THE ROLE OF PHYSIOLOGY IN THE FORMATION OF *PROCHLOROCOCCUS* SUB-SURFACE MAXIMA IN THE SARGASSO SEA

by

KIRSTEN LEIGH RHODES

(Under the Direction of Brian Binder)

ABSTRACT

Prochlorococcus marinus is a unicellular cyanobacterium that can account for a large share of primary production in oligotrophic environments. Using the contrast between shallow and deep *Prochlorococcus* concentrations in the summertime Sargasso Sea as a model system, we tested the importance of physiological factors in controlling *Prochlorococcus* abundance in this environment. *Prochlorococcus* growth rates were measured using a cell cycle-based approach. We found that growth rate did not vary consistently with depth, nor was it related to ambient *Prochlorococcus* concentration. Thus in some cases growth rate was highest at the subsurface *Prochlorococcus* maximum, while in others it was highest in shallower water, where *Prochlorococcus* concentrations were relatively low. We conclude that physiological factors alone are insufficient to explain depth-related differences in *Prochlorococcus* abundance, and that top-down factors such as grazing and viral lysis likely exert a strong influence on *Prochlorococcus* dynamics.

INDEX WORDS: cell cycle, cyanobacteria, diel, growth rate, *Prochlorococcus,* Sargasso, *Synechococcus*

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KIRSTEN LEIGH RHODES

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KIRSTEN LEIGH RHODES

Major Professor: Brian Binder

Committee: Brian Hopkinson
Liz Mann

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2012

DEDICATION

I would like to dedicate my Master's thesis to my grandparents. My father's parents, who did not get to see my degrees reach completion, have been an important inspiration in my life and I keep reminders of them around me at all times. My mother's parents, with their support and humor, have kept me determined and light-hearted. To my grandpa, no one makes me feel more confident and thankful as you always have. More than for anyone, this is for you, Walter.

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INTRODUCTION

Prochlorococcus marinus (Chisholm 1988) is found throughout the world's oceans between 40° N and 40° S. This small, unicellular cyanobacterium is often the numerically dominant phytoplankton group in open-ocean environments and can account for up to half of the photosynthetic biomass and primary production in oligotrophic regions (Li 1994, Vaulot et al. 1995, Campbell et al. 1997, DuRand 2001). Understanding the processes that influence the abundance and activity of *Prochlorococcus* is therefore important if we are to fully understand the dynamics of open-ocean ecosystems.

The distribution of *Prochlorococcus* in the world's oceans is relatively well-known, and has been reviewed elsewhere (Campbell et al. 1994, Campbell et al. 1997, Partensky et al. 1999, DuRand et al 2001, Johnson et al. 2006). Seasonal patterns and depth distributions have been intensively studied in the subtropical N. Atlantic (the Bermuda Atlantic Time-Series – BATS) and N. Pacific (the Hawaii Ocean Time-Series – HOT) (Olson et al. 1990, Campbell et al. 1997, DuRand et al. 2001, Malmstrom et al. 2010). At BATS, depth-integrated *Prochlorococcus* abundance varies seasonally, being highest in the late summer and fall and lowest in the winter and early spring (Olson et al. 1990, DuRand et al. 2001, Malmstrom et al. 2010). *Synechococcus*, another picocyanobacterium, shows the inverse pattern at this location, reaching a maximum in the winter / early spring and a minimum in summer / early fall.

The depth distribution of *Prochlorococcus* varies seasonally at BATS as well. In winter, *Prochlorococcus* abundance is relatively uniform over the top 100 m of the water column, whereas in summer a strong subsurface maximum (SSM) develops at around 60 – 80 m (DuRand

et al. 2001). The distributional patterns at HOT in the N. Pacific are somewhat different; here *Prochlorococcus* and *Synechococcus* concentrations vary much less over the course of the year, although a seasonal cycle in *Synechococcus* abundance does appear to occur (Campbell et al. 1997, Malmstrom et al. 2010). Dramatic subsurface maxima in *Prochlorococcus* abundance are also absent. These differences are taken to reflect physio-chemical differences between the two sites, i.e. the absence of regular wintertime deep mixing and higher PO_4 concentrations at HOT.

Overlaid on these seasonal and depth patterns of overall *Prochlorococcus* abundance are changes in ecotype abundance. So-called "high-light adapted" ecotypes (HL) are most abundant in the upper euphotic zone while "low-light adapted" ecotypes (LL) are more prominent in the lower euphotic zone (Rocap et al. 2003, Johnson et al. 2006, Zinser et al. 2006, Malmstrom et al. 2010). The overall result is a persistent layering of *Prochlorococcus* ecotypes that has been observed over large geographical areas (Johnson et al. 2006, West et al. 2001, Zinser et al. 2007). Although this ecotype layering persists unchanged throughout the year at HOT, deep winter mixing at BATS tends homogenize the distribution and in this area reproducible seasonal changes in the abundance of each ecotype are evident (Malmstrom et al. 2010).

What factors drive these abundance patterns in *Prochlorococcus*? The strong correspondence between the light and temperature physiology of ecotypes on one hand, and their geographical and depth patterns on the other argues strongly that light and temperature conditions are important determinants of the distribution of *Prochlorococcus* ecotypes (Moore et al. 1998, Moore and Chisholm 1999, Moore et al. 2002, Rocap et al. 2003, Moore et al. 2005, Johnson et al. 2006, Zinser et al. 2006, Zinser et al. 2007, Malmstrom et al. 2010). However, the extent to which these factors determine the overall abundance of *Prochlorococcus* remains unknown, and numerous other factors have been suggested as potential drivers of observed

seasonal and depth-related patterns. At BATS, for example, the seasonally-varying depth of the *Prochlorococcus* SSM is significantly correlated with the depth of the nitracline, suggesting that *Prochlorococcus* abundance profiles may be regulated by nutrient availability (Olson et al. 1990, DuRand et al. 2001). Copper toxicity has also been implicated as a potential regulating factor; cupric ion concentration, which is generally high at the surface and low at depth during the summer in the Sargasso Sea, may influence the development of sub-surface *Prochlorococcus* maxima and could possibly explain the generally higher concentration of *Prochlorococcus* in the oligotrophic North Pacific relative to the North Atlantic (Mann et al. 2002).

All of these potentially controlling factors - light, temperature, nutrient availability, and copper toxicity - are examples of "bottom-up" controls, affecting the growth of *Prochlorococcus*. However, the abundance of a *Prochlorococcus* population (like any microbial population) must ultimately be a reflection of both growth and mortality. The relationship between growth and loss terms on one hand and abundance on the other is not a simple one (e.g. see discussion in Landry et al. 1997). Generally speaking, grazing mortality has been found to approximately balance growth rate in natural *Prochlorococcus* populations (Landry et al. 1995, Liu et al 1995, Binder et al. 1996, Landry et al. 1997, Mann and Chisholm 2000, Llabrés et al. 2011), as would be expected for systems in quasi-steady state. Yet *Prochlorococcus* abundance clearly varies over time and space.

Mann and Chisholm (2000) assessed the effects of iron enrichment on *Prochlorococcus* growth and abundance in the context of the IronEx II study in the equatorial Pacific. In this study the addition of iron increased cell division rates, and *Prochlorococcus* populations grew faster and divided earlier in the day, often followed by a second division. However, this increase in population growth rate was not coupled to an increase in abundance; rather, grazing rates

appeared to increase concomitantly with growth rates, such that *Prochlorococcus* abundance remained unchanged. In this case then, whereas *Prochlorococcus* growth rate was clearly limited by iron availability ("bottom-up" control), *Prochlorococcus* abundance appeared to be regulated by grazing ("top-down" control).

The mechanisms by which *Prochlorococcus* abundance is regulated are clearly complex, and may involve both bottom-up and top-down factors. The goal of the current study was to assess the relative importance of the former in regulating the distribution and abundance of *Prochlorococcus*. To the extent that bottom-up controls act by affecting the physiological status of the population in question, such controls can be expected to be reflected in the growth rate of that population. Therefore we might predict that if bottom-up controls are the primary drivers underlying spatial and temporal patterns of *Prochlorococcus* abundance, then growth rate and abundance should be correlated. The summer-time *Prochlorococcus* vertical distribution in the Sargasso Sea represents a relatively simple system in which to explore this question; cell concentrations in the same water column at the same time typically vary by a factor of 10 or more between shallow depths and the SSM. Furthermore, by virtue of the tightly-phased cell division cycle of *Prochlorococcus*, estimation of its *in situ* growth rate is relatively straight-forward (Chang and Carpenter 1988, Vaulot et al. 1995). By closely examining the relationship between *Prochlorococcus* abundance and growth rate at different depths in this system, we can begin to disentangle the factors that underlie *Prochlorococcus* dynamics in the field..

METHODS

Sampling. Samples were collected in the western Sargasso Sea in May of 2009 and 2010 on the R/V *Cape Hatteras* (Table. 1). On each cruise, a station with a pronounced sub-surface *Prochlorococcus* maximum was occupied for approximately a week, over which time two or three 24 h sampling experiments were performed. For each experiment, samples were taken at 60 or 90 min intervals from four fixed depths, ranging from 20 to 125 m. Samples for flow cytometry were fixed with freshly titrated paraformaldehyde (pH 7.4 –8.1, 0.1% final concentration), held in the dark for 10 min, and then frozen in liquid nitrogen. Samples were kept in liquid nitrogen (2010 cruise) or transferred and stored in a -80°C freezer (2009 cruise) until analysis. A total of 13 time series distributed over 5 experiments and 2 – 4 depths were analyzed (Table 1). For the 2009 cruise, two depths were analyzed for each experiment: a shallow depth with relatively low *Prochlorococcus* abundance and a deeper depth associated with the subsurface *Prochlorococcus* maximum. For the 2010 cruise, two to four depths were analyzed. A small upwelling event (Fig. 1) during experiment 10-1 altered the dynamics of the water column for ~6 h (roughly 21:00-03:00) of the study, making analysis of sampling depths below 70 m unsuitable for analysis due to dramatic drops in *Prochlorococcus* abundance. The shoaling of isopycnals and isothermals moved the DCM upwards during this event, thus sampling at fixed depths would likely have resulted in different (possibly deeper or advected) water being sampled. *Prochlorococcus* in the upper 40 m could not be reliably analyzed under the dual-beam configuration used here (see below), thus these sampling depths were excluded from analysis.

Samples for nutrient and chlorophyll-a profiles were collected before each experiment. Nutrient samples were filtered immediately through 0.22 μm filters (Sterivex), and keep frozen at -20°C until analysis at the Woods Hole Oceanographic Institute Nutrient Analytical Facility or Oregon State University (for the 2009 and 2010 cruise, respectively). Chlorophyll samples (2 L seawater) were filtered through 47 mm GF/F filters and stored in liquid nitrogen prior to processing. Frozen filters were sonicated in methanol, extracted at -20°C overnight, and analyzed fluorometrically using the protocol of Welschmeyer (1994).

Flow cytometry. For pre-experiment depth profiles (Fig. 2), unpreserved seawater samples were analyzed on-board ship with single-beam flow cytometry using a modified Coulter-EPICS 753 flow cytometer (Beckman Coulter, Fullerton, CA, USA), as described previously (Binder et al. 1996). Preserved time series samples were analyzed by dual beam flow cytometry on the same instrument. Samples were chosen in random order (within each time series), defrosted in a 30°C water bath (just long enough to melt: ~5 min), and stained with the DNA-specific stain Hoechst 33342 (0.5 μg ml^{-1} final concentration) (Invitrogen, Carlsbad, CA, USA) for a minimum of 20 min in the dark. Prior to analysis, polystyrene fluorescent beads (Flow Check® 1.0 μm (YG) and 0.494 μm (BB); Polysicences Inc., Washington, PA, USA), were added to each sample, and used to normalize cellular light scatter, red (chlorophyll-derived) fluorescence, and Hoechst fluorescence. Samples were run at an infusion rate of 10 μl min^{-1} for 10 to 50 min, depending on cell abundance within the sample. A minimum of 10,000 *Prochlorococcus* cells were analyzed, except for samples in which low *Prochlorococcus* concentrations made this impractical.

Cell cycle analysis. DNA frequency distributions for *Prochlorococcus* cells were obtained from Hoechst-derived blue fluorescence. These frequency distributions were deconvoluted into their component cell cycle stages (G1, S, G2) using Modfit software (Verity Software House,

Topsham, ME, USA), and assuming a simple model comprised of two Gaussian populations (G1 and G2) and a broadened rectangle (S) (Bagwell 1992). Examples of deconvoluted histograms are shown in Fig 3.

Cell cycle stage progression was used to estimate *Prochlorococcus* growth rate according to the equation:

$$\mu = \frac{1}{t_d}\left(\frac{1}{24}\sum_{t_i=0}^{24}(\Delta t_i \cdot \ln(1 + f_i))\right) \quad \text{(Equation 1)}$$

Where μ is specific growth rate (d^{-1}), t_d is the duration of the S+G2 (i.e. terminal) phase (h), t_i is the sampling interval length (h), and f_i is the fraction of cells in S+G2 for that interval (McDuff & Chisholm 1982). The terminal phase duration (t_d) was estimated as twice the time lag between the S and G2 peaks for each time series (Chang and Carpenter 1990).

An alternative approach for calculating growth rate from cell cycle dynamics depends only on the maximum fraction of cells in the terminal phase over the course of 24 h (f_{max}):

$$\mu_{min} = \ln(1 + fmax) \quad \text{(Equation 2)}$$

Where f_{max} represents the maximum observed fraction of cells in S + G2 (Antia et al. 1990, Vaulot 1992). Note that because this approach is likely to underestimate the fraction of cells that undergo division on a given day, the estimate represents a minimum bound for the actual growth rate of the population.

RESULTS

Water Column Conditions and Cell abundance. Generally speaking, water column conditions were comparable, though not identical, in the 2009 and 2010 stations. In 2010 the deep chlorophyll maximum (DCM), in vivo fluorescence maxima (FM), and nutricline were more shallow and compressed than in 2009, particularly in 10-1, and appeared to broaden and deepen slightly by 10-3 (Fig. 2). Except as noted below, nutrient concentrations at all stations were similar. Preceding weather conditions for both cruises likely were responsible for the water column differences we encountered; this sort of effect was evident at station 10-1, during which an upwelling feature (perhaps an internal wave) passed (See Methods, Fig. 1). This event was followed by two days of high swells and rain. The resultant deepening of the surface mixed layer is obvious (Fig. 2, compare 10-1 and 10-2 density profile). Light attenuation coefficients were similar at all stations (not shown).

Prochlorococcus abundance showed clear subsurface maxima (SSM) near 100-110 m and 80 – 90 m in the 2009 and 2010 experiments, respectively (Figs. 2, 4). Cell concentrations at the SSM were 10 – 20 fold greater than near the surface. *Synechococcus* abundance was comparable to *Prochlorococcus* at the surface, but varied relatively little over the top 100 m (Fig. 2). Thus at the depth of *Prochlorococcus* SSM, *Prochlorococcus* was typically one order of magnitude higher in abundance than *Synechococcus*. Over the course of each cruise, *Prochlorococcus* abundance remained relatively stable, suggesting that the populations were in approximate steady-state (Fig. 5). A small weather event occurred between 10-1 and 10-2, which may be the cause of slight variations in surface populations during this time.

The depth of the *Prochlorococcus* SSM corresponded with the DCM in all experiments (±10 m) (Fig. 2). The FM ranged between 0 and 30 m below the DCM. The *Prochlorococcus* SSM typically fell at or slightly above the top of nutricline (as judged by the deepest sampling depth prior to a significant increase in Nitrite + Nitrate) (Fig. 2). Station 09-2 was the lone exception to this generalization: in this case the *Prochlorococcus* SSM was 40 m shallower than the apparent nutricline, which at 140 m was considerably deeper than that in the other experiments. PO_4 levels in this experiment were elevated and variable throughout the upper 100 m and the *Prochlorococcus* SSM was unusually broad. Mean forward angle light scatter (FALS) of *Prochlorococcus* cells increased significantly with depth (r^2=0.76, p=0.0001) (Fig. 6). Cellular FALS at the SSM was approximately 5 fold greater than at the surface. Generally speaking, we could discern no coherent diel patterns in mean FALS in this study. Interestingly, there were double populations noted in experiment 10-3 at 75 and 100 m. These populations were distinguishable by FALS but produced no abnormalities in the associated DNA histograms, indicating that the cell cycles of these sub-populations were well synchronized (see below).

Cell cycle dynamics. Prochlorococcus DNA frequency distributions at any given time point were bimodal, as expected (e.g. Fig. 3). Cell cycle dynamics over the course of the day for all stations and depths are shown in Figure 7. *Prochlorococcus* populations were predominantly in G1 phase from about 04:30 to 13:00, after which time S-phase cells began to appear. The fraction of S-phase cells peaked in the early evening, from 17:00 - 23:00, with shallow populations lagging deeper ones. G2 cells peaked between 22:00 and 02:00, between 1 - 5 h after the S peak. Populations generally returned to G1 phase 2 - 5 h after the peak in G2. In a few cases, however, G2 cells persisted until the end of the experiment. This behavior is particularly evident in experiment 10-1 (both depths), and appears to be associated with

secondary S peaks. Recall that an upwelling event occurred during the course of this experiment (see Methods, Fig. 1). This may have altered the physiology and/or composition of the *Prochlorococcus* populations at the depths in question.

Prochlorococcus growth rates. *Prochlorococcus* growth rates estimated from cell cycle dynamics ranged from 0.23-0.78 d^{-1} in this study (Figs. 7, 8). The general relationship between growth rate and depth appeared to differ between the two cruises. In 2009, growth rate was higher at 45 m than at 85 m in both experiments (0.38 d^{-1} and 0.1d^{-1} difference in 09-1 and 09-2, respectively). In contrast, in 2010 the highest growth rates were found at or below the SSM, although the trends varied between experiments. Within 10-1, growth rate at 70 m was essentially double that at 40 m. In 10-2, growth rate at 50 m and at the 80 m SSM were approximately equal, followed by a slight increase of 0.05 d^{-1} at 100 m. Finally, in 10-3 growth rates increased with depth down to 100 m, below which growth rate dropped sharply from 0.59 d^{-1} to 0.23 d^{-1} at 125 m. The lowest growth rate of all experiments was found at this 125 m depth. For comparison, the net *Prochlorococcus* growth rates at the shallow and SSM experimental depths over the course of each cruise were respectively 0.10 and 0.06 d^{-1} in 2009, and 0.05 and -0.03 d^{-1} in 2010 (estimated from regressions in Fig. 5).

Given the consistent trends in *Prochlorococcus* abundance versus depth on one hand, and the variable trends in growth rate versus depth on the other, no clear relationship between growth rate and abundance emerges from our results (Fig. 9). In 2009, the 2 fold increase in *Prochlorococcus* abundance from shallow to deep in both experiments is accompanied by a 1.3- to 1.75-fold *decrease* in growth rate (Fig. 9a). In contrast, in 2010 the increase in *Prochlorococcus* abundance from shallow depths to the SSM corresponded to no change or an increase in growth rate (Fig. 9b, solid arrows). The decrease in abundance immediately below

the SSM appeared to be accompanied by a continued increase in growth rate (Fig. 9b, broken lines); although at the greatest depth, both abundance and growth rate was low (Fig. 9b, dotted line).

In order to assess the validity of the calculated growth rates discussed above, those values were compared to estimates based on the f-max approach (see Methods). Note that this approach is based solely on the maximum fraction of cells in S + G2 observed over the course of the day, and is expected to yield lower-bound estimates of actual growth rate. Fig. 10 shows this minimum estimate in relation to our calculated growth rate estimates. Most estimates fell on or near a 1:1 line, and 85% of the data points for calculated growth rate were greater than or equal to the calculated minimum growth rate. The highest calculated growth rates were the strongest outliers in this test. The two lowest calculated growth rates were the only points that fell above the 1:1 line, but the difference in values was small.

Two pieces of information are used to estimate growth rates based on the Carpenter-Change approach: the time-integrated fraction of the population in S or G2, and the combined duration of S and G2 phases (t_d) (see Eq. 1). In this study, the latter factor appears to be dominant. Estimated growth rate is negatively correlated with S+G2 duration ($r = 0.79$, $p=0.0012$) (Fig. 11a), but uncorrelated with the integrated fraction in S or G2 (not shown). It is important to note the possibility of autocorrelation here, as growth rate is calculated as a function of t_d^{-1} (Eq. 1). However the relationship between *in situ* growth rate and S+G2 duration observed here is consistent with laboratory studies that measured S+G2 duration independently (Burbage and Binder 2007)(Fig. 11b).

DISCUSSION

Cell abundance and distribution. The depth profiles of *Prochlorococcus* and *Synechococcus* observed here were consistent with previous findings in the Sargasso Sea during this time of year (Olson et al. 1990, DuRand et al. 2001, Burbage and Binder 2007, Malmstrom et al. 2010). *Prochlorococcus* exhibited a consistent, pronounced SSM, whereas *Synechococcus* abundances remained relatively uniform throughout the upper euphotic zone (Fig. 2). At BATS (~780 km E/NE of our stations), the *Prochlorococcus* SSM develops somewhat later in the year, although the maximum cell concentrations observed here ($0.8 – 1.0 \times 10^5$ ml^{-1}) were comparable to the maximum reported at BATS (DuRand et al. 2001).

Daily averaged FALS of *Prochlorococcus* showed a clear trend of increasing cell size with depth: FALS increased approximately 5-fold from 40 m to 125 m over all the experiments. This pattern of increasing *Prochlorococcus* FALS with depth has been observed before (Chisholm et al. 1988, Olson et al. 1990, Binder et al. 1996). It is not clear whether it reflects depth-related changes in ecotype composition of the *Prochlorococcus* community or a more fundamental physiological response. This question might be addressed in the future by direct analysis of ecotype abundance in our samples using qPCR.

The *Prochlorococcus* SSM in the present study was approximately coincident with the deep chlorophyll maximum, and generally occurred just above the nitricline. This relationship between the depth of nitricline and the *Prochlorococcus* SSM (and/or median depth) has been noted previously, and has been taken as evidence that nitrate or a co-occurring nutrient limits *Prochlorococcus* abundance (Olson et al. 1990, DuRand et al. 2001). The role of nitrate *per se* is

unknown at present: although culture studies suggest that *Prochlorococcus* cannot utilize nitrate, and that only a restricted set of ecotypes can utilize nitrite (Rocap et el. 2003), more recent evidence based on both direct measures of N-assimilation in the field and metagenomic analysis strongly suggest that both N forms can be utilized by at least a fraction of *Prochlorococcus* (Casey et al. 2007, Martiny et al. 2009).

Notwithstanding the apparent influence of nutrient supply on the *Prochlorococcus* SSM, depth-integrated *Prochlorococcus* abundance has been shown to be directly related to nitricline depth (Olson et al. 1990, Partensky et al. 1999) such that well mixed, high-nutrient water columns are associated with lower *Prochlorococcus* concentrations. This observation seems to contradict the nutrient-limitation hypothesis, but it is important to note that nitricline depth is a function of water column stability, which is associated with a suite of environmental factors that could influence *Prochlorococcus*. Cavender-Bares et al. (2001) showed directly that surface *Prochlorococcus* concentrations were negatively related to nitrite +nitrate (and to phosphate) concentration in a transect between the Gulf Stream and the Sargasso Sea. *Prochlorococcus* likely benefits from scant nutrients in well-stratified water columns; its 0.5-0.8 μm diameter cell size reduces the threshold for diffusion limitation (Chisholm 1992). Under less oligotrophic conditions, species with higher growth rates could presumably out-compete *Prochlorococcus.* Cavender-Bares et al. (2001) suggest that *Prochlorococcus* may also benefit from low inorganic nutrient concentrations by virtue of their apparent ability to utilize organic N and P. Whatever the basis for the negative correlation between (inorganic) nutrients and *Prochlorococcus* abundance, this observation led these authors to conclude that the correspondence between nitricline depth and *Prochlorococcus* SSM was driven more by light availability than by nutrient supply.

Other environmental factors have also been hypothesized to control *Prochlorococcus* distributions. In addition to the negative correlation between (inorganic) nutrients and *Prochlorococcus* abundance, Cavender-Bares et al. (2001) found that surface *Prochlorococcus* abundance was positively correlated with sea surface temperature. Temperature has also been implicated in setting the upper latitudinal limits for *Prochlorococcus* (Johnson et al. 2006). More recently, Malmstrom et al. (2010) argued that at least with respect the ecotype distribution, temperature and light conditions are sufficient to explain the observed depth distribution dynamics. Finally, Mann et al. (2002) suggested that cupric ion toxicity could contribute to the formation of the *Prochlorococcus* SSM: during the summer in the Sargasso Sea free cupric ion concentration can be high enough at the surface (but not a depth) to inhibit *Prochlorococcus* growth during the summer.

All of these potential controlling factors are "bottom-up," i.e. they exert their influence through the physiology of the population in question. Thus, if any of them are primarily responsible for the formation of the *Prochlorococcus* SSM, one might expect that in the present case growth rate (reflecting overall physiological status) should be higher at depth where *Prochlorococcus* abundance is high, than at the surface where abundance is low. Thus by comparing the growth rate of *Prochlorococcus* populations at different depths, we can test the hypothesis that bottom-up controls play a major role in the formation of *Prochlorococcus* SSM in the Sargasso.

Cell Cycle & Growth Rate. Cell division in natural *Prochlorococcus* populations is tightly phased over the diel light:dark cycle (Fig. 7) (Vaulot et al. 1995, Partensky et al. 1996, Liu et al. 1997, Vaulot and Marie 1999, Jacquet et al. 2001a, Binder & DuRand 2002). The tight phasing of *Prochlorococcus* populations makes them amenable to a cell cycle-based approach for

estimating *in situ* growth rate (Chang and Carpenter 1988, Vaulot et al. 1995, Mann & Chisholm 2000, Blythe and Binder 2007). This approach is not without pitfalls, but it does provide estimates of *in situ* growth rate that are free of bottle artifacts and relatively insensitive to grazing and other loss terms.

The range of growth rate values observed in the present study was generally consistent with values from other studies (Goericke et al. 1993, Partensky et al. 1996, Binder et al. 1996, Binder and Burbage 2007). However, in contrast to previous studies that showed a decrease in growth rate with depth (e.g. Vaulot et al. 1995, Partensky et al. 1996) no consistent relationship between depth and growth rate emerged in the present study (Fig. 8). Generally speaking, growth rate appeared to decrease with depth in the 2009 experiments, but increase with depth in the 2010 experiments, at least down to 100 m. Such an increase in growth rate with increasing depth has rarely been reported, but is not without precedent: Mann et al. observed a similar phenomenon in the southern Sargasso Sea (personal communication). The reasons for the differences between cruises are not clear. Although conditions were similar in both years, the nitricline (and associated *Prochlorococcus* SSM) was deeper in 2009, and phosphate concentrations appeared to be elevated at the surface, at least during 09-2. However it is not obvious how these conditions might result in the different growth rate versus depth patterns observed.

As has been observed previously (Vaulot et al. 1995, Vaulot and Marie 1999, Jacquet et al. 2001b), cell cycle progression was slightly delayed at the surface. This is hypothesized to be a mechanism for avoidance of UV damage to DNA during replication (Llabrés and Agustí 2006, Kolowrat et al. 2010). Studies of cell death in the Atlantic Ocean and the Mediterranean Sea have found that the highest percent of dead cells is tied to the highest irradiance (Agustí 2004,

Agawin and Agustí 2005, Llabrés and Agustí 2006, Llabrés et al. 2010, Llabrés et al. 2011). Further, Llabrés et al. (2011) studied daily patterns of cell death in the Mediterranean Sea and found the highest percentage of dead *Prochlorococcus* cells were found in more shallow waters during the time of DNA synthesis, suggesting that any damage to the cell during DNA synthesis may lead to death if the cell does not recover quickly.

Comparison of our growth rate estimates with minimum estimates based on the F-max approach (see Methods) suggests that our estimates are reasonable (Fig. 10). The persistence of G2 cells in water showing the very highest growth rate (0.779 d^{-1} at 70 m during 10-1) may been a reflection of a second wave of S and G2, as has been observed in *Prochlorococcus* growing faster than 0.69 d^{-1} (Shalapyonok et al. 1998). Alternatively, the persistence of G2 may reflect perturbations resulting from physical mixing, or perhaps a compression of many populations and their respective cell cycle stages as the feature passed through. If introduced cells could not acclimate to different light or nutrient levels it is possible that the expected diel rhythm would be skewed (e.g. Vaulot et al. 1995, Kolowrat et al. 2010, Malmstrom et al. 2010). Deprivation of nutrients or light has been shown to arrest cells in G1, and depletion of P has shown cells to arrest in any of the cell cycle phases (Chisholm et al. 1986, Jacquet et al. 2001b, Parpais et al. 1996). The streamlining of the *Prochlorococcus* genome appears to have resulted in a loss of robustness of the circadian clock (Dufresne et al. 2003, Holtzendorff et al. 2008); thus the notion of a "well behaved" and predictable cell cycle in *Prochlorococcus* may need to be re-evaluated.

Some insight into the cell cycle behavior of *Prochlorococcus* can be gained by considering the relationship between growth rate and t_d (the combined duration of S + G2) (Fig. 11a). Given that the growth rate calculation includes a t_d^{-1} term (see Methods), this relationship could simply reflect "autocorrelation." However, the concordance between these results and

those of Burbage and Binder (2007) based on laboratory cultures strongly suggests that this relationship has biological significance (Fig. 11b). Note that Burbage and Binder calculated the duration of S and G2 based on the equations of Slater et al. (1977) for steady state populations, an approach wholly independent of the Carpenter and Chang (1988) approach used here.

Bottom-up Control of the Prochlorococcus SSM. The reproducible depth distribution of *Prochlorococcus* throughout this study was not reflected in our growth rate estimates: there appeared to be no consistent relationship between abundance and growth rate. In some experiments, growth rate was highest at (or below) the *Prochlorococcus* SSM, while in others, the trend was the opposite (Figs. 8, 9).

There is no fundamental reason to expect that growth rate should be strongly related to abundance. In fact, in the IronEx-II study discussed above, *Prochlorococcus* abundance remained unchanged in face of an iron-stimulated increase growth rate (Mann & Chisholm 2000). Nevertheless, as argued previously, if bottom-up forces were important in controlling abundance, a correlation between growth rate and abundance might be expected. It is important to note that this would only be the case if the population in question were in quasi-steady state, i.e. that the abundance of the population was approximately stable. In the absence of such stability, the observed level of abundance at any particular time might be a reflection of the growth rate at some previous time. Thus for example, if the population were in the waning phase of a bloom, high growth rate in the recent past may have led to high abundance, but growth rate at present might be low. Although we do not have any information regarding the state of the *Prochlorococcus* population at our stations prior to our arrival, the generally stable *Prochlorococcus* cell numbers we observed over the course of each cruise suggest that the assumption of quasi-steady state is not unwarranted (Fig. 5). Therefore, taken together our

results do not support the notion that *Prochlorococcus* abundance is related to physiological status – i.e. the most abundant cells are not always the fastest growing cells. This idea is reinforced by the observation that growth rate increased below the SSM (in the two experiments in which this can be assessed), even as *Prochlorococcus* abundance decreased (Fig. 9b, dashed lines).

The preceding doesn't imply that bottom-up controls are inconsequential with respect to *Prochlorococcus* abundance patterns, but it does suggest that a complete understanding of these patterns must include a consideration of top-down controls such as grazing and viral lysis. As discussed in the Introduction, micrograzers have been shown to graze *in situ Prochlorococcus* at a rate approximately equal to growth (Landry et al. 1995, Liu et al. 1995, Liu et al. 1997, Reckermann and Veldhuis 1997). However, little is known about the mechanisms by which the balance point between grazing and growth is regulated, i.e. by which the standing stock is set. Viral lysis may be a significant source of picocyanobacterial mortality as well (Brussard 2004). Although *Prochlorococcus* phages and resulting mortality rates have been described, their contribution to *Prochlorococcus* mortality appears minimal: this is likely a result of low encounter rates in oligotrophic environments (Sullivan et al. 2003, Baudoux et al. 2007). In addition to grazing and viral lysis, cell death from environmental stressors may also influence the abundance, and possibly growth rate of *Prochlorococcus* populations (Llabrés et al. 2011).

Prochlorococcus abundance must ultimately reflect the balance between growth rate and mortality. Bottom-up forces such as nutrient availability, light, and temperature affect the former, while top-down forces such as grazing, viral lysis, and cell death affect the latter. Disentangling the details and interrelationships of these controlling mechanisms will require a synthesis of various methodologies across many levels. Further studies of production and

standing stock can provide resolution for this complex issue of community structure, regulating features, and inter-woven dynamic cycles. Global climate change models suggest warming in the tropical regions will enhance oceanic stratification, thereby favoring *Prochlorococcus* (Bouman et al. 2011). Clearly, gaining an understanding of the factors that regulate *Prochlorococcus* populations is necessary if we are to be able to predict the fate of these populations in the future.

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TABLES AND FIGURES

Table 1. Experimental dates, locations, and sampling depths. Cruise refers to R/V Cape Hatteras cruise designation.

Exper.	Cruise	Depths Analyzed	Date	Lat˚N	Lon˚W
09-1	CH0409	45, 85 m	26-27 May 2009	30.172	71.999
09-2	CH0409	45, 85 m	29-30 May 2009	30.168	72.033
10-1	CH0510	40, 70 m	22-23 May 2010	30.704	72.679
10-2	CH0510	50, 80, 100 m	27-28 May 2010	30.746	72.718
10-3	CH0510	50, 75, 100, 125 m	30-31 May 2010	30.805	72.656

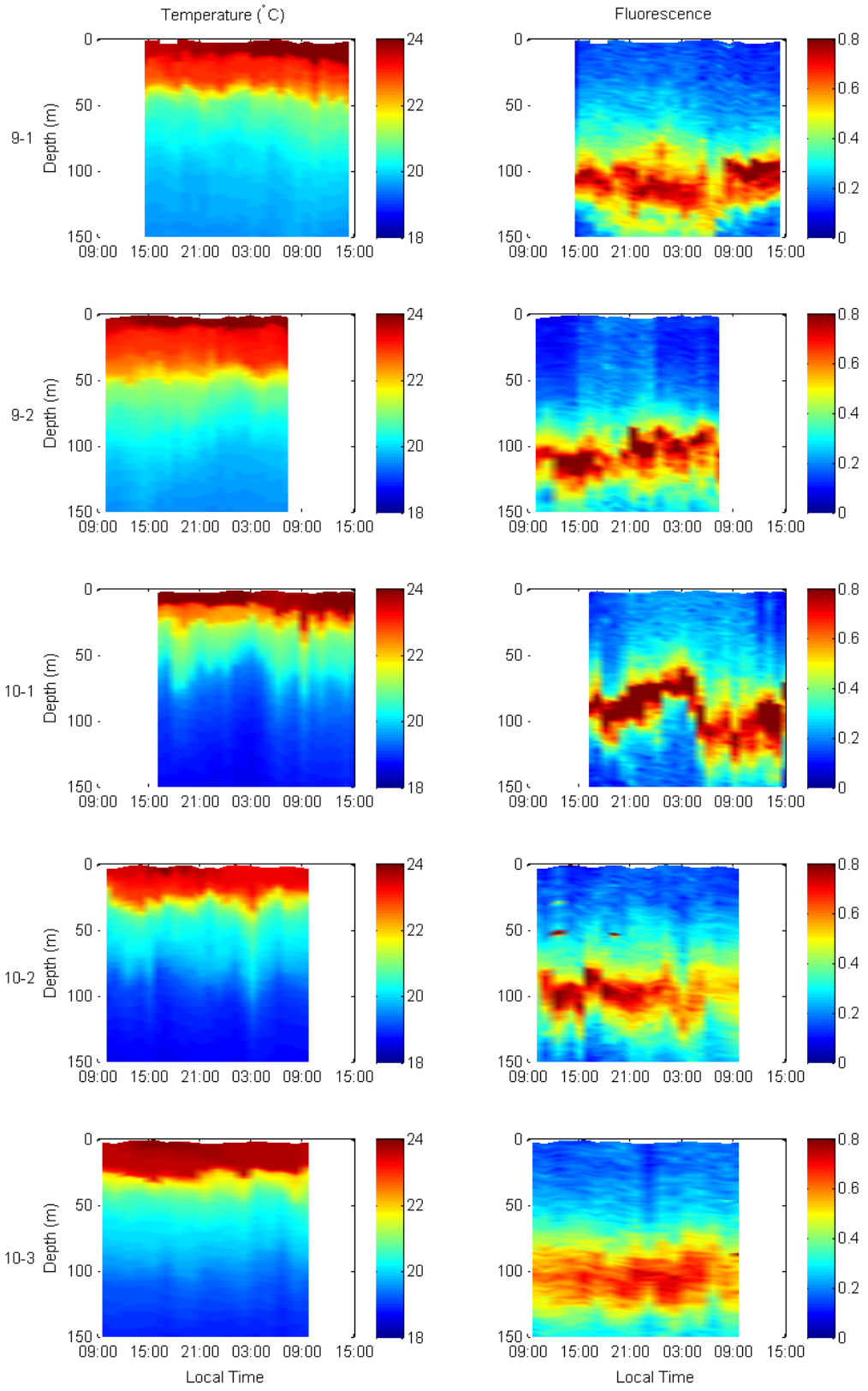

Fig. 1. CTD-based temperature and fluorescence versus depth and time over the course of each experiment.

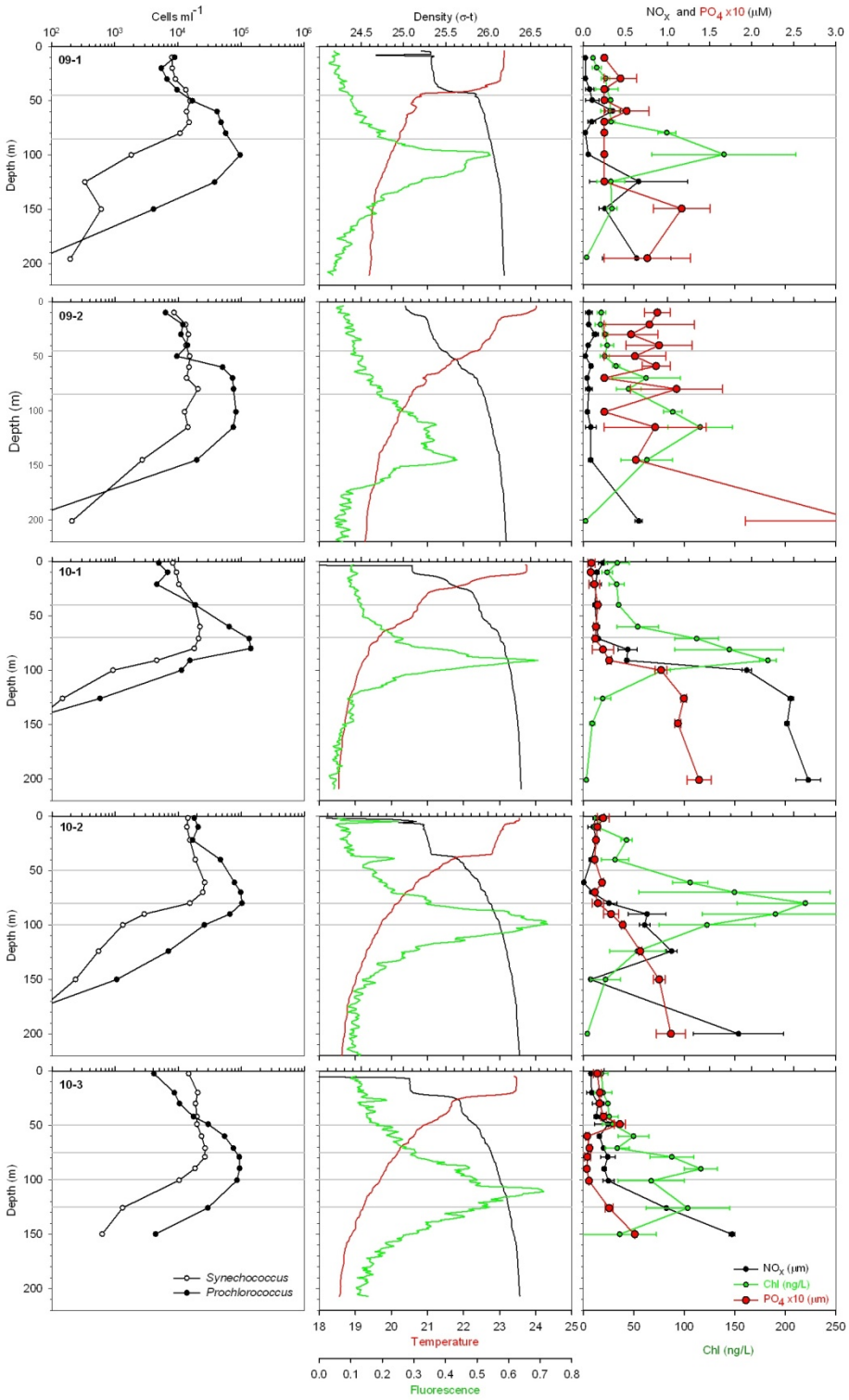

Fig. 2. Water column conditions prior to the start of each experiment. Left column: *Prochlorococcus* and *Synechococcus* cell concentrations; middle column: temperature, density, and chlorophyll fluorescence; right column: chlorophyll concentration, nitrate plus nitrite concentration, and phosphate concentrations ×10(all values are mean ± SE, n=2). Horizontal lines indicate depths analyzed in this study.

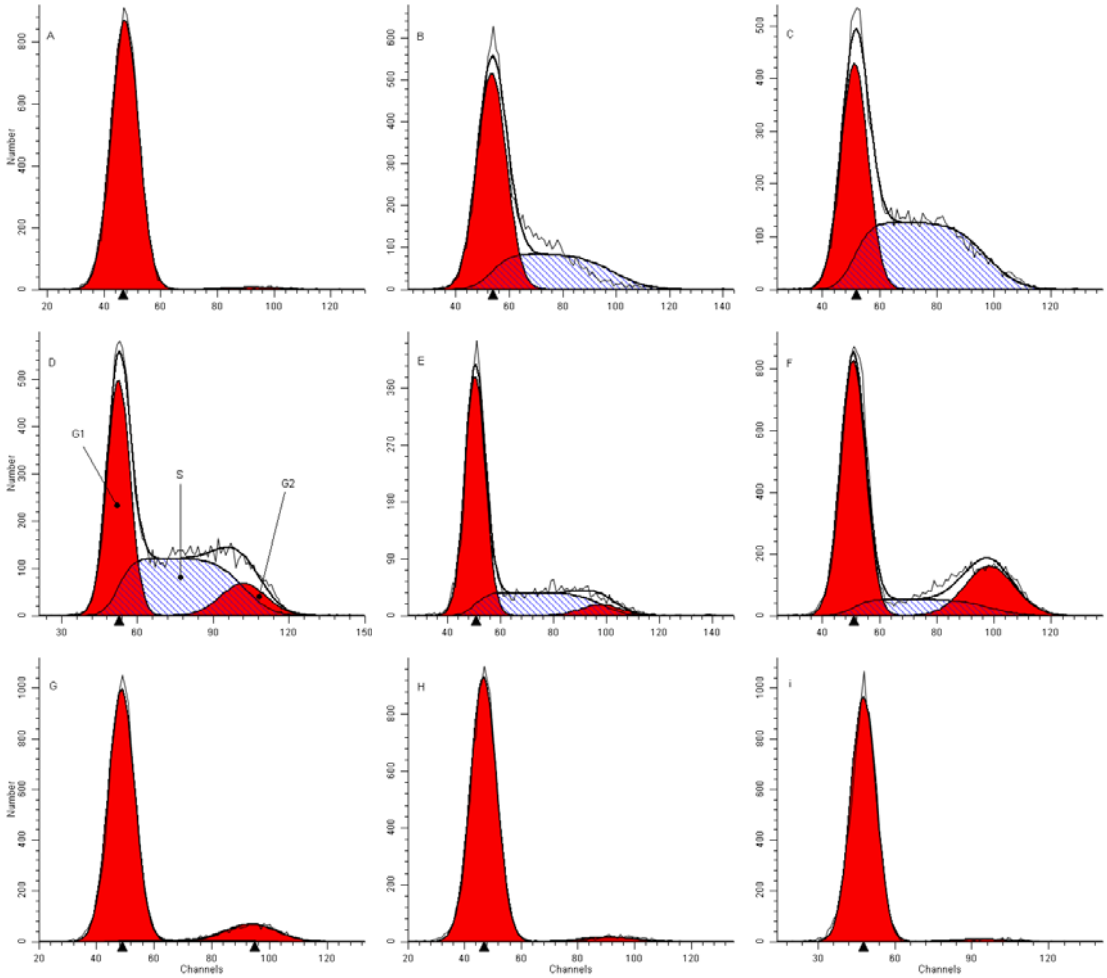

Fig. 3. Representative DNA frequency distributions, from 75 m in experiment 10-3 at different times of the day: A) 11:00 B) 17:00 C) 18:30 D) 20:00 E) 21:30 F) 23:00 G) 03:30 H) 06:30 and i) 09:30. Histograms show G1, G2 and S- solids and hatched, respectively. Horizontal axis represents relative DNA content and vertical axis represents cell number.

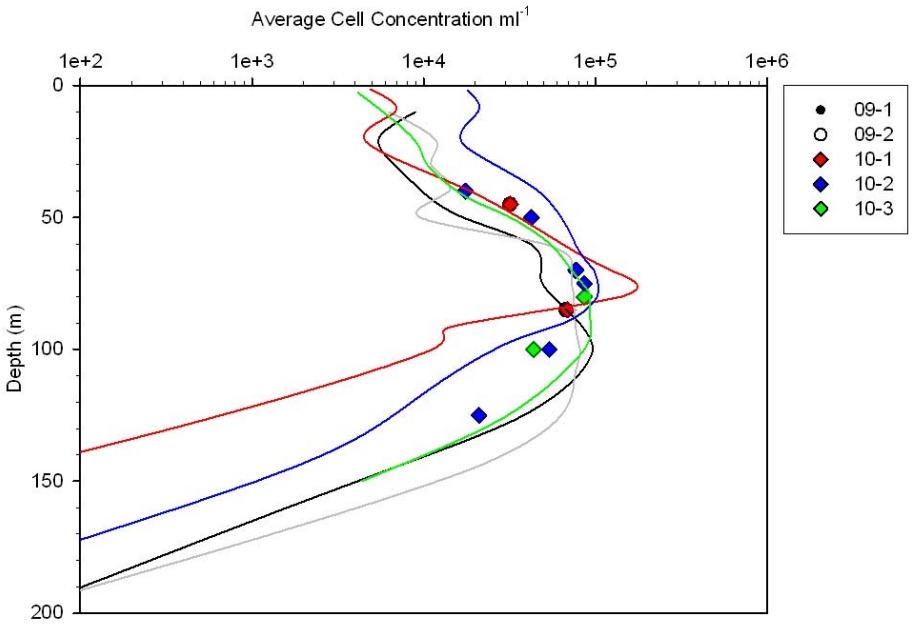

Fig. 4. Detail of *Prochlorococcus* cell concentration vs. depth. Pre-experiment depth profiles from Fig. 2 (solid lines) overlaid with mean cell concentration determined at each experimental depth over the course of each experiment.

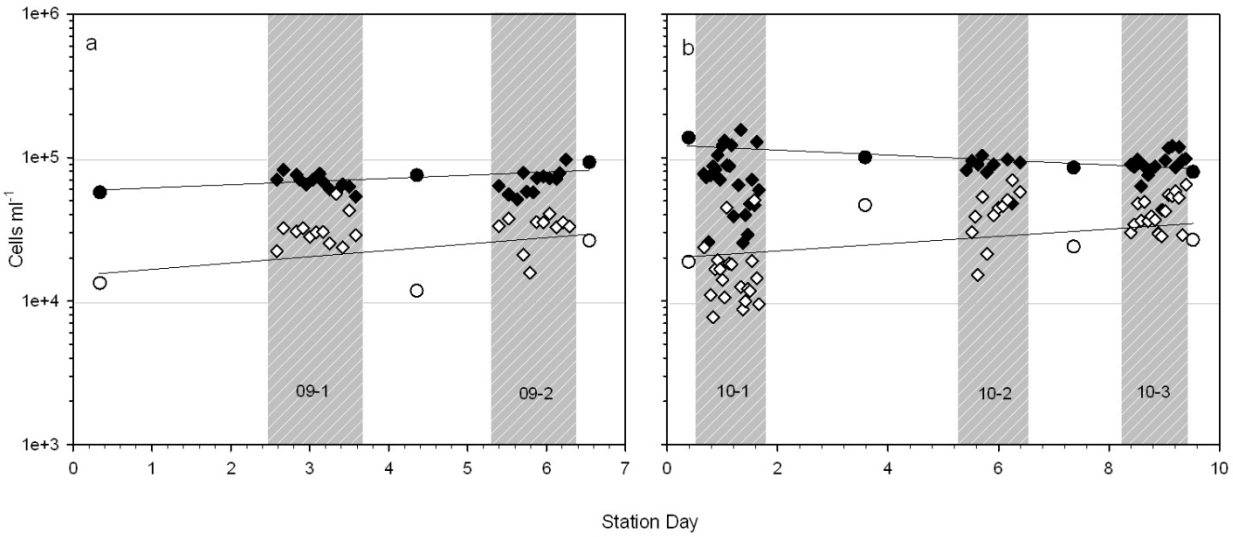

Fig 5. *Prochlorococcus* abundance at or near the experimental depths for the duration of each cruise. a) CH0409 at 45 m (open symbols) and 85 m (closed symbols); b) CH0510 at 45 m (open symbols) and 75 m (closed symbols). Circles represent profile data taken between experiments, diamonds represent cell abundance measured over the course of the experiments. Shaded grey areas denote the period of each experiment. Dark lines represent exponential fit based on the profile data and mean cell count for each experiment.

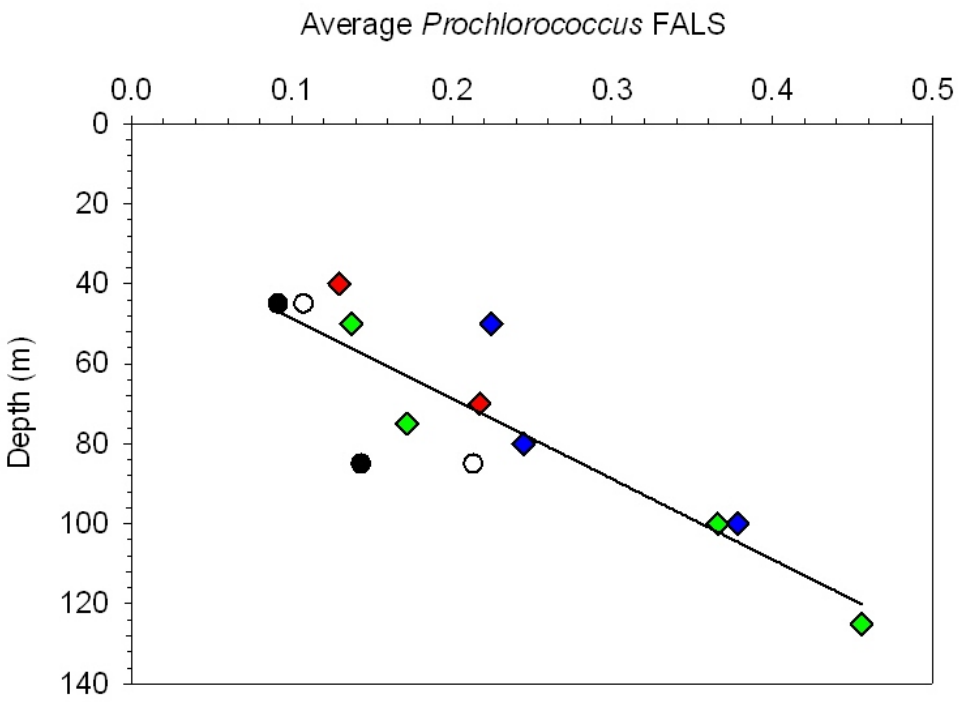

Fig. 6. Relationship between mean *Prochlorococcus* FALS (normalized to 0.494 μm diameter beads) and depth. See Fig. 4 for legend.

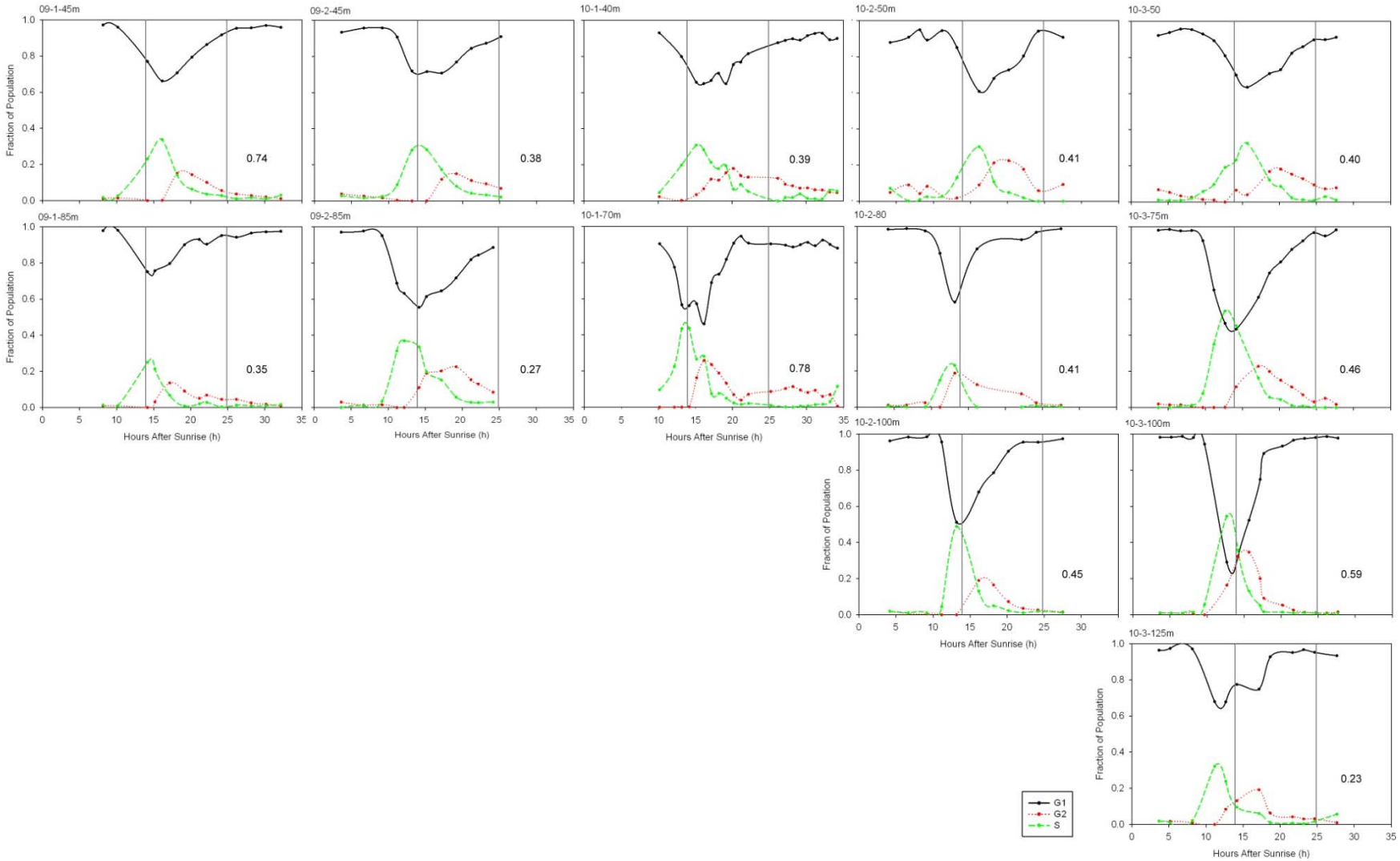

Fig. 7. Diel progression of *Prochlorococcus* cells through G1, G2 and S phases (solid black, dotted red, and dashed green lines, respectively) relative to hours after sunrise. Sunrise corresponds to 0 on x-axis, followed by vertical gray lines showing time of sunset and the subsequent sunrise. Fraction of population is given out of 1.0 (vertical axis). Experiment number and depth are noted in the upper left of each panel. Growth rate is noted in the lower right of each panel.

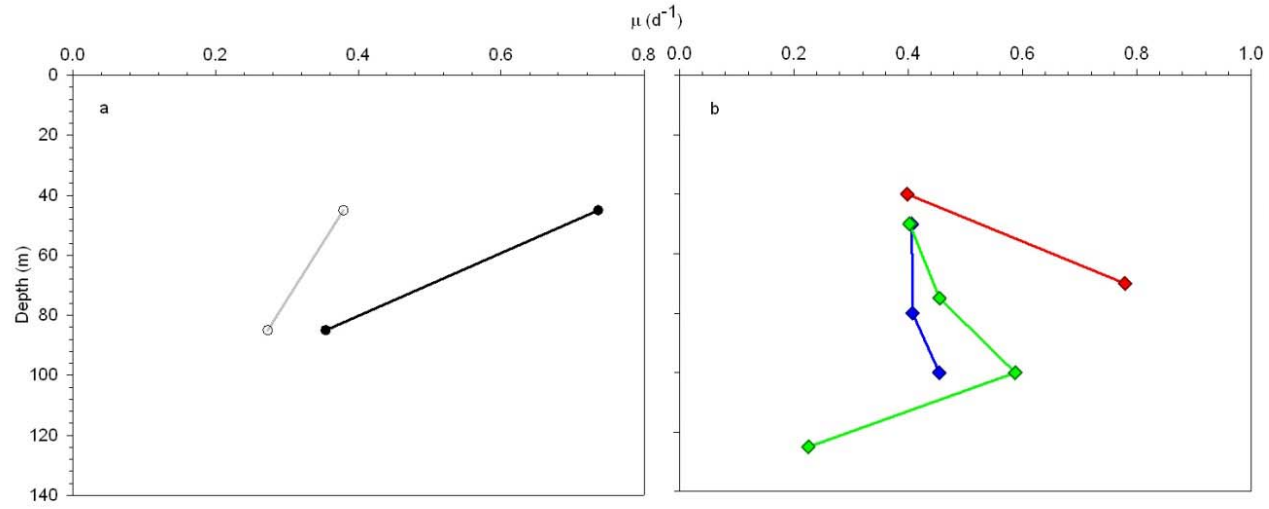

Fig. 8. *Prochlorococcus* growth rates in relation to depth, divided between a) CH0409 and b) CH0510. See Fig. 4 for legend.

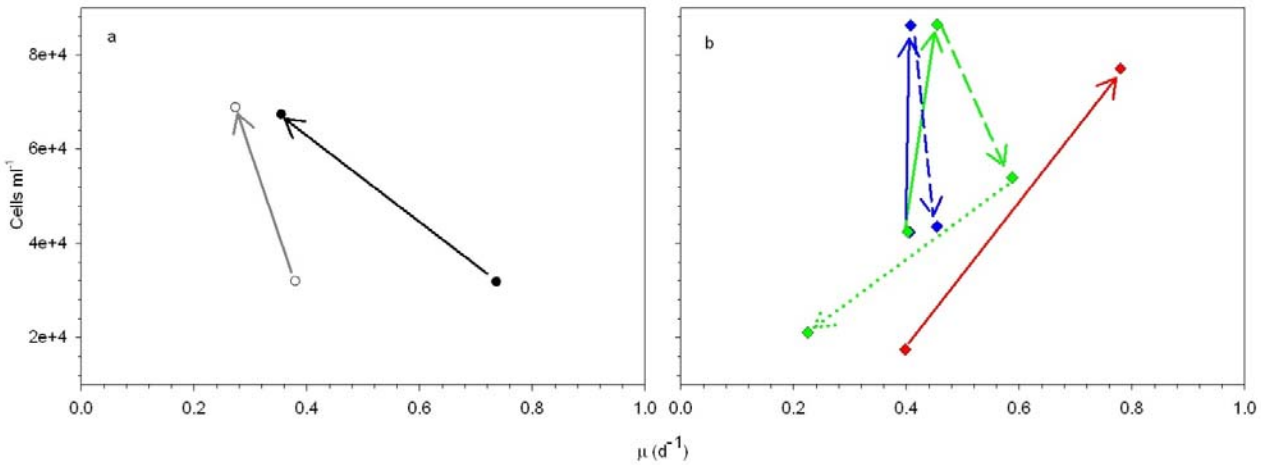

Fig. 9. *Prochlorococcus* growth rates in relation to cell abundance divided between a) CH0409 and b) CH0510. Arrows indicate progression downwards in the water column, beginning with the transition from shallow to SSM (solid lines), to below the SSM (dashed lines), into deeper water (dotted line). See Fig. 4 for legend.

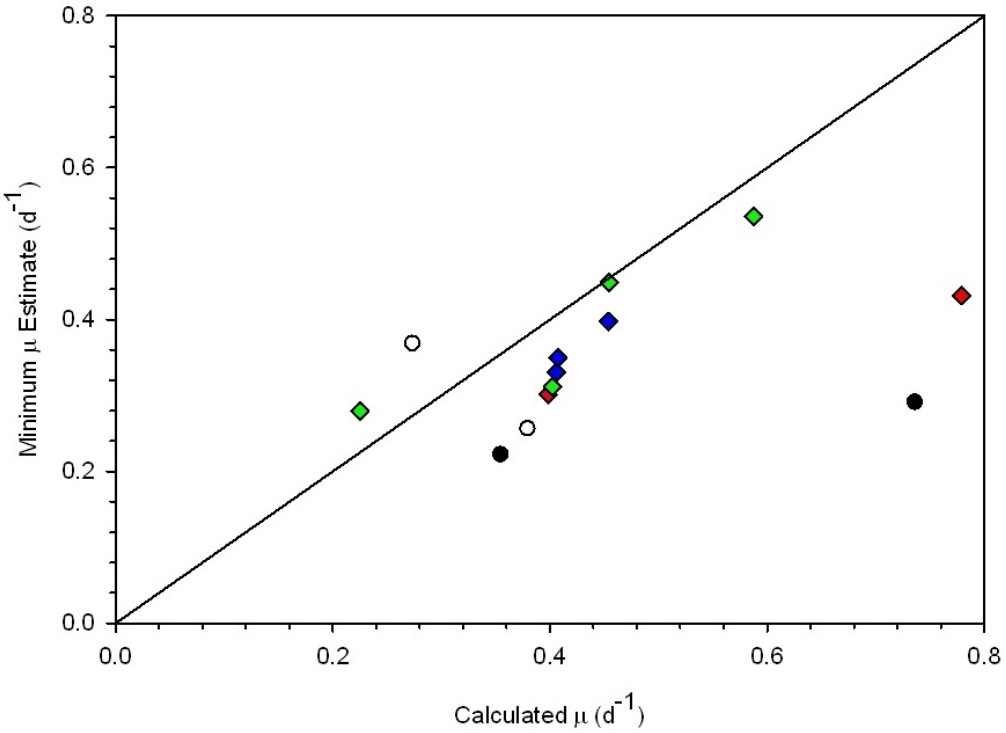

Fig. 10. The f-max approach: comparing minimum growth rate estimates to calculated growth rate estimates relative to a 1:1 line. See Fig. 4 for legend.

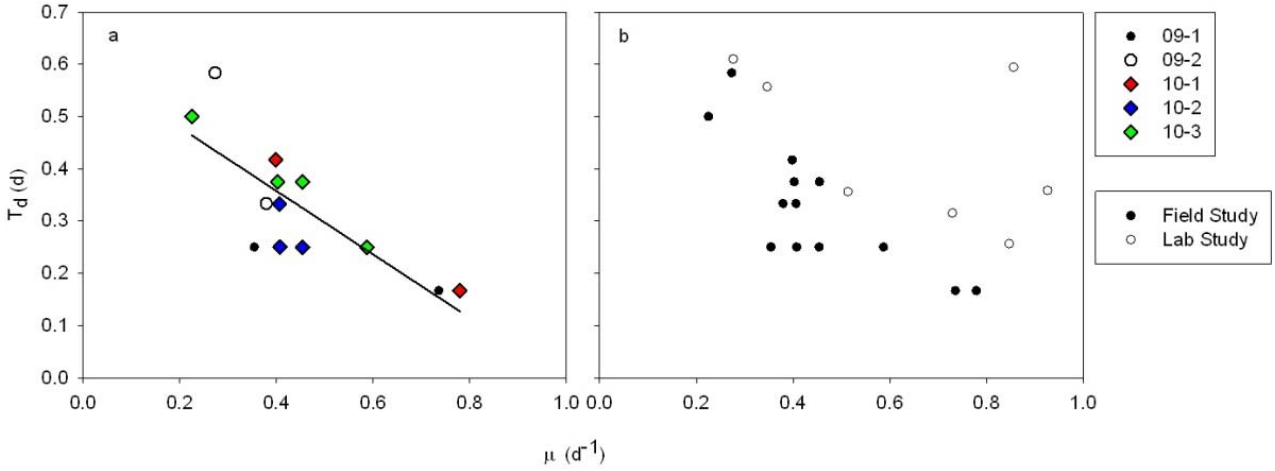

Fig. 11. The duration of S+G2 (t_d) vs. growth rate. (a) data from this study (r=0.794, p=0.0012); (b) same data (closed symbols), plotted with data from Burbage & Binder (2007) (open symbols).