Cyclic electron flow: powering unique motility and alternative nitrogen uptake in *Synechococcus* WH8102 during nitrogen limited growth

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Abstract

Global atmospheric carbon levels are continuing to rise from pre-industrial revolution levels. One of the main regulators of global atmospheric carbon levels, and thus climate, is the world's oceans. Unicellular marine cyanobacteria account for a large percent of total marine carbon fixation. We measured chlorophyll-specific ^{14}C uptake rates (P^b) and net primary production (NPP*) in continuous steady state cultures of *Synechococcus* WH8102 grown at three different nitrogen limited specific growth rates. P^b, NPP*, as well as cellular carbon, nitrogen, and Chl data revealed that NPP* was linearly dependent on nitrogen limited growth rate (R^2=0.99). P^b also increased with nitrogen limited growth rate for *Synechococcus* WH8102, but growth rates of $0.2d^{-1}$ and $0.5d^{-1}$ gave similar production values (R^2=0.89). The relationship between NPP* and P^b generally increased with decreasing nitrogen limitation, however, the two production measurements did not co-vary and P^b could not give a simple estimate for NPP*. Based on our experimental results, extensive literature search, and examination of *Synechococcus* WH8102's annotated genome, we conclude that cyclic electron flow around PS I is used by *Synechococcus* WH8102 to generate additional energy for uptake of alternative nitrogen sources and power for cellular motility.

Introduction:

Climate change is one of the greatest environmental challenges faced today. Effects of climate change are reflected by increasingly sporadic climate patterns, warming ocean surface temperatures, and decreasing mixing depths, thereby reducing seasonal phytoplankton blooms that are critically important in reducing atmospheric CO_2 concentrations (Behrenfeld et al. 2013 in press). The Keeling curve shows the large increase in global atmospheric CO_2 from preindustrial revolution levels to today (Keeling, 1978). One of the main regulators of atmospheric carbon, and thus climate, is the world's oceans as they are a major sink for atmospheric CO_2, sequestering up to a third of anthropogenically produced carbon (Winn et al. 1994). Up to 40% of the total carbon fixation in the world's oceans is due to primary production of unicellular marine cyanobacteria (Partensky et al. 1999). Primary production by these unicellular organisms is regulated by two main environmental factors: light and nutrient availability. Nitrogen is one of the most common forms of nutrient limitations in the world's oceans (Boynton et al. 1982; Graneli et al. 1984). The extent and duration of nutrient limitation is predicted to increase as the oceans warm (Behrenfeld et al. 2006). Warming oceans cause earlier initiation of oceanic stratification and reduce mixed layer depths. Shallower mixed layer depths effectively reduce nutrient cycling within each layer, thus limiting nutrient availability to photosynthetic organisms. Studying how nitrogen limitation affects cyanobacterial net primary production (NPP) will help us to better understand carbon cycling in the world's oceans. This information can then be used to increase the accuracy of models of oceanic primary production. Ideally, these models will help to predict oceanic ecosystem behaviors in response to climate change.

Currently there are no reasonably effective methods for measuring NPP in the field (NPP is defined as growth rate times cellular carbon, NPP=μ x C), so chlorophyll concentration (Chl) is used as a proxy. Chl can be quantified using remote satellite sensing technologies, enabling global estimates of NPP (Field et al. 1998). In these estimates Chl is related to NPP through the Chl-specific carbon fixation rate (P^b) (i.e. the amount CO_2 fixed into organic carbon per unit Chl). P^b is measured using radio-labeled bicarbonate ($NaH^{14}CO_3$), a method originally developed by Steemann and Nielson in 1952. In this method, a cell suspension is incubated with ^{14}C for a given length of time and the amount of ^{14}C incorporated into organic carbon is measured. This rate is normalized to the Chl content of the sample (^{14}C uptake (mg Chl x hr)$^{-1}$). Variability in P^b is often attributed to nutrient limitation effects on cell physiology (Geider et al. 1998). Indeed, in many studies, P^b was strongly dependent on nutrient limited growth rates (Thomas and Dodson 1972; Geider et al. 1998; Curl and Small 1965). For example, using continuous monocultures of *Dunaliella tertiolecta,* Thomas and Dodson (1972) showed a fivefold increase in the carbon assimilation rates per unit Chl in fast growing cells versus slow growing cells. These results led to the assumption that P^b can provide information about the nutrient status of the sample populations and can be used to estimate Chl-specific NPP (NPP*). However, this method linking Chl and NPP operates under the assumption that P^b and NPP* are directly related across all cellular growth rates. Thus, using the ^{14} C method to predict NPP requires that as a cell becomes nutrient limited thus decreasing its growth rate, NPP* also decreases (Fig. 1).

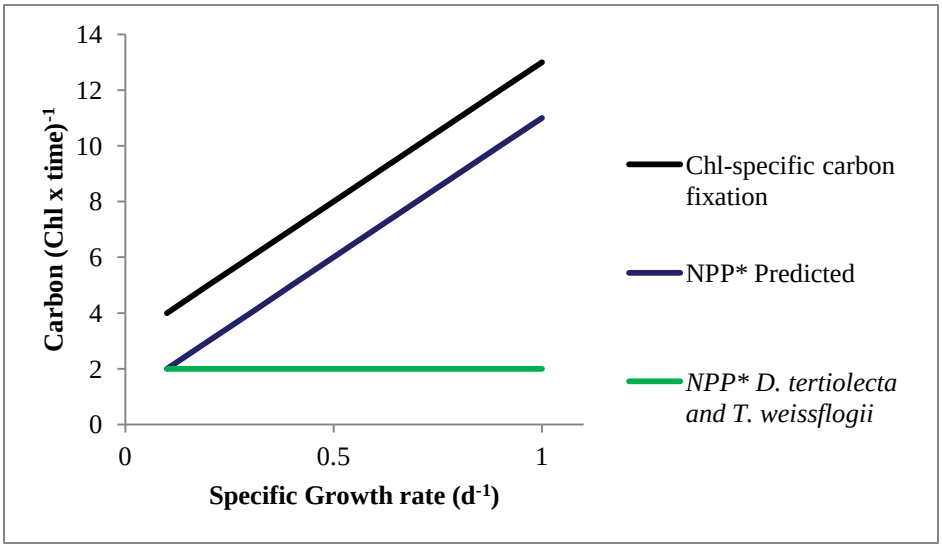

Fig. 1 Idealized schematic of the potential relationships between Chl-specific carbon fixation and NPP*. "NPP* predicted" is currently used in oceanic models of primary productivity.

Other culture-based studies demonstrated that P^b is independent of nutrient limited growth rate (Halsey et al. 2010; Laws and Bannister 1980). Similar to Thomas and Dodson (1972), Halsey et al. (2010; 2013) used continuous cultures of nitrate limited, steady state *D. tertiolecta* and *Thalassiosira weissflogii* and found that short term Chl-specific ^{14}C uptake was strongly dependent on growth rate, but NPP* was not (Fig. 1). Those studies concluded that differences in allocation of short and long term photosynthetic products are the basis for the observed incongruence in photosynthetic properties. It is reasonable to assume that these results apply to closely related species or similar groups of phytoplankton. The study described in this manuscript was undertaken to determine if these results can be generalized to cyanobacteria, possibly the greatest contributors to global marine NPP.

Synechococcus WH8102 is a ubiquitous cyanobacteria found in virtually all of the world's oceans, from areas of coastal upwelling to oligotrophic subtropical gyres, and thus is a

significant contributor to global oceanic primary production (Partensky et al. 1999). *Synechococcus* sp. is second in abundance only to *Prochlorococcus* sp., the most numerically abundant cyanobacteria. However, *Synechococcus* WH8102 is larger and grows faster, and therefore it may be a greater contributor to global NPP than *Prochlorococcus* sp. MED4 (Table 1) (Bertilsson et al. 2003). *Synechococcus* WH8102 is commonly found in oligotrophic environments where nutrient limitation, via either nitrogen or iron, is a constant environmental stress that reduces their overall growth rate. In addition, *Synechococcus* WH8102 exhibits a unique, and as of present, unknown form of motility that could complicate its metabolic regulation (Brahamsha 1996). Finally, this organism possesses genes that allow it to utilize less common sources of nitrogen, such as urea, oligopeptides, and some amino acids as additional sources of nitrogen (Palenik et al. 2006). As a result, *Synechococcus* WH8102 could possess unique strategies for energy allocation and carbon metabolism. Because of its near ubiquity in marine environments and potential for distinctive metabolic regulation, we selected *Synechococcus* WH8102 to further study the effects of nutrient limitation on the relationship between Chl-specific ^{14}C uptake and NPP*.

Table 1. Comparison of growth characteristics for *Synechococcus* WH8102 and *Prochlorococcus* MED4

	Average Abundance (Cells ml^{-1})	Size Range (μm)	Maximum Specific Growth Rate (day^{-1})
Synechococcus WH8102	10^4	0.6-2.1	1.13
Prochlorococcus MED4	10^5	0.5-0.9	1

Our results show that both P^b and NPP* were strongly dependent on nitrogen limited specific growth rate. Also, cellular carbon, but not Chl, varied strongly with growth rate, suggesting a different mechanism for pigment regulation than was previously shown in *D. tertiolecta* and *T. weissflogii*. These results reveal a fundamental difference in photosynthetic

efficiency in the globally important cyanobacteria *Synechococcus* WH8102 than was previously shown for the large green algae and marine diatom. We suggest this difference is due to this bacteria's capacity to rely on PSI cyclic electron transport for transport of alternative reduced nitrogen sources and to fuel motility at very low growth rates.

Materials and Methods:

Culture Conditions

Continuous steady state nitrogen limited cultures of *Synechococcus* WH8102 were grown in 300 ml chemostats at three different specific growth rates: 0.2,0.5, and 1.0 day^{-1}.This range of growth rates was selected to most closely replicate the spectrum of growth rates possible for this cyanobacterium in its natural environment. The cells were grown in L1 media made with natural seawater collected 5 miles off the Oregon coast.

Specific growth rate can be expressed as $\mu = \frac{D}{V}$

Where μ is specific growth rate in units of day^{-1}, D is the dilution rate of the chemostat in units of ml day^{-1} and V is total culture volume in units of ml.

$NaNO_3$ was added to the L1 media as the limiting nitrogen source. The cultures were grown at 20°C, continuously aerated, and grown in the constant presence of cool fluorescent lights at 220-240 μmol quanta $m^{-2}\ s^{-1}$ (as measured with a quantum meter fitted with a 4π spherical quantum sensor(Biospherical Instruments QSL-100)). After a minimum of 7 generations and when cell densities were stable for 3 consecutive days, cultures were considered to be in steady state and data collection was allowed to proceed. Measurements of cell density, size, and volume were made using a Multisizer 3 Coulter counter equipped with a 50 μm

aperture and a FACscan Flow Cytometer equipped with a blue laser. All measurements were collected in triplicate. Three independent chemostats were used for cultures grown at $0.2d^{-1}$ and duplicate chemostats were used for cultures grown at 0.5 and $1.0d^{-1}$.

Chlorophyll Extraction

Chl a concentrations were determined in triplicate by filtering 3 ml onto 25 mm glass fiber filters (Whatman GF/F). Filters were extracted for 24 to 48 hrs in 90% methanol at -20°C. The extract's absorptivity at 665nm (A_{665}) was measured with a spectrometer using A_{750} as the background. Chl a concentration was quantified using the equation of Jeffrey and Humphrey (1975).

$$\text{Chl a } (\mu g/ml) = ((12.94 \times (A_{665} - A_{750}))$$

Cellular carbon and nitrogen quotas

Cellular carbon and nitrogen were measured using an Exeter Analytical EA1 elemental analyzer. CHN analysis was carried out by filtering 1, 2, and 3ml of culture samples onto precombusted 25 mm glass fiber filters (Whatman, UK) to ensure a linear relationship between carbon or nitrogen, and volume filtered. A filter blank was subtracted. Calculated values were averaged to achieve the final figures for cellular carbon and nitrogen. NPP* was calculated using the equation below where μ is growth rate, C is cellular carbon, and Chl is chlorophyll concentration.

$$NPP^* = (C \times \mu) \times Chl^{-1}$$

Short term (20 min) ^{14} C uptake (P^b)

For short term ^{14}C uptake data, 5 ml culture sample was diluted with 8 ml L1 media with no added $NaNO_3$ (nitrate addition is know to cause rapid shift in metabolism from carbon to nitrogen reduction). 5 µCi of $NaH^{14}CO_3$ was added to the diluted culture samples and 1 ml aliquots were pipeted into 11 separate 7 ml scintillation vials. The samples were placed in a photosynthetron (CHPT Mfg Inc; Georgetown, DE) for 20 minutes at 20 °C at 10 different light levels (0-1750 µmol quanta m^{-2} s^{-1}). After incubation, 50 µl of 1M HCl was added to each scintillation vial and allowed to degas for a minimum of 24 hours. After degassing, samples were stored in a dark cabinet until measurement by scintillation counter. Total activity of ^{14}C added was quantified by addition of 50 µl phenelethylamine and 900 µl of water to 50 µl $NaH^{14}CO_3$ inoculated sample. Counts per minute (CPM) at each light level correspond to the amount of radioactive carbon assimilated by the cells during the incubation period. These rate values were used to construct a photosynthesis irradiance (PE) curve by measuring activity at each light level (Lewis and Smith 1983).

Statistical Analysis

Linear regression and ANOVA analyses were performed using Microsoft Excel 2007. Production measures from independent chemostats (3 at 0.2d^{-1}, 2 at 0.5 and 1.0 d^{-1}) were averaged and SE determined.

Results

Continuous, nitrate limited cultures of *Synechococcus* WH8102 were grown at three different specific growth rates of 0.2, 0.5, and 1.0 day^{-1}. For reference, a specific growth rate of 0.69 is approximately 1 cellular division per day. This range of specific growth rates was chosen to represent the spectrum of nutrient limited growth rates possible for these bacteria in marine environments. Steady state cellular characteristics and productivity measurements were collected from duplicate cultures growing at 0.5 and $1.0d^{-1}$, and triplicate cultures growing at $0.2d^{-1}$.

Particulate organic carbon per cell (pg C x $cell^{-1}$, or POC) for *Synechococcus* WH8102 slightly increased with increasing specific growth rate. Values were 0.93 +/- 0.05 pg C $cell^{-1}$ for the slowest growing cells, to 1.37 +/- 1.17 pg C $cell^{-1}$ for the fastest growing cells. Interestingly, cells growing at $0.5d^{-1}$ had only 0.57 +/- 0.22 pg C $cell^{-1}$ (Table 2). The error associated with the data increased with growth rate, such that POC was poorly correlated with nitrogen limited specific growth rates (R^2= 0.44, ANOVA p-value > 0.05). A similar study using continuous, nitrogen limited cultures of *Synechococcus* WH7803 also showed high variability in cellular carbon quotas across a similar range of growth rates (Hongbin et al. 1999). In contrast, POC decreased significantly with increasing growth rate in previously studied *D. tertiolecta* and *T. weissflogii*. (Halsey et al. 2010; Halsey et al. 2013).

Similar to POC, particulate organic nitrogen per cell (pg N x $cell^{-1}$ or PON) did not demonstrate a statistically significant correlation with growth rate. Values increased from 0.19 +/- 0.01 pg N $cell^{-1}$ for the slowest growing cells to 0.38 +/- 0.32 pg N $cell^{-1}$ for the fastest growing cells, but the middle growth rate was 0.13 +/- 0.04 pg N $cell^{-1}$ (Table 2). Again, error associated with the data increased with growth rate, such that PON was poorly correlated with

nitrogen limited specific growth rate (R^2= 0.69, ANOVA p-value > 0.05), a trend also in Hongbin et al. (1999).

Despite the poor POC and PON correlation with growth rate, the carbon to nitrogen ratio (C:N) was strongly correlated with nitrogen limited growth rate (R^2 = 0.96, ANOVA p-value <0.05). This strong correlation suggests that nitrogen assimilation and carbon metabolism are carefully coordinated during nutrient limited growth in *Synechococcus* WH8102. C:N decreased with decreasing nitrogen limitation, a trend observed for phytoplankton in other studies (Halsey et al 2010; Halsey et al. 2013; Hongbin et al. 1999). However, the slope of the relationship between C:N and growth rate in *Synechococcus* WH8102 was shallower than that of other organisms studied, where *m* = -1.51, -13.7, and -7.97 for *Synechococcus* WH8102, *T. weissflogii,* and *D. tertiolecta* respectively (Halsey et al. 2010; Halsey et al. 2013). Also, C:N across all growth rates were considerably lower than the Redfield ratio (approximately 6.6:1) and ranged from 4.8:1 in cells growing at $0.2d^{-1}$ to 3.6:1 in cells growing at $1.0d^{-1}$.

Chl per carbon ratio (Chl:C) increased with nitrogen limited growth rate (R^2 = 0.96, ANOVA p-value <0.05) (Fig. 2). Values ranged from 0.009 +/- 0.001 to 0.020 +/- 0.001 μg Chl (μg carbon)$^{-1}$ in cells growing at $0.2d^{-1}$ and $1.0d^{-1}$ respectively. This 2.2 fold increase demonstrates a strong relationship between pigment regulation and net carbon assimilation metabolism for *Synechococcus* WH8102. This Chl:C relationship has been observed in a wide range of phytoplankton (Halsey et al. 2010; Halsey et al. 2013; Laws and Banister 1980; Hongbin et al. 1999). However, total change in Chl per unit carbon across the entire spectrum of nitrogen limited growth rates for *Synechococcus* WH8102 was far less than previously studied *T. weissflogii,* and *D. tertiolecta* (Fig. 2) Chl:C dependence on growth rate was strongly driven by the increase in Chl per cell (Chl cell^{-1}) in cultures growing at 0.5 and $1.0d^{-1}$ (Table 2). However,

there was no significant difference in Chl per cell for cells growing at 0.2 and 0.5d^{-1} (ANOVA p value >0.05).

Table 2. Summary table of steady state characteristics for continuous, nitrate limited cultures of *Synechococcus* WH8102. Values are averages of duplicates with values in parentheses the range of measurements.

μ (day^{-1})	**POC $cell^{-1}$ (pg C)**	**PON $cell^{-1}$ (pg N)**	**C:N (pg pg^{-1})**	**Chl $cell^{-1}$ (fg Chl)**
0.2	0.932 (0.05)	0.193 (0.01)	4.82 (0.08)	8.34 (0.13)
0.5	0.572 (0.22)	0.139 (0.04)	4.11 (0.52)	7.41 (1.58)
1.0	1.373 (1.17)	0.384 (0.32)	3.57 (0.05)	32.41 (5.30)

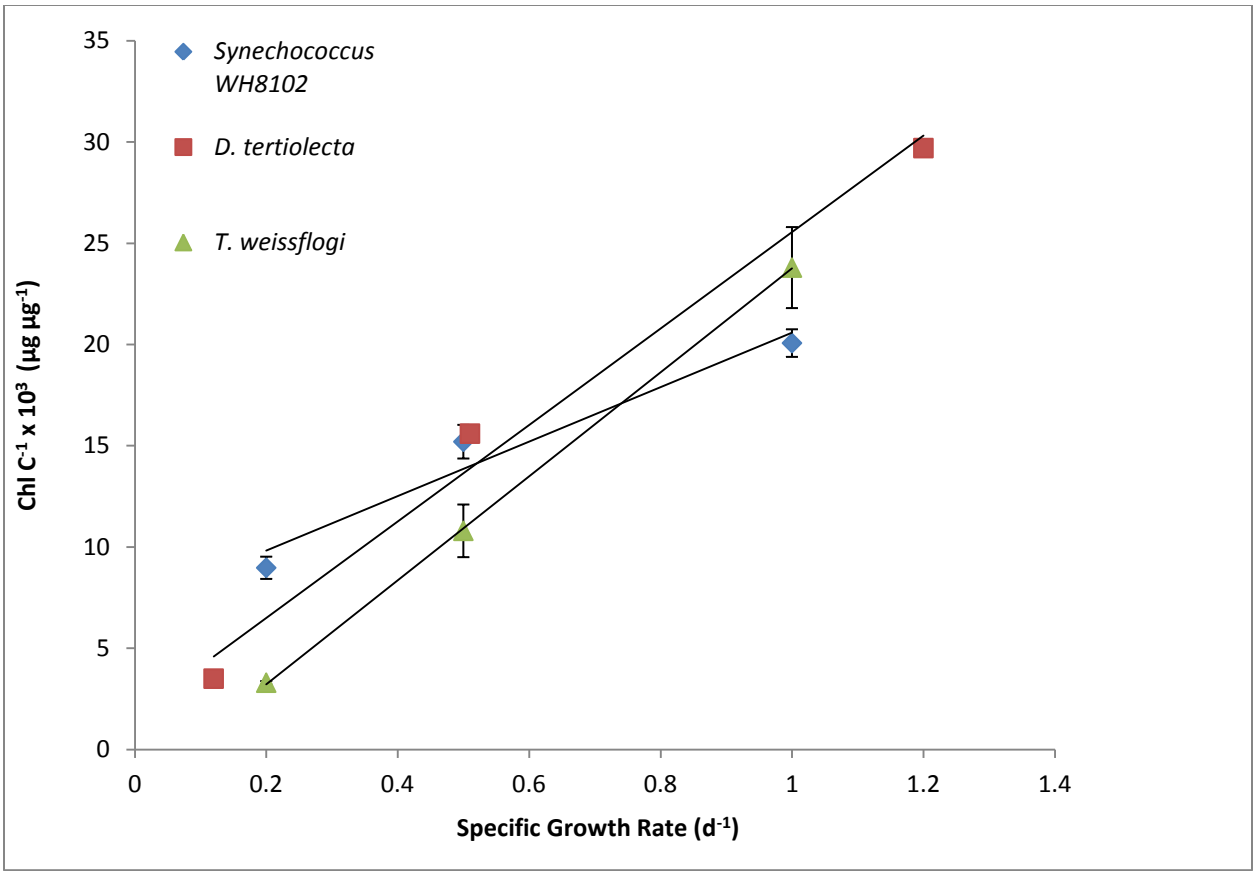

Fig 2. Comparison of Chl:C for *Synechococcus* WH8102 (blue diamonds), *D. tertiolecta* (red squares), and *T. weissflogi* (yellow triangles). Data points are averages of duplicate measurements. Bars are the range of measured values. *Synechococcus* WH8102 data is shown on the secondary axis. Data for *D. tertiolecta* and *T. weissflogi* from Halsey et al. (2010); Halsey et al. (2013).

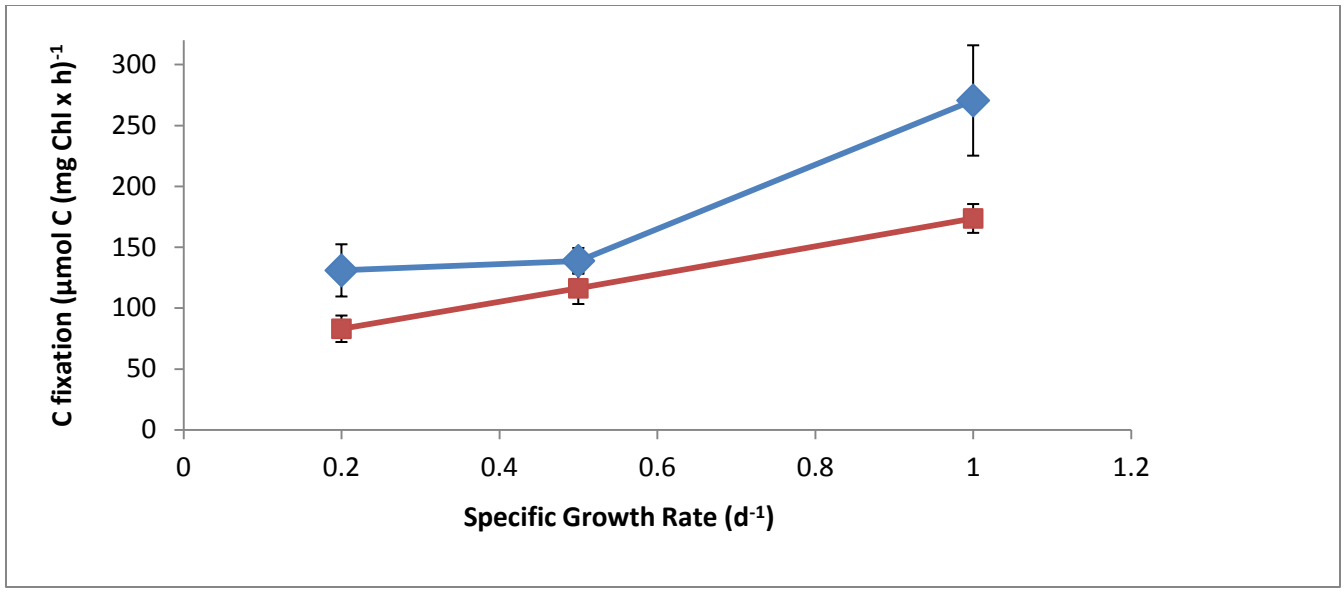

Fig. 3 Relationships between Chl-specific net primary production (NPP*), (red squares) and Chl-specific carbon fixation rates (P^{b}_{max}), (blue diamonds) at three different nitrogen limited growth rates. Where error bars not seen, they are contained within the data points.

NPP* for *Synechococcus* WH8102 was linearly correlated with nitrogen limited specific growth rate (R^2 = 0.99, ANOVA p-value <0.05) (Fig 3), a result that has not been previously reported for phytoplankton grown across a similar range of growth rates. NPP* for *Synechococcus* WH8102 ranged from 83.0 +/- 10.8 µmol C (mg Chl x h)$^{-1}$ in the slowest growing cells to 173.7 +/- 11.8 µmol C (mg Chl x h)$^{-1}$ in the fastest growing cells, a 2.1 fold increase in production efficiency (Fig. 3).

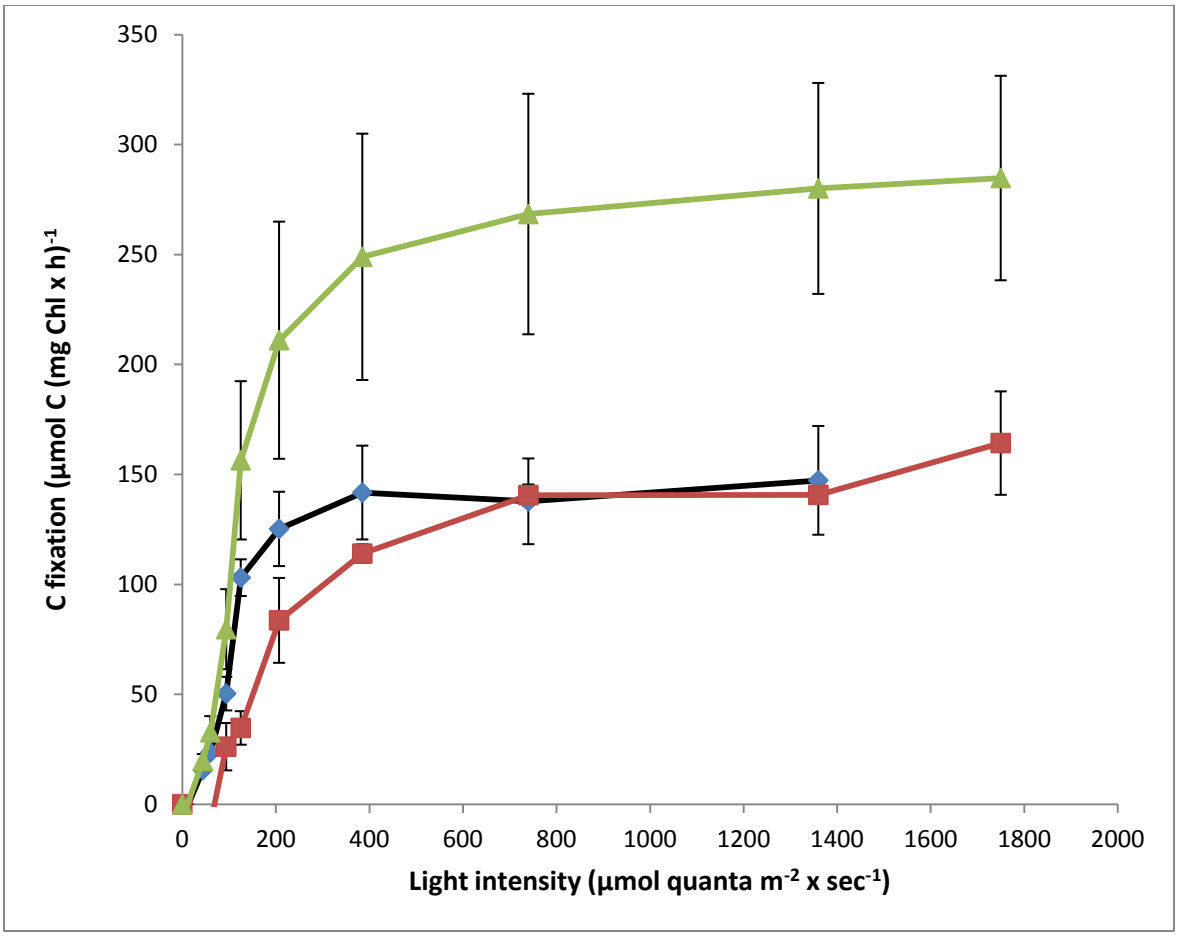

Fig. 4 PE curves for short term (20 min) Chl-specific ^{14}C uptake data in nitrogen limited *Synechococcus* WH8102 growing at 0.2d^{-1} (blue diamonds) 0.5 d^{-1} (red squares) and 1.0 d^{-1} (yellow triangles) are shown. 0.2 d^{-1} is an averaged value of triplicates, and 0.5 and 1.0 d^{-1} are averaged values of duplicate measurements. Error bars shown are range of measurements.

Photosynthesis irradiance (PE) curves generated from short term 20 minute, Chl-specific ^{14}C uptake measurements showed that Chl-specific carbon uptake rates at light saturation (P^b_{max}) was highest in cell growing at 1.0d^{-1}. In contrast, cells growing at 0.2d^{-1} and 0.5d^{-1} show similar P^b_{max} values (Fig 4). Light limited slope (α) for *Synechococcus* WH8102 was very similar for cells growing at 0.2 d^{-1} and 1.0^{-1} day while cells growing at 0.5d^{-1} gave a smaller α value (Fig. 4). P^b_{max} values are plotted in Fig. 3.

Discussion

Data presented here shows NPP* for *Synechococcus* WH8102 is dependent on its nitrogen limited growth rate (R^2= 0.99, AVOVA p-value <0.05), a behavior not observed in previously studied *D. tertiolecta* and *T. weissflogiii* (Laws and Banister 1980, Halsey et al. 2010; Halsey et al 2013). Our results show that the efficiency of net primary production in *Synechococcus* WH8102 increased with decreasing nitrogen limitation. P^b also increased with nitrogen limited growth rate in *Synechococcus* WH8102 (R^2=0.89) (Fig. 3): however the two production measurements do not co vary and therefore cannot give a simple estimate of NPP* for *Synechococcus* WH8102. The relationship between P^b and NPP* generally increased with decreasing nitrogen limitation. Variability in observed carbon assimilation rates has been attributed to the growth rate dependent allocation of newly fixed carbon between rapid oxidation pathways for energy production (e.g. TCA cycle, respiratory electron transport) and longer term storage (e.g. polysaccharides, lipids) (Halsey et al. 2010, Halsey et al. 2011). In *Synechococcus* WH8102, it appears that at slower growth rates the bulk of newly fixed carbon is allocated to rapid respiration pathways. At faster growth rates, carbon is allocated to longer term storage such as polysaccharides and lipids. This indicates that as the cellular growth rate increases, the amount of carbon immediately reduced but later catabolized for reductant and ATP generation increases.

Although nutrient limited carbon metabolism in *Synechococcus* WH8102 appears similar to the previously studied algae, some physiological characteristics in *Synechococcus* WH8102 appear to influence other photosynthetic properties observed in this study. For example, Chl:C and Chl:cell data show that Chl is retained even at the lowest growth rate. This excess Chl may

serve to absorb light energy that is needed to fuel metabolic processes that are present in *Synechococcus* WH8102 but not in *D. tertiolecta* and *T. weissflogii*.

Photosynthesis in *Synechococcus* WH8102 begins with light activation of Photosystem II (PS II) which splits water to produce O_2 and free electrons. Electrons are transferred from PS II to the plastoquinone pool (PQ) and then the cytochrome b_6f complex. Next, electrons are transferred from the b_6f complex via plastocyanin (PC) to Photosystem I (PS I). There, PS I is activated by light and ultimately acts to reduce $NADP^+$ to NADPH. NADPH is used in the Calvin cycle to reduce inorganic carbon for cellular biosynthesis and accumulation of biomass. Cyclic electron flow around PS I can function to regulate the NAD(P)H : ATP ratio of the cell. In this process, electrons are directed from PS I back to the cyt. b_6f complex in lieu of reducing $NADP^+$. Cyclic electron transport results in the enhancment of the proton motive force (PMF) for ATP synthesis without the reduction of $NADP^+$ to NADPH for the reduction of CO_2 . Thus, phytoplankton can use this mechanism to generate additional energy for cellular processes such as motility and transport.

Cyclic electron flow in cyanobacteria has been shown to increase under environmental stresses such as nutrient limitation (Bendall and Manasse, 1995). Interestingly, cyanobacteria have a high ratio of PS I to PS II when compared to other single celled photosynthetic phytoplankton (Sherman et. al 1994). For example, *Synechocystis* sp. PCC 6803 PS I/PS II ratio is approximately 5:1 (Shen et al. 1993) while nitrogen limited *D. tertiolecta* and *T. weissflogii* PS I/PSII ratios are 1:1 and 1:2 respectively (Berges et al. 1996). This high ratio may be important for cells that utilize cyclic electron flow as a major energy production pathway.

Chl:C for *Synechococcus* WH8102 increased linearly with respect to nitrogen limited growth (Fig. 2) implying a tightly controlled relationship between net carbon assimilation

metabolism and pigment regulation. However, in comparison to *D. tertiolecta* and *T. weissflogii* the total change of Chl per unit carbon across the same nitrogen limited growth rates was far less for *Synechococcus* WH8102. The total difference in Chl per unit carbon between the fastest and slowest growing cells for all three organisms was similar to their respective differences in PSI/PSII ratios. Change in Chl per unit carbon across the same range of nitrogen limited growth rates for *Synechococcus* WH8102, *D. tertiolecta*, and *T. weissflogii*, were 2.20 +/- 0.25, 3.95 +/- 0.74, and 7.20 +/- 0.86 respectively (Halsey et al 2010; Halsey et al. 2013) while PS I/PSII ratios are 2.0,1.0, and 0.5 respectively for *Synechococcus* sp. PCC 7002 (a closely related cyanobacterium), *D. tertiolecta* and *T. weissflogii* (Berges et. al 1996). This result may indicate that *Synechococcus* WH8102 is simply rerouting electron flow in response to nitrogen limitation in lieu of catabolizing or synthesizing chl.

ATP synthesis from cyclic electron flow can be used for a variety of cellular needs under stresses such as nitrogen limitation. Annotation of *Synechococcus* WH8102's genome has revealed multiple strategies for dealing with nitrogen limitation. Strategies include ATP dependent ABC transporters for alternative forms of several major nutrients such as nitrogen and phosphate. Genes encoding urea, cyanate, amino acid, and oligopetides transporters were identified, indicating the potential ability of *Synechococcus* WH8102 to use alternative sources of nitrogen (Palenik et al. 2003). Indeed, transport of a few amino acids has been demonstrated in this organism (Willey et al. 1989). Our results suggest that at very low growth rates *Synechococcus* WH8102 utilizes alternative forms of reduced nitrogen when exposed to low nitrate concentrations. A greater reliance on alternative nitrogen sources can effectively shut down electron allocation to nitrate reduction pathways. Thus, under these low light conditions, a greater fraction of electron flow is directed to carbon fixation resulting in a higher α in cells

growing at $0.2d^{-1}$ as opposed to cells growing at $0.5d^{-1}$ (Fig. 4). These physiological data suggest that under nitrogen limitation *Synechococcus* WH8102 uses cyclic electron flow to fuel ATP-dependent uptake of alternative forms of reduced nitrogen.

Synechococcus WH8102 exhibits a unique form of motility that responds to 10^{-10} M changes in nitrogen concentration (Willey et al. 1989). However, there is no physical appendage on the cyanobacterium that is indicative of motility, and how the cell is propelled forward remains a mystery. Furthermore, the energy source used to power its motility is also unknown. One study reported that a closely related cyanobacterium, *Synechococcus* WH8113, was powered by a sodium motive force, and when ATP levels were reduced to 2% of typical intercellular levels, the organism was still very motile (Willey et al. 1987). That study suggests that ATP, at least directly, is not responsible for providing the metabolic energy needed for motility. Thus, the sodium motive force is likely maintained by a Na^+/H^+ antiporter (Kogure, 1998). The retention of Chl at low growth rates may provide light harvesting capacity needed to drive cyclic electron transport that is in turn used to power Na^+/H^+ antiport. Finally, the resulting sodium motive force is used to drive swimming in *Synechococcus* WH8102. At higher growth rates, respiration of carbon supplies energy to set up the sodium motive force.

The relationships shown here between P^b, NPP*, cellular Chl, and nitrogen limited growth rate, suggest a different strategy for carbon metabolism, pigment regulation, and photosynthetic energy utilization in the globally abundant cyanobacterium *Synechococcus* WH8102, than has been described for larger green algae and marine diatoms. We find that in comparison to other algae at low growth rates *Synechococcus* WH8102 maintains higher levels of Chl that is known to be primarily associated with PS I. A high PS I/PS II can function to augment energy needs of the cell by supporting cyclic electron flow at PS I. We conclude that

the unique form of motility in *Synechococcus* WH8102 and ability to utilize alternative nitrogen sources are physiological processes that require significant energy investment via cyclic electron flow, particularly at low growth rates.

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