The loss of organic nitrogen during marine primary production may be significantly overestimated when using ^{15}N substrates

Kevin J. Flynn* and Lorraine S. Berry

Swansea Algal and Plankton Research Group, University of Wales—Swansea, Singleton Park, Swansea SA2 8PP, UK

Field studies indicate that natural phytoplankton populations may release very significant amounts (20–40%) of newly assimilated dissolved inorganic nitrogen (DIN) as dissolved organic nitrogen (DON). In laboratory cultures, however, it is usually possible to account for at least 90% of nitrogen added to the system as DIN plus cell nitrogen. Here we show that the bulk of the missing nitrogen may be accounted for as dissolved free and dissolved combined amino acids. In cultures (which usually have a biomass density at least an order of magnitude greater than is present in offshore waters), the contribution of DON to system nitrogen thus appears to be minor. It is proposed that this difference may be explained if the levels of DON represent equilibrium between release–leakage and transport back into the algae. It is demonstrated, using a dynamic model of algal nitrogen physiology, that this mechanism can explain both laboratory and field observations. Simulations of incubations with DI^{15}N reproduce the reported levels of loss in field incubations. However, because of isotope disequilibria between system components the ^{15}N protocol may significantly overestimate the net loss of nitrogen from the algal cells. The arguments apply equally to studies of bacterial production of DON and to questions concerning the release of other dissolved organics by healthy phytoplankton. The significance of dynamic equilibria between the organisms and the medium may be missed in laboratory studies conducted with high biomass cultures.

Keywords: organic nitrogen; primary production; ^{15}N uptake; bacteria; phytoplankton

1. INTRODUCTION

Marine primary production contributes 40% of global carbon fixation (Falkowski 1994). The proportion and absolute amount of production associated with the export of carbon from the atmosphere into the deep ocean is often estimated by the relative rates of assimilation of ‘new’ and regenerated dissolved inorganic nitrogen (DIN as nitrate and ammonium, respectively; Dugdale & Goering 1967) using ^{15}N substrates. One of the longest running debates in marine ecology concerns the extent of the release of organics by healthy marine phytoplankton (Sharp 1977). A significant net loss of organics from phytoplankton would enhance bacterial production, effectively shifting the fate of primary production away from the export processes. As bacteria not only contribute to the use and production of dissolved organic nitrogen (DON), but to the use of DIN (Wheeler & Kirchman 1986; Kirchman 1994), understanding the fate of DI^{15}N added during field experiments has become increasingly important (Bronk & Glibert 1994; Slawyk & Raimbault 1995; PujoPay *et al.* 1997). Other sources of DON may include predatory activity and cell lysis at death. Here we are only concerned with the activity of healthy cells.

It has been claimed that as much as 40% of DI^{15}N entering the phytoplankton may be released as DO^{15}N (Bronk & Glibert 1994; Bronk *et al.* 1994). Even accepting

that not all the organisms may have been healthy, these estimates from natural phytoplankton communities are greatly at odds with results from laboratory studies obtained using standard elemental and nutrient analyses. Typically, DIN plus particulate organic nitrogen (PON) remains constant $\pm 5\%$ during culture growth (e.g. Flynn *et al.* 1993). Indeed, PON may be estimated by mass balance in cultures (i.e. $\text{PON} = \text{system nitrogen} - \text{DIN}$; e.g. Harrison *et al.* 1989). Further, the concentrations of components of DON, such as dissolved free amino acids (DFAAs), though higher, are not too dissimilar to those found in nature and are certainly not in proportion with the much higher biomass levels in cultures (Flynn & Butler 1986; Flynn 1990).

The nature of the mechanisms for DON release from healthy cells and any resultant advantages for the organisms are not clear. What we do know is that phytoplankton are capable of taking up a wide range of organics, from amino acids to vitamins and no doubt some material of higher molecular weight (Admiraal *et al.* 1984; Lu & Stephens 1984; Flynn & Butler 1986; Flynn 1990; Antia *et al.* 1991; Bronk & Glibert 1993; Berg *et al.* 1997). It is logical to propose that the amount of DON in the environment represents a dynamic equilibrium between release and uptake, not only by bacteria (Middelboe *et al.* 1995) but also by the activity of the phytoplankton (Flynn & Butler 1986).

The aims of this paper are, first, to consider the nitrogen mass balance for cultures grown at biomass levels equating

*Author for correspondence (k.j.flynn@swansea.ac.uk).

to high coastal levels (40–60 $\mu\text{mol N l}^{-1}$). This included measurements of DFAAs and, for the first time, dissolved combined amino acids (DCAAs) and would thus account for all DON composed of primary amines (including most nitrogen in peptides and proteins). Second, the consequences of the operation of a dynamic equilibrium between release and recovery are explored as a function of biomass. This was considered using a model of algal nitrogen physiology that also enabled the implications for ^{15}N incubations to be considered.

2. MATERIAL AND METHODS

(a) *Experimental*

The diatom *Thalassiosira pseudonana* (CCAP 1409, Dunstaffnage Marine Laboratory, Oban, UK), raphidophyte *Heterosigma carterae* (HAIV, Instituto Español de Oceanografía, Vigo, Spain), and prasinophyte *Mantoniella squamata* (CCAP 1965/1), were grown in batch culture using ammonium (40–60 μM) in artificial seawater (Harrison *et al.* 1980). Phosphate was supplied at either 1 or 10 μM for low-phosphorus or high-phosphorus cultures, respectively. The N:P ratio in the low-phosphorus media results in a level of phosphorus stress in addition to nitrogen stress at stationary phase. Cultures were grown at 18 °C in a 12 L:12 D cycle of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (cool white fluorescent lamps) at the flask surface, shaken each day and had no aeration. At early stationary phase (typically 12 mg C l^{-1}), 10% (v/v) of the suspension was used to start the next batch by inoculation into fresh medium. Experiments were duplicated or triplicated with good reproducibility. The maximum carbon-specific growth rates in these cultures were 0.9 d^{-1} for the diatom and 0.65 and 0.5 d^{-1} for *H. carterae* and *M. squamata*, respectively. Samples were withdrawn every day via siphon tube. DFAAs and DCAAs were measured by HPLC using the method of Flynn & Flynn (1992) after the DCAA samples had first been subjected to acid hydrolysis (Keil & Kirchman 1991). Cell numbers and biovolume were determined in triplicate by an Elzone 282PC analyser, particulate carbon and nitrogen in duplicate by elemental analysis (Europa RoboPrep) and ammonium in duplicate by an Alpkem REA/2 microsegmented analyser.

(b) *Model development*

The ammonium–nitrate interaction model (ANIM; Flynn *et al.* 1997) is a dynamic mechanistic model of algal nitrogen metabolism describing the assimilation of nitrate and ammonium through glutamine to cell nitrogen. The maximum rates of ammonium and nitrate transport vary with nitrogen status and are described by parameters named, respectively, NH_4Tq and NO_3Tq (see also Flynn *et al.* 1999). Various feedback regulations from the size of internal nutrient pools control the rates of nutrient transport and assimilation.

For this work, the ANIM was modified by redefining the pool of glutamine (GLNP) as internal low molecular weight DON (InDONP). The maximum size of this pool was set at 0.03 g $\text{N g}^{-1} \text{C}$ (ca. 850 mM) to match a typical cell content (cf. Flynn 1990). Ammonium and nitrate transports were now controlled by the size of InDONP, rather than by GLNP as in the ANIM. Transport of DON into InDONP was made a hyperbolic function of the concentration of DON. The half saturation constant for transport of DON was set at 1 μM (cf. Flynn & Syrett 1986) and the maximum transport rate set at 10% of NH_4Tq , the maximum rate of ammonium transport (cf. Syrett *et al.* 1986). The control of release is problematical because of

our ignorance of the operating processes and the possibility that release of some forms of DON may be more important under different nutritional states. Eddy (1982) discussed the interaction between transport and leakage of compounds through the same porter; if this is a major route of DON loss (and it must be a route) then it follows that efflux will be a hyperbolic function of the internal substrate concentration. Here we made release a hyperbolic function of InDONP with a maximum rate of 5% of NH_4Tq . The overall response of the model, the residual level of DON outside the cells and the maximum gross release rates of DON (in the region of 25% of incoming DIN; cf. Bronk *et al.* 1994) are of the correct magnitude. A parallel model was constructed to enable the flow of ^{15}N to be followed through the system, assuming no isotopic discrimination. The model was run to simulate, at different nutrient loadings (1, 10 and 100 $\mu\text{mol N l}^{-1}$), steady-state growth under continuous light and the nitrogen-sufficient phase of batch growth in a light:dark cycle. Simulations were also run of ^{15}N experiments performed during the latter stages of exponential growth of the batch system.

3. RESULTS

(a) *Experimental*

Low- and high-phosphorus cultures showed similar patterns (figure 1) except that growth in low-phosphorus media was slower for *H. carterae*. Carbon-specific growth continued for several days after the ammonium was exhausted and the latter third of the time indicated in the plots in figure 1 was spent in the stationary phase. There was no evidence that low-phosphorus cells, which were relatively more nitrogen replete, released more DON. Variability in PON measurements after exhaustion of the ammonium, which explains most of the error in the calculated line fitted through the system nitrogen (figure 1), reflects sampling problems at low cell densities. If all system nitrogen is accounted for then this fitted line should be horizontal. There was only a significant failure to account for all system nitrogen for *M. squamata* in high-phosphorus (but not low-phosphorus) conditions (figure 1). This was at least in part due to adherence of cells onto the walls of the culture vessel, a common event in nitrogen-depleted cultures of prasinophytes (K. J. Flynn, unpublished data).

The total amount of DFAA nitrogen amounted to a few micromoles while the total of the combined and dissolved free amino acids (CDFAAs) amounted to up to 10% of system nitrogen for the flagellates. However, for the diatom *Thalassiosira*, the amounts of both DFAAs and CDFAAs increased gradually as cells passed into the stationary phase, eventually exceeding 20% of system nitrogen. The amount of DON did not rise in any culture in strict proportion with the increase in PON. Glutamate, serine, glycine and alanine (50–100 nM each) dominated the composition of DFAAs. Of the species used, *H. carterae* was not axenic; the filters used had a rated pore size of 1 μm that would allow bacteria to pass and so contaminate the CDFAA fraction. The level of CDFAAs is thus, if anything, an overestimate for this flagellate.

(b) *Simulations*

Simulations of nitrogen-limited chemostat systems (figure 2) indicated that the concentration of nitrogen as

Figure 1. Changes in system nitrogen, PON, CDFAAs, DFAAs and ammonium (DIN) during batch growth in high- and low-phosphorus media, respectively. (a,b) *Heterosigma carterae*, with symbol legend. (c,d) *Mantoniella squamata*. (e,f) *Thalassiosira pseudonana*. The change in system nitrogen is marked with a linear regression line with 95% confidence limits. The 95% confidence limits are within twice the symbol size for DFAAs and CDFAAs.

DON was small for all systems (figure 2c). However, the proportion of system nitrogen as DON and the proportion of DIN taken up and released as DON were both inversely proportional to the level of system nitrogen (figure 2a and 2d). In simulations of batch cultures, higher biomass systems attained higher residual levels of DON (figure 3a) but proportionately lower losses (figure 3b). In the lowest biomass system a greater period of time

was spent with a significant net loss of DON to the medium (figure 3c).

The results from the simulated ^{15}N method failed to estimate the net loss of nitrogen from the cells correctly (figure 4). The margin and degree of error varied both with the biomass of the system and with the duration of the incubation (which affected the degree of isotope disequilibrium). Generally, the net release was significantly overestimated

Figure 2. Simulations of steady-state growth at different growth rates (μ) in continuous light. Culture systems contained a total system nitrogen of 1 (dotted lines), 10 (dashed lines) or 100 (solid lines) $\mu\text{mol N l}^{-1}$. (a) Changes in the allocation of system nitrogen between PON (heavy lines) and DON. (b) External ammonium (DIN). (c) External DON. (d) Percentage of ammonium (DIN) taken into cells and subsequently released as DON.

for high and medium biomass systems. Release in the low biomass system could be of the correct order (depending on the duration of the incubation) but this was because the low biomass system was not at equilibrium with its environment at the time of testing, with the concentration of DON still rising during day 3 (figure 3a). Because of the dynamics of the system (time taken for the different internal and external pools to reach equilibrium) the rate of release as indicated by ^{15}N was usually also less than the real gross rate of release (figure 4, cf. figure 3c).

4. DISCUSSION

The results of the experimental work are entirely consistent with those we have obtained in the past (e.g. Davidson *et al.* 1992; Flynn *et al.* 1993). When using axenic or non-axenic cultures of $\geq 100 \mu\text{mol N l}^{-1}$ we account for $>95\%$ of system nitrogen as DIN + PON, while with the systems of *ca.* $50 \mu\text{mol N l}^{-1}$ described here we accounted for 80–90% of system nitrogen in these fractions (the balance as DON; figure 1). These values match those predicted by the model. Thus, the amount of system nitrogen present as DON amounted to $<5\%$ in the

$100 \mu\text{mol N l}^{-1}$ system (figure 3b) and the $10 \mu\text{mol N l}^{-1}$ system containing levels of DON (figure 3a) proportionately similar to those of CDEAAs in the experimental systems described earlier (*ca.* 10%; figure 1). In the simulated very low biomass system (more typical of oceanic populations) the concentration of DON was lowest but the proportion of nitrogen as DON highest (attaining 20%; figure 3), consistent with reports from field studies (e.g. Bronk *et al.* 1994).

From our experiments the bulk of DON released appears to be CDEAAs (figure 1). If there was a more significant release of non-refractory organic nitrogen in dense cultures (i.e. in line with the value of system nitrogen) then one may expect a very significant growth of bacteria (in terms of biomass). This is not our experience with high biomass non-axenic algal cultures, either in batch or chemostat systems.

Experimental sampling and incubation protocols disturb or destroy cell aggregates, removing mucilage that may retain leaked organics close to the cell. This problem may be of particular importance in low biomass suspensions, where large volumes of suspension must be filtered. Retention of mucilage with the PON, rather than

Figure 3. Simulations of batch growth in a 12 L:12 D cycle during nitrogen-sufficient growth. Culture systems contained a total system nitrogen of 1 (dotted lines), 10 (dashed lines) or 100 (solid lines) $\mu\text{mol N l}^{-1}$. DIN (ammonium) was exhausted by days 4 or 5. (a) Changes in the external concentrations of DON. (b) Contribution of DON to system nitrogen. (c) Gross (light lines) and net (heavy lines) release rates of DON relative to DIN transport.

being washed through into the DON fraction, depends on whether the shear stress during filtration carries it through the filter. However, PujolPay *et al.* (1997) reported that cell rupture could contribute little to the 'loss' of ^{15}N label and experiments conducted by us (not shown) also indicated that the amount of DFAAs which leak during harsh filtration protocols may add little to the external DON pool.

Figure 4. Percentage of DI^{15}N originally transported into the cells and now present outside the cells as DO^{15}N (light lines) in simulated batch cultures with total system nitrogen contents of 1 (dotted lines), 10 (dashed lines) or 100 (solid lines) $\mu\text{mol N l}^{-1}$ as shown in figure 3. The real net percentages lost as DON (heavy lines) are those indicated for these systems in figure 3c for this time period.

There are various direct and indirect forms of evidence for a dynamic equilibrium of organics from phytoplankton. The quantity and quality of DFAAs in new cultures rise rapidly to predictable levels, while additions of DFAAs are consumed down to similar levels (Flynn & Butler 1986; Flynn 1990; John & Flynn 1999). The concentrations of organics such as free amino acids within algae are 10^5 or more greater than outside the cell (Flynn 1990). As a consequence, some leakage may be expected, together with mechanisms to recover the organics. Only smaller uncharged forms of DON (such as glycine) may be expected to pass through the plasma membrane unaided (Raven 1980). Leakage of others will occur through transport proteins (Eddy 1982), as a function of the relative and absolute concentrations outside and inside the cell or, for larger molecules, perhaps through exocytosis. That a continual drain of energy may be required just to maintain a high constant internal concentration of low molecular weight metabolites suggests that the operation of porters is required simply to make good any leakage back through the porter (Eddy 1992). In our experiments, DFAAs constituted say 25–50% of the DON (figure 1), so there can be little doubt that at least this proportion of DON can or does participate in a dynamic exchange. In at least some instances there may be a clear sequence of significant loss and recovery of DON (Collos *et al.* 1992) such as DFAAs (Flynn & Gallon 1990; Glibert & Bronk 1994). Other organics (e.g. siderophores, transport-binding proteins and vitamins; Trick *et al.* 1983; Davies & Leftley 1985; Hunter *et al.* 1997) are also released from algae and then recovered.

In the simulations of a batch system, while the net loss from the higher biomass systems declined rapidly as levels of DON rose, the loss from the low biomass system remained higher for longer (figure 3). The timing reflects

the combined loss of DON from all cells, raising the external level of DON to a point at which a net loss no longer occurs. This concept would also apply where compounds are released but not retrieved if the unidirectional release was a function of the external concentration. It could explain the 'critical inoculum' problem encountered in culture work with certain species. Some cultures either do not grow or do so with a prolonged lag phase if only a small inoculum is used in subculturing work, perhaps because an inordinate amount of energy and materials are expended in balancing influx and efflux.

There are solid grounds to suspect that uptake and release are associated, at least for smaller compounds, through the bidirectional operation of porters (Eddy 1982). The model, employing such a bidirectional mechanism, can reproduce the results of both field and laboratory studies based on a dynamic equilibrium of organics between the cells and the external medium. The simulations indicate that under certain conditions the proportion of nitrogen being taken up as DIN and released as DON may indeed be high. The implications of these results may be extended to the original concerns over the release of dissolved organic carbon (Fogg 1977; Sharp 1977) and apply equally to the whole retrieval of compounds or part thereof through the activity of external enzymes (Palenik & Morel 1990).

However, it appears that ^{15}N assays may not give a reliable estimate of the net loss of DON (figure 4). Neither may they necessarily give good estimates of gross loss. Whether the assays yield results closer to net or gross loss depends on the duration of the assay and the dynamic equilibrium between the organism and its environment. The problems are exacerbated in longer incubations by external isotope dilution with nutrient regeneration and by processes such as surface adsorption of the organics (Bronk & Glibert 1991; Slawyk & Raimbault 1995). Estimates of release rates should be considered as maximal and not necessarily indicative of net rates of loss from the phytoplankton to their environment. Of equal concern is the resultant underestimation of phytoplankton production.

These problems also apply to studies of bacterial production. Indeed, as bacteria rather than phytoplankton (Kirchman 1994) may use a significant proportion of ^{15}N ammonium, bacterial activity is likely to be just as important for the loss of ^{15}N from the DIN + PON fraction (Bronk & Glibert 1994). Bacteria also release organics such as proteolytic enzymes and ^{15}N added as ammonium may be lost rapidly as DO^{15}N (Middelboe *et al.* 1995). In time this material will be recovered by microbes as the enzymes are degraded to DFAAs. On the other hand, however, the magnitude of the release of organics by algae will have been seriously underestimated by laboratory physiologists if the use of cultures of high biomass has minimized the impact of processes dependent on the establishment of equilibrium between the cells and their environment.

This work was supported by the University of Wales—Swansea, the Natural Environment Research Council's (UK) 'Testable models of aquatic ecosystems' special topic and European Union support for L.S.B.

REFERENCES

- Admiraal, W., Laane, R. W. P. M. & Peltier, H. 1984 Participation of diatoms in the amino acid cycle of coastal waters; uptake and excretion in cultures. *Mar. Ecol. Prog. Ser.* **15**, 303–306.
- Antia, N. J., Harrison, P. J. & Oliveira, L. 1991 The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. *Phycologia* **30**, 151–154.
- Berg, G. M., Glibert, P. M., Lomas, M. W. & Burford, M. A. 1997 Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. *Mar. Biol.* **129**, 377–387.
- Bronk, D. A. & Glibert, P. M. 1991 A ^{15}N tracer method for the measurement of dissolved organic nitrogen release by phytoplankton. *Mar. Ecol. Prog. Ser.* **77**, 171–182.
- Bronk, D. A. & Glibert, P. M. 1993 Application of a ^{15}N tracer method to the study of dissolved organic nitrogen uptake during spring and summer in Chesapeake Bay. *Mar. Biol.* **115**, 501–508.
- Bronk, D. A. & Glibert, P. M. 1994 The fate of the missing ^{15}N differs among marine systems. *Limnol. Oceanogr.* **39**, 189–195.
- Bronk, D. A., Glibert, P. M. & Ward, B. B. 1994 Nitrogen uptake, dissolved organic nitrogen release, and new production. *Science* **265**, 1843–1846.
- Collos, Y., Dohler, G. & Biermann, I. 1992 Production of dissolved organic nitrogen during uptake of nitrate by *Synedra planctonica*. *J. Plankt. Res.* **14**, 1025–1029.
- Davidson, K., Flynn, K. J. & Cunningham, A. 1992 Non-steady state ammonium-limited growth of the marine phytoflagellate *Isochrysis galbana* Parke. *New Phytol.* **122**, 433–438.
- Davies, A. G. & Leftley, J. W. 1985 Vitamin B_{12} binding by microalgae ectocrines: dissociation constant of the vitamin–binder complex determined using an ultrafiltration technique. *Mar. Ecol. Prog. Ser.* **21**, 267–273.
- Dugdale, R. C. & Goering, J. J. 1967 Uptake of new and regenerated forms of nitrogen in primary production. *Limnol. Oceanogr.* **12**, 196–206.
- Eddy, A. A. 1982 Mechanisms of solute transport in selected eukaryotic micro-organisms. *Adv. Microbial Physiol.* **23**, 2–78.
- Falkowski, P. G. 1994 The role of phytoplankton photosynthesis in global biogeochemical cycles. *Photosynth. Res.* **39**, 235–258.
- Flynn, K. J. 1990 Composition of intracellular and extracellular pools of amino acid utilisation of microalgae of different sizes. *J. Exp. Mar. Biol. Ecol.* **139**, 151–166.
- Flynn, K. J. & Butler, I. 1986 Nitrogen sources for the growth of marine microalgae: role of dissolved free amino acids. *Mar. Ecol. Prog. Ser.* **34**, 281–304.
- Flynn, K. J. & Flynn, K. 1992 Non-protein free amines in microalgae: consequences for the measurement of intracellular amino acids and of the glutamine/glutamate ratio. *Mar. Ecol. Prog. Ser.* **89**, 73–79.
- Flynn, K. J. & Gallon, J. R. 1990 Changes in intracellular and extracellular α -amino acids in *Gloeotheca* during N_2 -fixation and following addition of ammonium. *Arch. Microbiol.* **153**, 574–579.
- Flynn, K. J. & Syrett, P. J. 1986 Characteristics of the uptake system for L-lysine and L-arginine in *Phaeodactylum tricornutum*. *Mar. Biol.* **90**, 151–158.
- Flynn, K. J., Davidson, K. & Cunningham, A. 1993 Relations between carbon and nitrogen during growth of *Nannochloropsis oculata* (Droop) Hibberd under continuous illumination. *New Phytol.* **125**, 717–722.
- Flynn, K. J., Fasham, M. J. R. & Hipkin, C. R. 1997 Modelling the interactions between ammonium and nitrate uptake in marine phytoplankton. *Phil. Trans. R. Soc. Lond.* **B352**, 1625–1645.

- Flynn, K. J., Page, S., Wood, G. & Hipkin, C. R. 1999 Variations in the maximum transport rates for ammonium and nitrate in the prymnesiophyte *Emiliania huxleyi* and the raphidophyte *Heterosigma carterae*. *J. Plankt. Res.* (In the press.)
- Fogg, G. E. 1977 Excretion of organic matter by phytoplankton. *Limnol. Oceanogr.* **22**, 576–577.
- Glibert, P. M. & Bronk, D. A. 1994 Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria, *Trichodesmium*. *Appl. Environ. Microbiol.* **60**, 3996–4000.
- Harrison, P. J., Waters, R. E. & Taylor, F. J. R. 1980 A broad spectrum seawater medium for coastal and open ocean phytoplankton. *J. Phycol.* **16**, 28–35.
- Harrison, P. J., Parslow, J. S. & Conway, H. L. 1989 Determination of nutrient uptake kinetic parameters: a comparison of methods. *Mar. Ecol. Prog. Ser.* **52**, 301–312.
- Hunter, K. A., Kim, J. P. & Croot, P. L. 1997 Biological roles of trace metals in natural waters. *Environ. Monitor. Assess.* **44**, 103–147.
- John, E. H. & Flynn, K. J. 1999 Amino acid uptake by the toxic dinoflagellate *Alexandrium fundyense*. *Mar. Biol.* (In the press.)
- Keil, R. G. & Kirchman, D. L. 1991 Dissolved combined amino acids in marine waters as determined by a vapor-phase hydrolysis method. *Mar. Chem.* **33**, 243–259.
- Kirchman, D. L. 1994 The uptake of inorganic nutrients by heterotrophic bacteria. *Microbial Ecol.* **28**, 255–271.
- Lu, M. & Stephens, G. C. 1984 Demonstration of net influx of free amino-acids in *Phaeodactylum tricornutum* using high performance liquid chromatography. *J. Phycol.* **20**, 584–589.
- Middelboe, M., Borch, N. H. & Kirchman, D. L. 1995 Bacterial utilization of dissolved free amino acids, dissolved combined amino acids and ammonium in the Delaware Bay estuary: effects of carbon and nitrogen limitation. *Mar. Ecol. Prog. Ser.* **128**, 109–120.
- Palenik, B. & Morel, F. M. M. 1990 Comparison of cell surface L-amino acid oxidases from several marine phytoplankton. *Mar. Ecol. Prog. Ser.* **59**, 195–201.
- PujoPay, M., Conan, P. & Raimbault, P. 1997 Excretion of dissolved organic nitrogen by phytoplankton assessed by wet oxidation and ¹⁵N tracer procedures. *Mar. Ecol. Prog. Ser.* **153**, 99–111.
- Raven, J. A. 1980 Nutrient transport in microalgae. *Adv. Microbial Physiol.* **21**, 48–226.
- Sharp, J. H. 1977 Excretion of organic matter by marine phytoplankton: do healthy cells do it? *Limnol. Oceanogr.* **22**, 381–399.
- Slawyk, G. & Raimbault, P. 1995 Simple procedure for simultaneous recovery of dissolved inorganic and organic nitrogen in ¹⁵N tracer experiments and improving the isotopic mass-balance. *Mar. Ecol. Prog. Ser.* **124**, 289–299.
- Syrett, P. J., Flynn, K. J., Molloy, C. J., Dixon, G. K., Peplinska, A. M. & Cresswell, R. C. 1986 Effects of nitrogen deprivation on rates of uptake of nitrogenous compounds by the diatom, *Phaeodactylum tricornutum* Bohlin. *New Phytol.* **102**, 39–44.
- Trick, C. G., Andersen, R. J., Price, N. M., Gillam, A. & Harrison, P. J. 1983 Examination of hydroxymate-siderophore production by neritic eukaryotic marine phytoplankton. *Mar. Biol.* **75**, 9–17.
- Wheeler, P. A. & Kirchman, D. L. 1986 Utilisation of inorganic and organic nitrogen by bacteria in marine systems. *Limnol. Oceanogr.* **31**, 998–1009.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.