD) | Total P uptake (mmol P $m^{-2} d^{-1}$) | $> 2\ \mu m$ (mmol P $m^{-2} d^{-1}$) | $> 2\ \mu m$ (%) | $< 2- > 0.2\ \mu m$ (mmol P $m^{-2} d^{-1}$) | $< 2- > 0.2\ \mu m$ (%) |
|---|---|---|---|---|---|---|---|
| G1 | 16 April | 106 | 0.33 | 0.21 | 64 | 0.12 | 36 |
| G2 | 18 April | 108 | 0.27 | 0.17 | 63 | 0.10 | 37 |
| G3 | 19 April | 109 | 0.82 | 0.52 | 63 | 0.30 | 37 |
| F | 21 April | 111 | 0.66 | 0.39 | 59 | 0.27 | 41 |
| D1 | 23 April | 113 | 0.75 | 0.53 | 71 | 0.22 | 29 |
| A1 | 25 April | 115 | 0.58 | 0.29 | 50 | 0.29 | 50 |
| A2 | 26 April | 116 | 0.58 | 0.35 | 60 | 0.23 | 40 |
| B | 27 April | 117 | 0.46 | 0.21 | 46 | 0.25 | 54 |
| C1 | 28 April | 118 | 1.26 | 0.81 | 65 | 0.44 | 35 |
| D2 | 1 May | 121 | 1.35 | 1.13 | 84 | 0.22 | 16 |
| C2 | 2 May | 122 | 1.50 | 1.20 | 80 | 0.30 | 20 |

silicate concentrations suggest that there may have been some growth of diatoms prior to the cruise. All stations sampled before the storm had silicate concentrations of $< 2.5\ \mu mol\ l^{-1}$; the profiles were homogeneous in the surface 50 m and support the conclusion from the temperature profiles that the surface 50 m was well mixed. However, after the storm, silicate concentrations at stations A1, A2, B and C1 were 0.5–1 $\mu mol\ l^{-1}$ greater than the stations sampled a few days earlier. Only at stations C2 and D2, towards the end of April (Days 121 and 122), was there again evidence of utilisation of silicate in the surface mixed layer. These profiles are consistent with the rapid growth of a phytoplankton assemblage that includes diatoms and match the observed increases in chlorophyll concentrations (Fig. 4) and increases in species such as *N. delicatissima* and *T. nitzschioides*.

At this time, the phytoplankton assemblage was undergoing large changes, in production as well as in species composition. At the beginning of the cruise, in late winter conditions, size-fractionated samples showed that almost 50% of the phytoplankton consisted of picoplankton ( $< 2\ \mu m$). This is in agreement with earlier findings (Joint et al., 1986) for the region, that the winter months were dominated by small nanoflagellates and eukaryotic picoplankton. However, these cells were replaced by larger phytoplankton as soon as the water column stabilised. This presents something of a paradox. It is known that small phytoplankton cells have the most rapid and efficient mechanisms for the uptake of nutrients (Raven, 1986). At times of low nutrient concentration, their small size, with high surface area to volume ratios, ensure that the picoplankton can compete efficiently for available nutrient (Raven, 1986). Small phytoplankton cells also have shorter generation times, and allometric theory (Peters, 1983) suggests that the growth rate of small planktonic organisms will be greater than that of larger cells (Fenchel, 1974). Therefore, if small cells have the most rapid growth rates and also the most efficient nutrient uptake, why do they not continue to dominate the high nutrient regime at the onset of water column stabilisation? It is clear from this study that the production of the picoplankton size fraction remained constant, or even declined as the chlorophyll concentration increased. That is, larger phytoplankton species out-competed the nanoflagellates and picoplankton. Since nutrients, although at lower concentrations than in late winter, were not depleted at stations D2 and C2, where the bloom was most advanced, it seems unlikely that the failure of the picoplankton to respond is linked to nutrient supply. It must, therefore, result from a response to irradiance – the picoplankton may not be able to accommodate to higher PAR (Grande et al., 1991) as spring advances – or to grazing pressure that is much greater on small phytoplankton cells.

We assume that macrozooplankton cannot graze efficiently on small phytoplankton cells and that the primary grazers on picophytoplankton cells are protozoa. Since these organisms have short generation times, a tight coupling is possible between the primary producer and the grazer. In these circumstances, conditions that favour enhanced phytoplankton production may not result in an increase in picoplankton biomass, but there should be an increase in microzooplankton biomass. The biomass of microzooplankton did indeed change as the spring bloom developed, but not in the way predicted. At station B on Day 117, the microzooplankton biomass in the surface mixed layer was $189\ mg\ C\ m^{-2}$, but this declined to $90\ mg\ C\ m^{-2}$ at

station C2 on Day 122 (E.S. Edwards, Plymouth Marine Laboratory, pers. comm.). Therefore, it appears that the grazing pressure on the picoplankton might actually have declined in proportion to the declining picoplankton production rate. This suggests that if there was tight grazing control over the picoplankton fraction, something other than grazing was limiting the picoplankton production. Since grazing pressure was reduced and there was excess nutrient available, it seems probable that the decline in picoplankton biomass was a consequence of these organisms not being able to adapt to the increasing PAR as the season developed.

The difference in nutrient concentration between the surface mixed layer and below the thermocline gives an estimate of the quantity of nutrient utilised by the phytoplankton. At station D2, the spring bloom was developing when the station was sampled, but nutrients were not depleted. It is justifiable to assume that nutrient recycling was minimal. The difference in concentration between 10 and 50 m was 2.5 $\mu$mol $l^{-1}$ for nitrate, 0.2 $\mu$mol $l^{-1}$ for phosphate and 2.5 $\mu$mol $l^{-1}$ for silicate. The decline in nutrient concentrations was in the ratio 15.9 for N:P and 16 for Si:P. This N:P ratio is in agreement with the generally accepted Redfield ratio of 106C:16N:1P (Redfield et al., 1963).

The Redfield Ratio is a useful concept in studies of nutrient assimilation rates and is frequently used to link carbon and nutrient assimilation (Sambrotto et al., 1993a). However, there have been few studies in which carbon, nitrogen and phosphorus assimilation have been determined simultaneously. The data in Table 6 highlight the variability of this ratio, which appears to depend on the activity of the phytoplankton assemblage. Although the distinction is not clear-cut, the C:N:P ratios of actively growing assemblages towards the end of the cruise are different from those of the late winter populations sampled at the beginning.

During the period when phytoplankton growth rates were low (before Day 117), the C:P uptake ratios were generally close to the Redfield ratio – although a high value was obtained on Day 108 at station G2 and on Days 116 and 117. As the growth of the population increased after Day 118, the C:P ratios declined. Similarly, there were changes in the N:P uptake ratio. This was closest to the Redfield ratio for the actively growing phytoplankton at the end of the cruise and agrees well with the observed differences in nitrate and phosphate concentration above and below the thermocline at station D2. However, the uptake ratio was generally higher for the late winter populations. Indeed, at 2 stations (G1 and G2) the N:P ratio was extremely large. Although the C:P ratio was close to the Redfield Ratio, the nitrogen uptake rates were high, resulting in very low C:N ratios. C:N ratios increased on Day 115, immediately after the storm with the reintroduction of nutrients; maximum C:N ratios were recorded on days Days 116 and 117. The data in Table 6 are depth-integrated values, but similar ratios are obtained when data from individual depths are compared.

C:N uptake ratios have been reported to vary from $< 1$ to $> 20$ (Bouteiller, 1993). Most of the ratios in this study deviate only slightly from the expected Redfield ratio. The highest C:N ratios recorded were 7.5–9; similar ratios have been reported by Laws et al. (1989), who found atomic assimilation ratios of $7.1 \pm 0.6$ for the North Pacific subtropical gyre. At other stations, C:N ratios were very low, but other studies

Table 6
Atomic ratios for assimilation of carbon, nitrogen and phosphate; all data are depth-integrated values

| Station | Day (JD) | Carbon fixation ($mmol\,m^{-2}\,d^{-1}$) | Total N uptake ($mmol\,m^{-2}\,d^{-1}$) | P uptake ($mmol\,m^{-2}\,d^{-1}$) | C:N ratio | N:P ratio | C:P ratio | C:N:P ratio |
|---|---|---|---|---|---|---|---|---|
| G1 | 106 | 41.5 | 14.9 | 0.33 | 2.8 | 45.2 | 125.8 | 125:45:1 |
| G2 | 108 | 50.1 | 20.1 | 0.27 | 2.5 | 74.4 | 185.6 | 186:74:1 |
| G3 | 109 | 91.2 | 14.2 | 0.82 | 6.5 | 17.3 | 111.2 | 111:17:1 |
| F | 111 | 63.8 | 19.8 | 0.66 | 3.2 | 30.0 | 96.7 | 97:30:1 |
| D1 | 113 | 78.6 | 13.8 | 0.75 | 5.7 | 18.4 | 104.8 | 105:18:1 |
| A1 | 115 | 75.0 | 9 | 0.58 | 8.3 | 15.5 | 129.3 | 129:16:1 |
| A2 | 116 | 107.4 | 12 | 0.58 | 9.0 | 20.7 | 185.2 | 185:21:1 |
| B | 117 | 92.9 | 12.1 | 0.46 | 7.7 | 26.3 | 202.0 | 202:26:1 |
| C1 | 118 | 113.2 | 20.5 | 1.26 | 5.5 | 16.3 | 89.8 | 90:16:1 |
| D2 | 121 | 100.1 | 25.6 | 1.35 | 3.9 | 19.0 | 74.1 | 74:19:1 |
| C2 | 122 | 121.5 | 18.4 | 1.50 | 6.6 | 12.3 | 81.0 | 81:12:1 |

have reported comparable values; for example, in a study of a fjordic sea loch, Rees et al. (1995) found some C:N ratios $< 1$ at a number of stations in the spring. The first 2 stations had low C:N and high N:P ratios (Table 6); the C:P ratios were higher than the Redfield Ratio (much higher in the case of station G2), but the really anomalous values appear to result from relatively high rates of nitrate and ammonia uptake.

However, it is worth considering if constant assimilation ratios are to be expected. If a phytoplankton cell has an elemental composition close to the Redfield ratio, then assimilation ratios should also approximate to the Redfield ratio over the generation time of the organism. The assimilation of ammonia and phosphate may occur in the dark and is, therefore, de-coupled from photosynthesis; if the time-scale of the incubation is much shorter than the generation time of the organism, then deviations in uptake ratios from the Redfield ratio will occur. Clearly, this is most likely to be the case for those stations sampled early in the cruise, when growth rates of the phytoplankton were low. Also, we are not dealing with a single species but with a mixed assemblage of phytoplankton, with a range of generation times. This will also tend to result in the de-coupling of carbon, nitrogen and phosphorus assimilation rates. Therefore, from the data in Table 6, it is impossible to distinguish an assemblage that has an elemental composition given by the Redfield ratio, but with uncoupled C:N:P assimilation, from an assemblage that has a composition differing significantly from the Redfield ratio.

This cruise sampled in the spring, when the water column stabilised and the spring phytoplankton bloom was developing. The *f*-ratio (Table 4) demonstrates the importance of nitrate assimilation in the spring bloom. In late winter conditions before stabilisation, nutrient uptake rates were low; ammonium assimilation rates were comparable to the uptake rates of nitrate, even though ammonium concentrations were generally $< 0.01\%$ of the nitrate concentrations. As a result the *f*-ratio was about 0.5. However, with the development of the spring bloom, nitrate assimilation increased, ammonium assimilation remained largely unchanged (Table 4) and the *f*-ratio increased to $> 0.8$. Therefore, it appears that the small nanoplankton that dominated the water column before stratification were utilising ammonium rather than nitrate. Similar results were obtained by Sambrotto et al. (1993b), who observed that in the Iceland Basin, although phytoplankton $< 5$ μm accounted for over 70% of the particulate nitrogen, it was only responsible for 12% of the nitrate uptake. It appears that, although adapted to survive in winter in a deep mixed layer that exceeds the critical depth, picophytoplankton cells are not efficient utilisers of nitrate and cannot compete with diatoms and other large cells, which do utilise nitrate and grow rapidly when stratification becomes established.

It follows from the dominance of large phytoplankton species in the spring bloom that there is potential for a significant proportion of the carbon fixed to be exported out of the surface layer. Vertical flux at this time of year will be largely due to sedimentation, since the biomass of mesozooplankton is low in the Celtic Sea in April/May (Joint et al., 1999); this flux may also be enhanced by the loss of phytoplankton through vertical mixing, which can be considered as an agent for export (Ho and Marra, 1994). New production approximates to the sinking flux of particulate organic

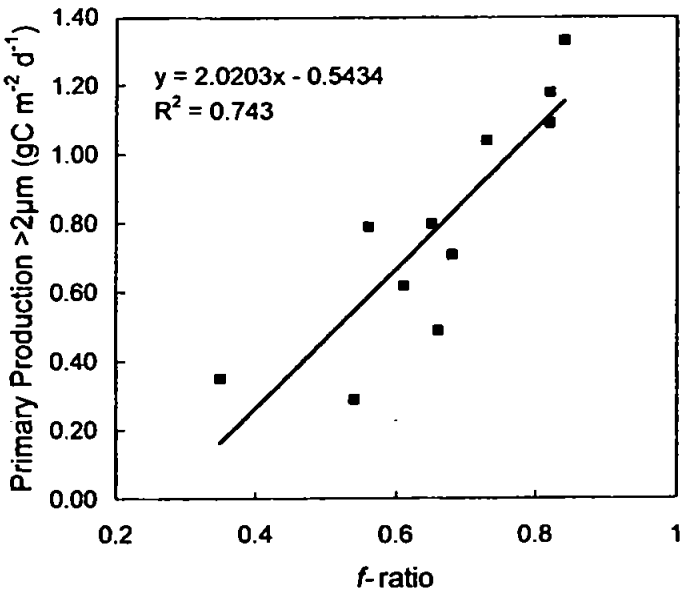


Fig. 7. Relationship between derived estimates of particulate vertical flux. Carbon fixed by phytoplankton larger than 2.0 µm against *f*-ratio.

matter to the deep ocean (Eppley and Peterson, 1979), where new production is total primary production (Table 3) multiplied by *f*-ratio (Table 4). Tremblay et al. (1997) have pointed out that new production is closely related to the size structure of the phytoplankton community and that high values of *f*-ratio occur at times when the phytoplankton assemblage is dominated by large phytoplankton cells; conversely, assemblages that are composed of picophytoplankton have low *f*-ratios. In agreement with the findings of Tremblay et al. (1997), in this study there is a strong correlation between primary production of $> 2$ µm phytoplankton and new production.

$$\text{Production by } > 2\,\mu\text{m phytoplankton} = 1.023 \times \text{New Production} + 0.064 (r^2 = 0.977).$$

At the time of the spring bloom, when primary production is known to be dominated by the larger phytoplankton and high *f*-ratios, this relationship is heavily influenced by the presence of a carbon fixation term in both variables. In order to investigate the validity of the approach, we have taken the independent variables and plotted $> 2$ µm primary production against *f* ratio (Fig. 7); the result remains a significant correlation ($r^2 = 0.743$, $p < 0.001$).

Therefore, with a knowledge of the size distribution of phytoplankton species in a natural assemblage, it is possible to estimate the approximate new production of that assemblage. If new production is assumed to be a measure of the vertical flux over an annual cycle, then the major sinking flux in the Celtic Sea will occur when large phytoplankton are the most productive; this occurs in the spring, small flagellates and picophytoplankton dominating the assemblages at all other times of the year (Joint et al., 1986).

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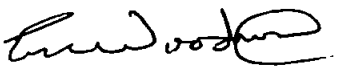
**Paper V**
Measurement of nitrate and ammonium uptake at ambient concentrations in oligotrophic waters of the north-east Atlantic Ocean.
*Marine Ecology Progress Series.* 187, 285-300, 1999.

**Rees A.,** Woodward M. & Joint I.

Contribution in terms of total effort by A.P. Rees on this work was: 75%

Signed: 
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NOTE

# Measurement of nitrate and ammonium uptake at ambient concentrations in oligotrophic waters of the North-East Atlantic Ocean

**Andrew Rees*, Malcolm Woodward, Ian Joint**

NERC Centre for Coastal and Marine Sciences, Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH, United Kingdom

ABSTRACT: Nitrate and ammonium measurements at low (4 to 650 nmol N $dm^{-3}$) nanomolar concentrations have been combined with $^{15}N$-nitrogen tracer experiments to determine uptake rates in oligotrophic regions of the NE Atlantic during the summer of 1996 between 50° and 36° N along the 20° W meridian. The highest nutrient and chlorophyll concentrations were measured at the north of the transect, where the nitrate concentration was 650 nmol $dm^{-3}$; at the southern, oligotrophic stations, nitrate concentration was ca 4 nmol $dm^{-3}$. Nitrate uptake has been determined at concentrations of nitrate (ambient plus added $^{15}N$ tracer) as low as 7 nmol N $dm^{-3}$. Nitrate uptake rate versus concentration along the transect can be described by a hyperbolic curve with parameter values $\rho_{max}$ = 2.77 nmol N $dm^{-3}$ $h^{-1}$ and $K_{NO_3}$ = 20 nmol N $dm^{-3}$. Along the transect, there was a decrease in the *f*-ratio from 0.39 to 0.02 and in the relative preference index for nitrate from 0.7 to 0.1. There was no direct indication of ammonium inhibition of nitrate uptake. The observed range in nitrate concentration of 2 orders of magnitude had the greatest influence over the interaction between ammonium concentration and nitrate uptake. There was an exponential decrease in nitrate uptake rate with increases in the ratio of ammonium:nitrate concentration.

KEY WORDS: $^{15}N$ uptake · Oligotrophic · Nanomolar nitrate/ammonium · *f*-ratio

Nitrate and ammonium concentrations in the oligotrophic oceans are typically less than 100 nmol $dm^{-3}$. In contrast to carbon uptake studies, the measurement of nutrient uptake at very low nutrient concentration levels has been limited by the lack of suitable methodologies. Many studies have measured nutrient uptake at concentrations greater than 50 to 100 nmol N $dm^{-3}$ (the typical detection limits for autoanalysis) and a number of algorithms have been applied to describe kinetic parameters and concentration dependent uptake rates for large areas of the world's oceans (e.g. Sahlsten 1987, Tamminen 1995, Harrison et al. 1996, Slawyk et al. 1997). However, measurements for nitrate and ammonium uptake under severe oligotrophic conditions have been hampered by the absence of reliable information on their relative concentrations.

Although sensitive methods have been developed to measure nitrate (Garside 1982) and ammonium (Jones 1991) at low nanomolar concentrations, there are no reports of the simultaneous use of these methods in conjunction with tracer experiments, although their potential has been recognised (Eppley et al. 1990, McCarthy et al. 1992, Rees et al. 1995). Determinations at ambient nitrate and ammonium concentration are essential to fully understand phytoplankton nitrogen dynamics, and in particular the inhibition by ammonium of nitrate uptake. Inhibition occurs at a wide range of ammonium concentrations, including nanomolar (Wheeler & Kokkinakis 1990, Harrison et al. 1996, Elskens et al. 1997); however Collos (1997) has reported, from a literature review of 76 studies, that 63% described covariation between ammonium and the assimilation of nitrate. Harrison et al. (1996), in recognising the limits of knowledge of nitrogen dynamics, described uptake kinetics for nitrate and ammonium at nanomolar concentrations. However, due to the inadequate detection limits of traditional ammonium analysis, in a number of cases they had to describe uptake or inhibition kinetics at unknown ambient concentrations plus added ammonium.

Flynn et al. (1997) modelled the interaction between ammonium and nitrate at concentrations which are typical of oceanographic concentrations; however, it

*E-mail: a.rees@ccms.ac.uk

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has been impossible to test the model predictions in the laboratory and, due to methodological insensitivities, to validate in the field. This paper describes a small number of data gathered during a recent cruise to the NE Atlantic where the analysis of nitrate and ammonium concentrations were made to nanomolar concentrations (detection limit 2 nmol N $dm^{-3}$) in parallel with uptake experiments using the $^{15}N$ tracer technique. The uptake of nitrate is described at ambient concentrations as low as 4 nmol N $dm^{-3}$ and is related to ammonium at concentrations of 44 to 110 nmol N $dm^{-3}$.

**Methods.** The positions of the stations along the 20° W meridian are shown in Table 1. Water samples were collected before dawn from depths approximating to the 33% irradiance level using 10 $dm^3$ Niskin bottles on a profiling CTD rosette and using a 30 $dm^{-3}$ go-flo (gf) bottle on 9 July. Nutrient concentrations were analysed within 1 h of sample collection. Nitrate was analysed by 1 of 2 methods depending on concentration. A Technicon autoanalyser using methods described by Brewer & Riley (1965) was used when ambient nitrate was >100 nmol N $dm^{-3}$ and a chemiluminescent method (Garside 1982) was applied when concentrations were below this concentration. Ammonium concentration was determined using the fluorometric method described by Jones (1991).

The original protocol for $^{15}N$ tracer experiments (Dugdale & Goering 1967) recommended the addition of tracer at approximately 10% of ambient concentration. In oligotrophic conditions, it has been recommended that additions should be made at the detection limits of the nutrient analysis system (McCarthy et al. 1992, IOC 1996). In this study, we made our lowest additions at 3 nmol N $dm^{-3}$ (Stn 97 only) and at 5 nmol N $dm^{-3}$, the remainder in the range 10 to 70 nmol N $dm^{-3}$ (Table 1). $^{15}N$-$NO_3$ and $^{15}N$-$NH_4$ were added to one each of two 2 $dm^3$ seawater samples which had been dispensed into clear polycarbonate bottles, prepared to JGOFS standards (IOC 1994). The bottles were transferred to an on-deck incubator, which simulated irradiance at 33% surface irradiance using a blue screen chosen for its spectral characteristic; temperature was controlled using a continuous flow of seawater from ca 2.5 m depth. The samples were incubated for <4 h, centred around local mid-day. The experiments were terminated by filtering through 47 mm GF/F filters (Whatman Ltd) and the filters were frozen until return to the laboratory. Analysis of particulate nitrogen and atom% $^{15}N$ were made by continuous flow mass spectrometry (Europa Scientific Ltd) using methods described by Barrie et al. (1989) and Owens & Rees (1989). Rates of uptake (ρN) were calculated from the equations of Dugdale & Goering (1967).

Chlorophyll concentration in 3 size fractions was measured by filtering 0.1 $dm^3$ sub-samples of the seawater used in the above experiments through a series of 3 polycarbonate filters (5.0, 2.0 and 0.2 µm) as described by Joint & Pomroy (1983) for fluorometric chlorophyll analysis according to Holm-Hansen et al. (1965).

**Results.** ***Analytical precision:*** Measurements are at the lower range of sensitivity for both mass spectrometer and nutrient analyses but analytical precision was not compromised. Quality control of the nutrient analyses was conducted to a standard decreed by participation in the QUASIMEME project (Aminot et al. 1997), and the precision of all analyses was better than 5%. Calculation of ρN requires values for the sample particulate nitrogen content and the $^{15}N$ atom%, both of which were obtained from the mass spectrometer. Results from a calibration of the instrument are shown in Fig. 1. Urea standards were analysed in the range 0.05 to 1.2 µmol N. The mean nitrogen content of the samples was 0.7 µmol and the minimum was 0.11 µmol. Therefore, the nitrogen content of the samples from the North Atlantic were analysed at concentrations where there was a linear relation between nitrogen content and the mass spectrometer output ($r^2$ = 0.99). High precision was

Table 1. Station location and sample depth for nitrogen uptake experiments during July 1996. For each station, ambient nitrate and ammonium concentrations are listed, with the tracer addition as a percentage of ambient concentration

| Stn | Date | Position | Depth (m) | Ambient $NO_3^-$ (nmol N $dm^{-3}$) | % $^{15}N$-$NO_3^-$ | Ambient $NH_4^+$ (nmol N $dm^{-3}$) | % $^{15}N$-$NH_4^+$ |
|---|---|---|---|---|---|---|---|
| 47 | 7 Jul | 50.3° N, 20.3° W | 10 | 650 | 11 | 110 | 9 |
| 49 | 8 Jul | 47.0° N, 20.0° W | 10 | 17 | 290 | 81 | 12 |
| Gf | 9 Jul | 44.5° N, 19.7° W | 10 | 8 | 63 | 65 | 8 |
| 53 | 10 Jul | 40.6° N, 19.4° W | 10 | 4 | 125 | 66 | 15 |
| 55 | 11 Jul | 37.0° N, 19.0° W | 25 | 9 | 56 | 44 | 11 |
| 67 | 13 Jul | 36.9° N, 19.2° W | 30 | 7 | 143 | 67 | 15 |
| 90 | 17 Jul | 36.3° N, 19.2° W | 25 | 6 | 167 | 80 | 13 |
| 97 | 18 Jul | 36.1° N, 19.2° W | 25 | 5 | 60 | 55 | 5 |

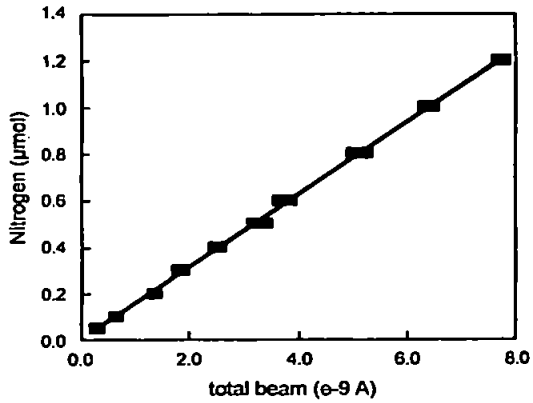


Fig. 1. Calibration curve for continuous flow isotope-ratio mass spectrometer response to urea standards in the concentration range 0.05 to 1.2 µmol nitrogen. Five replicates were analysed at each standard addition ($r^2 = 0.99$)

obtained with a coefficient of variation of atom% determination better than 2.37%.

***Transect from 50° to 36° N:*** The conditions sampled along the transect changed from relatively high nutrient and chlorophyll concentration to oligotrophy. Nitrate concentrations changed from 650 nmol N $dm^{-3}$ at 50.3° N to 4 nmol N $dm^{-3}$ at 40.6° N, ammonium concentrations varied between 110 and 44 nmol N $dm^{-3}$ and chlorophyll concentration from 1.27 µg $dm^{-3}$ to a minimum of 0.05 µg $dm^{-3}$ at the southern stations. Nitrate and ammonium uptake were measured at the stations listed in Table 1. Uptake rates are plotted against the total nitrate or ammonium available to the phytoplankton, i.e. the ambient concentration plus the nitrate or ammonium added as tracer. Fig. 2a shows the $\rho NO_3$ at each station in relation to the nitrate concentration in the experimental bottles. Although the data cannot be used to establish uptake kinetics since these are results obtained from different stations with individual phytoplankton assemblages, the data do fit a hyperbolic function, comparable to a typical Michaelis-Menten curve. The parameter values from this curve are $\rho_{max}$ = 2.77 nmol N $dm^{-3}$ $h^{-1}$ and $K_{NO_3}$ = 20 nmol N $dm^{-3}$; these values are very similar to those published by Harrison et al. (1996) for the North Atlantic.

At very low nitrate concentrations (<70 nmol $dm^{-3}$), the relationship between uptake rate and nitrate concentration is linear (Fig. 2b) with nitrate concentration, explaining 83% of the variance in nitrate uptake rate following a logarithmic transformation of both variables. The equation of the fitted line is $\log_{10}\rho NO_3 = 1.416\log_{10}NO_3 - 2.2321$ and there is a positive intercept of the abscissa at 5 nmol N $dm^{-3}$. Ammonium uptake rate did not appear to be dependent on ammo-

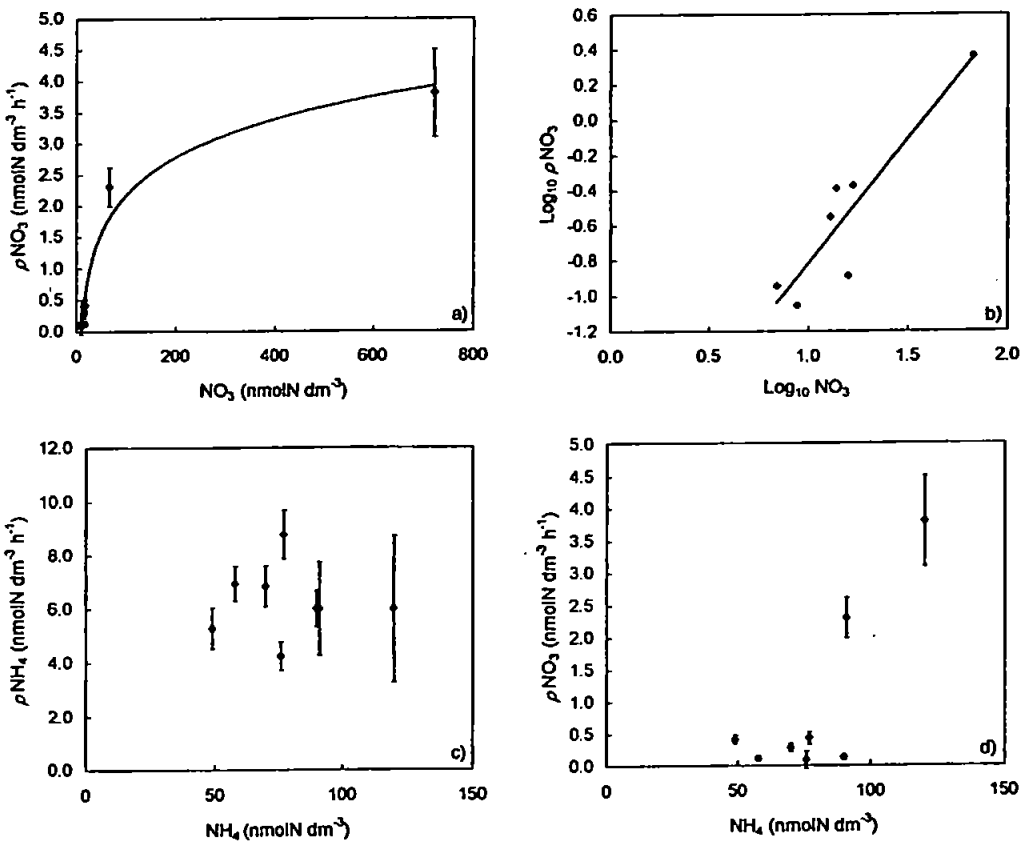


Fig. 2. Data obtained between 50° and 36° N along the 20° W meridian during July 1996 are plotted as (a) nitrate uptake ($\rho NO_3$) versus nitrate concentration ($NO_3$), (b) log transformation of $\rho NO_3$ versus $NO_3$, at concentrations <70 nmol N $dm^{-3}$, (c) $\rho NH_4$ versus $NH_4$, (d) $\rho NO_3$ versus $NH_4$. $NO_3$ and $NH_4$ concentrations are the sum of ambient plus $^{15}N$ added as tracer. Error bars were propagated through the rate equation based on a maximum 5% coefficient of variation (CV = standard deviation ÷ mean) on $NO_3$ and $NH_4$ analyses and from the standard deviation of $^{15}N$ atom% based on replicate mass spectrometer samples

nium concentration (Fig. 2c) but there did appear to be a positive correlation between $\rho NO_3$ and ammonium concentration (Fig. 2d). However, this correlation was largely explained by the influence of the high values for nitrate concentration of 722 and 67 nmol N $dm^{-3}$ at Stns 47 and 49 respectively; these stations also had the highest ammonium concentrations. If these 2 stations are excluded it is clear that there is no significant relationship between nitrate uptake and ammonium concentration.

**Discussion.** The observed variations in nitrate uptake along the transect from nutrient replete conditions in the north to severe oligotrophic conditions in the south offer new insights and parameter values which can be incorporated into models of the North Atlantic. Although these data were not obtained in typical kinetic experiments, the relationship between nitrate uptake and nitrate concentration at different stations approximates well to Michaelis-Menten kinetics (Fig. 2a), which are commonly used in plankton models to describe the uptake of nitrogen (Fasham et al. 1990, Haney & Jackson 1996). The parameters obtained are similar to those previously published for oligotrophic waters (Sahlsten 1987, Harrison et al. 1996).

In this paper, we have applied sensitive methods to determine nitrate and ammonium concentrations at very low nanomolar concentrations and have been able to measure uptake rates at previously unrecorded concentrations. The data help to reduce uncertainties regarding nitrate uptake at very low concentrations. McCarthy et al. (1992) described a linear relation between $\rho NO_3$ and $NO_3$ at concentrations between approximately 25 and 65 nmol N $dm^{-3}$. Extrapolation of this curve suggests that nitrate uptake would cease at the intercept value of 16 nmol N $dm^{-3}$. This implies that either a threshold for nitrate uptake exists or that there was an initial sigmoid relationship which the data of McCarthy et al. (1992) were not able to resolve. Fig. 2b demonstrates that nitrate uptake does occur at nitrate concentrations <16 nmol N $dm^{-3}$ and that the threshold, if it exists, is close to 5 nmol N $dm^{-3}$. However, these data cannot exclude the possibility that nitrate uptake occurs at ambient nitrate concentrations lower than 5 nmol N $dm^{-3}$ and that nitrate uptake may be best described by a sigmoid curve. In either case, it is clear that phytoplankton assemblages in the North Atlantic have extremely high affinities for nitrate.

There were changes in the size of the dominant phytoplankton groups along the transect, with an overall increase in the proportion of phytoplankton smaller than 5.0 µm (Table 2) from north to south. This may have been due to sampling of different biogeochemical regimes and/or may indicate several stages during the seasonal succession. Two measures of the importance of nitrate to phytoplankton, the *f*-ratio (Dugdale & Goering 1967) and the relative preference index (RPI) for nitrate ($RPI_{NO_3}$, McCarthy et al. 1977), declined along the transect, indicating the increased importance of ammonium for phytoplankton production. The RPI was first introduced by McCarthy et al. (1977) as a measure of the nitrogen sufficiency of the environment; they suggested that preference for nitrate would be indicated by values of $RPI_{NO_3}$ equal to or greater than unity. Recently Dortch (1990) and Stolte & Riegman (1996) have emphasised the need for caution in the use of the RPI and that it should not be used as an indicator of phytoplankton preference for a nitrogen species because the index is very dependant on nutrient concentrations. In support of this argument, we have found a positive correlation ($r^2 = 0.70$) for the least squares linear regression of $RPI_{NO_3}$ on $NH_4$:$NO_3$ ratio ($RPI_{NO_3} = -0.049NH_4{:}NO_3 + 0.532$).

Table 2. Percentage contribution of the nano- and picoplankton (<5.0 µm) to total chlorophyll concentration: *f*-ratio (= $\rho NO_3/\Sigma\rho N$) and the relative preference index ($RPI_{NO_3}$ = *f*-ratio/([$NO_3$]/[$\Sigma N$])) at the irradiance of 33% of that at the sea surface. nd: no data

| Stn | Chlorophyll (µg $dm^{-3}$) | Chlorophyll (% <5.0 µm) | *f*-ratio | $RPI_{NO_3}$ |
|---|---|---|---|---|
| 47 | 1.27 | 30 | 0.39 | 0.5 |
| 49 | 0.52 | 22 | 0.28 | 1.3 |
| GI | 0.32 | 37 | 0.04 | 0.2 |
| 53 | 0.07 | 71 | 0.02 | 0.2 |
| 55 | 0.06 | 75 | 0.07 | 0.3 |
| 67 | 0.05 | 75 | 0.04 | 0.2 |
| 90 | nd | nd | 0.02 | 0.1 |
| 97 | nd | nd | 0.02 | 0.1 |

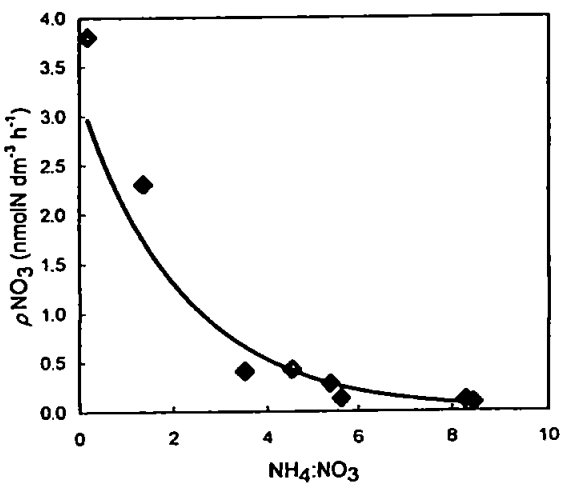


Fig. 3. The relationship between $\rho NO_3$ and the $NH_4$:$NO_3$ concentration ratio. The equation of the fitted curve is $\rho NO_3 = 3.19e^{-0.45(NH_4:NO_3)}$ ($r^2 = 0.93$)

The preference of phytoplankton for ammonium over nitrate is well documented and extends over the full spectrum of nitrogen concentrations (Harrison et al. 1996). Ammonium has also often been demonstrated to inhibit nitrate uptake in natural assemblages; for example, Wheeler & Kokkinakis (1990) found complete inhibition of nitrate uptake by ammonium concentrations of 100 to 300 nmol N $dm^{-3}$ in the subarctic Pacific. Generally, ammonium inhibition is considered to apply at higher concentration; Tamminen (1995) reported significant inhibition of nitrate uptake by ammonium concentrations of 350 nmol N $dm^{-3}$ when the ambient nitrate concentration was 4300 nmol N $dm^{-3}$.

The data presented here, although not derived from kinetic experiments, suggest that comparisons of different assemblages can provide valuable information on both the kinetics of nitrate uptake and of the effect of varying $NH_4$:$NO_3$ concentration ratios on $\rho NO_3$. Fig. 3 shows the influence of the ammonium:nitrate ratio on nitrate uptake at values ranging from 0.2 (120 nmol N $dm^{-3}$ $NH_4$ when the $NO_3$ was 722 nmol N $dm^{-3}$) to 8 (58 nmol N $dm^{-3}$ $NH_4$ to 7 nmol N $dm^{-3}$ $NO_3$). The relatively small change in ammonium concentration (120 to 49 nmol N $dm^{-3}$) was accompanied by a change of 2 orders of magnitude in nitrate. There was no direct evidence for ammonium inhibition and the greatest influence on nitrate uptake was the large decrease in nitrate concentration.

These data have quantified the relationship between nitrate uptake rate and nitrate and ammonium at concentrations typical of summer conditions in the NE Atlantic Ocean. The data should be useful in refining models of plankton production in 2 specific areas. Positive uptake of nitrate has been measured at concentrations of 5 nmol N $dm^{-3}$, hence defining the upper limit for any threshold nitrate uptake. Secondly, an exponential relationship is developed which describes nitrate uptake at changing $NH_4$:$NO_3$ ratios.

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**Paper VI**
Carbon, nitrogen and phosphorous budgets within a mesoscale eddy: Comparison of mass balance with in vitro determination.
*Deep-Sea Research II.* 48(4/5), 859-872, 2001

**Rees A.P.**, Joint I., Woodward E.M.S. & Donald K.M.

Contribution in terms of total effort by A.P. Rees on this work was: 70%

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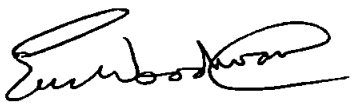
Signed: 
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DEEP-SEA RESEARCH
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# Carbon, nitrogen and phosphorus budgets within a mesoscale eddy: comparison of mass balance with in vitro determinations

Andrew P. Rees*, Ian Joint, E. Malcolm S. Woodward, Kirsten M. Donald

*NERC Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth, PL1 3DH UK*

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**Abstract**

A comparison is made of in vitro estimates of nitrate, ammonium and phosphate uptake with changes in ambient nutrient concentrations within a mesoscale eddy in the northeast Atlantic in June 1996. The stability of the eddy resulted in greatly reduced exchange with adjacent water masses, and hence a mass balance calculation was possible for these three nutrients. Over a 5-day period, there was a combined decline in nitrate and ammonium concentrations of 2.22 mmol N $m^{-3}$. In vitro estimates of nitrate and ammonium assimilation were estimated to be 1.69 mmol N $m^{-3}$, i.e. the in vitro estimates are 76% of the observed decrease in nitrate plus ammonium. In the case of phosphate, in vitro experiments estimated phosphate uptake to be 0.092 mmol P $m^{-3}$ over 3 days, and the measured change in dissolved phosphate concentration in the surface 12 m over the same period was 0.12 mmol P $m^{-3}$, i.e. the in vitro determinations were 77% of the observed changes in phosphate concentration. Thus, this study provides evidence that the two approaches of a mass balance calculation and an in vitro determination give similar answers, within a factor of 0.3, to the estimation of nutrient utilisation in the surface mixed layer. © 2001 Elsevier Science Ltd. All rights reserved.

## 1. Introduction

There are two fundamental approaches to the estimation of phytoplankton activity and the biogeochemical changes that result from that activity. The most frequently applied methodologies involve the containment of water samples in bottles and the measurement of changes over a period of time; such in vitro experiments usually involve the addition of a tracer such as $^{14}C$ or $^{15}N$, although changes in concentrations of oxygen or nutrients are also frequently measured. The alternative approach involves mass balance calculations within a water mass, again with changes in concentration with time being used to estimate the rate of a process. Examples of this approach are

* Corresponding author.

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described by Minas and Codispoti (1993), who summarise a number of estimates of primary production in different oceanic provinces based on changes in nitrate concentration.

Each approach has clear advantages but also considerable disadvantages. In vitro experiments are open to criticism because, by containing water samples, the plankton assemblage may behave differently to free-living organisms. The effect of the containment vessel — so called bottle effect — is a particular cause for concern, although the very process of sampling also may result in changes to the assemblage through the death of fragile organisms. However, in spite of the concerns about in vitro experiments, there is a widespread belief that the estimates obtained by this approach are not seriously in error for most procedures. Williams (1993) states that "fair agreement (i.e., within a factor of 2) can be obtained with the oxygen technique between the in vitro and in situ measurements"; in this case, the in situ measurements were done in a mesocosm experiment (Davies and Williams, 1984) and in a subtropical gyre of the North Pacific (Williams and Purdie, 1991).

The alternative approach involves mass balance calculations, based on changes in the concentration of the parameter under study. This methodology is also subject to criticism and the major problems of this approach relate to characterisation of the water mass involved and to spatial heterogeneity. Accurate estimates of a change in concentration are dependent upon quantification of the mixing and advective processes, because observed changes could result from vertical or lateral exchange with an adjacent water mass, or as a result of biological activity. Since the aim of the approach is to estimate the latter, it is essential to have good estimates of exchange, but this is difficult to achieve in most marine provinces. The two approaches also differ in their space and time scales, and there are obvious problems in relating measurements made over a few hours or days in small volumes of water (as in vitro measurements are inevitably constrained) with the mass balance approach, which considers much larger space scales over weeks or months.

Since global biogeochemical budgets rely on parameters made by one or other of these basic approaches, it is important to know if the two methodologies result in the same estimates. However, there have been very few simultaneous comparisons of in vitro and mass-balance estimates of phytoplankton production and nutrient utilisation. Experiments within large enclosures, or mesocosms, have been one approach to reconciling the two methodologies (e.g. Davies and Williams, 1984; Tamminen, 1989; Heiskanen et al., 1996), but the use of mesocosms is limited to investigations of coastal plankton communities and cannot be applied to oceanic provinces. Also, in spite of their relatively large size, most mesocosms still may have significant wall effects.

There is an urgent need to confirm the accuracy of production estimates, even for provinces which have been extensively studied. For example, the Northeast Atlantic was the subject of intensive sampling during the Joint Global Ocean Flux Study (JGOFS), which included the North Atlantic Bloom Experiment (NABE) in 1989 - 1990 (Ducklow and Harris, 1993). However, even in this intensively studied region, major uncertainties exist in the budgets, and Doney et al. (1996) commented that the "carbon budget cannot be closed to within 0.2–0.3 $Gt\,C\,y^{-1}$, and significant disagreements exist over the major term balances". Part of this uncertainty results from problems of reconciling datasets that originate from different methodologies and the lack of intercalibration of the estimates.

This paper reports the results of an intercomparison of the in vitro and mass balance approaches, which was done during a cruise to the northeast Atlantic in June 1996. The cruise sampled a cold-core eddy that was sufficiently stable (Martin et al., 1998) to allow the changes in nutrient concentration to be followed over consecutive days, whilst simultaneous *in vitro* measurements

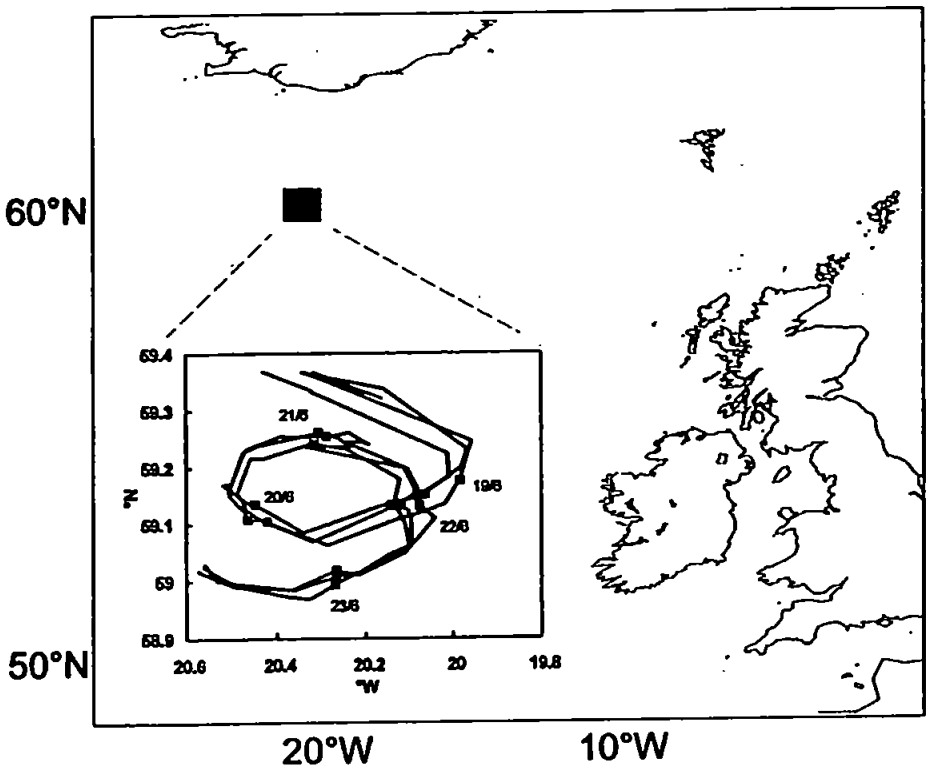


Fig. 1. Position of the PRIME eddy in the Iceland Basin of the northeast Atlantic at approximately 60°N 20°W. Inset is the trajectory paths of three Argos buoys placed close to the entre of the eddy between 17–18 and 24 June 1996. The approximate mid-day positions are marked.

where made of nitrate, ammonium and phosphate uptake. Mesoscale eddies offer an opportunity for mass balance estimates because of their discrete nature and reduced exchange with adjacent water masses. They are also important because they have the potential to transport substantial amounts of nutrients and organic matter from one water mass to another (The Ring Group, 1981).

This paper describes results obtained in an eddy in the North Atlantic (Fig. 1). This was first identified by satellite near-infra-red imagery of the sea-surface temperature (SST) as one of three similar structures existing in the Iceland Basin in the vicinity of 60°N 20°W during the six weeks prior to the cruise. The eddy was characterised by a towed body profiling CTD (Seasoar) and ship-mounted acoustic Doppler current profiler (ADCP) surveys; the results are described in detail by Martin et al. (1998, 2001). The eddy was approximately 60 km across and had an anticylonic circulation, with a cold surface temperature anomaly of approximately 1°C, and a shallow surface mixed layer of ca 12 m. Martin et al. (1998) suggest that the eddy was produced by the North Atlantic Current (NAC) rounding the southern end of the Hatton Bank and that the eddy may have transported significant amounts of salt and heat westwards across the easternmost front of the NAC.

When the eddy was located at the beginning of the cruise, a Lagrangian experiment was initiated. The details of the experiment are published elsewhere (Savidge and Williams, 2001). The area was marked by using one GPS and three ARGOS buoys drogued at 14 m and the trajectories and

mid-day positions are shown in Fig. 1. The study also involved the release of a tracer, sulphur hexafluoride ($SF_6$), close to the estimated centre of the eddy (Law et al., 2001). Following release of the $SF_6$, the eddy was sampled intensively over a 12-day period and $SF_6$ concentrations were measured. This mapping of SF6 dispersion permitted accurate estimation of the degree of vertical and horizontal advection within the eddy. Martin et al. (2001) describe the radial spreading of the $SF_6$ patch between 19 and 23 June and, on the basis of a simulation model, suggest that the $SF_6$ patch remained reasonably coherent over the 5-day period. They state that "the $SF_6$ data shows that, at least for the first 5 days, the surface waters within the eddy retained their identity quite well and that the sampling station remained in or close to the patch centre". However, during the early morning of 24 June, a storm resulted in the loss of about half the $SF_6$ within the eddy and deformation of the patch (Law et al., 2001). Therefore, in the period from the release of the $SF_6$ until the storm, any changes in nutrient concentration in the surface water should have been the consequence of phytoplankton assimilation and should not have resulted from dispersion and mixing of water masses. This 6-day period, therefore, offered an excellent opportunity to compare mass balance estimates of change in nutrient concentrations with estimates of nutrient assimilation made by in vitro determinations over the same time period.

## 2. Methods

Water samples were collected close to the eddy centre (Law et al., 2001; Martin et al., 2001) over a 6-day period. Samples for nutrient analyses were taken every 12 h, and measurements of chlorophyll concentration, carbon fixation and nutrient uptake were done daily. The rate measurements involved incubations for 24 h of samples taken before dawn. Daily experiments continued after the storm on 24 June, but the results are not considered in this paper. The physical structure of the water column was characterised using a profiling CTD. Water samples were collected with a rosette sampler from 8 depths, two or three of which were within the surface mixed layer. The samples were analysed for chlorophyll and the inorganic nutrients, nitrate, nitrite, ammonium, phosphate and silicate. Experiments were done to determine rates of carbon, nitrogen and phosphorus incorporation using $^{14}C$, $^{15}N$ and $^{33}P$ isotopes, respectively.

### *2.1. Nutrient concentrations*

Analyses of dissolved nutrient concentrations were done as soon as practicable after sampling and were always completed within 3 h of collection. Care was taken to avoid contamination when water was transferred from the rosette sampler to clean sample ampoules, and all samples were stored at 4°C in the dark until analysis. Nutrient concentrations were measured by colorimetric autoanalysis using a Technicon segmented flow colorimetric autoanalyser. The methods used were those of Brewer and Riley (1965) for nitrate, Grasshoff (1976) for nitrite, and Kirkwood (1989) for silicate and phosphate. Ammonium analyses were made, using the fluorometric method described by Jones (1991). Quality control of the nutrient determination is through the ongoing participation in the QUASIMEME project (Aminot et al. 1997), and the precision of all analyses was better than 5%.

### 2.2. Chlorophyll concentration

Chlorophyll concentrations were measured on board ship by fluorometric analysis of extracted pigments (Holm-Hansen et al., 1965). One-hundred millilitres samples were filtered through 0.2 μm polycarbonate filters and pigments were extracted in 90% acetone. Samples were stored in the dark at 4°C for ca 12 h before analysis in a Turner Designs fluorometer.

### 2.3. Carbon fixation

The rate of carbon fixation was estimated from the incorporation of $^{14}$C-bicarbonate over a 24 h period. Aliquots of water samples from each depth were transferred into three 60 ml clear polycarbonate bottles and a single black polycarbonate bottle; all bottles were cleaned following JGOFS protocols (IOC, 1994) to reduce trace metal contamination. Each bottle was inoculated with 370 kBq (10 μCi) $NaH^{14}CO_3$ and transferred to an on-deck incubation system before dawn. Incubations were done for 24 h in opaque boxes, with graded-light screens which allowed transmission of 97, 55 or 33% of incident irradiance; temperature in the incubators was regulated by the continuous flow of surface seawater. At dusk, the light screens were replaced with opaque screens to ensure incubations were unaffected by the ships lights. After 24 h, samples were filtered sequentially through 5, 2 and 0.2 μm polycarbonate filters. Filters were dried overnight over silica gel and the radioactivity of each filter was measured in a liquid scintillation counter, the efficiency of which was determined with an external standard, channels ratio method. The carbon fixation rates in this paper are for the total phytoplankton population i.e. the sum of the 5, 2 and 0.2 μm fractions.

### 2.4. Nitrate and ammonium uptake

The uptake rates for nitrate and ammonium were determined with the stable isotope $^{15}$N, using methods detailed by Rees et al. (1999). Two sets of triplicate samples from each depth were distributed into 0.62-l clear polycarbonate bottles and $^{15}N$–$NO_3^-$ and $^{15}N$–$NH_4^+$ were added at approximately 10% of the ambient concentration. Incubations were for 24 h as described above, and were terminated by filtration ( < 40 cmHg) onto ashed Whatman GF/F filters; each filter was then washed with filtered sea water and stored frozen until return to the laboratory. The filters were oven dried at 50°C before analysis and atom% $^{15}$N was measured by continuous flow nitrogen analysis-mass spectrometry (Europa Scientific Ltd., U.K.) using the techniques described in Barrie et al. (1989) and Owens and Rees (1989). Rates of assimilation were calculated from the equations of Dugdale and Goering (1967).

### 2.5. Phosphate uptake

Incorporation of $^{33}$P labelled orthophosphate was measured using a similar procedure to that for $^{14}$C uptake measurements. Water samples from each depth were distributed into three 60 ml clear and one dark polycarbonate bottles and incubated with 37 kBq $^{33}$P orthophosphate; incubations were for 24 h. At the end of the experiment, samples were size-fractionated by filtration through 5, 2 and 0.2 μm polycarbonate filters which had previously been boiled in 0.5 mol $l^{-1}$

lithium chloride made up in 1 mmol $l^{-1}$ phosphate buffer (pH 9.0). Filters were rinsed twice with 10 ml lithium chloride/phosphate buffer, following the method of Grillo and Gibson (1979), dried overnight in a desiccator and the radioactivity of each filter was measured in a liquid scintillation counter. Corrections were made for counting efficiency and for the half-life of $^{33}P$. The data in this paper are the uptake rates for the total population, i.e. the sum of the 5, 2 and 0.2 μm fractions.

## 3. Results

### 3.1. The eddy structure

Fig. 2a shows the surface temperature of the eddy structure at the beginning of the experiment on 14–17 June, as defined by a Seasoar survey. The centre of the eddy is clearly determined from the cold surface anomaly at the core. The nitrate (Fig. 2b) and phosphate (Fig. 2c) concentrations also show a similar distribution pattern, and elevated nutrient concentrations were associated with the cold core of the eddy. The nitrate concentration was $> 1\,\mu mol\,l^{-1}$ greater at the centre of the eddy than at the periphery, and the phosphate concentration was $> 0.1\,\mu mol\,l^{-1}$ higher than to the south of the survey area.

Surface temperature increased during the 2-week survey period, with an increase of approximately 1°C. Fig. 3 shows the vertical temperature structure during the 5 days before the storm.

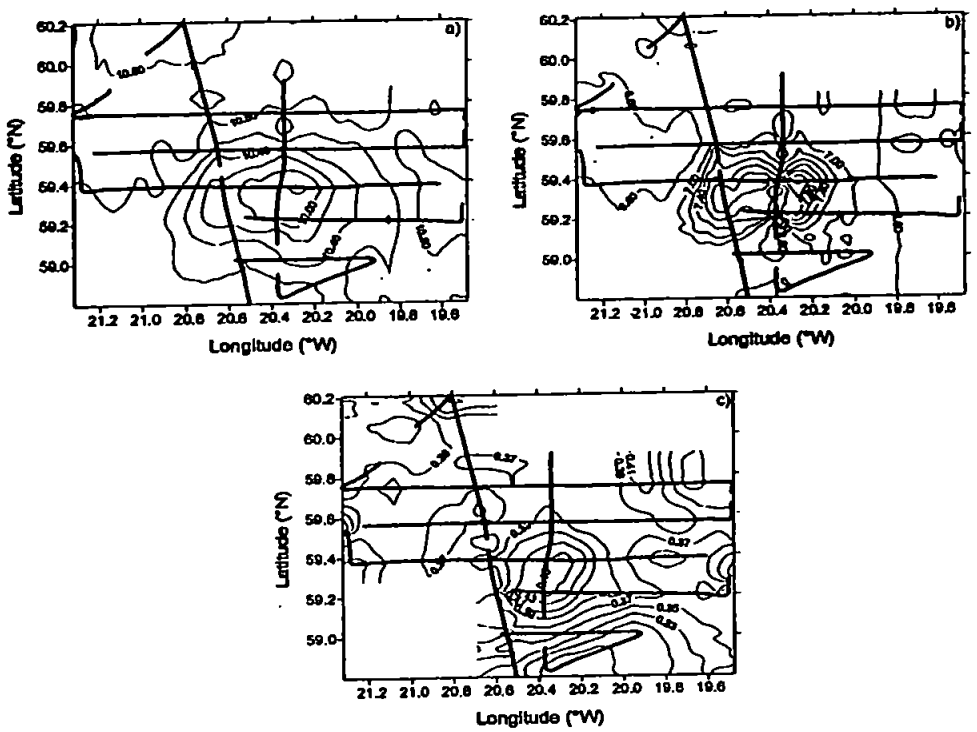


Fig. 2. (a) Sea surface temperature (°C), (b) nitrate (mmol $m^{-3}$) and (c) phosphate (mmol $m^{-3}$) distributions determined during a Seasoar survey at the beginning of the Lagrangian experiment on 15–17 June. The bold line is the cruise track.

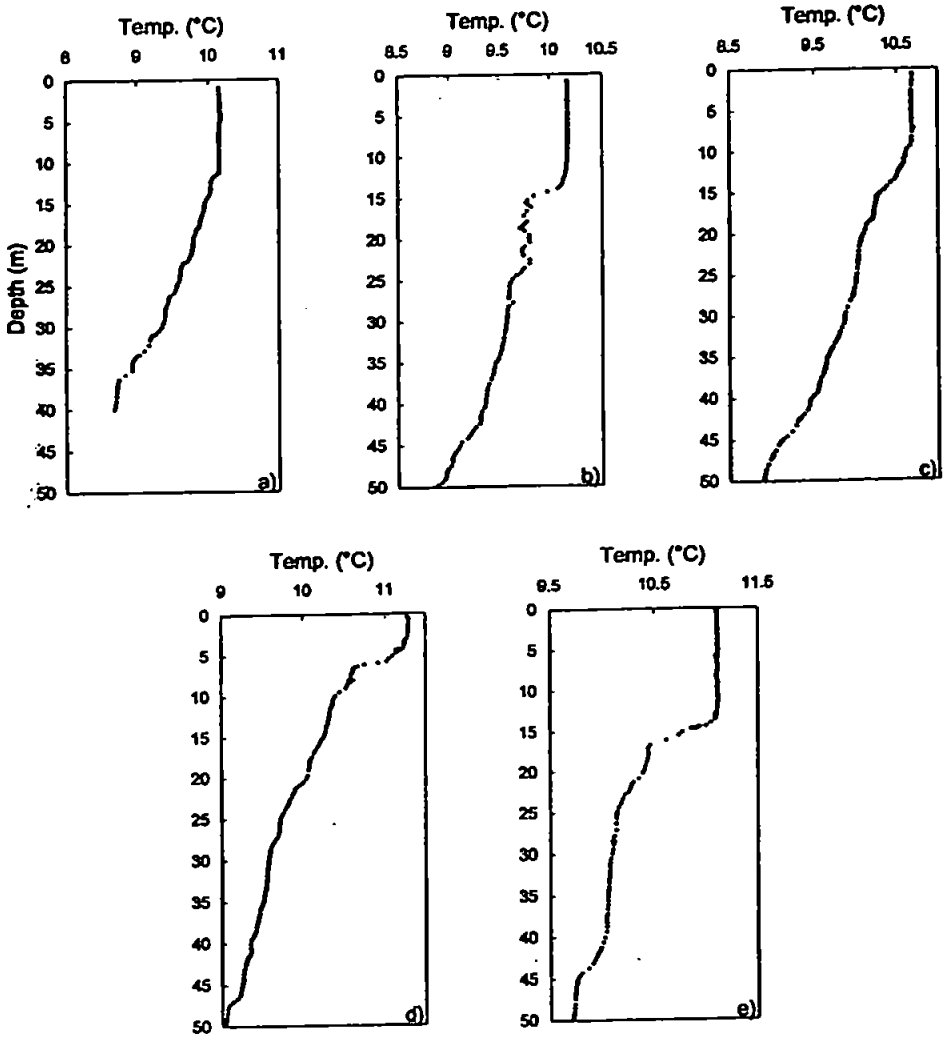


Fig. 3. Vertical temperature profiles at dawn on (a) day 2 (19 June) (b) day 3 (c) day 4 (d) day 5 and (e) day 6.

There were at least three thermoclines in the surface 50 m, but the shallowest and most stable was between 10 and 15 m; each CTD profile showed a homogeneous temperature structure in the near surface mixed layer. The mean depth of the uppermost thermocline over the period 14–23 June was 12 m, although there was significant shallowing on day 5 when the thermocline was at 6 m; it is not known whether this profile coincided with an internal wave or other transient phenomenon which resulted in a temporary change in the thermocline depth. On the following day, the thermocline was again measured at 15 m. The storm on Day 7 (24 June) resulted in deeper mixing of the surface mixed layer. Therefore, it is assumed that for the period 19–23 June, as well as having lateral coherence (Martin et al., 2001), there was minimal vertical exchange of the surface 12 m with deeper water. With this assumption, it is possible to attempt mass balance estimates for the surface waters of the eddy over the 5-day period from 18 to 23 June before the storm on 24 June. Since there is

some variability in the depth of the surface mixed layer, the mass balances are calculated in a volume ($m^{-3}$) basis rather than as a depth-integrated ($m^{-2}$) value for the surface mixed layer.

### 3.2. Changes in nutrient concentrations

During the 5-day period, nitrate concentrations decreased from 7.2 to 5.0 mmol N $m^{-3}$ at 10 m (Fig. 4a). The decrease in concentration was not linear, and there were some increase in concentration between days 2 and 3. However, there was a strong linear trend in the data that allows an

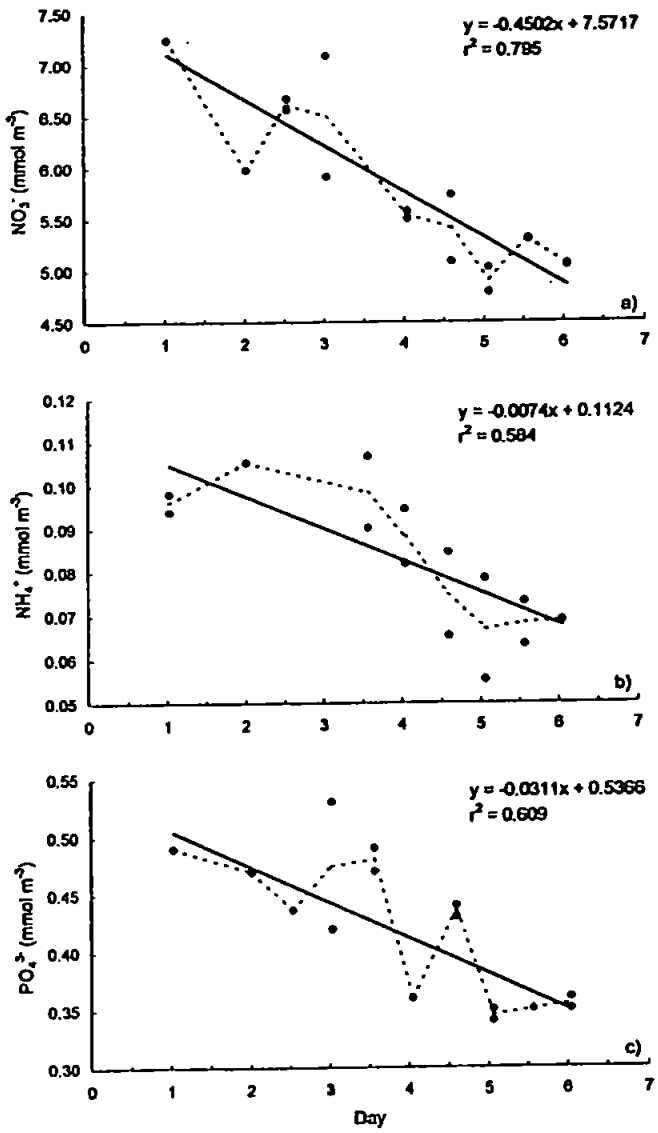


Fig. 4. Changes in dissolved inorganic nutrient concentrations in the surface mixed layer from day 1 (18 June) to day 6 (23 June) for (a) nitrate, (b) ammonium and (c) phosphate. The concentration at 2 and 10 m (●) on each day are shown, the dotted line connects the mean values of those 2 points and the solid line is fitted by a least-squares linear regression.

estimation of the rate of nitrate disappearance, which we assume is due to uptake by plankton. The fitted line (Fig. 4a) gives a mean rate decrease in nitrate concentration of 0.45 mmol N $m^{-3}$ $d^{-1}$ ($r^2 = 0.79$, $p < 0.001$). Ammonium and phosphate concentrations show similar decreases (Figs. 4b and c), but there is also variability from day to day. The ammonium concentration decreased from 0.10 to 0.07 mmol N $m^{-3}$ and phosphate declined from 0.50 to 0.35 mmol P $m^{-3}$. Linear regression analysis gives estimates of apparent ammonium utilisation of 0.007 mmol N $m^{-3}$ $d^{-1}$ ($r^2 = 0.58$, $p < 0.01$) and phosphate utilisation of 0.031 mmol P $m^{-3}$ $d^{-1}$ ($r^2 = 0.61$, $p < 0.01$).

In contrast to nitrate, ammonium and phosphate, nitrite and silicate showed no significant decline in concentration, but varied about mean values of 0.15 mmol N $m^{-3}$ and 2.46 mmol Si $m^{-3}$, by ± 10 and 12%, respectively. A more complete description of the inorganic nutrient regime for the whole water column is provided by Woodward and Rees (2001).

### 3.3. *Chlorophyll concentration*

During the 5-day period, chlorophyll concentrations increased over the first 3 days from 0.62 to 0.90 mg $m^{-3}$ but then decreased to 0.77 mg $m^{-3}$ by day 6 (Table 1). Between 54 and 56% of the chlorophyll biomass was due to phytoplankton cells larger than 5.0 μm. The assemblage was dominated by a bloom of the coccolithophore *Coccolithus pelagicus* and other nanophytoplankton, but few diatoms and dinoflagellates were present (Tarran et al., 2001).

### 3.4. *In vitro carbon, nitrogen and phosphorus assimilation*

The uptake rates of carbon, nitrate, ammonium and phosphate were determined at daily intervals. Samples were taken from 8 depths throughout the surface 50 m but only two or three of those samples were within the surface 12 m; therefore, mean values for those depths are used in this analysis of the data (Table 1) and the results are expressed as mmol $m^{-3}$ $d^{-1}$. The carbon fixation rate decreased throughout the period, even though there was an increase in chlorophyll concentration for the first 3 days. Nitrogen uptake ($\rho$N) rates were measured on days 2–5 inclusive. The maximum uptake rates of both nitrate and ammonium were measured on day 3 (Table 1). Although there were variations in the rate of assimilation of nitrate and ammonium, nitrate was the major nitrogen source for phytoplankton growth. The *f*-ratio (Dugdale and Goering, 1967) varied between 0.71 and 0.86 (Table 1). Phosphate assimilation ($\rho PO_4^{3-}$) was measured on only three occasions from day 4 and showed very little variation (Table 1).

## 4. Discussion

The stability of the mesoscale eddy, at least over the 5-day period before the storm, offered a rare opportunity to compare the rates of nutrient utilisation derived from in vitro incubations, with the observed changes in nutrient concentration. Although largely a consequence of phytoplankton activity, the two methods measure on different space and time scales. Changes in ambient nutrient concentrations result from past biological activity and may reflect variations in plankton assemblages as well as the consequence of their activity. In vitro experiments, particularly those involving radioisotope tracers, offer very precise estimates, but they are usually constrained to short-period incubations (Robertson et al., 1994). The object of this investigation is to consider whether or not the two approaches provide the same answer.

Table 1
Mean rates of phytoplankton carbon, phosphorus and nitrogen incorporation ( ± S. E.) and chlorophyll concentration for the surface mixed layer of the PRIME eddy in the Northeast Atlantic during June 1996

| Sample day | Date | No. of depths included | Chlorophyll ($mg\,m^{-3}$) | $^{14}C$ fixation ($mmol\,m^{-3}\,d^{-1}$) | $^{33}PO_4^-$ uptake ($mmol\,m^{-3}\,d^{-1}$) | $^{15}NO_3^-$ uptake ($mmol\,m^{-3}\,d^{-1}$) | $^{15}NH_4^+$ uptake ($mmol\,m^{-3}\,d^{-1}$) | *f*-ratio | C:N uptake ratio |
|---|---|---|---|---|---|---|---|---|---|
| 2 | 19 June | 3 | 0.62 | 4.83 ± 0.46 | nd[a] | 0.259 ± 0.012 | 0.065 ± 0.001 | 0.80 | 14.9 |
| 3 | 20 June | 3 | 0.78 | 4.24 ± 0.56 | nd[a] | 0.281 ± 0.008 | 0.114 ± 0.001 | 0.71 | 10.7 |
| 4 | 21 June | 2 | 0.90 | 3.04 ± 0.23 | 0.032 ± 0.003 | 0.271 ± 0.008 | 0.057 ± 0.002 | 0.83 | 9.3 |
| 5 | 22 June | 2 | 0.76 | 2.45 ± 0.54 | 0.029 ± 0.002 | 0.263 ± 0.004 | 0.044 ± 0.003 | 0.86 | 8.0 |
| 6 | 23 June | 2 | 0.77 | 1.63 ± 0.17 | 0.031 ± 0.004 | nd[a] | nd[a] | nd[a] | nd[a] |

[a]nd = not determined.



Table 2
Change (Δ) in nutrient concentrations determined from CTD casts at midnight, and in vitro uptake rates (ρ) for the period 19–23 June 1996

| Day | $\Delta NO_3^-$ ($mmol\,m^{-3}\,d^{-1}$) | $\rho^{15}NO_3^-$ ($mmol\,m^{-3}\,d^{-1}$) | $\Delta NH_4^+$ ($mmol\,m^{-3}\,d^{-1}$) | $\rho^{15}NH_4^+$ ($mmol\,m^{-3}\,d^{-1}$) | $\Delta PO_4^{3-}$ ($mmol\,m^{-3}\,d^{-1}$) | $\rho^{33}PO_4^{3-}$ ($mmol\,m^{-3}\,d^{-1}$) | *ΔDIC*[a] ($mmol\,m^{-3}\,d^{-1}$) | $\rho^{14}C$ ($mmol\,m^{-3}\,d^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 2 | − 1.26 | 0.259 | + 0.009 | 0.065 | — | — | − 8.32 | 4.83 |
| 3 | + 0.51 | 0.281 | − 0.007[b] | 0.114 | — | — | + 3.37 | 4.24 |
| 4 | − 0.95 | 0.271 | − 0.010 | 0.057 | − 0.12 | 0.032 | − 6.27 | 3.04 |
| 5 | − 0.64 | 0.263 | − 0.021 | 0.044 | − 0.01 | 0.029 | − 4.22 | 2.45 |
| 6 | + 0.15 | 0.269[c] | + 0.002 | 0.070[c] | + 0.01 | 0.031 | + 0.99 | 1.63 |
| $\sum(mmol\,m^{-3})$ | − 2.19 | 1.343 | − 0.027 | 0.350 | − 0.12 | 0.092 | − 14.45 | 16.19 |

[a]DIC estimated from change in $NO_3^-$ concentration and applying the Redfield ratio of C:N = 6.6.
[b]Data from midday CTD cast.
[c]Missing data, therefore mean value of previous four days used.

It is clear from the data in Table 1 that the uptake rates of carbon, nitrate, ammonium and phosphate varied from day to day and no constant ratio in nutrient uptake is apparent. Similar variations in C:N:P uptake ratios were found later in the cruise on a transect along 20°W to 37°N (Donald et al., 2001). The daily estimates of uptake ratios over this 5-day period (Table 1) do not conform to the Redfield ratio of 106C:16N:1P (Redfield et al., 1963). This can happen when the generation time of the phytoplankton cells in the assemblage is longer than the incubation period of the experiment, since it is only on a time scale equivalent to the generation time that a cell will have assimilated carbon and nutrients in proportion to its elemental composition (Thingstad et al., 1993). So, there can be uncoupling of nutrient uptake from cell growth. The uptake rates for each nutrient over the 5-day period ($\sum$mmol m$^{-3}$) is shown in Table 2 and they allow comparison over a longer time. The observed uptake ratios are 176C:18N:1P; although the N:P ratio is closer to the Redfield ratio, the carbon assimilation rates are high. The phytoplankton assemblage was dominated by a coccolithopore, part of the apparent carbon fixation measured by the $^{14}$C method will have been incorporated into the calcite of the coccoliths. However, a single experiment during this period showed no significant difference between acid-fumed and unfumed filters, suggesting that little $^{14}$C may have been incorporated into calcite. This was only a single observation, and independent measurements of calcite production rate were not measured on this cruise. Calcite production must remain a probable explanation for the high C:N and C:P ratios.

Table 2 also compares the daily changes in nutrient concentration ($\Delta$N or $\Delta$P) in the surface 12 m with estimates of in vitro uptake rates. If, on the time scale of one-day, there was close agreement between the two estimates, then the change in nutrient concentration should be the same as the in vitro determination made on the previous day. It is clear from Table 2 that this close correspondence does not apply. However, over the whole 5-day period of the experiment, some agreement does emerge. Nitrate concentration decreased by 2.19 mmol N m$^{-3}$, whereas the nitrate-utilisation rate is estimated to be 1.346 mmol m$^{-3}$; that is, the in vitro estimate appears to be only 61% of the mass-balance estimate. The in vitro experiments suggest that ammonium assimilation was 0.350 mmol m$^{-3}$ over the 5 days, but a minimal decrease in ambient ammonium concentration of 0.027 mmol m$^{-3}$ was observed. It appears, therefore, that the in vitro experiments overestimate ammonium uptake by a factor of 13. However, that analysis assumes that there is no cycling of nitrogen by ammonium uptake and regeneration. The chlorophyll concentration reached a peak at day 3 and decreased on subsequent days, suggesting that zooplankton grazing and subsequent excretion of ammonium was occurring. Since there was no observed increase in ammonium concentration, it is reasonable to assume that the regenerated ammonium was assimilated by the phytoplankton. The in vitro experiments certainly suggest significant ammonium uptake. Therefore, it is appropriate to consider both nitrate and ammonium in the mass balance calculations for nitrogen, and summing the change in nitrate and ammonium concentrations suggests that there was a decline in inorganic nitrogen of 2.217 mmol N m$^{-3}$ from 19 to 23 June. The combined in vitro estimates of nitrate and ammonium assimilation are estimated to be 1.696 mmol N m$^{-3}$ over the same 5-day period. That is, the in vitro estimates are 76% of the observed decrease in nitrate plus ammonium. This budget does not consider the possible contribution of dissolved organic nitrogen (DON), which decreased in concentration from 21 mmol m$^{-3}$ on day 1 to 7 mmol m$^{-3}$ on day 5 (A.E.J. Miller, pers. comm., Plymouth Marine Laboratory). If this DON was assimilated by phytoplankton, it would account for some of the apparent discrepancy between the two methods. However, it is probable that phytoplankton would release DON (Bronk and Glibert, 1994) and

that bacteria were responsible for most of the utilisation of DON; no measurements were made of this process during the cruise or of the release of ammonium by bacterial activity. It is, therefore, impossible to close this budget. Nevertheless, the agreement between the in vitro and mass balance approaches appears very good for nitrogen.

There was little or no correspondence in daily in vitro $^{33}P$ uptake rate estimates (which were very constant) with the observed changes in phosphate concentration (Table 2). However, over a 3-day period, the sum of in vitro experiments suggests a total phosphate uptake of 0.092 mmol $m^{-3}$, and the change in dissolved phosphate concentration in the surface 12 m over the same period was 0.12 mmol $m^{-3}$. Therefore, the in vitro determinations are 76% of the observed changes in phosphate concentration — identical to the difference obtained by the two methods for nitrate plus ammonium.

Carbon assimilation also was measured in vitro, but there were no comparable mass-balance estimates for carbon. However, an estimate of the potential change in dissolved inorganic carbon ($\Delta$DIC) is made in Table 2, based on the Redfield ratio of 6.6C:1N; this assumption is justified because the mean particulate C:N ratio measured during this period was 6.7 (R. Head, pers. comm., Plymouth Marine Laboratory). The in vitro estimate of carbon fixation by the $^{14}C$ method over this period was 16.25 mmol C $m^{-3}$. Applying the Redfield ratio to the observed change in nitrate concentration provides an estimate of the likely change in dissolved inorganic carbon of 14.45 mmol C $m^{-3}$, which is 89% of the in vitro estimate. In the absence of measurements of total DIC, this estimate is of little value for producing a mass balance of the carbon budget. However, it does suggest that the in vitro $^{14}C$ estimates over the 5-day period are consistent with the changes in inorganic nitrogen.

This experiment demonstrates that, within this mesoscale eddy and over a period of 5 days, it is possible to reconcile in vitro estimates of nitrate plus ammonium and phosphate uptake with the alternative method of calculating a mass balance. Indeed the agreement between the estimates is very good. Both in the case of nitrogen and phosphorus, the in vitro estimates are 76% of the observed change in nutrient concentration. This difference may be due to the two methodologies, but it also may reflect transport of nutrients across the thermocline or the fact that organic nutrient species were not measured in this experiment. Nevertheless, this study has shown that in vitro estimates are very close to the mass-balance estimates of nutrient change over a 5-day period. These results provide increasing confidence that the two approaches provide the same answers, at least within a factor of 0.3. There is justification for continuing to use either or both approaches in determining biogeochemical budgets of the oceans.

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# Appendix

**Full list of refereed scientific publications**

**By Andrew P. Rees**

1989

1. Owens N.J.P. and Rees A.P. 1989. Determination of Nitrogen-15 at sub-microgram levels of nitrogen using automated continuous-flow isotope ratio mass spectrometry. *Analyst*, 114, 1655-1657.
2. Owens N.J.P., Rees A.P., Woodward E.M.S. and Mantoura R.F.C. 1989. Size-fractionated primary production and nitrogen assimilation in the North-Western Mediterranean Sea during January 1989. In: Martin J-M and Barth H., editors. *EROS-2000 first workshop.* p. 126-135.
3. Law C.S., Rees A.P. and Owens N.J.P. 1989. Consumption and production of nitrous oxide in the Golfe du Lion. In: Martin J-M and Barth H., editors. *EROS-2000 first workshop.* p. 155-161.

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5. Woodward E.M.S., Owens N.J.P., Rees A.P. and Law C.S. 1990. Nutrient cycling and primary productivity in the Golfe du Lion. In: Martin J-M and Barth H., editors. *EROS-2000 second workshop.* p. 83-92.
6. Owens N.J.P., Woodward E.M.S., Aiken J., Bellan I.E. and Rees A.P. 1990. Primary production and nitrogen assimilation in the North Sea during July 1987. *Netherlands Journal of Sea Research*, 25, 143-154.

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7. Law C.S., Rees A.P. and Owens N.J.P. 1991. Temporal variability of denitrification in estuarine sediments. *Estuarine Coastal and Shelf Science, 33, 37-56.*

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8. Law C.S., Rees A.P. and Owens N.J.P. 1992. Nitrous oxide: estuarine sources and atmospheric flux. *Estuarine Coastal and Shelf Science.* 35. 301-314.
9. Owens N.J.P., Rees A.P., Plummer D.H., Woodward E.M.S. and Bellerby R.S. 1992. Phytoplankton nitrogen assimilation as new production in the North West Mediterranean Sea (April 1990). In: Martin J-M and Barth H., editors. *EROS-2000 third workshop.* p. 163-172.

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10. Law C.S., Rees A.P. and Owens N.J.P. 1993. Nitrous oxide production by estuarine epiphyton. *Limnology and Oceanography.* 38(2), 435-441.
11. Staples T.B., Lewis R.E., Owens N.J.P., Woodward E.M.S., Rees A.P. and Plummer D.H. 1993. A modelling study of the contribution of riverine and anthropogenic inputs of ammonia to a North Sea coastal area. *Estuarine Coastal and Shelf Science.* 37, 497-507.

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13. Rees A.P., Owens N.J.P. & Woodward E.M.S. 1995. Phytoplankton nitrogen assimilation at low nutrient concentrations in the NW - Mediterranean Sea (July 1993). In: Martin J-M and Barth H., editors. *EROS-2000 fifth workshop.* p. 141-148.

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14. Upstill-Goddard R.C., Rees A.P. & Owens N.J.P. 1996. Simultaneous high-precision measurements of methane and nitrous oxide in water and seawater by single phase equilibration gas chromatography. *Deep-Sea Research I*, 43, 1669-1682.

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15. Rees A.P., Owens N.J.P. & Upstill-Goddard R.C. 1997. Nitrous oxide in the Bellingshausen Sea and Drake Passage. *Journal of Geophysical Research: Oceans.* 102, 3383-3391.

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16. Shaw P.J., Purdie D.A., de Freitas P.S., Rees A.P. & Joint I. 1998. Nutrient uptake in a highly turbid estuary (the Humber, United Kingdom) and adjacent coastal waters. *Estuaries.* 21, 507-517.

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17. Rees A.P., Joint I. & Donald K.M. 1999. Early spring bloom phytoplankton –nutrient dynamics at the Celtic Sea shelf edge. *Deep-Sea Research.* 46, 483-510.
18. Woodward E.M.S., Rees A.P. & Stephens J.A. 1999. The influence of the south-west monsoon upon the nutrient biogeochemistry of the Arabian Sea. *Deep-Sea Research II*, 46(3/4), 571-591.
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21. Rees A.P., Woodward E.M.S. & Joint I.R. 1999. Measurement of nitrate and ammonium uptake at ambient concentrations in oligotrophic waters of the north-east Atlantic Ocean. *Marine Ecology Progress Series*, 187, 295-300.

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22. Rees A.P., Joint I.R., Woodward E.M.S. & Donald K.M. 2001. Carbon, nitrogen and phosphorus budgets within a mesoscale eddy: comparison of mass balance with in vitro determination. *Deep-Sea Research II.* 48(4/5), 859-872.

23. Woodward E.M.S. & Rees A.P., 2001. Nutrient distributions in an anticyclonic eddy in the north east Atlantic Ocean, with reference to nanomolar ammonium concentrations. *Deep-Sea Research II.* 48(4/5), 775-793.

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31. Archer S.D., Smith G.C., Nightingale P.D., Widdicombe C.E., Tarran G.A., Rees A.P. and Burkill P.H. IN PRESS. Dynamics of particulate dimethylsulphoniopropionate during a Lagrangian experiment in the northern North Sea. *Deep-Sea Research II.*

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