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Release of Dissolved Organic Nitrogen by Marine Diazotrophic Cyanobacteria, *Trichodesmium* spp.†

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***Trichodesmium* sp. is a filamentous, colonial cyanobacterium which contributes substantially to the input of nitrogen in tropical and subtropical oceanic waters through nitrogen fixation (N_2 fixation). We applied a ^{15}N tracer technique to assess the rate of release of dissolved organic nitrogen (DON) from this cyanobacterium and compared those rates with rates of N_2 fixation determined for the same assemblages at the same times of day. Rates of release of DON showed considerable variation within replicate experiments and were variable depending on time of day and duration of time course experiments. On average, rates of DON release were ca. 50% the rates of N_2 fixation. We also fractionated the DON released by using ultrafiltration and found that 60 to 80% of the total organic release was of the size class <10,000 Da. The release of these organic compounds by *Trichodesmium* spp. is likely a significant source of new nitrogen for the associated bacteria or the non-nitrogen-fixing filaments of the *Trichodesmium* colonies.**

Trichodesmium sp. is a filamentous, colonial cyanobacterium which is found frequently in abundance in tropical and subtropical waters of the world. As a pelagic nitrogen fixer, it can make a substantial contribution to both the input of new nitrogen and the flux of organic matter in warm oceanic surface waters. It has been estimated, for example, in the Caribbean Sea that *Trichodesmium* spp. can contribute up to 20% of the total primary productivity (10), and in the North Pacific Gyre the contribution of new nitrogen fixed by *Trichodesmium* spp. can be 50% of the total new nitrogen input to near-surface waters (14).

Tropical and subtropical regions of the oceans are typically impoverished with respect to dissolved inorganic nitrogen availability. By contrast, dissolved organic nitrogen (DON) concentrations of 5 to 10 μmol are common, making it the most abundant dissolved nitrogen form (24). Despite the large size of this pool, there is a general lack of quantitative information on both the production and fate of this pool. Most of the available information on DON is derived from studies of individual compounds, such as urea or dissolved free amino acids (1), or extrapolated from studies of dissolved organic carbon flux.

Over the past few years, we have been developing and applying a new ^{15}N labeling technique to measure the production of DON from autotrophs (3, 4). Such production may occur via passive release, cell death or lysis, or virus-induced lysis or during feeding and grazing by heterotrophs (4). In the present study, we present and apply a modification of this technique to the measurement of DON release from the diazotroph *Trichodesmium* sp. In conjunction with the results of Capone et al. (6), we demonstrate that this production can be a significant fraction of recently fixed nitrogen.

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MATERIALS AND METHODS

Sampling. Samples were collected during January and February 1992 aboard the R/V *Columbus Iselin* in the eastern Caribbean and Bahamas, on a transect from Monserrat to San Salvador (Fig. 1B in reference 9). Collections of *Trichodesmium* spp. were made with a 202-μm plankton net with a solid cod end, towed at 1 knot at depths of 1 to 30 m for ca. 10 min. Colonies of *Trichodesmium* spp. of similar size were picked from the tows with sterile inoculating loops and placed in filtered (0.2-μm pore size) seawater until the experiments were initiated. We did not differentiate the two common species of *Trichodesmium*, *Trichodesmium thiebautii* and *T. erythreum*, in these experiments. It was typically on the order of 1 h from the time of sample collection to the time the experiments were begun. Clean techniques (use of plastic gloves, acid washing and/or muffling at 500°C of all containers used, etc.) were used to minimize contamination of DON.

Experiments. The first step in our measurements was the preparation of water enriched with ^{15}N-labeled N_2 gas ($^{15}N_2$). We collected surface seawater and immediately filtered it through 0.2-μm-pore-size Nuclepore filters under a mild vacuum (<50 mm [ca. 6.7 kPa] mercury). A portion of the filtered seawater (ca. 750 ml) was decanted into a vacuum flask and degassed by vacuum pressure. We gently withdrew the degassed water into a 500-ml Hamilton gas-tight syringe, being careful not to inject bubbles of air. We then added a 5-ml bubble of $^{15}N_2$ gas (99 atom%; Cambridge Isotope Laboratories) into the Hamilton syringe and shook the syringe by hand until the bubble of gas was dissolved into the seawater (ca. 5 min).

While the $^{15}N_2$ gas-enriched water was being prepared, *Trichodesmium* colonies were isolated, enumerated, and held in small (5- to 10-ml) volumes of freshly filtered seawater. We then filled 125-ml Erlenmeyer flasks with 43 ml of the $^{15}N_2$-enriched water, added 20 to 100 *Trichodesmium* colonies per flask, and then filled the flasks with unenriched filtered seawater. The sample bottles had a $^{15}N_2$ enrichment of ca. 30 atom%. Flasks were capped with custom-built Teflon vacuum valves (Eldex), as described by Kana (13), and placed in on-deck, flowing-seawater incubators for 0.5 to 9 h (most experiments were 1 to 2 h) under 30% ambient irradiance. At

the end of the incubation, the samples were filtered through precombusted (500°C for 2 h) Whatman GF/F filters, and both the filters and filtrates were retained for further analysis as described below. When weather and ship motion allowed, samples were processed immediately. On other days, samples were separated into aliquots for the different types of analyses and frozen in cold acid-washed polyethylene bottles.

The extracellular DON concentrations and $DO^{15}N$ atom percent enrichment were measured as described by Bronk and Glibert (4). At the end of the incubation, samples were gently refiltered through 0.2-μm-pore-size Nuclepore filters, and the concentrations of NH_4^+, NO_3^-, and DON were measured on the filtrate. We then passed 27 ml of the 0.2-μm filtrate through ion retardation resin (Bio-Rad AG 11 A8) to separate the $DO^{15}N$ from the inorganic nitrogen as described by Bronk and Glibert (3); the resin attracts small charged molecules while allowing DON to pass. The eluates were neutralized to a pH of 7 to 8 and then boiled down to a few milliliters and transferred to a precombusted filter which was dried and analyzed by mass spectrometry; we refer to this fraction as total DON. The low-molecular-weight (LMW) DON was also isolated from the samples by passing 45 ml of the 0.2-μm filtrate through a Centriprep ultrafilter (Amicon 4305; 10,000-molecular-weight cutoff) prior to passing the filtrate through the ion retardation resin and proceeding as described above. Although the nominal molecular weight cutoff of the ultrafilters was 10,000, in practice they retained much smaller molecules.

The atom percent enrichment of the LMW DON within the intracellular pools of the colonies was measured as described by Bronk and Glibert (4). Rates of DON release were calculated as described by Bronk and Glibert (4).

Analyses. The concentration of DON was determined by the method of Valderamma (27), and concentrations of NH_4^+ and NO_3^- were determined by Technicon AutoAnalyzer. Mass spectrometry was done with a Nuclide 3″ 60° sector mass spectrometer. Particulate nitrogen contents of the *Trichodesmium* colonies were analyzed by a Control Equipment CHN analyzer after filtration of a known number of freshly collected colonies (10 to 20) onto a precombusted GF/F filter.

N_2 fixation rates. Although it had been our intent to compare N_2 fixation rates by *Trichodesmium* spp. determined by $^{15}N_2$ with rates determined by acetylene reduction (7) for samples taken from the same site and depth, such a comparison was only successfully completed on 1 day. We determined the $^{15}N_2$ incorporation rate for whole *Trichodesmium* colonies and for the LMW DON fraction of the *Trichodesmium* spp. For our comparisons of DON release rates with N_2 fixation rates in this report, we rely on N_2 fixation rates determined by acetylene reduction as reported by Capone et al. (6) for comparable times of day.

RESULTS

Relatively few direct comparisons of N_2 fixation by *Trichodesmium* spp. have been made with acetylene reduction and $^{15}N_2$ incorporation (8, 10, 18, 23). Estimates of the ratio of $^{15}N_2$ incorporation to acetylene reduction for *Trichodesmium* spp. for previous studies have ranged from 1.9:1 to 6:1. In our experiments, for whole *Trichodesmium* colonies, the mean N_2 fixation determined by $^{15}N_2$ incorporation during a midday time course was 182 ± 33 pmol of N colony^{-1} h^{-1} (Table 1), and the ratio of $^{15}N_2$ incorporation to acetylene reduction for these samples was 3.4:1; this ratio is in the middle of the range of previous studies. For the $^{15}N_2$ incorporation rate into LMW compounds only, the rate was 156 ± 27 pmol of N colony^{-1} h^{-1}. If a mean conversion factor of 3 mol of acetylene reduced mol of $^{15}N_2$ incorporated^{-1} is assumed, the rate of acetylene reduction was 159±7 pmol of N colony^{-1} h^{-1}; this is virtually equivalent to the rate of $^{15}N_2$ incorporated into LMW compounds.

TABLE 1. Rates of N_2 fixation by *Trichodesmium* spp. along a 2-h time course determined by the uptake of $^{15}N_2$ gas[a]

Sample[b]	Duration of incubation (h)	N_2 fixation rate (pmol of N colony^{-1} h^{-1})
Total N_2 fixation	0.50	154
	1.18	244
	1.23	162
	2.12	162
	2.15	189
N_2 fixation into LMW fraction only	0.70	137
	1.18	207
	1.23	137
	2.12	139
	2.15	159
Avg total N_2 fixation		182 ± 33
Avg fixation into LMW fraction		156 ± 27

[a] For comparison, the N_2 fixation rate determined in parallel by acetylene reduction was 159 ± 7 pmol of N colony^{-1} h^{-1} if a conversion factor of 3 mol of acetylene mol of N_2 reduced^{-1} is assumed.
[b] Samples were taken on 26 January 1992 at 1400 h.

Rates of total DON release by colonies of *Trichodesmium* spp. showed considerable variation within replicate experiments, along several time course experiments, and with the time of day at which individual experiments were initiated. Regardless of the time of day when experiments were conducted (no experiments were conducted at night, however), rates of DON release were highest during the first hour of incubation and then dropped rapidly; by 6 to 9 h of incubation the rates of DON release were only 20 to 25% of the rates initially measured (Fig. 1). We had difficulty measuring rates of release in experiments shorter than 1 h because insufficient label had accumulated in the DON pool to be accurately

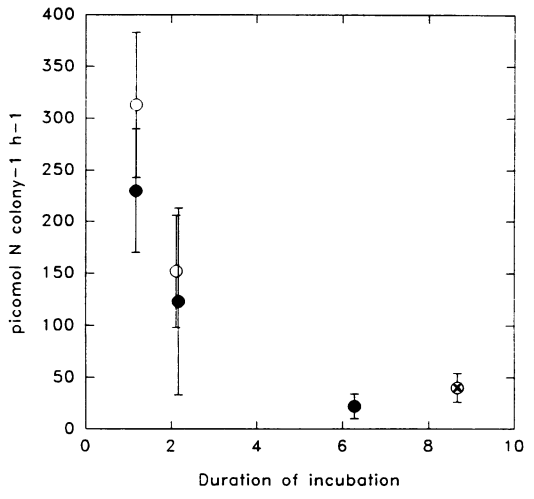

FIG. 1. Rates of release of total DON as a function of duration of incubation (hours). Data were pooled from several experiments: ○, 26 January 1991; ⊗, 29 January 1992; ●, 1 February 1992.

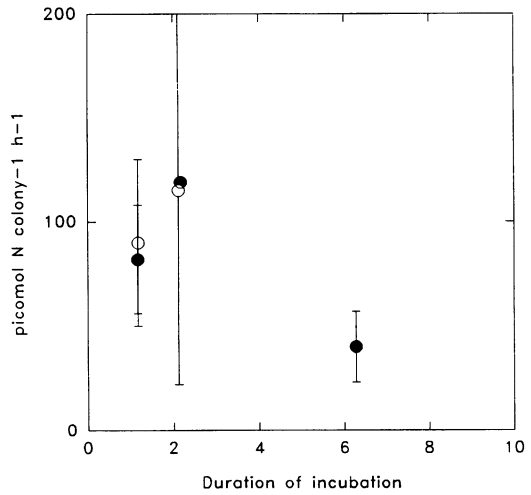

FIG. 2. Rates of release of LMW DON as a function of duration of incubation (hours). Data were pooled from two experiments: ○, 26 January 1992; ●, 1 February 1992.

detected. The high rates of DON release measured early in the time course may in part be due to stress the colonies may have experienced while the experiments were set up (11); we emphasize, however, that we used the gentlest techniques possible in all phases of the experimentation. The rapid decline in DON release, evident after our 1-h measurement point, was due to either a reduction in rates of release or the reincorporation of DON into *Trichodesmium* or associated microbes. While we also attempted in these experiments to measure the rates of DON uptake by *Trichodesmium* colonies by the method described by Bronk and Glibert (5), our sensitivity was very low for these samples. At best, we could estimate that uptake of DON was approximately equal to the rate of DON production in experiments of 2- to 4-h duration (data not shown).

The time course of LMW DON release (Fig. 2) did not reveal as large a change with duration of incubation as did the time course of total DON release. Rates of LMW DON release decreased through time such that at 6 h of incubation, rates were ca. 50% those measured at 1 to 2 h of incubation. Overall, rates of LMW DON release were 60% of the total DON release for incubations lasting for 1 to 2 h and 73% of the total DON release for all incubations of up to 6 h in duration.

In examining all available data as a function of time of day (Table 2), the mean rate of total DON release was 114 ± 45 pmol of N colony^{-1} h^{-1}, while the mean rate for those experiments conducted at midday only was 142 ± 57 pmol of N colony^{-1} h^{-1}. These rates represented 44 and 56% of the estimated rates of N_2 fixation, respectively. Rates of total DON release were not detectable at our 0600 sampling period. Although DON release may have been occurring during this period, it was not detectable with ^{15}N label because N_2 fixation rates were negligible, which would prevent any DON potentially released from being labeled with ^{15}N. Rates of total DON release averaged 37% of the rate of N_2 fixation during midmorning, and increased to 76% of N_2 fixation by mid-afternoon. The rates of N_2 fixation by *Trichodesmium* spp. rapidly decline in late afternoon (6, 7), and these percentages reflect that decline as well as a ca. 50% increase in the release rates of DON from morning to afternoon.

The average rate of LMW DON release for all times of day was 63 ± 22 pmol of N colony^{-1} h^{-1} (Table 2), and that for the measurements made at midday only was 78 ± 28 pmol of N colony^{-1} h^{-1}. Thus, LMW DON release rates were 44 to 76% of the total DON release and 21 to 45% of the estimated rates of N_2 fixation. Rates of LMW DON release were fairly constant from morning to afternoon, so the variability in the percentage of LMW release to N_2 fixation largely reflects diel changes in the N_2 fixation rates.

DISCUSSION

The results in this report, and those reported by Capone et al. (6), show that release of dissolved nitrogen-containing organic compounds by *Trichodesmium* spp. is a dynamic process which represents a significant fraction of the nitrogen fixed by these cyanobacteria. The similarity between our findings and those of Capone et al. (6), derived by completely independent methods, is striking and further underscores the importance of this flux.

It is now generally accepted that even healthy phytoplankton leak some organic compounds; with respect to carbon, several studies have demonstrated that for a wide range of phytoplankton groups, <10% of recently fixed photosynthate is released to the medium under normal growth conditions (16, 17, 19). For *T. thiebautii*, Shimura et al. (25) reported release of dissolved organic carbon to be 2 to 8% of photosynthetic rates,

TABLE 2. Rates of release of total and LMW DON from colonies of *Trichodesmium* spp. in incubation experiments of 1 to 2 h in duration and percentage of N_2 fixation represented by these rates[a]

Date (1992)	Time (h)	pmol of N colony^{-1} h^{-1}			% N_2 fixation	
		Release of total DON	Release of LMW DON	N_2 fixation rate[b]	Total DON	LMW DON
27 Jan.	0600	ND (1)	ND (1)	0	0	0
1 Feb.	1000	125 ± 105 (6)	73 ± 36 (5)	266	47	27
27 Jan.	1100	92 (1)	70 (1)	340	27	21
26 Jan.	1400	233 ± 102 (4)	102 ± 73 (4)	326	72	31
27 Jan.	1600	121 ± 19 (2)	71 ± 1.5 (2)	159	76	45
Avg						
All times		114 (5)	63 (5)	218	44	25
1000 to 1600 h		142 (4)	78 (4)	273	56	31

[a] Numbers in parentheses indicate numbers of samples analyzed for each date and time. ND, not detectable.
[b] N_2 fixation rate was estimated from corresponding time points reported by Capone et al. (6).

and the recent data of Capone et al. (6) on glutamine and glutamate release rates, when expressed on a carbon basis, represented 3% of photosynthetic production. Most of the release of LMW amino acids is thought to be exudation by passive diffusion across permeable cell membranes (2, 17).

The significant rates of release of DON suggest several possible mechanisms in addition to passive exudation. It should be underscored that because the production of these compounds was traced directly using $^{15}N_2$ gas, which enters the cells via N_2 fixation, this organic material represents a new flux from the cells within the time course of our experiments and not simply a diffusion of compounds from the interstitial water among the trichomes. Other potentially significant mechanisms of release of both LMW and high-molecular-weight DON are processes which rupture cells, including grazing and viral infection (3, 21, 26), or disruption of colonies during containment and/or filtration. Kirchman et al. (15) found that filtration can inflate measured rates of nitrogen release by phytoplankton and that release of dissolved primary amines increased even when gravity filtration was used. However, if the initial high rates of release of total DON that we observed during our time course experiments were due to artifactual cell breakage, the relative proportion of LMW DON to total DON would be expected to decrease through time, not increase as observed.

Although there are few known grazers of *Trichodesmium* spp., O'Neil and Roman (20) have demonstrated that the pelagic harpacticoid copepods *Macrosetella gracilis* and *Miracia efferata* ingest *Trichodesmium* spp. at average rates of 14.2 and 33.3 nmol of C copepod^{-1} h^{-1}, respectively. Assuming an average C/N molar ratio for *Trichodesmium* spp. of 5, these ingestion rates are equivalent to 2,840 and 6,660 pmol of N copepod^{-1} h^{-1} for the two species, respectively. There is considerable variability in these rates, reflecting past feeding history and physiological state of both the copepods and the cyanobacteria. It has been shown for numerous other planktonic systems that grazing by zooplankton can mediate significant release of dissolved organic and inorganic compounds (4, 12, 22), although no data which demonstrate the magnitude of DON release by harpacticoid feeding copepods are available. Nevertheless, the ingestion of *Trichodesmium* spp. can be considerable. In our experiments, we made no estimates of the abundance of copepods in the colonies we used, nor did we make any attempt to remove them from our samples. However, independently, it was observed that adult *Macrosetella* spp. or copepodites were found on virtually all colonies collected during this period (19a). Thus, while we cannot estimate the impact of these grazers on DON flux in our experiments, it is likely that they contributed to the measured DON release.

An additional potential mechanism of release of organic nitrogen, noted by Capone et al. (6), is that organic compounds may be actively released, thereby providing a source of fixed nitrogen for the non-nitrogen-fixing filaments of the colonies; the demonstrated uptake of glutamine and glutamate by *Trichodesmium* spp. (6, 9) adds credence to this hypothesis. Rapid cycling of organic acids within the colonies may possibly be an important nutritional strategy for these colonial organisms. Our time course data also may imply that much of the released DON may have been consumed within the time course of our experiments; in our longer time courses (Fig. 1), most of the release occurred in the first 1 to 2 h. Either no further release occurred after the first several hours of incubation, or released DON was subsequently consumed during the last few hours of incubation. We can further suggest that since the colonies were placed in filtered (0.2-μm pore size) seawater during our experiments, the *Trichodesmium* or associated bacteria consumed the organics, but we cannot discount the fact that very small unattached bacteria may also have been in our samples.

A comparison of the total DON release rates reported here with the rates of glutamine and glutamate reported by Capone et al. (6) strongly suggests that glutamine and glutamate make up the largest proportion of compounds that are released; their reported average sum of glutamine plus glutamate release rates were 105 pmol of N colony^{-1} h^{-1}, while our reported average rate of total DON release, assessed by a completely different technique, was 114 pmol of N colony^{-1} h^{-1}. Furthermore, Capone et al. (6) observed that internal pools of glutamate were ca. fourfold higher at their daily maximum, late afternoon, than were internal pool concentrations of glutamine at their maximum, which occurred at midday. This is consistent with our observations that the highest rates of DON release occurred from mid- to late afternoon.

Compared with ambient seawater concentrations of DON, the release of DON by *Trichodesmium* spp. is small except perhaps when blooms develop. Nevertheless, an average of 50% of the N_2 fixed is apparently released as DON by these cyanobacteria. Clearly, the fate of this organic material must play an important role in the nutrient-impoverished tropical and subtropical waters where *Trichodesmium* sp. is an important primary producer.

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REFERENCES

1. **Antia, N. J., P. J. Harrison, and L. Oliveira.** 1991. The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia **30**:1–89.
2. **Bjornsen, P. K.** 1988. Phytoplankton release of organic matter: why do healthy cells do it? Limnol. Oceanogr. **33**:151–154.
3. **Bronk, D. A., 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.
4. **Bronk, D. A., and P. M. Glibert.** 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.
5. **Bronk, D. A., and P. M. Glibert.** 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.
6. **Capone, D. G., M. D. Ferrier, and E. J. Carpenter.** 1994. Amino acid cycling in colonies of the planktonic marine cyanobacterium *Trichodesmium thiebautii*. Appl. Environ. Microbiol. **60**:3989–3995.
7. **Capone, D. G., J. M. O'Neil, J. Zehr, and E. J. Carpenter.** 1990. Basis for diel variation in nitrogenase activity in the marine planktonic cyanobacterium *Trichodesmium thiebautii*. Appl. Environ. Microbiol. **56**:3532–3536.
8. **Carpenter, E. J., and J. J. McCarthy.** 1977. Nitrogen fixation and uptake of combined nitrogenous nutrients by *Oscillatoria* (*Trichodesmium*) *thiebautii* in the western Sargasso Sea. Limnol. Oceanogr. **20**:389–401.
9. **Carpenter, E. J., J. M. O'Neil, R. Dawson, D. G. Capone, P. J. A. Siddiqui, T. Roenneberg, and B. Bergman.** 1993. The tropical

diazotrophic phytoplankter *Trichodesmium*: biological characteristics of two common species. Mar. Ecol. Prog. Ser. **95:**295–304.

10. **Carpenter, E. J., and C. C. Price.** 1977. Nitrogen fixation, distribution, and production of *Oscillatoria* (*Trichodesmium*) spp. in the western Sargasso and Caribbean seas. Limnol. Oceanogr. **22:**60–72.
11. **Fuhrman, J. A., and T. M. Bell.** 1985. Biological considerations in the measurement of dissolved free amino acids in seawater and implications for chemical and microbiological studies. Mar. Ecol. Prog. Ser. **25:**13–21.
12. **Glibert, P. M., C. A. Miller, C. Garside, M. R. Roman, and G. B. McManus.** 1992. NH_4^+ regeneration and grazing: interdependent processes in size-fractionated $^{15}NH_4^+$ experiments. Mar. Ecol. Prog. Ser. **82:**65–74.
13. **Kana, T. M.** 1990. Light-dependent oxygen cycling measured by an oxygen-18 isotope dilution technique. Mar. Ecol. Prog. Ser. **64:** 293–300.
14. **Karl, D. M., R. Letelier, D. V. Hebel, D. F. Bird, and C. D. Winn.** 1992. *Trichodesmium* blooms and new nitrogen in the North Pacific Gyre, p. 219–238. *In* E. J. Carpenter, D. G. Capone, and J. G. Reuter (ed.), Marine pelagic cyanobacteria: *Trichodesmium* and other diazotrophs. NATO ASI Series, vol. 362. Kluwer Academic Publishers, Dordrecht, The Netherlands.
15. **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.
16. **Larsson, U., and A. Hagström.** 1982. Fractionated phytoplankton primary production, exudate release and bacterial production in a Baltic eutrophication gradient. Mar. Biol. **67:**57–70.
17. **Mague, T. H., E. Friberg, D. Hughes, and I. Morris.** 1980. Extracellular release of carbon by marine phytoplankton; a physiological approach. Limnol. Oceanogr. **25:**262–279.
18. **Mague, T. H., F. C. Mague, and O. Holm-Hansen.** 1977. Physiology and chemical composition of nitrogen-fixing phytoplankton in the central North Pacific Ocean. Mar. Biol. **41:**213–227.
19. **Malone, T. C., H. W. Ducklow, E. R. Peele, and S. E. Pike.** 1991. Picoplankton carbon flux in Chesapeake Bay. Mar. Ecol. Prog. Ser. **78:**11–22.

19a. **O'Neil, J. M.** Personal communication.

20. **O'Neil, J. M., and M. R. Roman.** Ingestion of the cyanobacterium *Trichodesmium* spp. by the pelagic harpacticoid copepods *Macrosetella*, *Miracia*, and *Oculasetella*. *In* F. Ferrari and B. Bradley (ed.), Proceedings of the Fifth International Symposium on *Copepoda*. Hydrobiologia, in press.
21. **Proctor, L. M., and J. A. Fuhrman.** 1991. Roles of viral infection in organic particle flux. Mar. Ecol. Prog. Ser. **69:**133–142.
22. **Roman, M. R., H. W. Ducklow, J. A. Fuhrman, C. Garside, P. M. Glibert, T. C. Malone, and G. B. McManus.** 1988. Production, consumption, and nutrient cycling in a laboratory mesocosm. Mar. Ecol. Prog. Ser. **42:**39–52.
23. **Scranton, M. I., P. C. Novelli, A. Michaels, S. G. Horrigan, and E. J. Carpenter.** 1987. Hydrogen production and nitrogen fixation by *Oscillatoria thiebautii* during *in situ* incubations. Limnol. Oceanogr. **32:**998–1006.
24. **Sharp, J. H.** 1983. The distribution of inorganic nitrogen and dissolved and particulate organic nitrogen in the sea, p. 1–35. *In* E. J. Carpenter and D. G. Capone (ed.), Nitrogen in the marine environment. Academic Press, Inc., New York.
25. **Shimura, S., Y. Yamaguchi, Y. Aruga, Y. Fugita, and S. Ichimura.** 1978. Extracellular release of photosynthetic products by a pelagic blue-green algae, *Trichodesmium thiebautii*. J. Oceanogr. Soc. Jpn. **34:**181–188.
26. **Suttle, C. A., A. M. Chan, and M. T. Cottrell.** 1990. Infection of phytoplankton by viruses and reduction of primary productivity. Nature (London) **347:**467–469.
27. **Valderamma, J. C.** 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar. Chem. **10:** 109–122.