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1**Title:** Resource allocation by the marine cyanobacterium *Synechococcus* WH8102 in
2response to different nutrient supply ratios
3
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24

25**Running Title:** Elemental stoichiometry of *Synechococcus*

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30Instrumentation programs.

31

32**Abstract:**

33Differences in relative availability of nitrate vs. phosphate may contribute to regional
34variations in plankton elemental stoichiometry. As a representative of the globally
35abundant marine *Synechococcus*, strain WH8102 was grown in 16 chemostats up to 52
36days at a fixed growth rate with $N:P_{supply}$ ratios of 1 to 50. Initially, the phosphate and
37nitrate concentrations in the vessel decreased when the respective nutrient was limiting.
38~~Then, c~~Cell growth ~~was mostly~~generally stabilized, although several chemostats had
39apparent oscillations in biomass. We observed extensive plasticity in the elemental
40content and ratios. $N:P_{cell}$ matched the supply values between $N:P_{supply}$ 5 and 20. The
41$C:P_{cell}$ followed a similar trend. In contrast, the mean $C:N_{cell}$ was ~~at~~ 6.8 and did not vary
42as a function of supply ratios. We also observed ~~an~~ that induction of alkaline phosphatase,
43the fraction of P allocated to nucleic acids, and the lipid
44sulfoquinovosyldiacylglycerol:phosphatidyglycerol ratio inversely correlated with P
45availability. Our results suggest extensive plasticity in the elemental content and ratios
46that depends both on the external nutrient availability as well as ~~the~~ past growth history.

47Thus, our study provides a quantitative understanding of the regulation of the elemental
48stoichiometry of an abundant ocean phytoplankton lineage.

49**Main Text:**

50**Introduction:**

51 The elemental content of marine microbial communities is central to ocean
52biogeochemistry as cellular nutrient requirements link the global cycles of different
53elements (Redfield 1958). It has become apparent that the elemental composition of
54communities is not static but rather varies between different ocean regions. This variation
55includes elevated C:P, N:P, and C:N ratios in the high temperature but low nutrient gyres,
56and lower ratios in high nutrient regions like upwelling zones or high latitude waters
57(Martiny et al. 2013a; b). Such variations have a direct impact on our understanding of
58nutrient limitation patterns and rates of nitrogen fixation (Mills and Arrigo 2010).
59Furthermore, these regional differences in elemental stoichiometry are reflected in
60exported particles and thus may have long-term impacts on ocean nutrient ratios (Weber
61and Deutsch 2010; Teng et al. 2014).

62 Recent studies have demonstrated extensive differences in the elemental
63stoichiometry of bulk particles as well as within specific phytoplankton lineages (Geider
64and La Roche 2002; Martiny et al. 2013a; b). Part of this variation has been attributed to
65latitudinal differences in temperature, nutrient availability, and plankton diversity.
66Regional differences in the elemental stoichiometry between the ocean gyres have also
67been demonstrated (Martiny et al. 2013a; b). Such variations between the gyres do not
68appear to be linked to temperature and overall nutrient concentrations but may be due to
69changes in the relative availability of nitrogen vs. phosphorus.

70 There are multiple theoretical models for how phytoplankton regulate cellular
71composition in response to different environmental conditions (Droop 1973; Sterner and
72Elser 2002; Klausmeier et al. 2004; Bonachela et al. 2013). Some models directly
73describe the cell quota (*Q*) of C, N, and P, whereas others derive *Q* from the macro-
74molecular composition of the cell. The general concept is that nutrient and energy
75acquisition require N-rich proteins, growth requires P-rich ribosomes, and nutrient
76storage molecules may contain high N or P. According to the "growth rate hypothesis"
77(Sterner and Elser 2002), differences in growth rate imply differences in rRNA
78concentration and therefore cellular N:P ratios. Models also predict a large impact of N
79vs. P availability ($N{:}P_{supply}$) on cellular composition, whereby cells will match the $N{:}P_{supply}$
80(Droop 1973). Alternatively, cells may be more biochemically plastic at low growth rates, but
81more constrained at high growth rates (Klausmeier et al. 2004) or once the cell reaches
82maximum or minimum physiological quotas (Bonachela et al. 2013).

83 The experimental support for these predictions almost exclusively comes from
84chemostats as such a setup allows for separation of growth vs. $N{:}P_{supply}$ effects. Some
85analyses show that N:P and C:P ratios decrease as a function of growth rate (Elrifi and
86Turpin 1985; Makino et al. 2003), whereas other studies have found the opposite trend
87depending on $N{:}P_{supply}$ (Goldman et al. 1979). There is also some evidence that phytoplankton
88stoichiometry matches the nutrient supply ratio (Rhee 1978; Leonardos and Geider 2004).
89However, unique maximum and minimum cell quotas have been found for some species,
90which ultimately may limit elemental ratios (Rhee 1978).

91 Although there are some studies of phytoplankton stoichiometry, extrapolating the
92results to the global ocean remains difficult due to the limited diversity of strains studied.

93So far, experiments have mostly focused on either large eukaryotic phytoplankton (e.g., 94*Dunaliella*, *Rhinomonas*, and *C. muelleri*) and/or freshwater species (e.g., *Selenastrum* or 95*Scenedesmus*). Many of these lineages are rare or absent in the ocean and we know little 96about how the elemental composition of the small and abundant marine Cyanobacteria 97like *Prochlorococcus* and *Synechococcus* respond to different nutrient supply ratios.

98 The minimum N and P quotas required for growth vary with cell size (Edwards et 99al. 2012), so small phytoplankton lineages may have unique stoichiometric responses to 100nutrient supply. We hypothesize that *Synechococcus* elemental and biochemical 101composition would track nutrient supply ratios at intermediate values, but will deviate at 102extreme supply ratios due to physiological constraints. To address this hypothesis, we 103analyzed the cellular composition of *Synechococcus* strain WH8102 isolated from the 104open ocean in response to different nutrient supply ratios. Specifically, we asked (i) what 105is the elemental content of *Synechococcus* WH8102 when growing under different 106nutrient supply ratios and (ii) what is the concentration of key macromolecules in the cell 107under these different nutrient conditions? We used a chemostat approach to examine 108$N{:}P_{supply}$ effects on *Synechococcus* elemental composition independent of growth rate.

109

110Methods

111**Culture conditions:** An axenic culture of an open ocean *Synechococcus sp.* strain 112WH8102 (CCMP 2370) was cultured in modified SN medium at 24°C. The medium 113differs from the regular SN medium by the nitrate and phosphate concentrations as listed 114in Table 1. But as in SN, trace metals are buffered with citrate rather than EDTA and the 115salinity of the natural seawater is reduced by adding 25% dH_2O (Waterbury et al. 1986).

116Seawater collected at the San Pedro Ocean Time-series (33.3° N, 118.2° W), was filtered
117at 0.22 µm, diluted to 75% with milli-Q water, and autoclaved before being enriched with
118nutrients and trace elements.

119 The absence of heterotrophic bacteria contamination was assessed before and
120during experiments by checking for growth in marine LB broth (autoclaved mix of
121seawater, tryptone (10 g/L) and yeast extract (5 g/L) adjusted to a pH of 7.8). Presence of
122bacteria was also checked by flow cytometry (Accuri C6 Flow Cytometer, BD
123Biosciences, San Jose, California) using a blue laser and different detectors (side and
124light scatter - green and red fluorescence 530/30BP and 670LP). Before the reading,
125samples were fixed with glutaraldehyde at a final concentration of 0.1% (Polysciences,
126Warrington, Pennsylvania) and incubated in the dark for 15 min with 10000x diluted
127SybrGreen (Life Technologies, Grand Island, New York).
128
129**Chemostat setup and parameters:** Four experiments of four continuous cultures each
130were run for 50 days (Figure S1, www.aslo.org/lo/toc/vol_xx/issue_x/xxxxa1.pdf). Each
131culture included 3 polycarbonate Nalgene (Lima, Ohio) laboratory bottles: a vessel of 8 L
132of sterile fresh medium, 4 L of culture, and a waste reservoir. The incubator maintained a
133constant temperature of 24°C and was programmed for a 12:12 light-dark cycle using a
134photon flux density of 35 µE/m^2/s during the day. On one side of the incubator were
135placed 4 medium reservoirs and on the other side 4 culture vessels were placed on stirring
136plates. 4 waste reservoirs were stored outside the incubator. Vessels were connected to a
137peristaltic pump (PumpPro, Watson Marlow, Wilmington, Massachusetts) by Masterflex
138tygon pump tubing (Cole-Parmer Vernon Hills, Illinois) and manifold marprene long life
139process tubing (Watson Marlow, Wilmington, Massachusetts). Pump rate varied around
14070 rpm. Culture vessels were bubbled with an air pump (Penn-Plax Inc., Hauppauge,

141New York). Cultures were grown at a constant dilution rate of 0.5 d^{-1} and sampled three

142times per week into 250 ml flasks from a sampling port located just before waste

143reservoirs.

144

145**Cell counts:** Duplicates of 1 ml diluted samples were incubated for 15 min in the dark

146with glutaraldehyde (final concentration 0.1%, Polysciences, Warrington, Pennsylvania)

147and enumerated on a flow cytometer at a flow rate of 14 µL/min. Cells and pigments

148were excited at 488 nm by a blue laser and discriminated based on their orange and red

149fluorescence using 584/40BP and 670LP filters.

150

151**Particulate organic matter:** Particulate organic carbon (POC), nitrogen (PON) and

152phosphorus (POP) samples were collected in duplicate by filtration of 50 ml of culture

153onto precombusted (5 h, 500°C) GF/F filters (Whatman, Florham Park, New Jersey) and

154stored at -20°C. To quantify POC and PON, filter samples were thawed and allowed to

155dry overnight at 65°C. Filters were then packed into a 30 mm tin capsule (CE Elantech,

156Lakewood, New Jersey) and analyzed for C and N content on a FlashEA 1112 nitrogen

157and carbon analyzer (Thermo Scientific, Waltham, Massachusetts) (Sharp 1974). POC

158and PON concentrations were calibrated using known quantities of atropine and peach

159leaves in each run. The amount of POP was determined in each sample using a modified

160ash-hydrolysis method (Lomas et al. 2010).

161

162**Nutrients:** 50 ml of nitrate and phosphate samples were collected by filtration through a

1630.2 µm syringe filter and stored at -20°C. Dissolved inorganic phosphate concentrations

164were determined using the MAGIC-SRP method and calculated against a potassium

165monobasic phosphate standard (Lomas et al. 2010). Nitrate samples were treated with

166ethylenediamine-tetraacetate solution and passed through a column of copperized

167cadmium filings according to the Bermuda Atlantic Time-Series study methods 168(http://bats.bios.edu/methods/chapter9.pdf). Detection limits of nitrate and phosphate 169measurements were respectively 80 and 40 nmol L^{-1}.

170

171**Nucleic acid content:** DNA and RNA were quantified as previously described 172(Zimmerman et al. 2014a). In brief, replicate 50 ml samples from each vessel were 173collected on precombusted (5 h, 500°C) GF/F filters (Whatman, Florham Park, New 174Jersey). Filters were placed into a bead beater tube with 0.65 g of 0.1 mm glass beads 175(MO BIO Laboratories Inc., Carlsbad, California), flash frozen in liquid nitrogen, and 176stored at -80˚C until analysis. Nucleic acids were released from filters by mechanical 177lysis (MP FastPrep-24 bead beater, MP Biomedicals, Solon, Ohio) in a mix of 800 µL of 178Tris buffer (5 mM) and 200 µL of RNA preservative (saturated ammonium sulfate 179solution). Sample supernatant was used to prepare assays in 96-well microplates with the 180Qubit dsDNA or RNA HS Assay kits (Life technologies, Eugene, Oregon). Fluorescence 181was measured on a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, 182California). The fraction of P in each nucleic acid was estimated based on the average 183molecular weight of a nucleotide in DNA and RNA (340 and 330 g/mol, respectively).

184

185**Enzyme activity:** To characterize the impact of different $N{:}P_{supply}$ on alkaline phosphatase 186activity, potential enzyme activity was quantified for each chemostat (Allison et al. 1872012). Briefly, 50 µL of varying concentrations (2-200 µmol L^{-1}) of fluorometric 188substrate (4-methyl-umbelliferyl phosphate, Sigma-Aldrich) were combined with 200 µL 189of fresh culture sample in a black 96-well microplate (Greiner Bio-One) and incubated

190for 1 hour at room temperature. During the incubation, the microplates were measured at 191360 nm excitation/460 nm emission in a fluorometer (BioTek Synergy 4) at 0, 15, 30, 45, 192and 60 minutes. Sample blanks (200 μL culture + 50 μL DI water) were included to 193account for the background fluorescence of each sample, and substrate blanks (200 μL 194un-inoculated media + 50 μL substrate solution) were included to account for 195autohydrolysis of the substrate during the assay incubation. To determine conversion of 196fluorescence to product concentration and to account for quenching of fluorescence by 197the sample, 50 μL of standard solution (4-methyl-umbelliferone, Sigma-Aldrich) was 198added at a final concentration of 10 μmol L^{-1} to sample or uninoculated media.

199 The concentration of reaction product in the sample wells was determined based 200on the standard, after correcting for the substrate blank and sample blank described. The 201fluorescence values used for samples and substrate blanks represent the means of 3 or 4 202replicate wells, while the values used for the standard and sample blanks represent the 203means of 8 replicate wells. Enzyme activity values that showed substrate inhibition at 204high substrate concentrations were dropped prior to the regression analysis, and V_{max} and 205standard error values were estimated by fitting a hyperbolic curve to the resulting 206activities.

207

208**Lipids:** To determine the lipid composition of the cells, 3 replicates of 150 mL culture 209were filtered on precombusted (5 h, 500°C) GF/F filters (Whatman, Florham Park, New 210Jersey) and analyzed for three classes of glycolipids (monoglycosyldiacylglycerol, 211MGDG, diglycosyldiacylglycerol, DGDG and sulfoquinovosyldiacylglycerol, SQDG), 212three classes of phospholipids (phosphatidylglycerol, PG, phosphatidylethanolamine, PE

213and phosphatidylcholine, PC), and three classes of betaine lipids (diacylglyceryl 214trimethylhomoserine, DGTS, diacylglyceryl hydroxymethyl-trimethyl-β-alanine, DGTA 215and diacylglyceryl carboxyhydroxymethylcholine, DGCC). Lipids were analyzed using 216high performance liquid chromatography/electrospray-ionization triple-quadrupole mass 217spectrometry (HPLC-ESI-TQMS) with a Hewlett Packard 1200 HPLC instrument and 218Thermo TSQ Vantage mass spectrometer (Popendorf et al. 2013). The canonical 219cyanobacterial lipids are MGDG, DGDG, SQDG, and PG (Wada and Murata 1998), but 220other lipids were monitored nonetheless to assess the presence of heterotrophic bacteria 221and samples with a high concentration of non-cyanobacterial lipids were eliminated from 222analysis.
223

224**Results:**

225 In order to identify the cellular response to different nutrient conditions, we grew 226*Synechococcus* WH8102 in chemostats with a dilution rate of 0.5 d^{-1} at 16 different 227nutrient supply ratios ($N:P_{supply}$) ranging from $N:P_{supply}$ 1 to 50 (Table 1). We monitored 228cell abundances, dissolved nutrient concentrations, and cellular nutrient quotas (and 229ratios) up to 52 days for all 16 chemostats (Figure S1). For chemostats with lower 230$N:P_{supply}$, the nitrate concentration (i.e., $nitrate_{vessel}$) dropped during the first 10 days as 231cells nearly exhausted the N source (Figure S2). However, $nitrate_{vessel}$ rarely reached 232detection limits. In contrast to $nitrate_{vessel}$, $phosphate_{vessel}$ was high at very low $N:P_{supply}$. At 233$N:P_{supply} \geq 10$, *Synechococcus* WH8102 was capable of taking up all the available 234phosphate and $phosphate_{vessel}$ dropped below the detection limit. On average, the 235chemostats supported ~ 10^{10} cells/L and cell abundances largely followed the same 236temporal trend in all chemostats. Specifically, cell numbers increased initially until N

237and/or P was low in the chemostat and then dropped. In some chemostats ($N{:}P_{supply}$ 5, 7, 23810, 12, 15, 22, 35, and 38), cell abundances appeared to reach an equilibrium. In other 239chemostats, we observed that cell abundances declined following the exhaustion of 240nutrients. This decline led to oscillations with no apparent steady-state biomass 241concentration (e.g., $N{:}P_{supply}$ 20) for the duration of the chemostat run.

242 The concentrations of total cellular carbon and nitrogen (i.e., POC and PON) 243followed a similar temporal trend as cell abundances (Figure S2). POC and PON 244increased initially and then stabilized at concentrations of 200 – 600 µmol C L^{-1} and 20 – 24580 µmol N L^{-1}, respectively. Thus, POC and PON did not vary as strongly temporally as 246the cell abundances. In contrast, the temporal variability in POP did not always match 247POC and PON and especially at high $N{:}P_{supply}$, all inorganic P was incorporated into 248cellular biomass. We also examined the cell quota (Q) and found large temporal intra-249chemostat variations. For example, Q_C tripled over a ten-day period in the chemostat with 250$N{:}P_{supply}$ 5. The same was observed for Q_N and Q_P. Such temporal changes in Q were 251observed across all chemostats. We also compared Q_C to the flow cytometry forward 252scatter and observed that these two independent assessments of cell size were highly 253correlated ($r_{spearman}$ = 0.74, $p < 1 \times 10^{-7}$) and showed very similar temporal trends (Fig. 254S3). The increase in cell size and biomass was related to growth physiology, whereby 255cells were larger during periods of high nutrient availability and just prior to increases in 256cell abundances. We examined cellular elemental ratios and found limited differences in 257$C{:}N_{cell}$, whereas $N{:}P_{cell}$ and $C{:}P_{cell}$ typically increased initially and then dropped once 258nutrients in the chemostat were exhausted.

259 Despite some temporal variability across chemostats, we quantified the mean cell
260quotas and elemental ratios across the last 20 days of each chemostat (Figure 1). The
261mean cell quotas across all $N{:}P_{supply}$ were 211 fg C, 36 fg N, and 6 fg P (Figure 1 A-C and
262Table 1). Across chemostats with different $N{:}P_{supply}$, Q_P indicated a decrease while Q_N and
263Q_C do not. This was in part due to considerable temporal and between-chemostat
264variations in cell size. The mean $C{:}N_{cell}$ was 6.8 and thus close to Redfield proportions
265(Figure 1D). Further, this ratio did not vary significantly across different $N{:}P_{supply}$. In
266contrast, we saw significant responses for both $C{:}P_{cell}$ and $N{:}P_{cell}$ (Figure 1E and F).
267$C{:}P_{cell}$ increased from 50 to 180 for $N{:}P_{supply}$ 1 to 22. At higher $N{:}P_{supply}$, there was little
268change in the $C{:}P_{cell}$. With a similar shape, $N{:}P_{cell}$ ranged from 5 to 30 and thus largely
269matched the $N{:}P_{supply}$ at lower ratios and then stabilized at high $N{:}P_{supply}$.

270 We examined the impact of different $N{:}P_{supply}$ on the expression of alkaline
271phosphatase, nucleic acid content, and lipid profiles (Figure 2). The alkaline phosphatase
272expression for each chemostat was quantified using fluorometric substrates and reported
273as the maximum reaction velocity (V_{max}). For alkaline phosphatase, we found a significant
274positive induction of enzyme activity in relation to increasing $N{:}P_{supply}$ (Figure 2A). At
275$N{:}P_{supply} \leq 10$, V_{max} was close to detection limits. Above this supply level, V_{max} rose
276steadily as a function of $N{:}P_{supply}$ and reached a maximum at $N{:}P_{supply}$ 42. V_{max} was also
277linked to $N{:}P_{cell}$ ($r_{spearman}$ = 0.629, p = 0.0116) suggesting that internal nutrient
278requirements could contribute to the level of phosphatase expression. Thus, the degree of
279P limitation (and change in $N{:}P_{cell}$) had a quantitative effect on enzyme V_{max}.

280 The mean concentrations of DNA and RNA were 3.7 and 3.9 fg/cell and each
281nucleic acid contributed on average 9% of total cellular P (Table 1). The fraction of

282cellular P in nucleic acids varied between 2 and 27%, and this fraction increased

283significantly with $N:P_{supply}$ (Fig. 2B). In contrast (data not shown), we did not observe a

284significant relationship between the absolute level of nucleic acids and $N:P_{supply}$.

285 Finally, we quantified the lipid content of *Synechococcus* WH8102 across

286chemostats and found that phosphorus stored in lipids accounted for on average 4 % (1.1

287– 9) of Q_P (Table 1). We then examined the ratio of sulfo- to phospholipids (SQDG:PG)

288as this has been demonstrated to change with P stress (Van Mooy et al. 2009) (Figure

2892C). We observed a clear correlation between Q_P whereby cells with a low Q_P had a high

290SQDG:PG. However, the lipid ratio was not only linked to $N:P_{supply}$. For example, the

291ratio was 33.2 on week 4 and 9.6 on week 5 in a chemostat with a constant $N:P_{supply}$ of 35.

292Thus, it appeared that a combination of past growth history and nutrient availability

293affected the lipid content.

294

295**Discussion:**

296 Consistent with our initial hypothesis, we found that the elemental stoichiometry

297of a cultured representative of *Synechococcus*– a very abundant phytoplankton in the

298ocean (Flombaum et al. 2013) – changes as a function of $N:P_{supply}$. Flexibility in elemental

299composition is consistent with past studies of phytoplankton (Rhee 1978), but our work

300also suggests lower and upper limits of elemental ratios specifically in *Synechococcus*.

301Limits are especially clear at high $N:P_{supply}$ ratios, where cell stoichiometry approximates

302a constant value (Fig. 1). This plateau is mostly driven by the phosphorus quota, Q_P,

303which reaches a minimum. Thus, our data suggests a physiological limit constraining

304cellular stoichiometry and resource allocation strategies (Pahlow and Oschlies 2009;

305Bonachela et al. 2013). It is worth noting that there is extensive genomic diversity within 306marine *Synechococcus* that can influence nutrient uptake, growth physiology, and 307ultimately their C:N:P ratios (Scanlan et al. 2009). Furthermore, strain WH8102 was 308isolated more than 30 years ago and may have undergone some genetic change in the 309laboratory. However, in a recent comparison of two *Synechococcus* strains, little 310variability was detected suggesting that our results are generally applicable to this genus 311(Kretz et al. 2015).

312 Studies with a single nutrient have shown a link between external nutrient 313concentration and nutrient-uptake protein expression (Tetu et al. 2009). However, the 314linked response of alkaline phosphatase expression to $N{:}P_{supply}$ and $N{:}P_{cell}$ supports a 315regulation of biochemical allocation to nutrient acquisition (Fig. 2A), whereby N and P 316may interact at the cellular level and determine the protein expression. Such an 317interaction is consistent with results for nitrogen-related V_{max} using various growth rates 318and supply ratios (Rhee 1978), and indicates that internal nutrient requirements influence 319the expression of nutrient-acquiring enzymes (Bonachela et al. 2013). More specifically, 320in condition of a high level of cellular N, low *P* content in the cell allows phytoplankton 321to up-regulate the synthesis of nutrient-uptake proteins therefore increasing the uptake 322rate of such nutrient, whereas high levels of *P* allow the cell to down-regulate *P*-uptake 323protein synthesis and allocate those resources to other cellular functions.

324 We found that between 5 and 30% of cellular P is tied up in nucleic acids and 325between 1 and 9 % in polar lipids. These fractions are consistent with other recent studies 326of marine microorganisms and communities (Van Mooy et al. 2006; Zimmerman et al. 3272014a; b) and shows that the majority of P is present in other cellular fractions. These

328may include storage compounds such as poly-phosphate (Martin et al. 2014). We also 329observed that with increasing $N:P_{supply}$, nucleic acids constitute an increasing fraction of 330cellular P (Fig. 2B) but with no trend in absolute concentrations. Again, this result is 331consistent with field observations (Zimmerman et al. 2014b) and suggests that absolute 332nucleic acid concentrations may be more strongly tied to growth rate than the availability 333of a specific nutrient. Our observations and previous studies also suggest that other P 334fractions may be declining as a function of P availability. The distribution of the data also 335confirm that a subset of cultures can have lower SQDG:PG lipid ratios in cells with low 336Q_P . The exact mechanism for this is unclear but it is consistent with past observations of 337*Synechococcus (Van Mooy et al. 2006)*. Thus, *Synechococcus* WH8102 responds to 338declining P availability by increasing enzyme mediated nutrient acquisition as well as 339reducing allocations to lipids and unknown P pools.

340 *Synechococcus* WH8102 cell quotas quantified in this study are within the bounds 341of previous observations but also suggest extensive plasticity. In a past analysis of the 342elemental content of *Synechococcus* strains, Bertilsson et al. (2003) detected cell quotas 343of 92 to 244 fg C, 20 to 50 fg N, and 0.47 to 3.34 fg P. Similarly, Heldal et al. (2003) 344found cell quotas of 120 to 250 fg C, 17 to 36 fg N, and 2.6 to 7.9 fg P. We also observed 345a three-fold variation in the cellular carbon content, a result confirmed independently by 346flow cytometry. The mechanisms controlling this change in biomass are unknown but 347may be related to nutrient availability and growth physiology as has been seen in other 348organisms (Schaechter et al. 1958). It appears that cells are largest during periods of 349increasing cell abundances and thus when the population growth rate is higher than the 350dilution rate.

351 We found strong temporal differences in both cell abundances and carbon cell
352quotas of *Synechococcus* WH8102, and not all chemostats reached a clear steady-state.
353Part of the temporal variation appears to be related to nutrient concentrations, whereby
354cell abundances drop following the exhaustion of nutrients (Figure S2). This may be due
355to an extensive time-lag in physiological acclimation to low nutrient conditions as
356previously seen in both *Prochlorococcus* and *Synechococcus* (Martiny et al. 2006; Tetu et
357al. 2009). Theoretical models also predict that such a response time-lag can cause
358oscillations in biomass and not lead to a single equilibrium value (Xia et al. 2005).
359Unstable population densities have also been observed for other species (Caperon 1969).
360Overall, this result suggests that the physiological history of the cell can have a large
361impact on the observed elemental content and ratio and thus partly disguise the link
362between environmental conditions and cellular content.

363 We examined the cell physiology of *Synechococcus* in response to differences in
364the relative availability of N vs. P. By using a chemostat setup, we further isolated the
365effect of nutrient supply ratios without a confounding effect of changes in average growth
366rate. We found extensive plasticity in the N or P cell quotas that make it difficult to
367interpret the absolute cell quotas in relation to nutrient availability. This variation appears
368to be caused by significant changes in overall cell size and biomass driven by the past
369growth history. Similarly, the levels of nucleic acids showed considerable variability that
370are likely linked to these putative cell size changes and possibly growth history. In
371contrast, lipid profiles, alkaline phosphatase induction, and cellular nutrient ratios are
372excellent biomarkers for nutrient stress. Furthermore, our study identifies novel controls
373on elemental stoichiometry and biochemical allocation in relation to the relative

374availability of N and P. Considering the large contribution of marine Cyanobacteria to 375ocean primary production (Flombaum et al. 2013), such information can be important for 376understanding the role of nutrient availability in controlling the elemental stoichiometry 377of ocean communities and their contribution to productivity.

378

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482

483Table 1: Mean (and standard deviation) of chemostat variables based on the last 20 days

484of operation.

485

$N{:}P_{supply}$	P_{supply} (µM)	N_{supply} (µM)	Q_C (fg/cell)	Q_N (fg/cell)	Q_P (fg/cell)	[cells] (10^{10} cells/L)	C:N (mol/mol)	C:P (mol/mol)	N:P (mol/mol)	DNA (fg/cell)	RNA (fg/cell)	V_{max} (amol/h/cell)	lipids %P_{lipid}	SQDG/PG
1	40	40	103 (13)	20 (3)	6.9 (4.9)	4.5 (1.1)	6.0 (0.5)	51 (25)	8.8 (4.7)	2.5 (0.3)	3.0 (0.04)	4 (0.1)	10 (1)	
3	13.3	40	237 (50)	40 (9)	14 (3.8)	2.2 (0.8)	7.0 (0.7)	44 (5)	6.4 (0.5)	3.1 (0.02)	3.6 (0.3)	13 (0.3)	8 (1)	1.1 (0.1)
5	8.0	40	460 (249)	83 (40)	18 (9.9)	0.7 (0.8)	6.3 (0.7)	68 (13)	11 (3.1)	6.4 (0.3)	8.9 (0.2)	18 (5)		
7	5.7	40	163 (33)	27 (6)	5.4 (1.4)	3.4 (0.7)	7.1 (0.4)	80 (9)	11 (1.1)	3.7 (0.1)	4.0 (0.1)	9 (1)	27 (6)	2.2 (0.7)
10	4.0	40	181 (52)	32 (9)	4.0 (1.6)	2.5 (0.5)	6.2 (0.7)	113 (19)	19 (4.2)	3.1 (0.1)	4.9 (0.3)	13 (1)	17 (4)	3.8 (0.4)
12	3.3	40	146 (23)	25 (5)	3.7 (1.1)	3.0 (0.8)	7.0 (0.9)	103 (14)	15 (1.8)	3.3 (0.4)	2.7 (0.2)	117 (15)	40 (3)	3.0 (0.9)
15	2.7	40	269 (83)	43 (15)	7.0 (3.2)	2.3 (0.9)	7.4 (0.9)	107 (21)	15 (2.6)	2.6 (0.1)	2.4 (0.2)		15 (2)	4.7 (0.2)
18	2.2	40	313 (251)	46 (41)	4.1 (9.5)	1.3 (0.5)	8.2 (1.2)	126 (28)	20 (4.5)	6.7 (0.4)	7.1 (1.0)	23 (1)	46 (8)	2.5 (0.6)
20	2.0	40	170 (37)	30 (7)	2.2 (0.9)	2.5 (0.5)	6.1 (0.5)	146 (15)	24 (1.7)	3.1 (0.2)	4.1 (0.8)	95 (8)	14 (1)	9.0 (1.9)
22	2.0	44	107 (29)	18 (6)	1.7 (0.9)	3.8 (2.3)	6.9 (0.4)	190 (59)	27 (7.5)	2.0 (0.04)	2.1 (0.3)	407 (36)	11 (1)	
28	2.0	56	305 (102)	51 (23)	9.2 (4.4)	0.7 (0.1)	7.1 (1.1)	92 (13)	13 (2.3)	7.2 (0.8)	4.6 (0.6)	443 (31)	9 (1)	2.6 (0.4)
30	2.0	60	104 (27)	18 (4)	1.9 (0.8)	3.8 (1.0)	6.5 (0.3)	161 (47)	25 (7.3)	2.6 (0.7)	3.2 (0.9)	863 (219)		
35	2.0	70	214 (68)	32 (15)	5.3 (3.0)	1.3 (1.0)	8.4 (1.2)	123 (43)	15 (3.5)	3.1 (0.4)	1.7 (1.2)	346 (23)		4.9 (0.9)
38	2.0	76	148 (69)	27 (12)	3.2 (2.1)	3.4 (1.6)	6.5 (0.7)	144 (49)	22 (7.4)	3.6 (0.4)	3.3 (0.3)	922 (180)		
42	2.0	84	268 (119)	48 (24)	5.6 (2.8)	1.3 (0.5)	6.4 (0.6)	129 (21)	20 (3.5)	4.1 (0.1)	4.3 (0.3)	1020 (102)		6.6 (0.6)
50	2.0	100	184 (86)	36 (13)	2.9 (1.6)	1.8 (1.3)	6.0 (0.5)	173 (30)	29 (6.0)	2.3 (0.29)	1.9 (0.5)	240 (20)	13 (2)	5.6 (1.5)
Mean			**211** (95)	**36** (16)	**6.0** (4.5)	**2.4** (1.2)	**6.8** (0.7)	**116** (42)	**18** (4.7)	**3.7** (1.6)	**3.9** (1.9)	**525** (361)	**12** (13)	**4.2** (2.3)

486

487Figure Legends:

488Figure 1. Mean cellular nutrient contents and ratios across 16 chemostats with different
489N:P nutrient supply ratios. (A) Carbon cell quota (Q_C), (B) nitrogen cell quota (Q_N), and
490phosphorus cell quota (Q_P). Molar ratios of (D) carbon to nitrogen, (E) carbon to
491phosphorus, and (F) nitrogen to phosphorus. A 1:1 line is added to Fig. 1F to guide a
492comparison between $N:P_{supply}$ and $N:P_{cell}$. The inserted statistics represent Spearman
493correlations between the variables. The error bars represent one standard deviation and
494are based on measurements for the last 20 days of operation of each chemostat.
495
496Figure 2. Mean cellular macromolecule content across 16 chemostats with different
497$N:P_{supply}$. (A) Alkaline phosphatase maximum activity (V_{max}) plotted with the residual
498phosphate concentration ($phosphate_{vessel}$). (B) Fraction of P allocated to the nucleic acids
499DNA and RNA. (C) Molar ratio of sulfoquinovosyldiacylglycerol (SQDG) to
500phosphatidylglycerol (PG) lipids as a function of the P cell quota (Q_P). The inserted
501statistics represent Spearman correlations between the variables.
502
503Supplementary Information:
504Figure S1. Overview of chemostat design.
505
506Figure S2. Temporal changes in cell and nutrient concentrations in all 16 chemostats with
507different nutrient supply ratios. This includes cell abundance, residual nitrate ($nitrate_{vessel}$)
508and phosphate ($phosphate_{vessel}$) concentration in the chemostats, C, N, and P cell quotas,
509total particulate organic carbon, nitrogen, and phosphorus and associated ratios. (Note
510that some cell count data are missing due to technical issues).
511
512Figure S3. Temporal changes in carbon cell quota (Q_C) and forward scatter (FSC)
513measured using flow cytometry in all 16 chemostats with different nutrient supply ratios
514(A – P). (Q) This illustrates the overall comparison between Q_C and FSC across all
515chemostats (red dots). The inserted statistics represent Spearman correlations between the
516variables.