Comparison of two models in the estimation of nitrogen uptake rates using data from 15-N incubation experiments*

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Abstract

This paper compares two uptake rate models, Dugdale & Goering's (D&G) model and Elskens' model. The aim is to provide an insight into how estimates of uptake processes, i.e. regeneration and loss rates from both dissolved and particulate nitrogen pools, influence the total uptake rates when the two models are compared. The uptake rates of three nitrogenous nutrients (nitrate, ammonium and urea) from 15-N incubation experimental data were compared. The comparison indicated that the D&G model underestimated nitrate uptake rates by about 34%, implying a significant regeneration and loss rates of the nutrient. Elskens' model further showed that the loss rates from the dissolved phase were about 40% and 25% for the ammonium and urea pools, respectively, indicating that the D&G model underestimated the experimental uptake rates of the nutrients. On

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average, nitrification made up about 30% of the total ammonium uptake flux, whereas the sinks from particulate nitrogen and dissolved nitrogen were estimated at 36% and 56%, respectively. The D&G model sometimes overestimated the f-ratio values to about 60% and higher as a result of ammonium and urea uptake rates underestimation. This paper also shows that detritus adsorption, bacterial uptake and cell lysis are equally important processes.

1. Introduction

Dugdale & Goering (1967) developed a model, now in common use, for calculating nitrogen uptake rates by phytoplankton in the marine environment. Based on the application of the nitrogen isotope (15-N) by means of incubation experiments, the model was developed on the assumptions (Nees et al. 1962) that (a) isotopic fractionation during the uptake process is negligible, (b) nitrogen is not regenerated or recycled during the incubation period, and (c) the disappearance of nitrogen from the substrate pool is balanced by the appearance of an equal amount of particulate nitrogen. Apart from these assumptions, the concept of new and regenerated production (Dugdale & Goering 1967) was also recognised as being important in the nutrition of phytoplankton; this implies that regenerated nitrogen is not negligible.

Although ammonium was the identified regenerated nutrient, the concept draws attention to the importance of nutrient regeneration processes. It is worth noting that decreasing the incubation time has been recommended when using the Dugdale & Goering (D&G) 15-N incubation method in order to avoid nutrient regeneration. But this is not necessarily a good solution given the uncertainties of the analytical methods, especially when the calculations involve concentration differences; as a result, random errors may be extremely large. Because of these limitations, several other models have been developed with the aim of improving the determination of uptake rates.

Blackburn (1979) and Caperon et al. (1979) introduced linear differential equations for estimating rates of ammonium uptake and remineralisation by considering isotopic balances in the dissolved phase. Overall, their equations predict that the biological production of unlabelled ammonium within the system results in an exponential decrease of 15-N atom enrichment with incubation time. However, Glibert et al. (1982) reported some limitations when ambient ammonium is near the limit of detection, or when the concentration is not significantly different at the beginning or end of the incubation. They therefore introduced a correction method for isotope dilution of the ammonium pool directly into the D&G equation. Another modification was by Garside & Glibert (1984), who used a computer-based

model to consider isotopic balance in both dissolved and particulate phases. In practice, this computational approach essentially yields the same results as the calculations of Glibert et al. (1982) but with a number of advantages, the greatest of them being its predictive ability, which permits several experimental designs to be tested before the actual experiments are done.

An alternative model for estimating the influence of ammonium (from the process of ammonification) was developed by Lancelot & Billen (1985), who focused on bacterial processes as sources of ammonium. Indeed, from measurements of bacteria production and protozoa grazing rates, Goeyens et al. (1991) observed that there was an agreement between the prediction of the model and the values measured by the 15-N dilution experiment, implying that a large part of ammonium regeneration was due to bacteria and grazers rather than exudation by plankton.

Laws (1984) suggested other processes affecting the determination of uptake rates: nitrification, adsorption to the walls of polycarbonate containers and/or clay suspended particles, and the release of dissolved organic nitrogen (DON) by plankton. Literature supports this argument since there are a number of field data suggesting that an average of 25 to 41% of the dissolved inorganic nitrogen (NH_4^+ and NO_3^-) taken up by phytoplankton are released as DON in oceanic, coastal and estuarine environments (Bronk & Glibert 1991, 1993, Bronk et al. 1994, Slawyk & Raimbault 1995). Collos (1998) reported that the effect of nitrite excretion in estimating nitrogen uptake rates could be as great as or even greater than the effect of DON release. Furthermore, it is also recognised that in nature, several nitrogen sources may be present and utilised simultaneously by primary producers during incubation; hence, the unlabelled nitrogen could lead to significant underestimations of uptake rates (Collos 1987).

The processes mentioned in the above models are a series of important findings that require the underlying assumptions of 15-N models to be re-examined. Elskens et al. (2002) provided a general framework for studying the propagation of errors via numerical modelling. This approach was used to check the estimation performance of various models: Dugdale & Goering (1967), Blackburn (1979), Glibert et al. (1982), Garside & Glibert (1984), and Laws et al. (1985). It was concluded that the accuracy of the estimated N-flux rates, and to some extent their precision, were both poor. The main reason is that the models give insufficient consideration to bias, and to conditions which could lead to error magnification.

It is clear that a better analysis of nitrogen fluxes in the marine environment requires the quantification of the largest possible number of processes. In practice, however, quantifying these processes is unlikely to

be a routine procedure; therefore, evaluation of the data obtained from the classical 15-N-tracer experiment is important. This constitutes the main objective of this paper.

2. Material and methods

We examined data obtained from 15-N incubation experiments on the basis of the performance of the Elskens model (Elskens et al. 2002). Data were used to calculate uptake rates using both the D&G model and the Elskens model.

Seawater was sampled in 1996, 1997 and 1998 from the North Sea using the normal uptake rate incubation procedure in order to determine the uptake rates of NO_3^-, NH_4^+ and urea by phytoplankton. The experiments were initiated by determining the concentrations of NO_3^-, NH_4^+ and urea following the filtration of the subsamples through Whatman glass-fibre filters (GF/F). Three 2-litre polycarbonate bottles, one for each of the three nutrients, were filled with the sample water, to which labelled nutrients were then added, i.e. NO_3^- (99.5% 15-N), NH_4^+ (99.8% 15-N) and urea (99% 15-N) at concentrations of less than 10% of their corresponding ambient concentrations. Incubations were done by using a shore-floating incubator at natural light irradiance for 24 hours. The final concentrations of particulate matter (PM) from each of the incubation bottles were collected on Whatman glass-fibre filters (GF/F) pre-combusted at 450°C. The particulate nitrogen (PN) in the PM was converted to nitrogen by a modified version of Duma's method (Fiedler & Proksch 1975) and its ^{15}N abundance was measured by emission spectrometry using Jasco NIA-1 or N-151 ^{15}N Analysers. Calibration was done with certified standards (Goeyens et al. 1985).

At the end of each incubation experiment, the concentrations of each nutrient were again analysed in order to monitor the net change of dissolved nutrient concentrations. Nitrate concentrations were determined by an automated diazotation method (D'Elia 1983). The ammonium concentrations were determined by the indophenol-blue method (Koroleff 1969), the urea concentrations by an adapted diacetylmonoxime method (Goeyens et al. 1998). The uptake rates of NO_3^-, NH_4^+ and urea were calculated using the D&G model. Other uptake rate parameters were established from the difference in concentrations before and after the incubation time, that is, the total concentration change divided by the total incubation time. This was based on the original assumptions of the model that in the absence of external influencing factors (regeneration, nitrification, adsorption, etc.), the two methods for calculating uptake rates would give similar uptake values. By using the same data the uptake rates have been recalculated using the Elskens model.

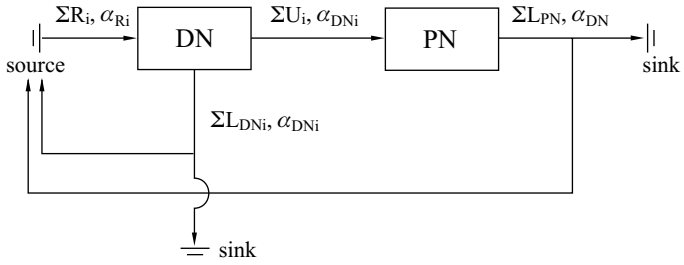
2.1. Data validation

Bearing in mind the limitations of the D&G model in providing accurate uptake rates, the data were worked up using the Elskens model in order to take into account the following effects as summarised in the scheme below:

(i) Simultaneous uptake of three different nitrogen sources (nitrate, ammonium and urea);

(ii) Regeneration processes of the dissolved nitrogen pools;

(iii) Loss rates from the dissolved and particulate nitrogen pools



where α_i is the isotopic enrichment (ratios of 15-N:14+15-N) associated with the corresponding nitrogen flux rates; R_i = regeneration, U_i = uptake; L_{DNi} = Dissolved nitrogen (DN_i) loss and L_{PN} = Particulate nitrogen (PN) loss.

It is noted that R_i, L_{DNi} and L_{PN} involve a variety of processes, the importance of which is closely dependent on experimental conditions. From the above scheme, the source term is explained as representing all processes giving rise to the regeneration of dissolved nutrient pools during incubation, such as excretion of nitrogen (organic and inorganic) by the planktonic community, bacterial transformation from one nitrogen source to another (e.g. ammonification, nitrification), whereas the sink term represents all the processes responsible for the nitrogen lost during the time of incubations. These may include the adsorption of nitrogen to clay particles and/or to container walls, nitrogen uptake by bacteria passing through the GF/F filters used in 15-N studies, and the break-up of cells containing 15-N as a result of filtration stress. The differential equations describing the system for both 14+15-N and 15-N isotopes can be followed from the model (Elskens et al. 2002).

The results from the Elskens model were calibrated in order to determine the interval of mathematically possible solutions for the uptake rates from the ecological point of view by establishing maximum and minimum values of possible solutions. Logically, all solutions (maximum and minimum) do not have the same possibility of being correct values. Therefore, an external

calibration method was required to verify which of the model solution sets was a realistic prediction. This was achieved by comparing the model results with those of the ^{14}C incorporation experiments (V. Rousseau, personal communication) through the calculation of C/N uptake ratios. The method was applied to the 1998 data only, since no results of ^{14}C incorporation for 1996 and 1997 were available.

3. Results

3.1. Uptake rate analyses and validity

In Fig. 1, the uptake of the three nutrients (ammonium, nitrate and urea) indicated that the D&G model predicted values were not linearly correlated to the uptake rates calculated from concentration differences (between initial and final concentrations at the end of the incubation time). This is additional evidence for the inconsistency with the assumptions made under the model calculations. It indicated that the results did not fit the mass balance for both dissolved and particulate nitrogen pools. During the incubation period there was a greater decrease in nutrient concentrations that could not be accounted for by the calculated uptake rates. It is clear from the figure that uptake rates from the D&G model were lower than

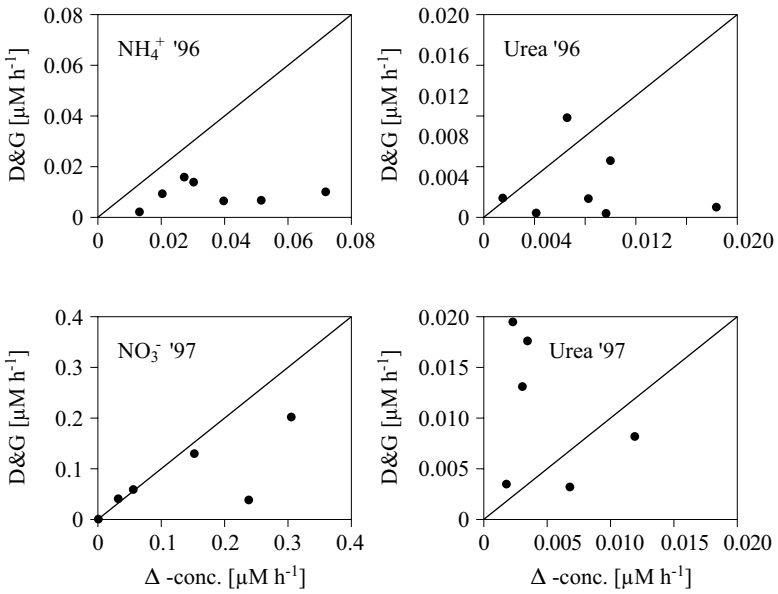


Fig. 1. Scatter plot of some of the observed divergence in the uptake rates derived from Dugdale & Goering's (D&G) and those derived from the differences between the initial and final concentrations of the nutrients (Δ-conc.) during incubation. The bisect lines indicate the expected linearity of the data sets

those calculated from the concentration differences, indicating that the D&G model did not account for some of the processes that lead to the disappearance of the dissolved nutrient pool. Although the divergence from the linear relationship applied to all three nutrients, ammonium uptake rates appeared to be below the points of expected linearity. The scatter of nitrate uptake rates was less compared to ammonium; the rates of urea uptake displayed the widest scatter.

While the use of the isotope dilution models explained in the introduction can help us to account for ammonium regeneration, Fig. 1 none the less shows a lack of balance between initial and final (after the incubation) concentrations even for nitrate and urea.

3.2. Calibration of results

By using the Elskens model various uptake parameters were recalculated for comparison with values initially calculated by the D&G model. When the C/N ratios were plotted (Fig. 2a) the minimum estimates were the most likely correct values, although with the limited data available, the points fluctuate along a mean of 8.5, slightly above the Redfield ratio. It should be noted that most of the data lie between the warning lines, drawn two standard errors from the mean (sem $= \sigma/\sqrt{\mathrm{n}}$) at 2.7 and 26.1 respectively, within the variations of C/N ratios (3–20) usually reported for marine phytoplankton (Caperon & Meyer 1972, Laws & Wrong 1978) and the ratios of the suspended matter (6–12) of our sample from the same study area over the period 1996–1999. When considering the C/N ratios corresponding to the maximum estimates (Fig. 2b), the points fluctuate along a centre line of 1.3 with warning lines drawn at 0.2 and 6.9. Contrary to appearances, the probability of obtaining such a value estimated by the binomial distribution is only 0.18. These results lead to the rejection of the maximum estimates in favour of the minimum values. Note that this does not mean that the minimum estimates of the Elskens model are 'true' uptake rates, rather that they merely provide reasonable values given the expected variability in the C/N uptake ratios. In this context, two interesting observed patterns merit our attention:

(i) The C/N uptake ratios in Fig. 2a display an inverse relationship with the nitrogen concentration (Table 1), being low at the highest ambient levels of nitrogen (> 18 μM) and increasing with nitrogen diminishing to 0.1 μM.

(ii) In April, the C/N uptake ratio was substantially higher than the range usually reported in the literature (3–20).

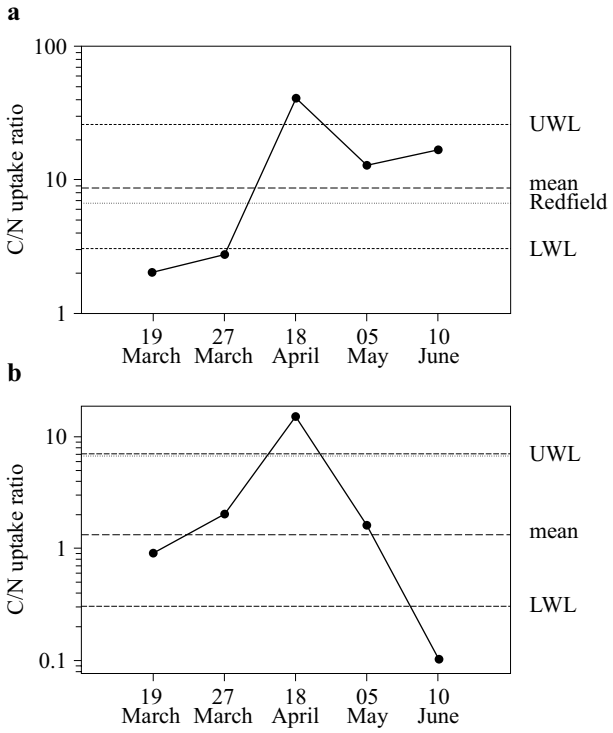


Fig. 2. Calibration of Elskens model results using minimum (a) and maximum estimates (b) of the solution locus in calculating C/N uptake ratios. Note that a log scale was used on the y-axis because the sampling distribution of the C/N uptake ratio is approximately log-normally distributed. UWL – upper warning line; LWL – lower warning line

Table 1. Concentrations of individual nutrients measured during experiments and the corresponding total N concentrations

Date	Concentration [μM]				
	NH_4^+	Urea	NO_3^-	NO_2^-	Total N concentration
19 March 1998	1.25	0.32	19.02	0.39	20.98
27 March 1998	0.39	0.24	17.41	0.00	18.04
18 April 1998	0.27	0.21	0.13	0.00	0.61
05 May 1998	1.02	0.74	0.00	0.00	1.76
10 June 1998	0.86	0.37	0.19	0.00	1.42

4. Discussion

It has been a tradition to emphasise ammonium regeneration in order to explain the balance of nitrogen between the dissolved and particulate pools. However, in the present study, this was insufficient to explain the balance between concentrations before and after incubation, not just for ammonium, but also for nitrate and urea. Dugdale & Wilkerson (1986) drew attention to a similar problem, and although they managed to correct

their results for isotope dilution, they were unable to provide a balance for the differences between the initial and final concentrations. In fact, this problem has also been tackled by other authors (Laws 1984, Price et al. 1985, Williams & Fisher 1985, Ward et al. 1989). However, it has been assumed hitherto that the lack of balance is due to combined effects, including the release of nitrogen by the plankton community, regeneration, grazing and bacterial uptake. Elskens' model was applied to the data in order to explain the possible cause of these observations. Application of the model gives two sets of uptake rates, maximum estimates and minimum estimates.

The calibration of results favoured the minimum estimated values. However, two points have been highlighted as a result of comparing C/N uptake ratios in Figs 2a and 2b. Firstly, the fact that the ratios were inversely related to the nitrogen concentrations can be explained as being the result of a general physiological adaptation of phytoplankton. This was demonstrated by Lancelot & Mathot (1985), who reported the results of unicellular algae growing in nitrogen-deficient conditions, where as an adaptation, they synthesised reserve products with low nitrogen content. The second point was the abnormally high C/N ratio. This can be explained in relation to the predominance of *Phaeocystis* sp. in the phytoplankton assemblages during the period. Primary production was excessively high, as it coincided with the bloom period of *Phaeocystis* sp. From the literature, it is known that no bloom of single cells has ever been reported; rather, the bloom was of a colonial form of *Phaeocystis* sp. (Lancelot et al. 1998). Further, during the bloom maximum, Lancelot et al. (1987) estimated that the mucilaginous matrix makes up approximately 90% of the colony's biomass. Because mucilage is polysaccharide in nature (Van Boekel 1992, Lancelot & Rousseau 1994), *Phaeocystis* sp. must require more carbon during the bloom period. This may have raised the C/N ratio above the Redfield proportion.

4.1. Significance and validity limits of the D&G model

Since the D&G equation does not consider processes such as regeneration and loss rates, the major questions are therefore by how much uptake rates are biased and what the resulting impact on the N-uptake parameters, e.g. f-ratios, will be. The results of this intercomparison exercise are summarised in Fig. 3. It appears that estimates of uptake rates by the D&G equation are consistently underestimated (paired t-test) with respect to the output of the Elskens model. However, both model results show a similar seasonal trend. In addition, it is noted that the pattern of underestimation varies from one nitrogen source to another.

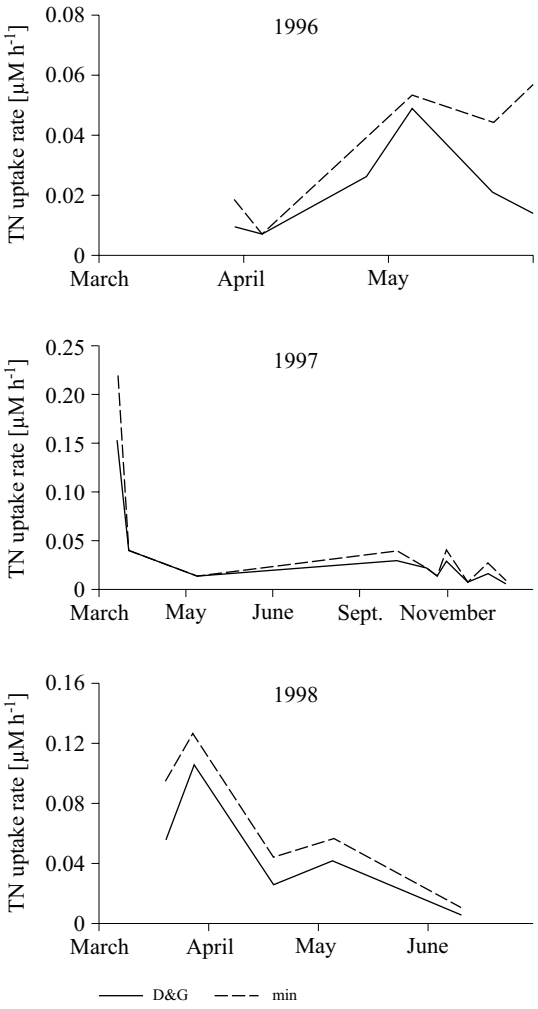


Fig. 3. Trends of TN uptake rates calculated using Dugdale & Goering's model (D&G) and minimum estimates from Elskens' model

The range of deviation for nitrate was less than that of ammonium or urea (Fig. 4). The respective nitrate, ammonium and urea maximum underestimations were 34%, 78% and 95%. In general, the underestimation median ranges for all nutrients lay below 60%. The values (Fig. 3) collectively implied that with regard to ammonium and urea, uptake rates contributed significantly to the underestimation of nitrogen uptake rates by the D&G model. However, in the total uptake trends the underestimation is masked by higher uptake rates for nitrate than for ammonium and urea. This is demonstrated by the trend of f-ratios (Fig. 5) in which the ratios obtained from the Elskens model indicated that by using the D&G model,

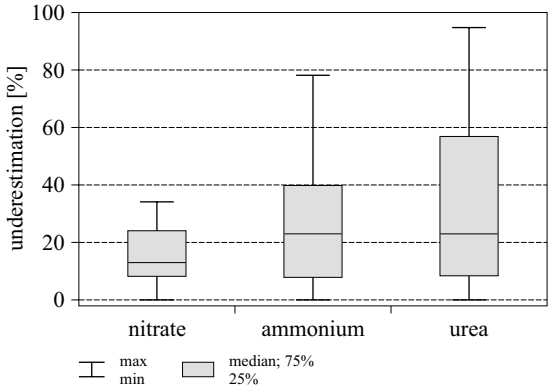


Fig. 4. Deviations of uptake rates calculated by the D&G equation from the minimum estimates of the model by Elskens et al. (2002)

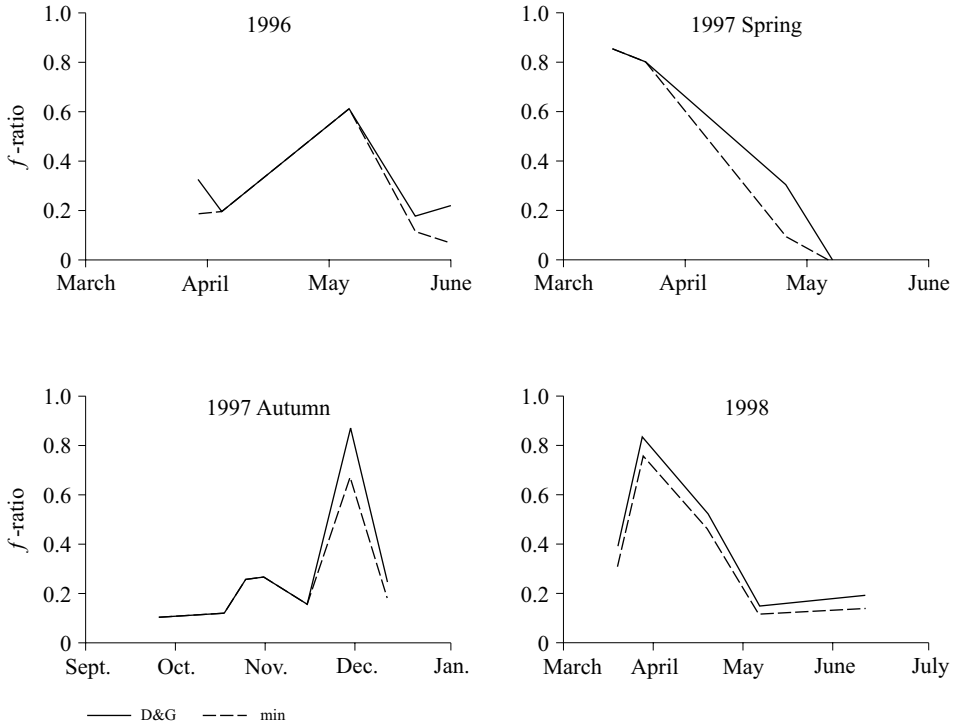


Fig. 5. Comparison of f-ratios and variations for the uptake rates calculated by the D&G model and for the minimum estimated uptake rates obtained using Elskens' model

the status of nitrate and its importance in primary production is sometimes considerably overstated, for example, by 64% on 31 May 1996 and by 66% on 25 April 1997 (calculated from the ratio differences). Other calculations indicated overestimation of f-ratios over a range of about 1–40%.

4.2. Nitrogen fluxes

The Elskens model provided estimates of uptake rates together with regeneration (R_i), loss rates from the dissolved (L_{DNi}) and the particulate (L_{PN}) nitrogen pools. Synopses of these rates are illustrated in Fig. 6. Overall, these results can be summarised thus:

(i) Regeneration and loss rates were not negligible in relation to uptake rates for most of the 15-N tracer experiments.

(ii) Nitrogen demand exceeded regeneration in all experiments, implying that changes in nutrient concentrations with time ($\partial_t NH_4^+$, $\partial_t NO_3^-$ and ∂_turea) < 0 over the incubation period. The average ratios of production to consumption were 0.96, 0.22 and 0.32 for ammonium, nitrate and urea, respectively.

(iii) Loss rates from the dissolved nutrient pools represented a significant fraction of the total nitrogen consumption fluxes with average values of 40, 33 and 25% for ammonium, nitrate and urea, respectively. Since physico-chemical adsorption is likely to be minimal for nitrate and urea, these results suggested an important role for bacteria during the incubation experiments via direct assimilation and/or transformation reactions from one nitrogen source to another (e.g. ammonification, nitrification, etc).

(iv) The loss rate from the urea pool was 4.8 nM h^{-1}. This is assumed to be indicative partly of an ammonification rate which was in fact comparable to that calculated from the model of Lancelot & Billen (1985) – an average value of 4.5 nM h^{-1}. It is noted that both estimates are substantially lower than the average ammonium regeneration rate of 34.5 nM h^{-1} provided by the model, which suggests that direct excretion of ammonium by zooplankton was the most important process during the experiments. Note also that this process is mainly responsible for urea regeneration (Cho & Azam 1995). Hence, it is estimated that ammonium and urea excretions respectively represented 49% and 14% of the loss rate from the particulate nitrogen pool.

(v) The source-sink processes were estimated on an average basis to be 57% and 43% of the total nitrogen loss rates (L_{DNi} + L_{PN}). As already pointed out, loss rates from the dissolved and particulate nitrogen are source-sink processes with respect to the equation for N-mass conservation: the source term represents all processes giving rise to the regeneration of nutrients during the incubation period, while the sink term represents the nitrogen lost within the time of incubation, as a result of e.g. bacterial uptake, adsorption, cell lysis and mortality.

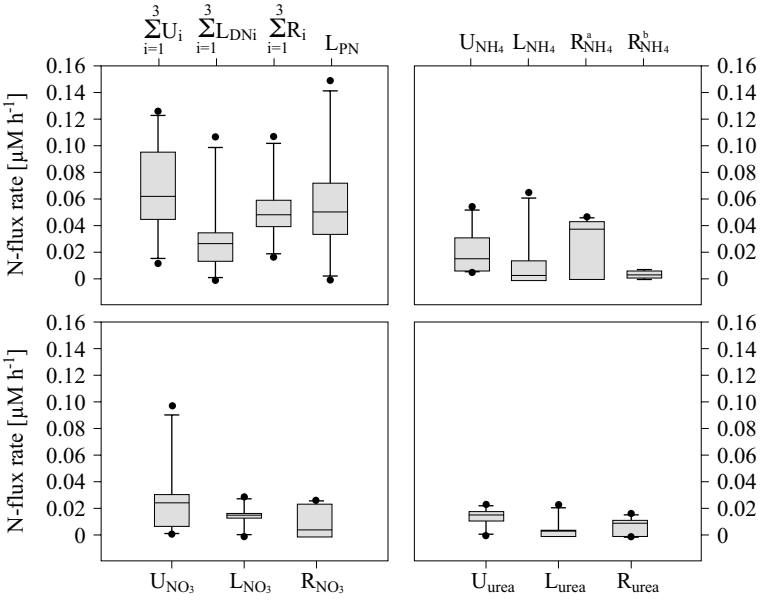


Fig. 6. Box and whisker plots of N-flux rates for 1998 from the Elskens model approach. The values correspond to the minimum estimates of the model. U_{NH4}, U_{NO3} and U_{urea} – corresponding nutrient uptake rates; L_{NH4}, L_{NO3} and L_{urea} – corresponding nutrient loss rates; L_{PN} – loss rate of PN; R_{NO3} and R_{urea} – corresponding nutrient regeneration rates; $R^a{}_{NH4}$ – ammonium regeneration estimated by Lancelot & Billen's model; $R^b{}_{NH4}$ – ammonium regeneration estimated by Elskens' model; $\sum_{i=1}^{3} U_i$, $\sum_{i=1}^{3} L_{DNi}$ and $\sum_{i=1}^{3} R_i$ – sum of uptake, loss and regeneration rates of the three nutrients

(vi) Finally, the particulate nitrogen sink was estimated to be 36% of the L_{PN} flux, which stands in agreement with the range of values usually reported in the literature (25 to 41%; Bronk et al. 1994), whereas the dissolved nitrogen sink term, which is required to close the mass balance equation, would then represent 56% of the total L_{DNi} fluxes.

These exchanges between the dissolved and particulate nitrogen pools are summarised in Fig. 7. It is important to stress that 15-N tracer experiments were not performed in the replicates owing to practical handling limitations. Hence, the low number of degrees of freedom impedes significance testing, and the values given in Fig. 7 are therefore only indicative of the general behaviour of the system and the variability of the two models. The variations in corresponding fluxes are as shown in Fig. 6. They illustrate the complexity of the system to be tackled by the oversimplified approach of Dugdale & Goering model (1967).

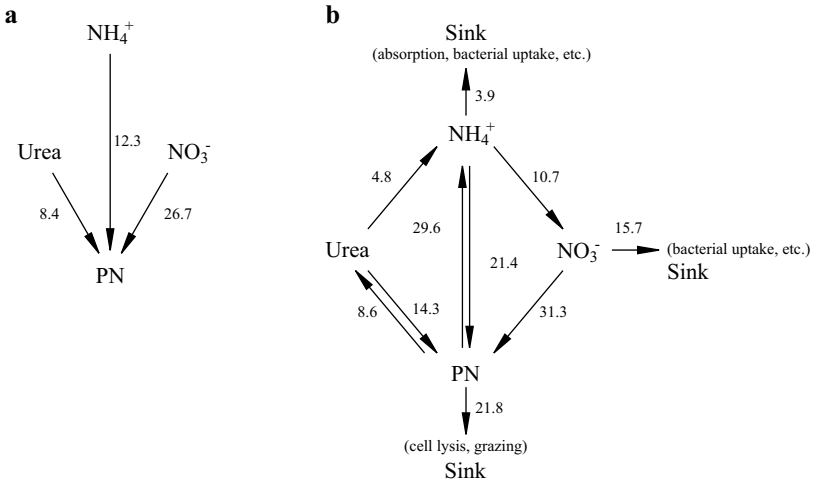


Fig. 7. Comparison of nitrogen flux rates as estimated from the equation of (a) Dugdale & Goering (1967) and (b) the Elskens model (2002). Nitrogen flux rates are presented as mean estimated values expressed in nM h^{-1}

5. Conclusion

From this comparison, it is apparent that the 15-N technique for measuring uptake rates needs refinement in order for more parameters to be accommodated in routine measurements. Similarly, uptake rates calculated by the D&G model need to be corrected if precise values are to be used. The underestimation seems to be important as regards the uptake rates of ammonium and urea, and this explains why the f-ratios estimated by the D&G model were higher than those estimated by the Elskens model. Nevertheless, the underestimation does not affect the seasonal trend of uptake rates, and f-ratios suggesting that any conclusion made based on the trend of concentrations are acceptable. Great emphasis has commonly been placed on ammonium regeneration, but as this data comparison has shown, we cannot neglect urea regeneration and nitrification. Likewise, we cannot ignore the loss rates of dissolved nitrogen through detritus adsorption, bacterial uptake, and that possibly escaping through the GF/F filter and cell lysis.

With reference to the 1998 results, it is shown that we need to establish biological parameters (more than flux rates) of the phytoplankton communities in the environment under investigation, for example, the variable POC:PON ratios at different growth stages of phytoplankton. This is important especially for a dynamic environment like the North Sea, where the two major phytoplakton communities (diatoms and *Phaeocystis* sp.)

display significant physiological differences and behave quite differently in the same environment. Even within the *Phaeocystis* sp. there are known differences depending on the development stage which show that organic carbon derived from *Phaeocystis* colonies has a C/N ratio of about 27, whereas the ratio from solitary cells is about 7 (S. Becquevort, pers. comm.).

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