

COMMENT

Limnol. Oceanogr., 45(8), 2000, 1879–1883
© 2000, by the American Society of Limnology and Oceanography, Inc.

Magnitude of dissolved organic nitrogen release relative to gross nitrogen uptake in marine systems

The magnitude and ecological significance of rates of dissolved organic nitrogen (DON) production and release in aquatic systems have long been the subject of investigation and controversy. Recently, Slawyk et al. (1998) reported “low ^{15}N losses to the DON pool” in a laboratory and field study. It is important to note that though the amount of ^{15}N that ultimately passes into the DON pool may be small, it can translate into a large DON release rate relative to the rate of gross nitrogen uptake. Slawyk et al. (1998) further note that “these loss rates are considerably lower (by a factor of 3–6) than those previously reported from culture (Collos 1992; Collos et al. 1992) and field work (Bronk et al. 1994).” In Bronk et al. (1994), percent release values were presented that represented the full range of values that had been measured by the authors at that time. Subsequent researchers have tended to focus only on the low or, more commonly, on the high end of the range. When the ratio of DON release to gross nitrogen uptake is computed from all available published reports, however, the range of values encompass the range published in Bronk et al. (1994) and demonstrate that DON is consistently a significant flux.

The objectives of this comment are to clarify confusions in current terminology and calculation protocols, to review available DON release data in light of the contention that DON release is a small percentage of gross nitrogen uptake (Slawyk et al. 1998), to offer insight into the various methodologies that should be considered when comparing DON release rate data, and to bring additional experimental data to bear on theoretical calculations presented in Slawyk et al. (1998). We close with a general discussion of DON release in marine systems.

As a starting point, the terminology used to describe nitrogen uptake and release is reviewed because it is in a state of some confusion (Table 1). Dugdale and Goering (1967) introduced the ^{15}N -based nitrogen uptake rate (ρ). Bronk et al. (1994) specified this traditionally measured rate a “net” uptake rate to denote that it is a measure of the rate of nitrogen uptake into cells that remains in the cells at the end of the incubation, also referred to as particulate nitrogen (PN) production (Table 1). This net uptake rate is in contrast to a gross uptake rate (ρ_G), which is defined as DON production plus PN production (Bronk et al. 1994).

With regard to release processes, Bronk and Glibert (1991) introduced a DON release rate that was a measure of the release of DON from an intracellular organic nitrogen (ON) pool to the extracellular DON pool (Bronk and Glibert 1991, Eq. 2; Bronk and Glibert 1993). Bronk (1999) suggested that this rate be specified the intracellular pool (IP)

DON release rate to indicate that intracellular pools are used in its calculation. Slawyk et al. (1998, Eq. 3) refer to this rate as ρ_{DON} .

A much less labor intensive means of measuring DON release rates was later introduced by Bronk et al. (1994). This new calculation protocol was based solely on the change in the ^{15}N atom percent enrichment of the extracellular DON pool and did not require isolation and measurement of the intracellular ON pool (Bronk et al. 1994, note 7). This DON release rate was calculated as the difference between the gross nitrogen uptake rate (ρ_G) and the traditionally determined net nitrogen uptake rate (ρ).

$$\text{DON release} = \rho_G - \rho, \quad (1)$$

with the gross nitrogen uptake rate (ρ_G) calculated as follows:

$$\rho_G = \frac{(\text{PN} \times \text{PN at \%xs}) + (\text{DON} \times \text{DON at \%xs})}{\text{DIN at \%xs} \times \text{Time}} \quad (2)$$

where PN and DON at %xs are the ^{15}N atom percent enrichments of the PN and DON pools minus the atom percent of an atmospheric nitrogen standard, DIN at %xs is the atom percent excess enrichment of the dissolved inorganic nitrogen (DIN) pool, and time is the period of incubation (Bronk et al. 1998, Eq. 3). The DON release rate in Eq. 1 above is equivalent to the rate termed $\rho_{\text{DIN}}^{\text{loss}}$ by Slawyk et al. (1998, Eq. 2).

Bronk (1999) discussed the differences in the two types of DON release rates and distinguished them as the IP DON release rate versus the extracellular pool (EP) DON release rate. Though in general the two rates are similar, short-term variations in the atom percent enrichment of the intracellular ON pool would be expected to cause some variations between them (Bronk 1999). It was also noted that the IP DON release rate is very labor intensive and therefore is unlikely to be widely applied. It also requires a host of assumptions and therefore is less robust than the EP DON release method. As a result, the EP DON release rate has become more common. The IP and EP distinctions are only necessary when both types of rates are being discussed.

In the interest of consistency in terminology and therefore to facilitate comparison of data, we suggest that the previously established terminology be adopted. Specifically, the more common EP DON release rate should be taken as “the DON release rate” (Bronk et al. 1994). In the special case when the transfer of nitrogen from the intracellular to the extracellular DON pool is being measured, that rate should be specified an IP DON release rate as originally described

Table 1. Terminology used to describe different nitrogen flux rates.

Rate	Description	References
ρ (net uptake)	Particulate nitrogen (PN) production	Dugdale and Goering (1967); Bronk et al. (1994, 1998)
ρ_G (gross uptake)	PN plus dissolved organic nitrogen (DON) production	Bronk et al. (1994, 1998)
IP DON release	Rate of DON release from the intracellular organic nitrogen pool to the extracellular DON pool	Bronk and Glibert (1991, 1993); Bronk (1999)
DON release	Equivalent to ρ_{DON}	Slawyk et al. (1998)
	Difference between rates of gross and net N uptake ($\rho_G - \rho$). Can be specified an extracellular pool (EP) DON release rate.	Bronk et al. (1994, 1998)
	Equivalent to $\rho_{\text{DIN}}^{\text{loss}}$	Slawyk et al. (1998)

(Bronk 1999). Further, the designation of ρ_{DON} should not be used to describe the IP DON release rate (Slawyk et al. 1998) because ρ has been used to define uptake rates since the ^{15}N method's introduction (Dugdale and Goering 1967). As a result, ρ_{DON} suggests a DON uptake rate rather than a release rate. Along these same lines, we suggest that $\rho_{\text{DIN}}^{\text{loss}}$ not be used to denote a DON release rate because DON is not specified in this notation and there are other possible nitrogen pools to which ^{15}N label could be lost in addition to DON.

Turning attention to the contention of Slawyk et al. (1998) that their DON loss rates are much less than others previously reported, the available literature data are reviewed in Table 2. DON release, as a percentage of gross DIN uptake, generally varies from ~ 10 to 35% in a wide range of environments (mean for all measured rates in Table 2 is $25 \pm 21\%$, $n = 30$). In the past, direct comparisons of release data have been difficult because many researchers do not present actual DON release rates (Table 2). To allow some direct comparisons, rates of DON release were calculated using data from a 24-h time course conducted in the North Atlantic, presented in Slawyk and Raimbault (1995), and the equations presented in Slawyk et al. (1998) for calculating DON release (i.e., $\rho_{\text{DIN}}^{\text{loss}}$). The data used to calculate the rates were derived from DON measurements of GF/F filtrates. Bronk and Glibert (1994) note that in some cases GF/F filtrate can result in an overestimation of rates when NH_4^+ is the substrate; in a wide range of environments, no such overestimation has ever been demonstrated when NO_3^- is the substrate. For incubation times comparable to the incubation times in Bronk et al. (1994 and 1998), the ratio of DON release to gross uptake was 39 ± 15 and $36 \pm 8\%$ for incubations with ^{15}N -labeled NH_4^+ and NO_3^- respectively; as noted above, the NH_4^+ release may be overestimated to some degree but the NO_3^- rates can be taken as robust. When the entire time course is averaged, the percent DON release was 24 and 29% for NH_4^+ and NO_3^- , respectively (Table 2). These data do not support the general conclusion that DON release, as a percentage of gross nitrogen uptake, is low. In another publication that presented a larger data set from the same field study reported by Slawyk et al. (1998), Raimbault et al. (1999) note that "DON release via excretion, cell lysis, or sloppy feeding [in their study] might represent at least ~ 20 –100% of the new production" in the equatorial Pacific. Though no actual DON release rates were presented, these

estimates of percent DON release as a function of gross nitrogen uptake are comparable and even higher than those published in the past. Taken at face value, this statement again appears in conflict with the conclusion of Slawyk et al. (1998). The body of work to date presents a consistent picture where DON release is indeed a significant fate for DIN in planktonic marine systems.

Although the majority of published DON release rates vary in the range of ~ 10 to 35% of gross nitrogen uptake, occasionally the percent release is much higher or lower (Table 2). We suggest that these higher and/or lower rates likely represent the presence or absence of different trophic interactions such as grazing (Ward and Bronk unpubl. data). As with any outliers, however, a first impulse is to look for experimental artifacts. With respect to DON release measured with ^{15}N , there are three potential artifacts in particular that must be considered: filtration stress, environmental stress, and problems in isolating the DON pool for isotopic analysis.

First, if cells rupture or leak during the filtration process (e.g., Goldman and Dennett 1985; Kirchman et al. 1989), release of DOC and DON will be overestimated. This process could explain the occasional outliers in the data sets summarized in Table 1. However, when one compares a given system through time, depths throughout a single vertical profile, or one environment with another and observes consistent trends of higher or lower rates of DON release, it is imprudent to dismiss these trends as the result simply of filtration. The novel approach of Slawyk et al. (1998) removes the need for filtration and will allow gross uptake rates to be estimated without this potential artifact; unfortunately it does not remove the filtration step from the measurement of DON release. The question of filtration artifacts remains one of the most pressing issues that needs to be resolved.

Second, any environmental stress during sample collection or incubation also has the potential to result in an overestimate of DON release rates. One example of this was the possible light stress during sample collection suggested as one possible explanation for the very high DON release rates observed at the base of the euphotic zone in Monterey Bay (Bronk and Ward 1999; Lomas and Glibert 1999). To obtain accurate DON release rates, every effort must be made to maintain samples at in situ light and temperature conditions at all times and to avoid any additional environmental stresses.

Table 2. Published rates of DON release and ratios of DON release to gross DIN uptake determined using ¹⁵N tracer techniques. Methods include ion retardation by Bronk and Glibert (1991; IRC), wet chemical by Slawyk and Raimbault (1955; WC-SR), and wet chemical by Bronk and Ward (1999; WC-BW). Data are mean ± standard deviation. NP, indicates no data presented. When ranges are presented, the mean percentage release was calculated using the median value.

Location	Date/ culture	Substrate	DON release rate ng-at N L ⁻¹ h ⁻¹	DON release: gross uptake (%)	Method	References
Oceanic						
Caribbean Sea	Nov 1988	NH ₄ ⁺	9.8 ± 3.0	27.8 ± 8.0	IRC	Bronk et al. (1994)
Southern Calif. Bight	Oct 1992	NH ₄ ⁺	26.4 ± 2.1	50.0 ± 0.9	IRC	Bronk et al. (1994)
Southern Calif. Bight	Oct 1992	NO ₃ ⁻	4.2 ± 1.5	74.1 ± 1.3	IRC	Bronk et al. (1994)
Ross Sea, Antarctica	Nov/Dec 1994	NO ₃ ⁻	66.5*	19.0 ± 13.9	IRC	Hu and Smith (1998)
Ross Sea, Antarctica	Dec/Jan 1995/6	NO ₃ ⁻	27.2*	8.0 ± 3.8	IRC	Hu and Smith (1998)
North Atlantic	Sep/Oct 1992	NH ₄ ⁺	1.6 to 3.1†	24.1 ± 4.9†	WC-SR	Slawyk and Raimbault (1995)
North Atlantic	Sep/Oct 1992	NO ₃ ⁻	0.2 ± 0.5†	28.8 ± 10.4†	WC-SR	Slawyk and Raimbault (1995)
Equat. and oligo. Pacific	Nov 1994	NH ₄ ⁺ NO ₃ ⁻	NP	15.3 ± 13.4	WC-SR	Slawyk et al. (1998)
			Mean ± std	30.9 ± 21.4		
Coastal						
Southern Calif. Bight	Oct 1992	NH ₄ ⁺	12.4 ± 3.2	20.5 ± 1.6	IRC	Bronk et al. (1994)
Southern Calif. Bight	Oct 1992	NO ₃ ⁻	3.4 ± 0.2	34.3 ± 3.6	IRC	Bronk et al. (1994)
Monterey Bay, California	Mar 1993	NH ₄ ⁺	61.9 ± 47.2	16.2 ± 12.0	WC-BW	Bronk and Ward (1999)
Monterey Bay, California	Mar 1993	NO ₃ ⁻	1.4 ± 0.7	22.2 ± 33.6	WC-BW	Bronk and Ward (1999)
Monterey Bay, California	Sep 1993	NH ₄ ⁺	20.8 ± 20.1	64.7 ± 22.1	WC-BW	Bronk and Ward (1999)
Monterey Bay, California	Sep 1993	NO ₃ ⁻	12.2 ± 2.2	85.7 ± 14.3	WC-BW	Bronk and Ward (1999)
Akkeshi Bay, Japan	May 1998	NH ₄ ⁺	NP	3.6 ± 0.1‡	WC-SR	Hasegawa et al. in press
Akkeshi Bay, Japan	Jun 1998	NH ₄ ⁺	NP	4.9 ± 0.4‡	WC-SR	Hasegawa et al. in press
Akkeshi Bay, Japan	Aug 1998	NH ₄ ⁺	NP	2.7 ± 0.1‡	WC-SR	Hasegawa et al. in press
			Mean ± std	28.8 ± 28.5		
Estuarine						
North Chesapeake Bay	Apr 1989	NH ₄ ⁺	51.8	34.0	IRC	Bronk et al. (1994)
North Chesapeake Bay	Apr 1989	NO ₃ ⁻	60.6	11.0	IRC	Bronk et al. (1994)
South Chesapeake Bay	Apr 1989	NH ₄ ⁺	36.8 ± 32.1	26.3 ± 12.6	IRC	Bronk et al. (1994)
Mid-Chesapeake Bay	May 1988	NH ₄ ⁺	71.6 ± 59.2	27.8 ± 18.3	IRC	Bronk et al. (1998)
Mid-Chesapeake Bay	Aug 1988	NH ₄ ⁺	55.3 ± 47.0	14.2 ± 8.3	IRC	Bronk et al. (1998)
Mid-Chesapeake Bay	Oct 1988	NH ₄ ⁺	33.4 ± 39.1	28.4 ± 25.4	IRC	Bronk et al. (1998)
Choptank River	Aug 1990	NH ₄ ⁺	193.1 ± 118.4	12.6 ± 8.8§	IRC	Bronk and Glibert (1993)
			Mean ± std	22.0 ± 9.2		
Culture						
<i>Trichodesmium</i>	Jan/Feb 1992	N ₂ gas	114.0 ± 83.4	44.4 ± 31.8	IRC	Glibert & Bronk (1994)
<i>Synechococcus</i> 7803		NH ₄ ⁺	0.018 ± 0.016¶	10.4 ± 5.9	IRC	Bronk (1999)
<i>Synechococcus</i> 8018		NH ₄ ⁺	0.016 ± 0.009¶	11.8 ± 5.6	IRC	Bronk (1999)
<i>P. tricornutum</i> and <i>D. tertiolecta</i>		NO ₃ ⁻	NP	3.9#	WC-SR	Pujo-Pay et al. (1997)
<i>Dunaliella tertiolecta</i>		NO ₃ ⁻	NP	6 to 21	WC-SR	Slawyk et al. (1998)
<i>Microcystis novacekii</i>		NH ₄ ⁺	106.7 ± 35.1	20.9 ± 3.7**	IRC	Nagao and Miyazaki (1999)
			Mean ± std	17.6 ± 14.2		

* Calculated from uptake and percent release data.

† Calculated using raw data for the complete time-course in Slawyk and Raimbault (1995) and equations in Slawyk et al. (1998).

‡ Calculated using data from the <90 μm fraction and so grazing is likely reduced or absent.

§ Calculated from gross uptake rates from the >202 μm size fraction for the first 6 h of incubation.

|| Measurements were made with picked *Trichodesmium* colonies during a cruise in the Caribbean, units are pmol N colony⁻¹ h⁻¹.

¶ In units of fg-at N cell⁻¹ h⁻¹.

Estimated using the ¹⁵N mass balance.

** Cells preserved with formalin prior to filtering.

Third, overestimation of DON release is not the only potential problem. Very low or undetectable DON release rates should also be a cause for concern due to experimental artifacts in the isolation of DON for isotopic analysis. The method introduced by Slawyk and Raimbault (1995) requires that samples be incubated under basic conditions and elevated temperatures for two weeks. Slawyk and Raimbault (1995) report a recovery of 91.2 and 98.9% of the DON pool for seawater from the coast of Marseille. In a study in Akkeshi Bay, however, Hasegawa et al. (2000) used the Slawyk and Raimbault (1995) method and report that final recovery of DON was "seldom higher than 80%." In other trials using the Slawyk and Raimbault (1995) method, only $42.7 \pm 8.8\%$ of the total DON pool, from two rivers in Georgia and the South Atlantic Bight, was recovered at the end of the incubation (Bronk unpubl. data). Loss of DON would be expected to vary depending on the composition of the DON pool, which could explain these different recovery efficiencies.

The problem in the isolation is likely the lengthy diffusion step under hot, basic conditions that is used to remove NH_4^+ and $\text{NO}_3^-/\text{NO}_2^-$ from solution. If dissolved organic matter is heated for an extended period of time under basic conditions, base hydrolysis is likely to occur, which could result in loss of amino nitrogen as ammonia. Indeed, some researchers use a diffusion step very similar to the Slawyk and Raimbault (1995) method as a way of removing labile DON before isolation of NO_3^- (Sigman et al. 1997). A similar loss of DON was also observed during the development of the wet chemical isolation technique used in Bronk & Ward (1999). It was found that a brief period of heating of the sample on a hot plate with a buffer was less destructive to the DON than the weeklong incubation under basic conditions. Regardless of the method used, variations in DON isolation efficiency must always be considered when analyzing DON release data. If recently released labile DON is lost from the DON pool, the small change in the atom percentage enrichment can result in a large underestimate of DON release.

In an attempt to put some constraints on sustainable rates of DON release, Slawyk et al. (1998) presented a series of theoretical calculations aimed at constraining estimates of sustainable rates of DON release. The approach was sound but experimental data indicates a different conclusion. The argument is that a 50% loss of ^{15}N to the DON pool ($\rho_{\text{DIN}}^{\text{loss}}$) relative to gross DIN uptake will result in a highly detectable loss of particulate organic nitrogen (PON) such that the PON pool would totally dissolve into DON in ~ 3 d (Slawyk et al. 1998). To make this argument, the assumption is made that the atom percent enrichment of the intracellular ON pool is approximately equal to the atom percent enrichment of the total PON pool. Based on direct measurements, however, the atom percent enrichment of the intracellular ON pool is significantly more enriched than the total PON pool. In the culture studies presented by Bronk (1999), the ratio of the atom percent enrichments of the intracellular ON pool relative to the total PN pool was 20.5 ± 9.9 ($n = 10$) at the end of a 1-h incubation. Even for a highly productive estuarine system such as Chesapeake Bay, the intracellular ON pool takes $\gg 6$ h to approach the atom percent of the PN pool (Fig. 1). These observations seem intuitively correct because the intracellular dissolved nitrogen pools would

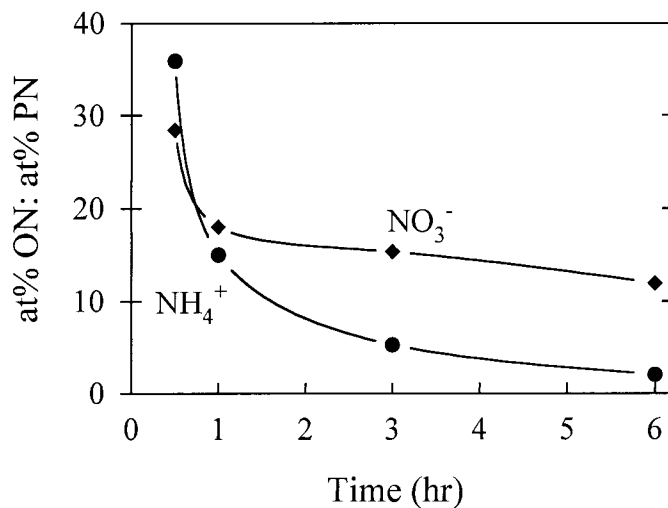


Fig. 1. The ratio of the atom percentage excess enrichment of the intracellular organic nitrogen (ON) pool to the atom percentage excess enrichment of the particulate nitrogen (PN) pool during a time-course experiment in April 1990 in Chesapeake Bay.

have much shorter turnover times than the entire pool of cellular nitrogen such that as the incubation continued, the atom percent enrichment of the intracellular DON pool and total PON pool should become more similar over time but would take many hours to reach isotopic equilibrium. This is analogous to the long period of time required for the various intracellular carbon pools to reach isotopic equilibrium in ^{14}C studies.

With these data in hand, one can repeat the calculations of Slawyk et al. (1998) assuming a more realistic factor, e.g., $R_{\text{DONi}} = 20 R_{\text{PON}}$. Repeating the calculations for the worse case 50% loss scenario, holding other assumptions constant, the required DON release rate would then be 15 nM d^{-1} or 25% of gross DIN uptake. This rate is lower than could be detected as a loss of PON and is well within the range of sustainability with our present understanding of plankton dynamics and within the range of rates reported in Table 2.

In conclusion, the microbial community in marine and aquatic systems is dependent on dissolved organic matter, including DON, produced and released by primary producers. That these rates of DON release vary temporally and spatially is to be expected. That these rates can occasionally be a large percentage of the nitrogen taken up by cells is very likely an indication of the physiological condition of the cells themselves and, more importantly, their relationship with other trophic levels. When compared overall, the body of work on DON release published to date provides a consistent picture that indicates DON release is a significant flux through marine and aquatic systems (Table 2). We agree with Slawyk et al. (1998) that high ratios of DON release to gross uptake ($> 50\%$) are likely a result of cells dying (such as at the end of a bloom) or during intense grazing and sloppy feeding. It is counterintuitive that healthy cells would excrete significant amounts of DON (Sharp 1977) unless they are being affected by grazing or viral infection. Clearly there is much work to be done to define the mechanisms that produce these observations, such as sloppy feeding, viral infec-

tion, and physiological limitation. There is also a need to incorporate the cycling of DON into the bigger picture of DOC flux under a range of conditions.

Deborah A. Bronk¹

Department of Marine Sciences, School of Marine Programs
University of Georgia
Athens, Georgia 30602-3636

Bess B. Ward

Department of Geosciences
Princeton University
Princeton, New Jersey 08544

References

- BRONK, D. A. 1999. Rates of NH_4^+ uptake, intracellular transformation, and dissolved organic nitrogen release in two clones of marine *Synechococcus* spp. *J. Plankton Res.* **21**: 1337–1353.
- , AND P. M. GLIBERT. 1991. A ^{15}N tracer method for the measurement of dissolved organic nitrogen release by phytoplankton. *Mar. Ecol. Prog. Ser.* **77**: 171–182.
- , AND ———. 1993. Contrasting patterns of dissolved organic nitrogen release by two size fractions of estuarine plankton during a period of rapid NH_4^+ consumption and NO_2^- production. *Mar. Ecol. Prog. Ser.* **96**: 291–299.
- , AND ———. 1994. The fate of the missing ^{15}N differs among marine systems. *Limnol. Oceanogr.* **39**: 189–195.
- , ———, T. C. MALONE, S. BANAHAN, AND E. SAHLSTEN. 1998. Inorganic and organic nitrogen cycling in Chesapeake Bay: Autotrophic versus heterotrophic processes and relationships to carbon flux. *Aquatic Microb. Ecol.* **15**: 177–189.
- , ———, AND B. B. WARD. 1994. Nitrogen uptake, dissolved organic nitrogen release, and new production. *Science* **265**: 1843–1846.
- , AND B. B. WARD. 1999. Gross and net nitrogen uptake and DON release in the euphotic zone of Monterey Bay, California. *Limnol. Oceanogr.* **44**: 573–585.
- COLLOS, Y. 1992. Nitrogen budgets and dissolved organic matter cycling. *Mar. Ecol. Prog. Ser.* **90**: 201–206.
- , G. DOHLER, AND I. BIERMANN. 1992. Production of dissolved organic nitrogen during uptake of nitrate by *Synedra planctonica*: Implications for estimating new production in the oceans. *J. Plankton Res.* **14**: 1025–1029.
- DUGDALE, R. C., AND J. J. GOERING. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnol. Oceanogr.* **12**: 196–206.
- GLIBERT, P. M., AND D. A. BRONK. 1994. Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria *Trichodesmium* spp. *Appl. Environ. Microbiol.* **36**: 3996–4000.
- GOLDMAN, J. C., AND M. R. DENNETT. 1985. Susceptibility of some marine phytoplankton species to cell breakage during filtration and post filtration rinsing. *J. Exp. Mar. Biol. Ecol.* **86**: 47–58.
- HASEGAWA, T., I. KOIKE, AND H. MUKAI. 2000. Release of dissolved organic nitrogen by size-fractionated natural planktonic assemblages in coastal waters. *Mar. Ecol. Prog. Ser.* **198**: 43–49.
- HU, S. H., AND W. O. SMITH. 1998. The effects of irradiance on nitrate uptake and dissolved organic nitrogen release by phytoplankton in the Ross Sea. *Cont. Shelf Res.* **18**: 971–990.
- KIRCHMAN, D. L., R. G. KEIL, AND P. A. WHEELER. 1989. The effect of amino acids on ammonium utilization and regeneration by heterotrophic bacteria in the subarctic Pacific. *Deep-Sea Res.* **36**: 1763–1776.
- LOMAS, M. W., AND P. M. GLIBERT. 1999. Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms. *Limnol. Oceanogr.* **44**: 556–572.
- NAGAO, F., AND T. MIYAZAKI. 1999. A modified ^{15}N tracer method and new calculation for estimating release of dissolved organic nitrogen by freshwater planktonic algae. *Aquat. Microb. Ecol.* **16**: 309–314.
- PUJO-PAY, M., P. CONAN, AND P. RAIMBAULT. 1997. Excretion of dissolved organic nitrogen by phytoplankton assessed by wet oxidation and N-15 tracer procedures. *Mar. Ecol. Prog. Ser.* **153**: 99–111.
- RAIMBAULT, P., AND OTHERS. 1999. Carbon and nitrogen uptake and export in the equatorial Pacific at 150°W: Evidence for an efficient regenerated production cycle. *J. Geophys. Res.* **104**: 3341–3356.
- SHARP, J. H. 1977. Excretion of organic matter by phytoplankton: Do healthy cells do it? *Limnol. Oceanogr.* **22**: 381–399.
- SIGMAN, S. D., M. A. ALTABET, R. MICHNER, D. C. MCCORKLE, B. FRY, AND R. M. HOLMES. 1997. Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: An adaptation of the ammonia diffusion method. *Mar. Chem.* **57**: 227–242.
- SLAWYK, G., AND P. RAIMBAULT. 1995. Simple procedure for simultaneous recovery of dissolved inorganic and organic nitrogen in ^{15}N -tracer experiments and improving the isotopic mass balance. *Mar. Ecol. Prog. Ser.* **124**: 289–299.
- , ———, AND N. GARCIA. 1998. Measuring gross uptake of ^{15}N -labeled nitrogen by marine phytoplankton without particulate matter collection: Evidence for low ^{15}N losses to the dissolved organic nitrogen pool. *Limnol. Oceanogr.* **43**: 1734–1739.

Acknowledgements

This research was supported by NSF grant OPP-9530732 to D.A.B. and NSF grant OCE-9115940 to B.B.W.

Received: 18 October 1999
Amended: 17 February 2000
Accepted: 20 February 2000