

Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synechococcus* populations in oligotrophic environments

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ABSTRACT: Dilution experiments were used to examine growth and grazing mortality rates among *Prochlorococcus* and *Synechococcus* populations in the Sargasso Sea and California Current. In these experiments, deviation from linearity in the relationship between dilution and net growth rate was significant in a large number of cases. An alternative, more conservative approach for estimating growth and grazing mortality rates (independent of the shape of this relationship) was therefore employed. Growth rates estimated by this approach ranged from 0.32 to 0.76 and 0.37 to 0.67 d⁻¹ for *Prochlorococcus* and *Synechococcus*, respectively. Grazing mortality rates ranged from 0.25 to 0.85 and 0.13 to 0.51 d⁻¹, respectively. Cell-cycle-based growth rate estimates were consistent with these dilution-based rates. Nutrient amendment had little effect on picocyanobacterial growth rates, but did stimulate grazing mortality (and in some cases changed the apparent functional response of the grazer community) in a number of experiments. We hypothesize that improved food quality in nutrient-replete picoplankton cells may be responsible for these changes. Diel patterns of picocyanobacterial abundance in the Sargasso Sea experiments suggest that grazing activity varied strongly over the diel cycle, with low grazing activity during the first half of the light period. Growth rate and abundance were not positively correlated among or within picocyanobacterial groups, as might be expected if physiologically mediated controls were the dominant forces regulating these populations.

KEY WORDS: *Prochlorococcus* · *Synechococcus* · Picoplankton growth rates · Dilution method · Grazing mortality · Food quality · Flow cytometry · Functional responses

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INTRODUCTION

Prochlorococcus and *Synechococcus* are responsible for a large portion of the primary production in oceanic systems (Chavez 1989, Li et al. 1992) and are considered important components of marine pelagic food webs. Although a significant amount is known regarding their distribution, abundance, and biology (see e.g. Waterbury et al. 1986, Partensky et al. 1999), much less is understood regarding the biological and/or environmental factors that regulate these populations, or the

relative strengths of those factors. In particular, the relative importance of grazing (top-down) versus resource (bottom-up) controls in regulating the abundance of *Prochlorococcus* and *Synechococcus* populations remains largely unexplored.

Among the few analytical approaches currently available for assessing growth and/or grazing mortality rates among picophytoplankton in the field, the Landry-Hassett dilution technique is attractive because it can be applied to any identifiable picoplankton group, it yields both growth and grazing mortality rates, and in the case of grazing estimates it is based on the consumption of the *in situ* prey (Landry & Hassett 1982, Landry 1993). This method decomposes net phytoplankton population growth into true growth and

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grazing mortality: progressively more dilute samples are assumed to suffer progressively less grazing impact, allowing extrapolation to growth rate in the absence of grazing. The difference between this true growth rate (μ) and the observed net growth rate in the undiluted treatment is then taken as the grazing mortality rate (g). Operationally, these estimates of μ and g are most commonly calculated as the y -intercept and slope of the linear regression of observed net growth rate against experimental dilution (ranging between 0 and 1), although other formulations have been used (see Gallegos 1989).

The dilution approach (as commonly implemented) rests on 2 fundamental assumptions: (1) that phytoplankton growth rate is independent of dilution, and (2) that grazing impact is a linear function of the experimental dilution (i.e. of prey concentration) (Landry et al. 1995b). Assumption (1) may be violated if the reduced grazing activity at higher dilutions results in reduced nutrient recycling, leading to reduced nutrient availability in these treatments. In practice, this issue is often addressed by adding low concentrations of nutrients to all treatments; the stimulatory effect on growth rate of such additions is assessed by comparing the net growth rates in nutrient-amended and non-amended treatments at ambient phytoplankton concentrations (i.e. in undiluted treatments). Calculated μ is then corrected for by the observed offset, if any (Landry et al. 1995b). To our knowledge, the appropriateness of this correction has not been tested.

Violations of assumption (2) may arise if, for example, the functional response among relevant grazers is nonlinear over the range of prey concentrations subtended by the experimental dilutions, or if the abundance of grazers changes substantially over the course of the experiment (Gallegos 1989, Evans & Paranjape 1992, Landry et al. 1995b). Nonlinear dilution experiment results have been reported on numerous occasions (e.g. Gallegos 1989, Landry et al. 1993, Lessard & Murrell 1998), and are generally assumed to reflect nonlinear functional responses in those experiments. Gallegos (1989) showed that under certain circumstances these results could be used to gain information about the underlying functional responses, and proposed a '3-point' approach for estimating overall growth and grazing mortality rates that is less sensitive to such nonlinearities. Landry et al. (1995b) directly tested the assumption that grazing rate is proportional to dilution by independently measuring the former in their dilution treatments using fluorescently labeled bacterial prey. In that study, the assumption was shown to be valid.

The dilution technique has been applied to *Synechococcus* and/or *Prochlorococcus* populations in the equatorial, subtropical, and subarctic Pacific, the trop-

ical and subtropical North Atlantic, and the Arabian Sea (Landry et al. 1995a, Reckermann & Veldhuis 1997, Landry et al. 1998, Lessard & Murrell 1998, Rivkin et al. 1999, Kuipers & Witte 2000). Here we use experiments conducted in the Sargasso Sea and the California Current to assess the applicability and behavior of dilution experiments as applied to these important picophytoplankton. We examine the linearity of the experimental results, present a simple approach for estimating μ and g that is insensitive to the response of grazing to dilution, and use independent measures of growth rate to validate this approach. Furthermore, by comparing nutrient-amended and non-amended dilution series, we examine the influence of nutrient additions that are typically employed in these experiments. Finally, we address the potential importance of diel variation in grazing activity.

MATERIALS AND METHODS

Experiment locations. Two incubation experiments were performed on each of 3 different oceanographic research cruises in 1998. The first 2 cruises were to the Sargasso Sea in February/March and June/July, and the third to the California Current (CALCOFI program) in September (Table 1). For Sargasso Sea cruises, nitrogen (as nitrate plus nitrite) was measured using the chemoluminescence method of Garside (1982) and phosphorus (as soluble reactive phosphorus) by a modification of the MAGIC method (Karl & Tien 1992, Bulldis-Thompson & Karl 1998). Chl *a* was analyzed on shore using the methods of Welschmeyer (1994). For the California Current cruise, nitrogen (nitrate and nitrite) and phosphorus were measured using procedures similar to those described in Atlas et al. (1991). Chl *a* was extracted with 90% acetone and measured fluorometrically according to the methods described on the CALCOFI website (available at: http://calcofi.org/data/1990s/1998/9807_int.html). Nutrient and chlorophyll data from the Sargasso Sea are provided by Kent Cavender-Bares (MIT, Cambridge, MA), and from the California Current by the CALCOFI program (<http://calcofi.org/data/>).

Dilution experiments and diel sampling. Dilution experiments were based on a modification of the methods of Landry & Hassett (1982). In all experiments, 5 different dilutions were employed, corresponding to fractions of unfiltered seawater equal to 1.0, 0.6, 0.4, 0.3 and 0.2. This series of dilutions received nitrogen and phosphorus additions (500 nM NH_4 and 30 nM PO_4 ; final concentrations). Note that none of the stations were in areas in which iron or other trace metals are thought to be limiting. A second, abbreviated series of dilutions (corresponding to fractions of unfiltered seawater equal

to 1.0, 0.4, and 0.2) received no such supplements. This series was used as a control to evaluate the effects of nutrient additions in dilution experiments. Finally, a separate set of undiluted bottles, with and without nutrient additions, was used for time course sampling in selected experiments (see below). Replicate bottles were prepared for each treatment in all series.

During the Sargasso Sea cruises, seawater was collected from 50 m depth in trace metal-clean 10 l Go-Flow bottles. The light level at this depth (approx. 12% incident light) corresponded to that in the on-deck water bath used on these cruises. In the California Current cruise, water was collected in 10 l Niskin bottles from depths of 12 m (eastern station, CC-SUMMER-E) and 25 m (western station, CC-SUMMER-W) in order to be within the mixed layer (above the thermocline), where both *Synechococcus* and *Prochlorococcus* could be expected to be relatively abundant. In all cases, seawater for filtration (to be used for dilution) was collected first, filtered through a 0.2 μm sterile acid-rinsed Gelman Supor filter capsule and distributed to acid-cleaned 1 l polycarbonate bottles. Approximately 1.5 h after the initial water collection, seawater for the unfiltered fraction was collected, sieved through 70 μm Nitex-mesh (to include nanoplanktonic grazers but exclude their predators), and distributed to the bottles. Nutrient supplements were added just prior to Time-0 sampling. Bottles were placed randomly in Plexiglas on-deck water baths cooled with surface seawater. Incubator light-levels were adjusted to reproduce the light intensities from which the water was sampled using blue Plexiglas or neutral density screening (in the Sargasso Sea and California Current cruises, respectively). Bottles were placed in the incubator around dawn, and incubations were terminated after 24 h. Initial and final time point samples were taken from every bottle immediately prior to placement in the water bath and at the end of the 24 h period, respectively. On any given cruise, bottles were not reused between experiments; instead,

clean bottles were used each time to reduce the risk of trace-metal contamination.

Time course sampling was performed in separate bottles equipped with custom-built sampling caps, in which Teflon sampling tubes and air vents were installed. A sterile syringe was used to withdraw water through the sampling tube at each time point. A 3 ml aliquot was withdrawn and discarded to ensure that the sample was not compromised by sequestration of water in the tubing, and then a second 3 ml aliquot was withdrawn and preserved. During sampling, the bottles were vented to the atmosphere through 2 stacked Acrodisk filters (0.2 μm); ganged polypropylene valves prevented water from entering (or re-entering) the bottle. These bottles were sampled at approximately 2 h intervals over the course of the experiment, with the exception of the summer Gulf Stream station (GS-SUMMER), which was sampled on a less frequent basis.

Samples were preserved with 0.2% paraformaldehyde (final conc.) within approximately 15 min of sampling, incubated for 10 min in the dark, and subsequently frozen in liquid nitrogen for later analysis on shore (Vaulot et al. 1989). In some cases a 10 ml volume of preserved sample was held on ice for up to 40 min prior to aliquoting and freezing in liquid nitrogen.

Flow cytometry. Flow cytometric analysis was performed using a modified Coulter EPICS 753 (Binder et al. 1996). Sample was delivered at rates between 2 and 10 $\mu\text{l min}^{-1}$ using a syringe pump, and data acquisition was triggered on forward angle light scatter (FALS). Sample count rates were usually less than 300 s^{-1} and never exceeded 1000 s^{-1} . Samples for cell abundance measurements were analyzed using 488 nm excitation at 750 mW of laser power. Fluorescence was collected through a 580 nm band pass filter (30 nm bandwidth) for orange (phycobiliprotein) fluorescence, and a 680 nm band pass filter (40 nm bandwidth) for red (chlorophyll) fluorescence. Just before analysis, 0.47 μm diameter green/orange-fluorescing latex

Table 1. Time and location of the experiments as well as ambient nutrient and chlorophyll concentrations at the experimental depth. Dates are start dates of the experiments. SMLD: surface mixed layer depth (approximated from temperature profiles at each station); na: not available; Calif. Curr.: California Current

Date (1998)	Location	Stn	Latitude	Longitude	Depth (m)	Chl <i>a</i> ($\mu\text{g l}^{-1}$)	Nitrogen (nM)	Phosphorus (nM)	SMLD (m)
1 Mar	Sargasso Sea	SS-WINTER-S	26° 00' N	70° 00' W	50	0.10 ^a	3.3 ^a	1.8 ^a	100
6 Mar	Sargasso Sea	SS-WINTER-N	31° 40' N	64° 11' W	50	0.29	238	4.8	175
26 Jun	Sargasso Sea	SS-SUMMER-S	35° 54' N	69° 12' W	50	na	3.8	3.6	10
28 Jun	Gulf Stream	GS-SUMMER	38° 25' N	72° 55' W	50	na	1335	213	15
13 Sep	Calif. Curr.	CC-SUMMER-E	32° 30' N	118° 12' W	12	0.57	110	350	15
16 Sep	Calif. Curr.	CC-SUMMER-W	30° 10' N	122° 55' W	25	0.19	100	200	50

^aConcentration was not measured at 50 m but was the same value just above and below this depth

beads (Polysciences) were added to the sample for later signal normalization. Samples for cell cycle analysis were stained with the DNA-specific stain Hoechst 33342 (0.5 $\mu\text{g ml}^{-1}$ final conc.) for a minimum of 45 min (Binder et al. 1996). In this case, 100 mW of UV excitation was used, combined orange and red auto-fluorescence was collected through a 590 nm long pass filter, and Hoechst (blue) fluorescence was collected between 408 and 470 nm. Under these conditions, *Prochlorococcus* and *Synechococcus* could be easily separated on the basis of their different FALS versus orange/red fluorescence signatures. A mixture of 0.47 μm diameter green/orange-fluorescing and 0.46 μm diameter blue-fluorescing beads was used for signal normalization in these cell-cycle samples.

Data analysis. *Prochlorococcus* and *Synechococcus* were identified and enumerated based on light scatter and fluorescence signals as described previously (Olson et al. 1990, 1993), and net growth rate was calculated for each group in each bottle based on the Time-0 and 24 h cell counts. Specific growth and grazing mortality rates were taken as the y -intercept and slope, respectively, from the linear regression of these observed net growth rates versus dilution, as is standard practice (e.g. Landry et al. 1995b). In order to assess the appropriateness of linear regressions for describing these data, ANOVA was used to test the significance of deviations from such regressions as compared to the variability among replicate bottles (Sokal & Rohlf 1981). We also applied an alternative method for estimating growth and grazing mortality rates from these data that was independent of the particular shape of the relationship between net growth rate and dilution (Gallegos & Jordan 1997). In this case, the observed net growth rate for the 0.2 dilution bottle was used as a conservative estimate of the specific growth rate, and grazing mortality was estimated as the difference between the net growth rate at dilutions of 0.2 and 1.0. These estimates represent lower bounds of both rates because net growth rate can reasonably be expected to remain constant or increase, but not decrease, as dilution approaches zero (at which point net growth rate is equivalent to true growth rate, by definition). In diluted bottles with very low cell counts (below 150 cells per analysis), initial cell abundance was calculated based on measurements from the undiluted bottles and the nominal dilution. Variations due to inexact bottle filling (i.e. departures from the nominal dilution in individual bottles) would be unaccounted for in this analysis and could be a source of error in the calculated growth rate. This approach was taken for *Synechococcus* at the Sargasso Sea winter southern station (SS-WINTER-S) and CC-SUMMER-W, and for *Prochlorococcus* at GS-SUMMER.

Populations in 3 experiments exhibited unexpected behavior associated with nutrient enrichment. At SS-

WINTER-S, *Prochlorococcus* and *Synechococcus* did not grow in the nutrient-enriched bottles (i.e. apparent growth rate in the 0.2 dilution was -0.36 and -0.33 d^{-1} for *Prochlorococcus* and *Synechococcus*, respectively) and the FALS distributions for these populations were uncharacteristically broad (indicative of senescing cells). *Prochlorococcus* cells also did not grow in the nutrient treatment at CC-SUMMER-E, with negative growth in the 0.2 dilution. Lastly, nutrient-enriched samples at CC-SUMMER-W had a considerable amount of flow cytometric 'noise' in the orange channel, making unambiguous analysis of *Synechococcus* populations impossible. These experimental series were not used in subsequent analyses.

Cell-cycle analysis was used to provide an independent estimate of specific growth rates in 2 experiments. Hoechst-stained samples were used to acquire DNA distributions for *Prochlorococcus* and *Synechococcus* populations. (*Synechococcus* DNA was collected as a log signal, linearized using a calibration for the corresponding log amplifier [Schmid et al. 1988], and re-binned into a linear histogram.) These distributions were decomposed into G1, S, and G2 subpopulations using non-linear curve fitting (Modfit, Verity Software House). Note that for the purpose of discussion, we refer to cells with 1 apparent genome equivalent as G1, cells with between 1 and 2 genome equivalents as S, and those with 2 genome equivalents as G2. This terminology is borrowed from the eukaryotic cell cycle and is employed here as a simple way of referring to sub-populations of cells with different DNA content, not to refer to cell-cycle phases *per se* (Binder & Chisholm 1995). Growth rates were calculated according to Liu et al. (1997) using the equation:

$$\mu = (T_{\text{duration}})^{-1} \int_0^{24} \ln[1 + f_{\text{S+G2}}(t)] dt$$

where T_{duration} is the duration of the terminal phase (i.e. S + G2) estimated as twice the distance between the peaks of S and G2 cells, and $f_{\text{S+G2}}(t)$ is the fraction (f) of S + G2 cells at time t . The value of the integral is calculated as the area under the curve of $\ln[1 + f_{\text{S+G2}}(t)]$ versus time.

RESULTS

Oceanographic regimes of the experiment locations

The experiments were conducted at sites with different water column structure and nutrient concentrations (Table 1). Inorganic nitrogen concentrations at the experimental depths ranged from less than 10 nM (in the southern Sargasso Sea stations) to greater than

1000 nM (at the Gulf Stream station) while phosphorus ranged from less than 10 nM (in the Sargasso Sea stations) to 350 nM (in the California Current). *Prochlorococcus* outnumbered *Synechococcus* at the experimental depth at all stations except GS-SUMMER (Table 2).

Dilution experiments

The statistical significance of deviations from linearity in our dilution experiments was tested for all experimental series in which the number of treatments exceeded 2, using ANOVA (see 'Materials and methods'). For this analysis, data were treated separately for the nutrient-amended and non-amended treatments, and for *Prochlorococcus* and *Synechococcus*, yielding an array of 16 data sets that could be tested. In 7 out of these 16 data sets, deviations from the regression were significantly greater than that expected from the observed within-treatment variability (Table 2). Deviations from linearity were very common for *Prochlorococcus* (6 out of 8 series), and relatively uncommon for *Synechococcus* (1 out of 8 series). The results of this

analysis are consistent with the non-linearities that are apparent from the data (Fig. 1), and suggest that for these experiments, estimating growth and grazing mortality rates from linear regression coefficients is inappropriate. For this reason, and for the sake of internal consistency, the alternative approach of using the net growth rates at 0.2 and 1.0 dilutions to calculate conservative growth and grazing mortality rates was employed for all treatments in all experiments (see 'Materials and methods', and 'Discussion'). These estimates are used throughout the discussion that follows. In those cases where deviations from the regression were not significant, the regression-based values of growth and grazing mortality rates are also presented in Table 2. These regression-based rates were on average 10% higher than the conservative estimates we use.

Over all the dilution experiments, *Prochlorococcus* growth rates ranged from 0.32 to 0.76 d⁻¹, and mortality rates from 0.25 to 0.85 d⁻¹ (Table 2). On average for all the stations, *Prochlorococcus* growth (mean ± SD: 0.49 ± 0.13 d⁻¹) was in approximate balance with grazing mortality (0.41 ± 0.18 d⁻¹). *Synechococcus* growth

Table 2. Abundance, growth rate (μ), grazing mortality rate (g , determined from dilution experiments) and net growth rate (k , $\mu - g$) for *Prochlorococcus* and *Synechococcus*. Abundance was recorded at the experimental depth of sampling, at ca. 8 h prior to the experiment. Growth and mortality rates were determined from the 1.0 and 0.2 dilution treatments exclusively (see text for details). na: treatment not available (see text). Significance of deviation from the linear regression (see text for details); nd: not determined (only 2 dilution treatments performed); ns: not significant ($p > 0.05$); * $p < 0.05$ (significant); ** $p < 0.01$ (highly significant)

Stn	Abundance ($\times 10^3$ ml ⁻¹)	Nutrient treatment	—2-point analysis—			—Linear regression—			
			μ	g	k	Deviation	μ	g	r^2
<i>Prochlorococcus</i>									
SS-WINTER-S	59	–	0.44	0.31	0.13	ns	0.46	0.35	0.66
		+		(na)					
SS-WINTER-N	22	–		(na)					
		+	0.76	0.85	–0.090	*			
SS-SUMMER-S	82	–	0.52	0.25	0.27	ns	0.49	0.22	0.66
		+	0.40	0.47	–0.070	*			
GS-SUMMER	2.6	–	0.58	0.37	0.21	nd	0.67	0.46	
		+	0.58	0.43	0.15	*			
CC-SUMMER-E	49	–	0.37	0.39	–0.020	**			
		+		(na)					
CC-SUMMER-W	92	–	0.45	0.32	0.13	*			
		+	0.32	0.29	0.03	**			
<i>Synechococcus</i>									
SS-WINTER-S	7.0	–	0.39	0.42	–0.030	ns	0.45	0.49	0.82
		+		(na)					
SS-WINTER-N	15	–		(na)					
		+	0.54	0.51	0.03	*			
SS-SUMMER-S	9.0	–	0.47	0.13	0.34	ns	0.42	0.09	0.46
		+	0.37	0.34	0.03	ns	0.45	0.40	0.77
GS-SUMMER	42	–	0.62	0.30	0.28	nd	0.69	0.37	
		+	0.56	0.42	0.14	ns	0.60	0.50	0.85
CC-SUMMER-E	9.5	–	0.65	0.26	0.39	ns	0.68	0.30	0.90
		+	0.67	0.25	0.42	ns	0.75	0.34	0.90
CC-SUMMER-W	2.2	–	0.56	0.34	0.22	ns	0.63	0.46	0.99
		+		(na)					

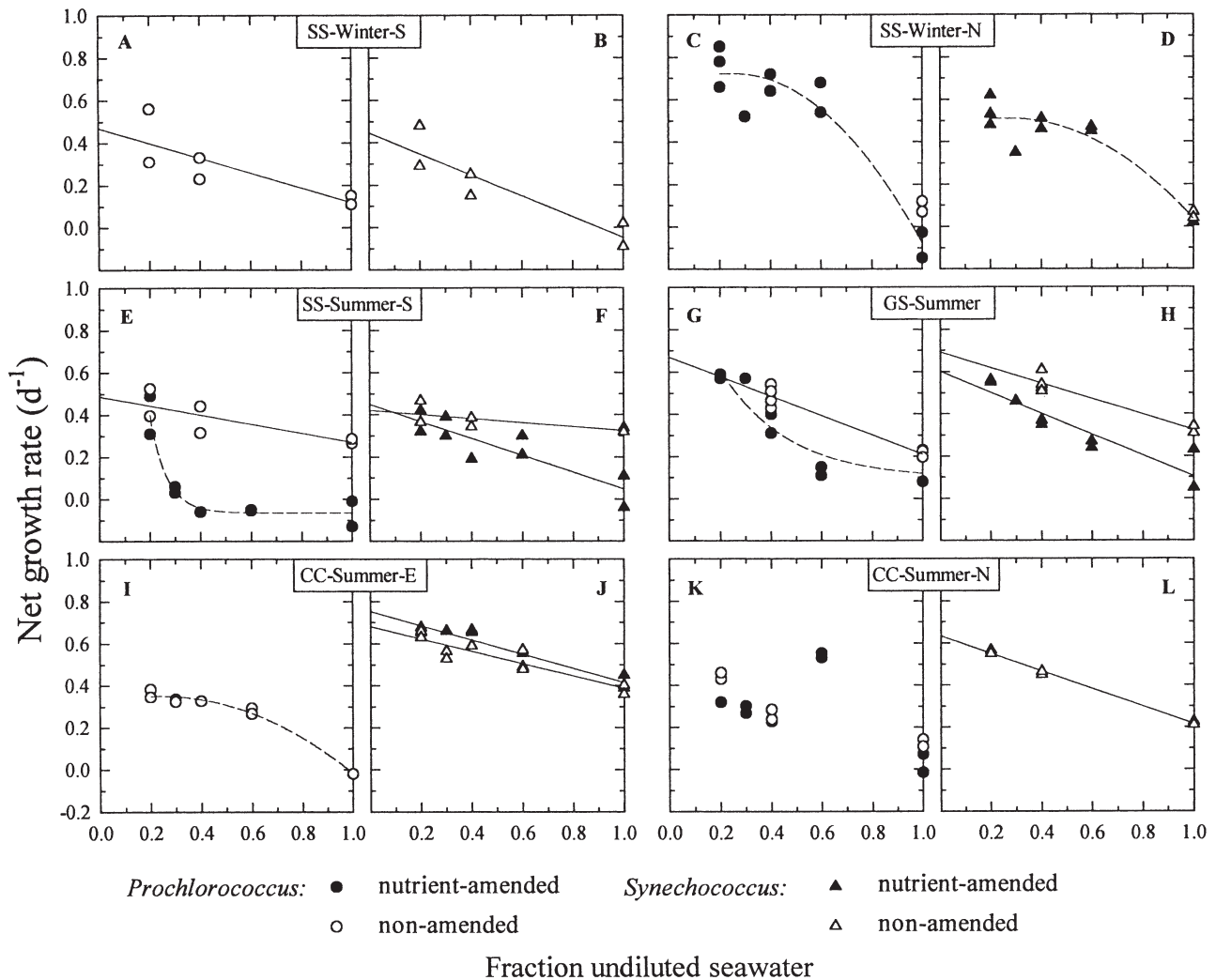


Fig. 1. Apparent growth rate of (A,C,E,G,I,K) *Prochlorococcus* and (B,D,F,H,J,L) *Synechococcus* as a function of fraction unfiltered seawater in the dilution experiments at the stations indicated. Solid lines show linear regressions (where deviation therefrom is not significant); dashed lines (drawn by eye) represent nonlinear trends

and grazing mortality rates ranged from 0.37 to 0.67 and 0.13 to 0.51 d^{-1} , respectively. Overall *Synechococcus* growth rates were slightly higher than their grazing mortality rates (0.54 ± 0.11 and 0.33 ± 0.11 d^{-1} , respectively).

Effects of nutrient amendment

Because we included unamended treatments at a range of dilutions in our experiments, the influence of nutrient additions on both growth and grazing can be assessed. There was a strong correlation between *Prochlorococcus* and *Synechococcus* growth rates in nutrient-amended versus non-amended treatments for those experiments in which the comparison can be

made ($r = 0.963$, $p < 0.01$) (Fig. 2A). Surprisingly, growth rates in nutrient-amended treatments were generally equal to or lower than those in corresponding non-amended treatments (Fig. 2A). In contrast, grazing mortality rates in amended and non-amended treatments were not correlated ($r = 0.179$), and mortality rates in amended treatments were generally equal to or greater than those in corresponding non-amended treatments (Fig. 2B).

At 2 stations (SS-SUMMER-S, GS-SUMMER) cellular fluorescence of both *Prochlorococcus* and *Synechococcus* increased dramatically in the nutrient-amended treatments (Fig. 3). Similar increases were not observed at other stations. Differences in cell size (as reflected by FALS) were less pronounced, although cells in the nutrient-amended treatments were gener-

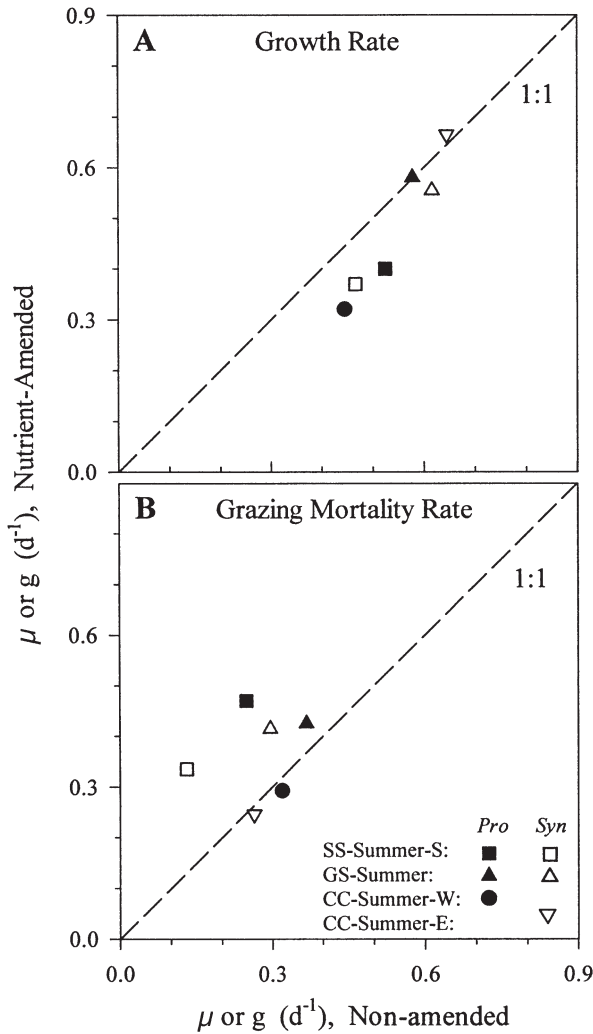
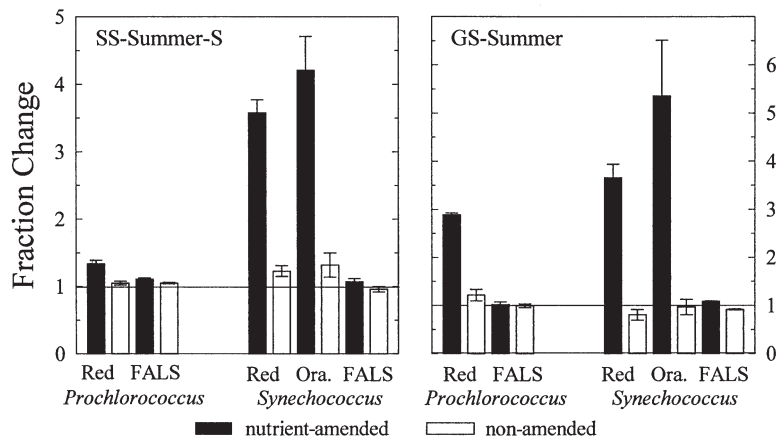


Fig. 2. Relationship between *Prochlorococcus* (Pro) and *Synechococcus* (Syn) (A) growth rate (μ) and (B) grazing mortality (g) in nutrient-amended versus non-amended treatments. Data are from all experiments for which both treatments are available, as indicated in legend. Dashed line represents a 1:1 relationship between the 2 treatments

ally larger than those in unamended treatments (Fig. 3). Note that the 2 stations at which fluorescence and FALS responded to nutrient amendment were also the ones at which nutrient addition appeared to result in increased grazing mortality (Fig. 2B).

Fig. 3. Fractional change in red and orange fluorescence and in forward angle light scatter (FALS) over the course of the experiment, in the nutrient-amended versus non-amended treatments at SS-SUMMER-S and GS-SUMMER. Error is calculated by combining the standard deviations of time-initial and time-final parameter measurements, according to Meyer (1975)



Cell-cycle analysis

Cell-cycle analysis was performed on time-course samples from undiluted treatments in the 2 Sargasso Sea winter experiments. *Prochlorococcus* populations were well-phased at both stations, with a distinct peak in S cells occurring at sunset, and a peak in G2 cells occurring a few hours thereafter (Fig. 4A,C). At SS-WINTER-N, $T_{duration}$ was estimated to be 8 h in both the nutrient-amended and non-amended treatments. The cell-cycle-based estimates of *Prochlorococcus* growth rate were $0.71 \pm 0.03 \text{ d}^{-1}$ (mean \pm SD) and 0.69 d^{-1} (single determination), in amended and non-amended treatments respectively. These estimates compare quite well with the dilution experiment estimate of 0.76 d^{-1} (for the nutrient-amended treatment) at this station. At SS-WINTER-S, the G2 maximum occurred somewhat earlier than at the northern station, reflecting a lower $T_{duration}$ of 5 h (Fig. 4C). The cell-cycle-based estimate of *Prochlorococcus* growth at this station (in the non-amended treatment) was 0.39 d^{-1} , compared to the dilution-based estimate of 0.44 d^{-1} .

In general, cell-cycle analysis can be difficult to perform on *Synechococcus* because natural populations of these cyanobacteria do not always stain efficiently, are often not as well-phased as *Prochlorococcus*, and may display strain-specific variations in cell cycle behavior (Binder & Chisholm 1995). Nevertheless, because *Synechococcus* populations stained fairly well at SS-WINTER-N, we undertook the estimation of growth rate via cell-cycle analysis for this population as well (Fig. 4B). The peak in *Synechococcus* S cells occurred 4 h prior to sunset in both treatments, and was followed closely by the peak in G2 cells. $T_{duration}$ was estimated to be 4 h in both the nutrient-amended and non-amended treatments. Throughout most of the experiment (and in both treatments) an apparent low-DNA population was present, ranging from 0 to 38% of the total *Synechococcus* population (square symbols,

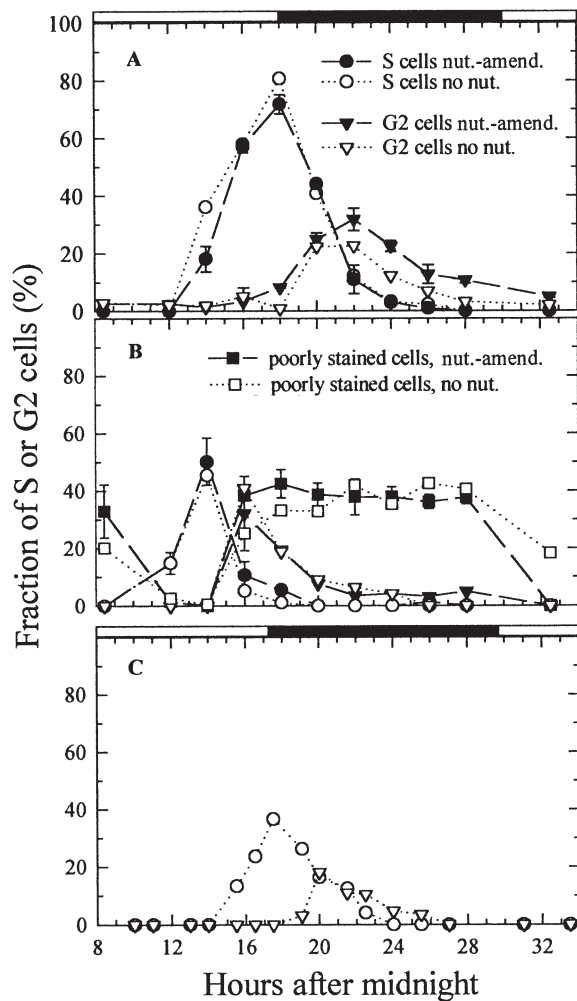


Fig. 4. Percentage of S cells (cells with between 1 and 2 genome equivalents), G2 cells (cells with 2 genome equivalents) and poorly stained cells in (A) *Prochlorococcus* at SS-WINTER-N, (B) *Synechococcus* at SS-WINTER-N, and (C) *Prochlorococcus* at SS-WINTER-S. Mean \pm SD shown for nutrient-amended treatments (nut.-amend.); non-amended treatments (no nut.) were unreplicated. Open and closed bars indicate light and dark periods, respectively

Fig. 4B). The appearance of these cells coincided with the G2 maximum and with increasing abundance of the *Synechococcus* population. We suspect that this population represents freshly divided cells that for some reason did not stain well. A similar phenomenon was observed by Binder & Chisholm (1995) in cultured *Synechococcus* grown on a light:dark cycle. Therefore this population was included with G1 cells for the purpose of estimating growth rates. Cell-cycle-based μ estimates for *Synechococcus* were $0.67 \pm 0.02 \text{ d}^{-1}$ (mean \pm SD) and 0.67 d^{-1} for the nutrient-amended and non-amended treatments, respectively. The dilution-based μ estimate for *Synechococcus* at this station (in

the nutrient-amended treatment) was 0.54 d^{-1} (Table 2). At SS-WINTER-S, *Synechococcus* cell concentrations were too low to obtain robust DNA distributions.

Diel variations in abundance

Variations in abundance over the course of the day were evaluated in undiluted bottles for all the Sargasso Sea experiments. The most striking of the experiments was SS-WINTER-N in which *Prochlorococcus* and *Synechococcus* abundance dropped precipitously between 02:00 and 08:30 h, the time period directly following the bulk of *Prochlorococcus* cell division (Fig. 5A). The decline in cell numbers during this time period amounted to consumption of at least 83 to 90 % of the daily *Prochlorococcus* cell production and 70 % of *Synechococcus* cell production. This heavy mortality period was well replicated among bottles and between nutrient treatments, but was less striking or absent in the other experiments (Fig. 5B–D). It should be noted that the lack of dramatic change in *Prochlorococcus* and *Synechococcus* abundance over the course of the day at the other Sargasso Sea stations suggests significant diel variation in grazing pressure at these stations, given the well-established diel variation in cell division rate (see 'Discussion').

DISCUSSION

Interpreting dilution experiments

Non-linear responses

As discussed in the 'Introduction', the dilution approach for evaluating phytoplankton growth and grazing mortality rates rests on 2 important assumptions: (1) that grazing impact is a linear function of the experimental dilution (i.e. of prey concentration.), and (2) that phytoplankton growth rate is independent of dilution (Landry et al. 1995b). Together these assumptions predict that net phytoplankton growth rate will increase linearly as experimental dilution increases. We tested this prediction by using ANOVA to assess the significance of the deviation from linear regression in the present series of experiments. This analysis is very similar to the goodness-of-fit test outlined by Evans & Paranjape (1992), differing only in the degrees of freedom associated with the residual mean square values. (This difference arises because those authors were interested in establishing the range of μ estimates that were consistent with a given grazing model, whereas we are interested in testing the conformity of

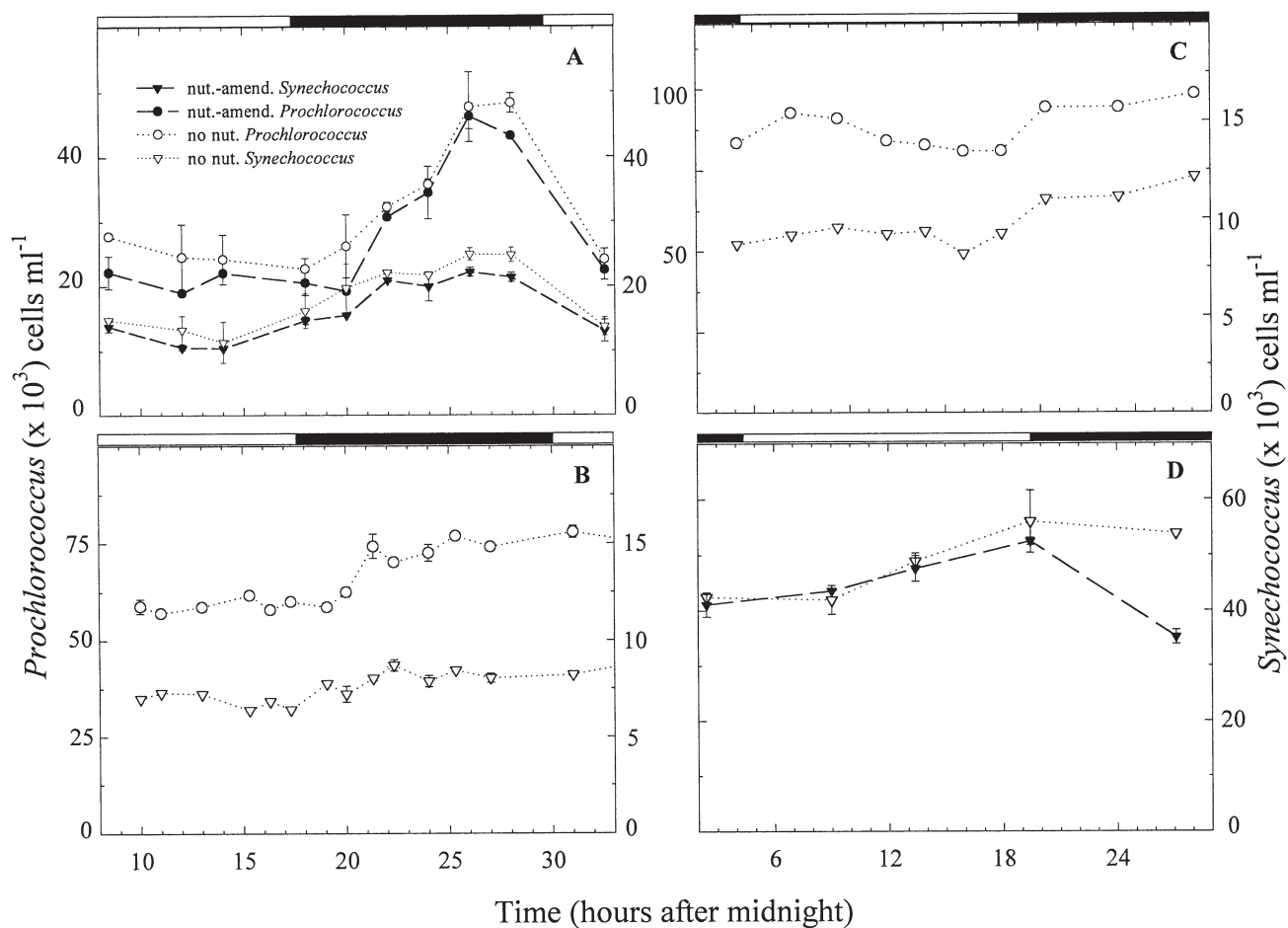


Fig. 5. Cell concentration as a function of time at (A) SS-WINTER-N, (B) SS-WINTER-S, (C) SS-SUMMER-S, and (D) GS-SUMMER. Symbols with error bars represent the mean \pm SD of cell concentrations in replicate bottles. Open and closed bars indicate light and dark periods, respectively. nut. amend.: nutrient-amended treatments; no nut.: non-amended treatments

the data to the specified model.) Our analysis indicates that in almost half of the cases, the results departed significantly from linearity, suggesting that interpretation of these experiments with linear regression is inappropriate (Table 2). Non-linear behavior has been observed in other dilution experiments (e.g. Gallegos 1989, Landry et al. 1993, Lessard & Murrell 1998). The ANOVA utilized here provides a simple means to objectively assess the significance of apparent deviations from linearity, and allows the researcher to determine when more conservative approaches for estimating growth and grazing mortality rates are appropriate.

Non-linearity of the sort observed here and elsewhere does not invalidate the dilution approach generally. At its core, this approach uses dilution to separate prey from their grazers so that the growth rate of the former can be estimated in the absence of the mortality inflicted by the latter. At issue in the case of nonlinear

results is the best way to extrapolate the observed values of net growth rate at finite dilutions to the value of μ , the 'pure' growth rate, at infinite dilution. While the assumption of linearity in the response of grazing rate to prey concentration is perhaps the simplest one statistically, from a biological point of view it is not surprising that such an assumption should be violated: it is equivalent to assuming a linear functional grazing response. In fact, as pointed out by Evans & Paranjape (1992), even when the linear model appears to fit the data, other models that are biologically more realistic might be expected to yield growth and grazing estimates that are more accurate.

Gallegos (1989) addressed this problem by using a '3-point' method for analyzing dilution experiments. Under this approach, the 2 most dilute treatments are exclusively used to extrapolate to zero grazing. The rationale behind this approach is that the extrapolation to infinite dilution will be most accurate if only the

points closest to this condition are used. Our strategy, which may be termed a 'modified 2-point' method (to distinguish it from earlier 2-point approaches that nevertheless assumed linearity over all dilutions), takes this approach a step further, and uses only the most dilute treatment (0.2 in the present experiments) to estimate μ . The disadvantage of this approach is that it does not account for any further reductions in grazing rate that might be realized at yet higher dilutions. In essence it assumes that at the dilution in question, grazing has been reduced to zero, or close to it. On the other hand, the approach offers the advantage that it represents a firm lower estimate of μ : given the biologically reasonable assumption that grazing mortality will not increase at higher dilutions, it can be concluded that μ cannot be lower than the net growth rate observed at that dilution. The magnitude of the potential underestimation of true growth rate under this approach could be reduced in the future by including more dilute treatments, as recommended previously by Gallegos (1989). Gallegos & Jordan (1997) showed that for such high dilutions (0.05 in their case), estimates of μ based on the modified 2-point approach were identical to those derived from linear regression. Although analysis of such high dilutions can be difficult in oligotrophic environments (owing to the low ambient cell concentrations), it is possible (Binder unpubl.), and would yield much more robust estimates of growth and grazing mortality rates when non-linear responses are present.

Nutrient additions

As discussed in the 'Introduction', low levels of nutrients are often added to dilution experiments to compensate for any reduction in nutrient availability at higher dilutions. In order to control for any artifactual stimulation of growth rate by such additions, separate undiluted treatments that receive no such amendment are also included. The growth rate calculated from the nutrient-amended series is then adjusted by the offset between the net growth rate observed in the undiluted treatments with and without nutrients. Grazing mortality rates are assumed to be insensitive to the presence or absence of additional nutrients.

In the present study, we included non-amended treatments at a number of dilutions (rather than at just 1.0). Comparison of nutrient-amended and non-amended dilution series suggests a response to nutrient additions that is more complex than that outlined above. Contrary to our expectations, nutrient additions appeared to have little effect on the growth rate of *Synechococcus* or *Prochlorococcus* in our experiments; as the fraction of unfiltered seawater

decreased, the difference between net growth rates with and without nutrients decreased as well: at dilution 0.2 (the point from which we estimated growth rate), the 2 rates were generally very similar (Table 2, Figs. 1 & 2A). This lack of growth rate stimulation could indicate that the rates of cell division among the extant picocyanobacterial populations in these waters were not nutrient-limited, or that these rates respond to nutrient enrichment on time scales greater than 24 h. As mentioned in the 'Materials and methods', a number of nutrient-enriched dilution series failed entirely (i.e. showed negative net growth at all dilutions), suggesting that the added nutrients can actually have a deleterious effect on *Prochlorococcus* or *Synechococcus* populations. It is unlikely that such effects result from contamination artifacts, as in all cases other experiments on the same cruises using the same nutrient stocks showed no such growth inhibition. It may be that the final concentration of added nutrients, though relatively low, can still represent a 'shock' to picocyanobacterial populations under certain conditions.

In contrast to their effect (or lack thereof) on growth rate, and again against expectation, nutrient additions appeared to have a strong positive influence on grazing mortality rates in some experiments (Table 2, Fig. 2B). This may reflect changes in the 'food quality' of *Prochlorococcus* and *Synechococcus* in nutrient-limited versus nutrient-replete cells, as discussed below. Note that under the traditional experimental scheme (in which the sole non-amended treatment is undiluted), these same phenomena would be incorrectly interpreted as a reduction in growth rate in response to nutrient addition. Clearly, comparing net growth rates with and without added nutrients at a single dilution is an insufficient basis for interpreting the response to nutrients in dilution experiments.

Despite the apparent insensitivity of growth rates to nutrient additions in our experiments, cellular fluorescence and (to a much lesser degree) FALS among *Prochlorococcus* and *Synechococcus* increased in nutrient-amended treatments at the Sargasso Sea and Gulf Stream summer stations (SS-SUMMER and GS-SUMMER; Fig. 3). This suggests that these populations responded physiologically to the increased nutrient concentrations. Nutrient status could affect picoplankton fluorescence by affecting cellular pigment content and/or photophysiology (e.g. Sosik et al. 1989), and could affect FALS through growth rate-mediated changes in size (e.g. Binder & Liu 1998, Worden & Binder unpubl.). It is unlikely that the observed changes were the result of differences between experimental and ambient light intensity or other experimental artifacts, because such artifacts would presumably be expressed in both nutrient-amended and

non-amended treatments. Furthermore, cellular fluorescence and FALS in non-amended treatments were in most cases similar to (or slightly less than) that at the start of the experiment (Fig. 3), again suggesting that uncontrolled changes associated with the experiment itself were not significant. The observation of cellular fluorescence increases in the absence of growth rate increases raises concerns about the application of pigment analysis to dilution experiments (e.g. Landry et al. 1993, Latasa et al. 1997). If these increases in cellular fluorescence reflect increases in per-cell pigment content, then growth rates calculated from changes in bulk pigment concentration would be inflated.

***Prochlorococcus* and *Synechococcus* growth rate estimates**

Despite the potential problems associated with dilution experiments, the estimates of *Prochlorococcus* growth rates derived from our modified 2-point analyses were comparable to estimates based on cell-cycle analysis within the same bottles. At SS-WINTER-N, for example, estimates of *Prochlorococcus* growth rate (in the nutrient-amended treatment) were 0.76 and 0.71 based on the dilution experiment and cell cycle analysis, respectively. The dilution-based *Prochlorococcus* and *Synechococcus* growth rates are also generally consistent with the limited number of published estimates available for the Sargasso Sea. Our SS-WINTER-S *Prochlorococcus* growth rate (0.44 d⁻¹ dilution based; 0.39 d⁻¹ cell-cycle based) agrees well with the *in situ* growth rate estimated by cell cycle analysis (0.40 d⁻¹) for the same station and depth (Mann 2000), suggesting that the dilution experiment was not influenced significantly by bottle effects or other artifacts. Likewise, the *Synechococcus* growth rates reported here were similar to the dilution-based estimates of Lessard & Murrell (1998) for the Sargasso Sea in the summertime (0.54 and 0.87 d⁻¹). Our results are also consistent with the growth rates estimated by Goericke (1998) based on ¹⁴C incorporation into pigments. In that study, picocyanobacterial growth rate was found to average approximately 0.60 and 0.35 d⁻¹ (at depths comparable to those used in the present study) in the winter and summer, respectively. The *Prochlorococcus* growth rates reported in Kuipers & Witte (2000) for stations in the tropical and subtropical north Atlantic were generally lower than those reported here, but those estimates were based on dilution experiments involving populations from much greater depths. To our knowledge there are no reported growth (or mortality) rates for either *Prochlorococcus* or *Synechococcus* in the California Current.

***Prochlorococcus* and *Synechococcus* grazing mortality**

Averaging over all treatments, experiments, and seasons, the grazing mortality rates for *Prochlorococcus* and *Synechococcus* were 0.41 and 0.33 d⁻¹, respectively. These grazing mortality rates are of the same order as those estimated by Caron et al. (1999) for the general picoplankton community south of Bermuda (which ranged from 0 to 0.61 d⁻¹), based on fluorescently labeled prey (FLP) measurements. The dilution-based estimates of *Synechococcus* grazing mortality rates at Bermuda Atlantic Time Series (BATS) by Lessard & Murrell (1998) are somewhat lower (ranging between undetected and 0.33 d⁻¹), although they overlap considerably with the estimates reported here. FLP-based estimates of *Synechococcus* grazing mortality north of BATS by Sanders et al. (2000) are lower still (0.05 to 0.22 d⁻¹). These low rates may reflect discrimination against fluorescently labeled *Synechococcus* prey. Recent work has demonstrated that although grazers ingest both live prey and inert particles at similar rates, inert particles (as well as certain prey species) are then selectively egested or rapidly excreted and therefore underestimate overall grazing mortality (Boenigk et al. 2001a,b).

As discussed above, grazing mortality was either unaffected or enhanced for both *Prochlorococcus* and *Synechococcus* in nutrient-amended treatments compared to non-amended treatments (Fig. 2). In particular, increased grazing mortality was observed for both groups at SS-SUMMER-S and GS-SUMMER, the 2 stations at which cellular fluorescence and FALS increased in response to nutrient additions (Fig. 3). We assume that the increase in cellular fluorescence and FALS in the nutrient-amended treatments was an indication of improved physiological condition, as discussed above. Because these changes are likely to be reflected as improved food quality of these cells for protistan grazers (e.g. through decreased cellular C:N), we hypothesize that the increased grazing mortality observed in nutrient-amended treatments was mediated by grazer responses to the physiological condition of the prey. The fact that food quality (including factors such as cell size and cell surface properties) can influence protozoan grazers and grazing rates is well established (e.g. Porter 1984, Monger & Landry 1992, Šimek et al. 1994, Christaki et al. 1998, Monger et al. 1999). It should be noted that because the grazers in dilution experiments have at their disposal a number of alternate potential prey items (e.g. heterotrophic bacteria), prey-switching may come into play (see below). Prey quality may also influence grazing rate indirectly, by influencing the rate of increase of the grazers themselves over the course of the experiment: if higher food

quality in nutrient-amended treatments resulted in higher grazer reproduction, then overall grazing rate could increase even while grazer-specific ingestion remained constant. Although no such differential increases in protozoa concentration were observed in a series of dilution experiments at locations similar to our experimental sites (Binder & Burbage unpubl.), this does not exclude the possibility that the sub-set of the protozoa that actively graze *Prochlorococcus* and/or *Synechococcus* may have increased (Landry et al. 1995b).

It is possible that viral lysis could contribute to the mortality of picophytoplankton in our experiments. Wilson et al. (1996) concluded that P-replete *Synechococcus* cells were more susceptible to lysis than were P-depleted cells; therefore it is worth considering whether the increased mortality we observed in nutrient-amended treatments could have resulted from increased viral lysis. We believe that this is unlikely to be the case because (1) the relatively short duration of the experiments (24 h) makes it likely that cell lysis would result mainly from infections that occurred prior to the start of the experiment, and therefore would not vary with dilution; and (2) even if viral lysis from new infections were important, viral particles would presumably pass the 0.2 μm pore filter employed for making the filtered seawater diluent, and viral concentration would therefore not vary among dilution treatments. In both these cases, and in general, lysis rates should be independent of dilution, and would therefore be perceived as decreased growth rates rather than increased mortality.

Our data clearly indicate that for the closely related *Prochlorococcus* and *Synechococcus*, grazing mortality rates are frequently quite different even at the same station. In laboratory studies, Christaki et al. (1999) likewise documented differential behavior of predators toward *Prochlorococcus* and *Synechococcus*. Furthermore Boenigk et al. (2001b) have shown that several different grazers ingest and then rapidly excrete *Synechococcus* while ingesting and then digesting several other types of bacteria, demonstrating a high level of prey selectivity. These results emphasize the danger of assuming that grazing rates generated either at the community level or from measurements on a single prey species are representative of grazing rates for (other) specific picoplankton populations.

Grazer functional responses

The non-linearities we observed in the dilution experiments can be interpreted in the context of grazer functional responses. Gallegos (1989) showed that the

grazer-specific ingestion rate at a given dilution ($I[D]$, prey grazer⁻¹ h⁻¹) is related to the net growth rate at that dilution ($R[D]$, h⁻¹) as follows:

$$R(D) = \mu - \frac{Z}{P} I(D)$$

where D = the fraction of unfiltered seawater, μ is the specific growth rate as defined previously (assumed here to be constant, and equal to the mean observed net growth rate at $D = 0.2$), and Z and P are the concentrations of zooplankton grazers and phytoplankton prey in undiluted seawater, respectively. Rearranging, and recognizing that specific grazing mortality rate at a given dilution, $g(D) = \mu - R(D)$, we find:

$$I(D) = g(D) \frac{P}{Z}$$

Thus, in a given experiment, the per-grazer consumption rate at any given dilution is proportional to the specific mortality rate of the prey at that dilution. This equation allows us to calculate a relative grazer-specific ingestion at each dilution in a given experiment, and thereby to construct the grazer functional response. These calculations are based on the assumption that grazer abundance does not change appreciably over the course of the experiment, and that the functional response of the grazers depends on the prey concentration at the start of the experiment (Gallegos 1989). Gallegos (1989) and Evans & Paranjape (1992) discuss the implications of these assumptions.

Two different non-linear behaviors are apparent among our dilution experiments. The first involves a relatively steep slope (i.e. strong response) in net growth rate at higher values of D , and a much shallower slope (weak response) at lower values (e.g. Fig. 1C,D,I). As shown in Fig. 6A, this behavior would result from a functional response in which consumption rate is low (or zero) below a certain prey density threshold, but increases when prey density exceeds that threshold (e.g. Type IIa or III responses; see e.g. Steele 1974, Gallegos 1989). Lessard & Murrell (1998) suggested that herbivores in the Sargasso Sea may exhibit such behavior. In contrast, the second observed class of non-linear behavior involves relatively little change in net growth rate among higher values of D , and a very rapid increase at lower values (e.g. Fig. 1E,G). This behavior would result from a saturating functional response (e.g. Type I or II) (Fig. 6B). At one other station (CC-SUMMER-W), the observed non-linear response of net growth rate to dilution was less systematic, and does not clearly fall into either of these 2 groups.

We are unable to determine a clear underlying reason for the differences in apparent grazer functional responses among experiments. In general, differences

in functional responses can be expected to be mediated by differences in prey quality, grazer community structure, grazer condition, and the range of prey concentrations under consideration. Linearity in the dilution experiment data was much more common for *Synechococcus* than for *Prochlorococcus* (7/8 vs 2/8 of the testable experiments, respectively), presumably reflecting a general trend toward linearity in the functional response of grazers to *Synechococcus* (but not to *Prochlorococcus*), at least over the range of cellular concentrations encountered in this study. This difference could reflect differences in the perceived food quality of these prey items by a single group of grazers, or in the behavior of different sub-sets of grazers that specialize on specific prey. Note that the within-treatment variance of observed net growth rates was not different among *Prochlorococcus* and *Synechococcus*; therefore, any deviations from linear regression should have been detected with equal efficiency for both groups.

In the context of grazing on *Prochlorococcus*, the threshold response would be expected at stations where the ambient cell abundance was lowest, since dilutions would be more likely to result in prey concentrations below the feeding threshold. Conversely, the saturating response would be expected to be more common at stations with high ambient cell concentrations. With the exception of the Gulf Stream station, this expectation largely held true: *Prochlorococcus* concentrations were lowest at SS-WINTER-N and CC-SUMMER-E (2.2×10^4 and 4.9×10^4 ml⁻¹, respectively), the 2 stations at which the threshold response was observed. At SS-SUMMER-S, *Prochlorococcus* abundance was high (8.2×10^4 ml⁻¹) and the saturating response was the most pronounced (Fig. 1E). At GS-SUMMER, although *Prochlorococcus* abundance was low (2.6×10^3 ml⁻¹) an obvious saturating response was observed (Fig. 1G). This may reflect a difference in the *Prochlorococcus* strains and/or the grazers that are present in these different environments.

Nutrient status appears to exert a strong influence on functional response under some circumstances. At SS-SUMMER-S, nutrient additions resulted in a dramatic increase in mortality rates, presumably reflecting increased food quality among nutrient-replete prey, as discussed above. In this experiment, functional responses in nutrient-amended and non-amended treatments can be compared directly, since the initial prey concentration, grazer community, and grazer abundance are all identical. Nutrient amendment resulted in a clear change in grazer behavior reflected not only in increased ingestion rates, but in the shape of the functional response as well (Fig. 6B). The dramatic increase in *Prochlorococcus* ingestion rate in the nutrient-amended treatment could be the result of a simple

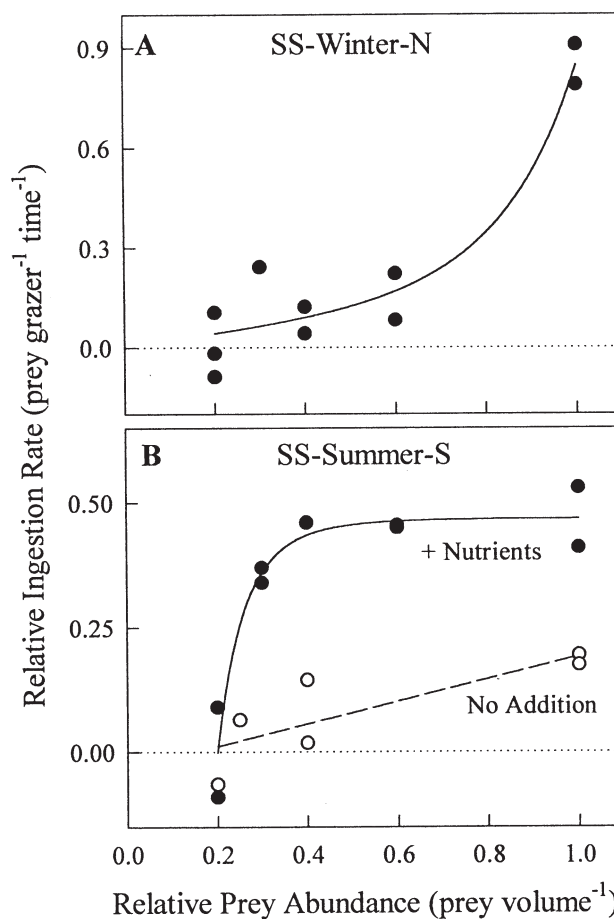


Fig. 6. Representative functional responses calculated from the data in Fig. 2, as described in the text. Solid lines are drawn by eye; broken line is linear least squares regression. Note that the calculations force the mean relative ingestion rate to 0 at a relative prey abundance of 0.2

increase in grazer activity in response to increased food quality. Alternatively, it might reflect prey switching among grazers that prefer heterotrophic bacteria in the non-amended situation, but prefer nutrient-replete *Prochlorococcus* cells in the amended treatment.

Diel periodicity in grazing mortality

The diel patterns of abundance in the Sargasso Sea and Gulf Stream experiments provide evidence of diel variability in grazing mortality among *Prochlorococcus* and *Synechococcus*. At SS-WINTER-N, both *Prochlorococcus* and *Synechococcus* abundance decreased dramatically during the second half of the night, such that the bulk of the daily cell production was consumed within approximately 6.5 h (between 02:00 and 08:30 h) (Fig. 3A). The rate of grazing required to account for this reduction in abundance is

high, but not unreasonable. Given a maximum clearance rate for marine nanoflagellates of 8×10^{-5} ml grazer⁻¹ h⁻¹ (Fenchel 1982a,b), and assuming a nanoflagellate abundance of 10^3 ml⁻¹ (Caron et al. 1999), 50% of a prey population could be consumed in approximately 9 h, a rate of consumption on the same order as observed here.

Cell cycle data from SS-WINTER-N indicate that cell division (which can roughly be assumed to coincide with the presence of G2 cells) in both *Prochlorococcus* and *Synechococcus* occurred largely in the late afternoon and early evening, as documented previously (e.g. Waterbury et al. 1986, Vaultot et al. 1995, Vaultot & Marie 1999) (Fig. 4). Therefore, the lack of significant decreases in abundance during the morning hours in this experiment must reflect low grazing mortality during this time. This same logic applies to SS-WINTER-S, at least for *Prochlorococcus*: although no significant diel variation in *Prochlorococcus* abundance was observed, the cell cycle data clearly demonstrate that *Prochlorococcus* cell division is restricted to the early evening. Therefore, grazing mortality must have increased during this time. Assuming that the timing of cell division at SS-SUMMER-S and GS-SUMMER was similar to that above, and in light of the modest abundance changes over the course of these experiments, grazing mortality appears to have varied over the day at these stations as well. Diel variation in picocyanobacterial grazing has been reported in other studies (e.g. Liu et al. 1997, Dolan & Šimek 1999, Kuipers & Witte 2000), and could be related to diel changes in cell size (resulting from cell division) (Dolan & Šimek 1999), in the availability of alternate prey (Kuipers & Witte 2000), or in grazer abundance and/or behavior.

Influence of growth and grazing on picocyanobacterial community structure

Among the experiments reported here, ambient *Prochlorococcus* and *Synechococcus* abundances ranged over more than an order of magnitude; the abundance ratio of the 2 groups over almost 3 orders of magnitude (Table 2). Numerous 'physiological' factors have been suggested to influence the relative abundance of *Prochlorococcus* and *Synechococcus* over spatial and temporal scales. These include sensitivity to cupric-ion toxicity, temperature response, and light adaptation (see review in DuRand et al. 2001). The influence of these sorts of factors would presumably be reflected in differences between the growth rates of the 2 groups under different conditions. In that case, we might expect to find a positive correlation between growth rate and abundance within each group, or

between the ratios of *Prochlorococcus* and *Synechococcus* abundance on one hand and *Prochlorococcus* and *Synechococcus* growth rate on the other. However, this was not the case. The only significant correlation among these rates and standing stocks was a negative one between *Prochlorococcus* growth rate and abundance ($r = -0.679$, $p < 0.05$). Although the spatial and temporal coverage of these experiments is limited, these results nevertheless suggest that physiological or growth-rate-related controls alone are insufficient to explain the often contrasting patterns of abundance between *Prochlorococcus* and *Synechococcus*. Other processes such as resource partitioning (e.g. Moore et al. 2002), loss terms, and food-web effects likely play a role as well. The factors influencing the strength and relative importance of these processes, as well as the interaction between them, must be addressed if we are to gain a better understanding of the regulation of *Prochlorococcus* and *Synechococcus* populations in the environment.

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