environmental microbiology

Environmental Microbiology (2018) **20**(8), 2743–2756 doi:10.1111/1462-2920.14111

Mixotrophic metabolism by natural communities of unicellular cyanobacteria in the western tropical South Pacific Ocean

Solange Duhamel [1]* France Van Wambeke,[2] Dominique Lefevre,[2] Mar Benavides[2,3†] and Sophie Bonnet[2,3]

[1]*Lamont Doherty Earth Observatory, Division of Biology and Paleo Environment, PO Box 1000, 61 Route 9W, Palisades, NY 10964, USA.*

[2]*Aix Marseille Université, Université de Toulon, CNRS, IRD, MIO UM 110, 13288, Marseille, France.*

[3]*Aix Marseille Université, Université de Toulon, CNRS, IRD, MIO UM 110, 98848, Noumea, New Caledonia.*

Summary

Cyanobacteria are major contributors to ocean biogeochemical cycling. However, mixotrophic metabolism and the relative importance of inorganic and organic carbon assimilation within the most abundant cyanobacteria are still poorly understood. We explore the ability of *Prochlorococcus* and *Synechococcus* to assimilate organic molecules with variable C:N:P composition and its modulation by light availability and photosynthetic impairment. We used a combination of radiolabelled molecules incubations with flow cytometry cell sorting to separate picoplankton groups from the western tropical South Pacific Ocean. *Prochlorococcus* and *Synechococcus* assimilated glucose, leucine and ATP at all stations, but cell-specific assimilation rates of N and P containing molecules were significantly higher than glucose. Incubations in the dark or with an inhibitor of photosystem II resulted in reduced assimilation rates. Light-enhanced cell-specific glucose uptake was generally higher for cyanobacteria (~50%) than for the low nucleic acid fraction of bacterioplankton (LNA, ~35%). Our results confirm previous findings, based mainly on cultures and genomic potentials, showing that *Prochlorococcus* and *Synechococcus* have a flexible mixotrophic metabolism, but demonstrate that natural populations remain primarily photoautotrophs. Our findings indicate that mixotrophy by marine cyanobacteria is more likely to be an adaptation to low inorganic nutrient availability rather than a facultative pathway for carbon acquisition.

Received 9 August, 2017; revised 12 March, 2018; accepted 17 March, 2018. *For correspondence. E-mail sduhamel@ldeo.columbia.edu; Tel. 845-365-8774; Fax 845-365-8150. †Present address: Marine Biological Section, Department of Biology, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark.

Introduction

Unicellular marine cyanobacteria (e.g., *Prochlorococcus* and *Synechococcus)* are major contributors to primary production and carbon (C) export in the open ocean (Li *et al*., 1983; Richardson and Jackson, 2007; Johnson and Lin, 2009; Martiny *et al*., 2009). Cyanobacteria are aerobic oxygenic prokaryotes that use a chlorophyll-based light-harvesting complex and CO_2 as a C source. Therefore, marine representatives have been classically considered photoautotrophs (Karl, 2007), and *Prochlorococcus* and *Synechococcus* have long been considered to be unable to use organic C sources such as glucose (Waterbury *et al*., 1986; Béjà and Suzuki, 2008). However, the simple picture that marine cyanobacteria are purely photoautotrophic is actually much more complex. Indeed, recent molecular evidences indicate that organic compound uptake genes are ubiquitous within marine picocyanobacteria (Yelton *et al*., 2016), suggesting that these photosynthetic microorganisms may present mixotrophic metabolism. *Prochlorococcus* and *Synechococcus* have the genetic capacity to use not only organic molecules with key limiting chemical elements such as nitrogen (N) and phosphorus (P), but also molecules devoid of such elements, such as glucose (Gomez-Baena *et al*., 2008; Gao and Xu, 2012; Muñoz-Marín *et al*., 2013; 2017; Yelton *et al*., 2016). Unicellular marine cyanobacteria are, thus, potentially capable of degrading and assimilating a wide range of organic molecules, but only a handful of studies have measured assimilation rates of some selected organic molecules such as amino acids, adenosine-5′-triphosphate (ATP) or dimethylsulfoniopropionate (Michelou *et al*., 2007; Mary *et al*., 2008a,b; Duhamel *et al*., 2012; Ruiz-Gonzalez

et al., 2012b; Björkman *et al*., 2015; Evans *et al*., 2015). Results suggest that marine picocyanobacteria may obtain nutrients mixotrophically via the uptake of N and/or P-containing organic molecules when facing nutrient limitation (Yelton *et al*., 2016).

Direct evidences that marine picocyanobacteria use organic molecules to obtain energy or C are scarce, particularly under *in situ* conditions. To date, only one study by Muñoz-Marín *et al*. (2013) demonstrated that *Prochlorococcus* in the Atlantic Ocean can take up organic C lacking other essential nutrients at nanomolar concentrations in the light (using radiolabelled glucose). A limited number of studies have attempted to quantify the relative contribution of mixotrophy compared to photoautotrophy in C assimilation by natural communities of marine cyanobacteria. First attempts to quantify the contribution of glucose uptake to total C assimilation in *Prochlorococcus* indicated that it may be very small ($<$ 1%) (Muñoz-Marín *et al*., 2013). However, measurements of C assimilation from inorganic and organic substrates were done separately (in the same sampling area but on separate cruises and by different users) and only one taxon was considered, making comparison between fluxes and taxonomic groups difficult (Paoli *et al*., 2008; Muñoz-Marín *et al*., 2013; Benavides *et al*., 2017). Thus, it is still unclear if organic C assimilation by marine unicellular cyanobacteria is ubiquitous, and how it contributes to total C uptake to different groups of picocyanobacteria (e.g., high-light and low-light adapted *Prochlorococcus* and *Synechococcus*, Partensky and Garczarek, 2010) in comparison to CO_2 fixation.

Additional critical gaps in our understanding of picocyanobacteria mixotrophic metabolism concern regulating factors. In particular, we still do not know how organic C assimilation by marine unicellular cyanobacteria depends upon light availability and photosynthetic electron transport in natural settings (Moore, 2013). Recent findings showed that light enhances picocyanobacteria uptake rates of amino acids, ATP or dimethylsulfoniopropionate (Michelou *et al*., 2007; Mary *et al*., 2008a; Duhamel *et al*., 2012; Ruiz-Gonzalez *et al*., 2012b; Björkman *et al*., 2015; Evans *et al*., 2015). Interestingly, organic nutrient assimilation by the low nucleic acid bacterioplankton (LNA), which has been shown to be numerically dominated by the photoheterotrophs SAR11 (Mary *et al*., 2006; Hill *et al*., 2010; Gómez-Pereira *et al*., 2013; Zubkov *et al*., 2015), was enhanced to a comparable extent to that by *Prochlorococcus* (Gómez-Pereira *et al*., 2013). However, the mechanisms of this light enhancement are not well understood. Recently, Muñoz-Marín *et al*. (2017) explored the potential role of photosynthetic electron transport in the regulation of glucose uptake by *Prochlorococcus* SS120 in laboratory cultures. Using different photosynthesis inhibitors, they showed that glucose uptake was significantly reduced or even inhibited. This remains untested with natural populations.

To improve our understanding of marine unicellular cyanobacteria utilization of organic molecules and answer pressing questions about their mixotrophic metabolism in the wild, we conducted a set of experiments in the WTSP during the OUTPACE cruise (Oligotrophy to UlTra-oligotrophy PACific Experiment). Based on the ubiquity of picocyanobacterial organic compound uptake genes (Yelton *et al*., 2016), but low glucose uptake rates by *Prochlorococcus* in the Atlantic Ocean (Muñoz-Marín *et al*., 2013), we hypothesize that mixotrophy by *Prochlorococcus* and *Synechococcus* is an adaptation to nutrient limitation rather than a facultative pathway for carbon acquisition. To test this hypothesis, we measured group-specific assimilation rates of organic molecules containing C only, C and N or C, N and P (glucose, leucine and ATP respectively) in *Prochlorococcus* and *Synechococcus*. We then compared C assimilation from glucose and sodium bicarbonate to test how much C is assimilated via autotrophic or mixotrophic pathways. We also compared the light enhancement of leucine, ATP and for the first time, glucose, by natural *Prochlorococcus*, *Synechococcus* and LNA and tested the effect of the photosystem II inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). We hypothesize that as for organic nutrient assimilation, organic C uptake is enhanced in the light, and that it is partially tied to photosynthetic electron transport.

Results

Description of the study area

Three incubation experiments were carried out in the WTSP at the long duration (LD, 72 h) stations A, B and C (thereafter LDA, LDB, LDC respectively) chosen for their contrasted biogeochemical conditions (Table 1) (Moutin *et al*., 2017). At LDA and LDB, seawater was sampled within the well-lit top mixed layer (ML at 54% PAR, 7 and 9 m depths respectively) and at the deep chlorophyll maximum (DCM, 0.3% PAR, 70 and 90 m respectively), while LDC was sampled at 60 m depth (10% PAR), to compare results between microbial communities adapted to different light levels and nutrient conditions. The ML was 14, 21 and 34 m deep, at LDA, LDB and LDC respectively (Moutin *et al*., 2018).

Station LDA was relatively oligotrophic (Table 1), with moderately high chlorophyll *a* concentrations compared to typical open ocean regional values (0.36 ± 0.05 $\mu g\ l^{-1}$) (Benavides *et al*., 2017). Station LDB was sampled in an elevated chlorophyll *a* patch, about twofold the concentration at LDA in the ML (0.83 ± 0.07 $\mu g\ l^{-1}$) (Benavides *et al*., 2017; de Verneil *et al*., 2017). Compared to LDA, LDB exhibited similar low nutrient concentrations in the ML but depleted inorganic nutrients at the DCM (Table 1). *Prochlorococcus*, *Synechococcus* and LNA abundances were 2.3-, 1.4- and 3.6-fold higher in the ML of LDB than at LDA; and, respectively, were 2.3- to 6.1-, 13.1- to 31.8-

Table 1. Ancillary data Characteristics of the seawater samples collected at stations LDA, LDB and LDC used in experiments.

Name	Depth	Coordinates		PAR	*T*	10^3 cell ml^{-1}			nmol l^{-1}				
	m	Long.	Lat.	%	°C	Pro	Syn	LNA	PO_4^{3-}	NO_3^-	Glc	Leu	ATP
LDA-ML	9	164°41E	19°12S	54	29.4	255	28.6	195	40	< 20	1.93	1.78	1.14
LDA-DCM	90			0.3	25.2	112	0.9	128	240	820	1.43	1.80	1.12
LDB-ML	7	170°51W	18°14S	54	29.9	598	39.2	699	30	< 20	0.56	0.52	0.65
LDB-DCM	70			0.3	25.3	98	3.0	156	90	< 20	0.45	0.40	0.81
LDC	60	165°45W	18°40S	10	26.4	145	1.2	156	110	20	0.52	0.56	0.48

Latitude (Lat.), longitude (Long.), fraction of surface photosynthetically active radiation (PAR, %) received at the corresponding depth (m), temperature (*T*, °C), *Prochlorococcus* (Pro), *Synechococcus* (Syn) and LNA cell abundances (10^3 cell ml^{-1}), phosphate and nitrate concentrations (PO_4^{3-} and NO_3^- nmol l^{-1}) and ambient concentrations of glucose (Glc), leucine (Leu) and ATP (nmol l^{-1}).

and 1.5- to 4.5-fold higher in the ML than at the DCM (Table 1). LDC presented characteristics of the oligotrophic South Pacific gyre (lower surface chlorophyll concentrations and deeper DCM) (Claustre *et al.*, 2008; Moutin *et al.*, 2017). Glucose concentrations were on average 2.6- to 4.3-fold higher at LDA than at LDB and LDC. Leucine and ATP concentrations were on average 1.4- to 4.5-fold higher at LDA than at LDB and LDC (Table 1).

Assimilation rates of organic molecules in light incubations

The assimilation of radiolabelled organic molecules with C alone, or C with N or P (glucose, leucine and ATP respectively) was measured in separate incubations. Flow sorting of radiolabelled cells after incubation in the light showed that *Prochlorococcus*, *Synechococcus* and LNA assimilated glucose, leucine and ATP at all sampled stations and depths (Fig. 1). Group-specific assimilation rates of glucose followed trophic gradients and were generally higher at LDB > LDA > LDC, and higher in the ML than at the DCM (Fig. 1A and B). Cell specific rates of glucose assimilation by LNA were 4.8- and 1.7-fold higher than *Prochlorococcus* in the ML at LDA and LDB, respectively, and 2.3-fold higher at LDA-DCM, but *Prochlorococcus* presented higher cell specific rates than LNA at LDB-DCM and LDC-60 m (4.1 and 1.3 times, respectively, Fig. 1A). *Synechococcus* presented 1.4- ± 0.2-fold higher cell specific assimilation rates than *Prochlorococcus* at LDB ($p < 0.05$) and LDC, but about half the rate by *Prochlorococcus* at LDA, although differences were not significant at LDA and LDC. Because *Synechococcus* was much less abundant than *Prochlorococcus* and LNA (on average *Prochlorococcus*, *Synechococcus* and LNA represented 46% ± 5%, 2% ± 2% and 52% ± 7% of the cumulated abundance (*Prochlorococcus* + *Synechococcus* + LNA) respectively; Table 1), the contribution of the *Synechococcus* group to glucose assimilation rates was at least an order of magnitude lower than that of *Prochlorococcus* and LNA groups (0.90 ± 1.12, 0.08 ± 0.11 and 1.95 ± 2.52 pmol Glc l^{-1} h^{-1} on average, for *Prochlorococcus*, *Synechococcus* and LNA groups, respectively, Fig. 1B). Consequently, *Synechococcus* accounted for < 3% of the total glucose assimilation by the three combined sorted groups (*Prochlorococcus* + *Synechococcus* + LNA), while LNA accounted for > 65% at LDA and LDB-ML, and *Prochlorococcus* contributed to 70% and 54% at LDB-DCM and LDC. Thus, *Prochlorococcus* can be a large contributor to glucose assimilation rates in comparison to SAR11-like photoheterotrophic groups. But compared to the total microbial community glucose assimilation (i.e., bulk rates), *Prochlorococcus* accounted for only 4.9% ± 3.3%, similar to results obtained in the Atlantic Ocean (Muñoz-Marín *et al.*, 2013).

Group-specific assimilation rates of leucine and ATP were also higher at LDB and LDA than at LDC, and were higher in the ML than at the DCM ($p < 0.05$, Fig. 1). On average, *Prochlorococcus* and LNA groups accounted for 50% ± 23% and 21% ± 7% of leucine assimilation by the total microbial community respectively; and 17% ± 12% and 40% ± 17% of ATP assimilation by the total microbial community respectively. The *Synechococcus* group contributed to < 1% of the leucine and ATP assimilation by the total microbial community. Cell specific leucine and ATP assimilation rates by *Synechococcus* were roughly an order of magnitude lower than by *Prochlorococcus*, except for ATP at LDB and LDC, where assimilation rates by *Synechococcus* were similar to those by *Prochlorococcus*.

Light-enhanced uptake of organic molecules and relationship to photosynthesis

Flow sorting of labelled cells incubated in light or dark bottles showed that light enhanced the cell specific uptake of all radiolabelled organic molecules tested here, including glucose, and in most cases to a larger extent in *Prochlorococcus* and *Synechococcus* as compared to LNA (Fig. 2). On average, incubations in the light represented an enhancement of 44% ± 18%, 57% ± 30% and 35% ± 11% of dark glucose uptake, 73% ± 23%, 57% ± 30% and 35% ± 11% of dark leucine uptake and 56% ± 25%, 35% ± 24% and 43% ± 30% of dark ATP uptake by

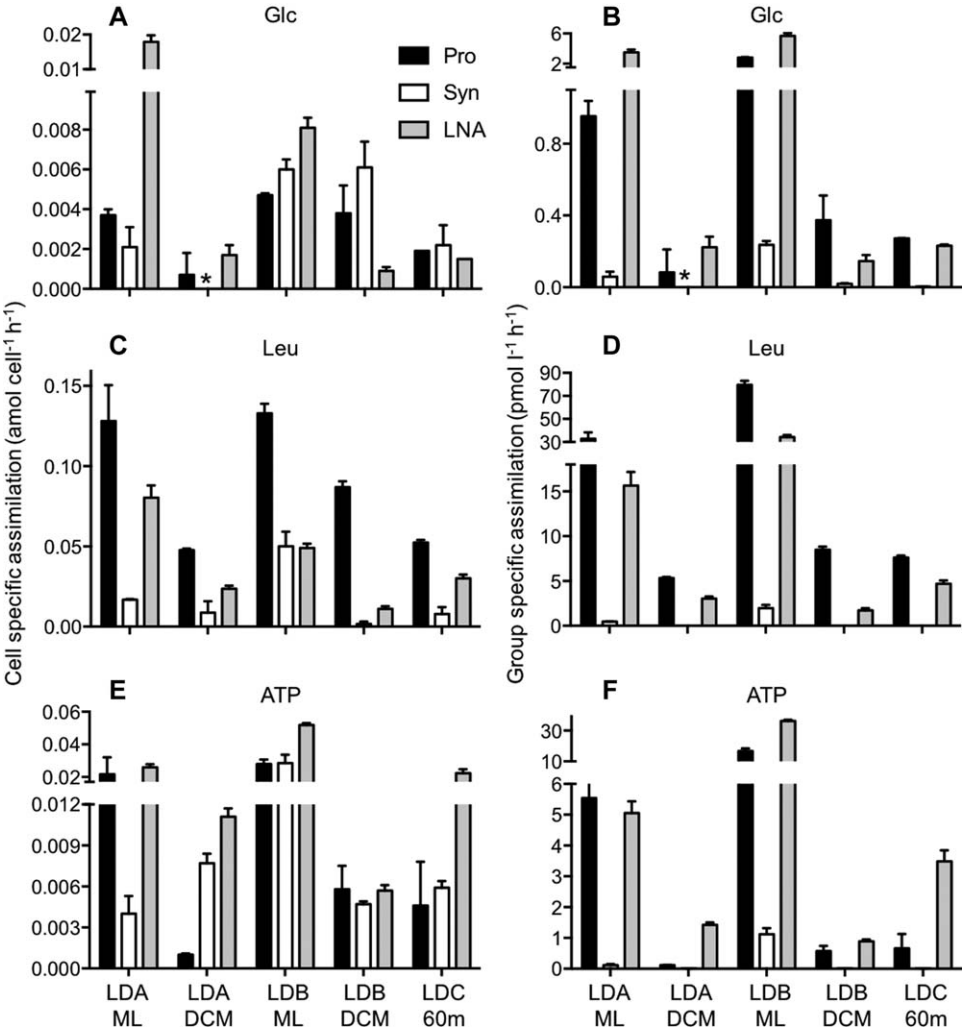

Fig. 1. Cell specific (A, C, E, amol cell^{-1} h^{-1}) and group specific (B, D, F, pmol l^{-1} h^{-1}) assimilation rates of glucose (A, B), leucine (C, D) and ATP (E, F) by *Prochlorococcus* (Pro, black bars), *Synechococcus* (Syn, white bars) and LNA bacteria (LNA, grey bars) in incubations in the light. Error bars represent standard deviation on triplicate samples. * indicate nonmeasurable rates (killed control corrected rates $\leq$ killed control).

Prochlorococcus, *Synechococcus* and LNA respectively. In comparison, incubations in the light represented an enhancement of 92% $\pm$ 11% and 99% $\pm$ 2% of dark ^{14}C-sodium bicarbonate uptake by *Prochlorococcus* and *Synechococcus* respectively; where dark uptake was negligible (Fig. 2G). Interestingly, the light enhancement of organic molecule uptake rates was higher at the DCM than in the ML for *Prochlorococcus* and *Synechococcus* (light to dark ratios of glucose, leucine and ATP uptake were 1.3–2.1 (ML) versus 2.2–5.5 (DCM), 1.1–4.1 (ML) versus 2.2–11.4 (DCM) and 1.9–10.5 (ML) versus 1.5–1.7 (DCM) respectively; Figs 3–5). In comparison, light enhancement of organic molecule uptake by LNA was mostly similar between ML and DCM. In SYBR stained samples, high-DNA-content bacteria (HNA) could be properly distinguished from *Prochlorococcus* only in samples from the DCM and differences between light and dark incubations were either not significant or higher in the dark (*t*-test, $p < 0.05$, Fig. 2B, D and F), and thus this group is not further discussed here. However, these results indicate that the light enhancement of organic molecule assimilation by *Prochlorococcus* could not be a result of by-sorting HNA bacteria overlapping in size with *Prochlorococcus*.

Additions of the photosystem II inhibitor DCMU resulted in reduced *Prochlorococcus*, *Synechococcus* and LNA glucose uptake to a level not statistically different from rates in the dark, except for *Prochlorococcus* and LNA at LDB-ML where uptake was higher in DCMU compared to the dark (Fig. 3). Cell specific leucine uptake was lower when DCMU was added, significantly for *Prochlorococcus* and LNA at all stations/depths but only in the ML for *Synechococcus* (Fig. 4). For *Prochlorococcus*, DCMU resulted in higher or equal rates than in the dark while for LNA, DCMU resulted in lower rates than in the dark. In most cases, DCMU resulted in decreased cell specific ATP uptake for the three groups compared to light samples, but differences between dark and DCMU were not significant (except for *Synechococcus* at LDC, Fig. 5).

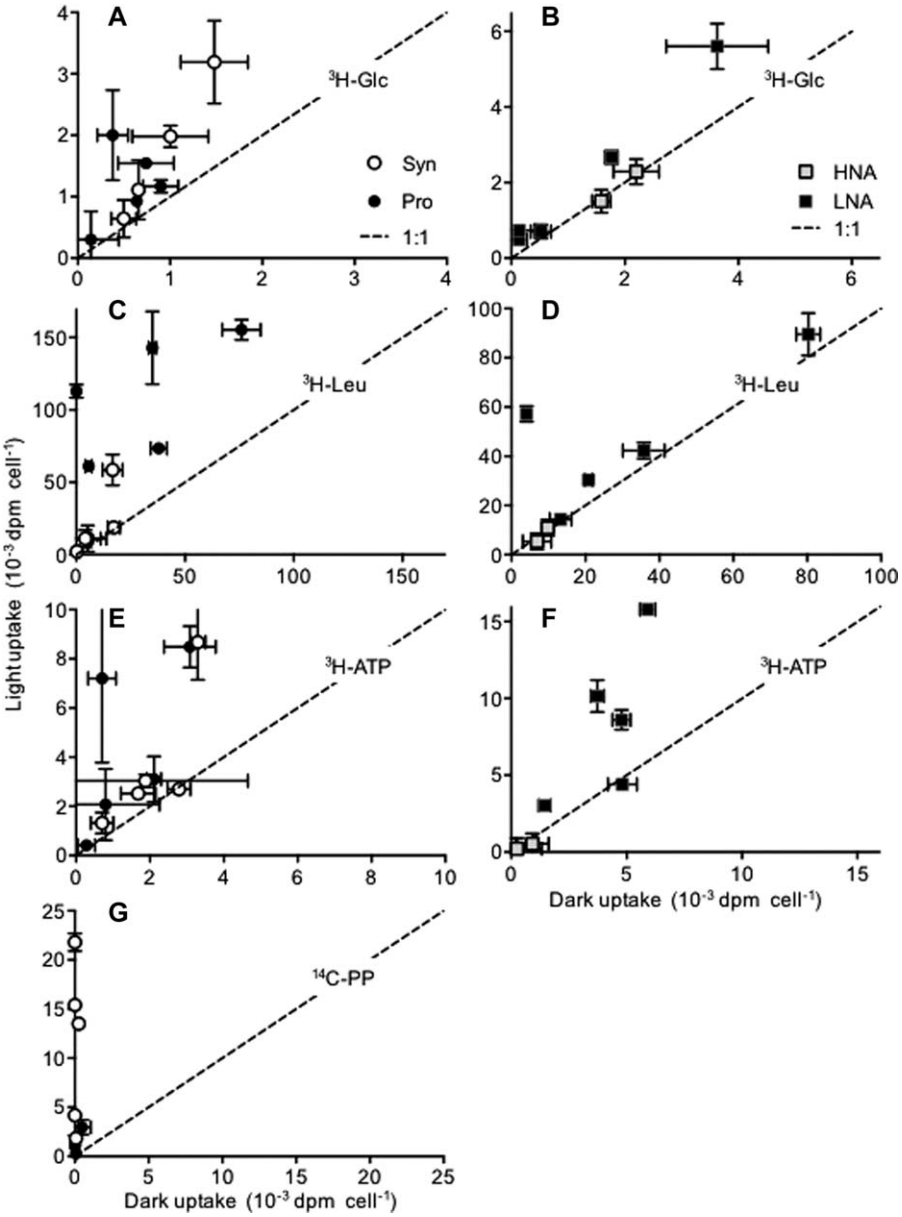

Fig. 2. Scatter plots comparing cell specific uptake (10^{-3} dpm cell^{-1}) in the light (ordinate) and in the dark (abscissa) by picocyanobacteria (Pro: black filled circles, Syn: white filled circles; A, C, E, G) and bacteria (LNA: black filled squares, HNA: grey filled squares; B, D, F), for ^{3}H radiolabelled glucose (^{3}H-Glc, A, B), leucine (^{3}H-Leu, C, D) and ATP (^{3}H-ATP, E, F) and for ^{14}C radiolabelled sodium bicarbonate (^{14}C-PP, G). Error bars represent standard deviation on triplicate samples. The dotted lines represent the 1:1 ratio.

Bacterial production

Because bacterial production is commonly measured using leucine or thymidine assays, the effect of light and DCMU on the incorporation rates of leucine and thymidine into trichloroacetic acid (TCA) insoluble material (Leu_{inc} and Tdr_{inc} respectively) was estimated in separate incubations. Light affected Leu_{inc} to a larger extent than Tdr_{inc} (Fig. 6A, B and E). Leu_{inc} and Tdr_{inc} rates were 12%–57% (40% ± 21%) and 2%–27% (11% ± 14%) lower in the dark than in the light respectively (Fig. 6E). The addition of DCMU resulted in an average decrease of 68% ± 10% and 49% ± 23% in Leu_{inc} and Tdr_{inc} rates in the light respectively (Fig. 6A and B). The Leu_{inc} to Tdr_{inc} ratio was on average 1.6 ± 0.5 times higher in the light than in the dark, 1.5 ± 0.3 times higher in the light than with DCMU (Fig. 6C).

Discussion

Characterization of Prochlorococcus *and* Synechococcus *mixotrophic metabolism*

Owing to their capability to utilize sunlight and atmospheric CO_2 for growth, *Prochlorococcus* and *Synechococcus* are commonly considered photoautotrophs. Yet, recent evidence has shown that the uptake of organic N- (leucine, amino acids) and P- (ATP) molecules by *Prochlorococcus* and *Synechococcus* is enhanced in the light (Michelou *et al.*, 2007; Mary *et al.*, 2008b; Duhamel *et al.*, 2012;

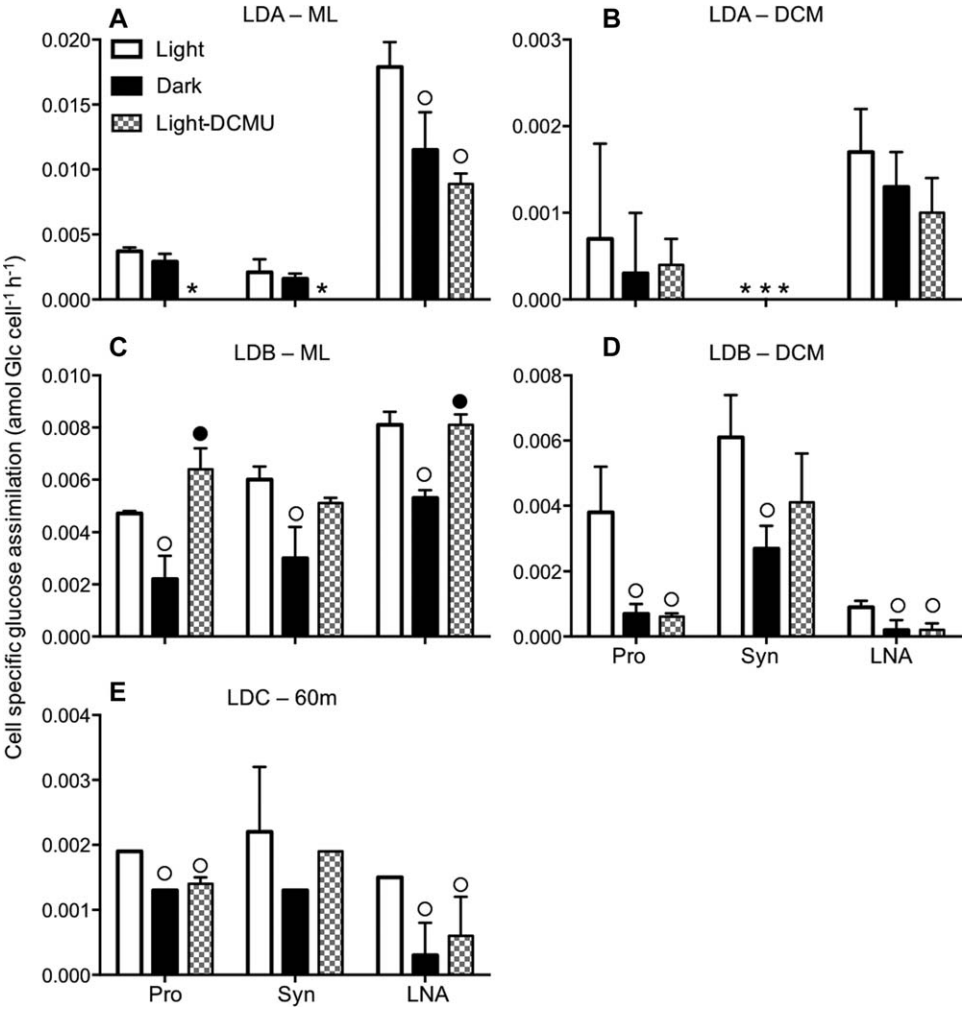

Fig. 3. Cell specific glucose assimilation (amol Glc cell^{-1} h^{-1}) at LDA – ML (A), LDA – DCM (B), LDB – ML (C), LDB – DCM (D) and LDC – 60 m (E), in incubations in the light (white bars), in the dark (black bars) and in the light with DCMU (checker board pattern) for *Prochlorococcus* (Pro), *Synechococcus* (Syn) and LNA bacteria (LNA). Error bars represent standard deviation on triplicate samples. One-way ANOVA multiple treatment comparison results are represented by white or black circles when values are significantly ($p < 0.05$) different from the light or the dark treatments respectively. * indicate nonmeasurable rates (killed control corrected rates $\leq$ killed control).

Gómez-Pereira *et al*., 2013). Therefore, previous studies (e.g., Zubkov *et al*., 2003; Michelou *et al*., 2007; Zubkov, 2009; Gómez-Pereira *et al*., 2013; Moore, 2013; Muñoz-Marín *et al*., 2013; 2017; Björkman *et al*., 2015) since the early work of Rippka (1972) have commonly defined this nutritional plasticity in marine cyanobacteria as photoheterotrophy, although *sensus stricto* this term defines organisms that use light for energy, but cannot use CO_2 as their sole C source. However, these studies demonstrating light-enhancement of N or P-containing organic molecules uptake did not directly verify if marine cyanobacteria could also use organic molecules containing only C (e.g., glucose) and if light also enhances organic C assimilation. To the best of our knowledge, only Muñoz-Marín *et al*. (2013) demonstrated that *Prochlorococcus* could assimilate glucose, a molecule devoid of heteroatoms (N or P), in natural seawater. Our results geographically expand these findings from the Atlantic Ocean and demonstrate that not only *Prochlorococcus* but also *Synechococcus* assimilate glucose in biogeochemically distinct marine environments of the WTSP. Interestingly, cell- and group- specific assimilation rates of glucose appeared to follow trophic gradients, similar to organic C uptake by *Trichodesmium* (Benavides *et al*., 2017). Further, we demonstrate that light enhanced cell specific glucose uptake by nearly 50% for *Prochlorococcus* and *Synechococcus*, suggesting that variability in light availability (e.g., changes in light intensity due to euphotic layer PAR gradient, diel sunlight rhythm, cloud coverage) could largely impact organic C assimilation by these cyanobacteria. Similar results were also found in cultures of the low-light *Prochlorococcus* SS120 strain in which 24 h incubation in the dark induced a 40% decrease in glucose uptake (Gomez-Baena *et al*., 2008). Thus, marine cyanobacteria may be a significant competitor of heterotrophic bacteria for this labile molecule, especially during the day, potentially creating temporal patterns between strictly heterotrophic and mixotrophic bacterioplankton. Expanding this finding to all labile C molecules, such temporal patterns could affect dissolved organic matter (DOM) remineralization and C sequestration via the microbial C pump.

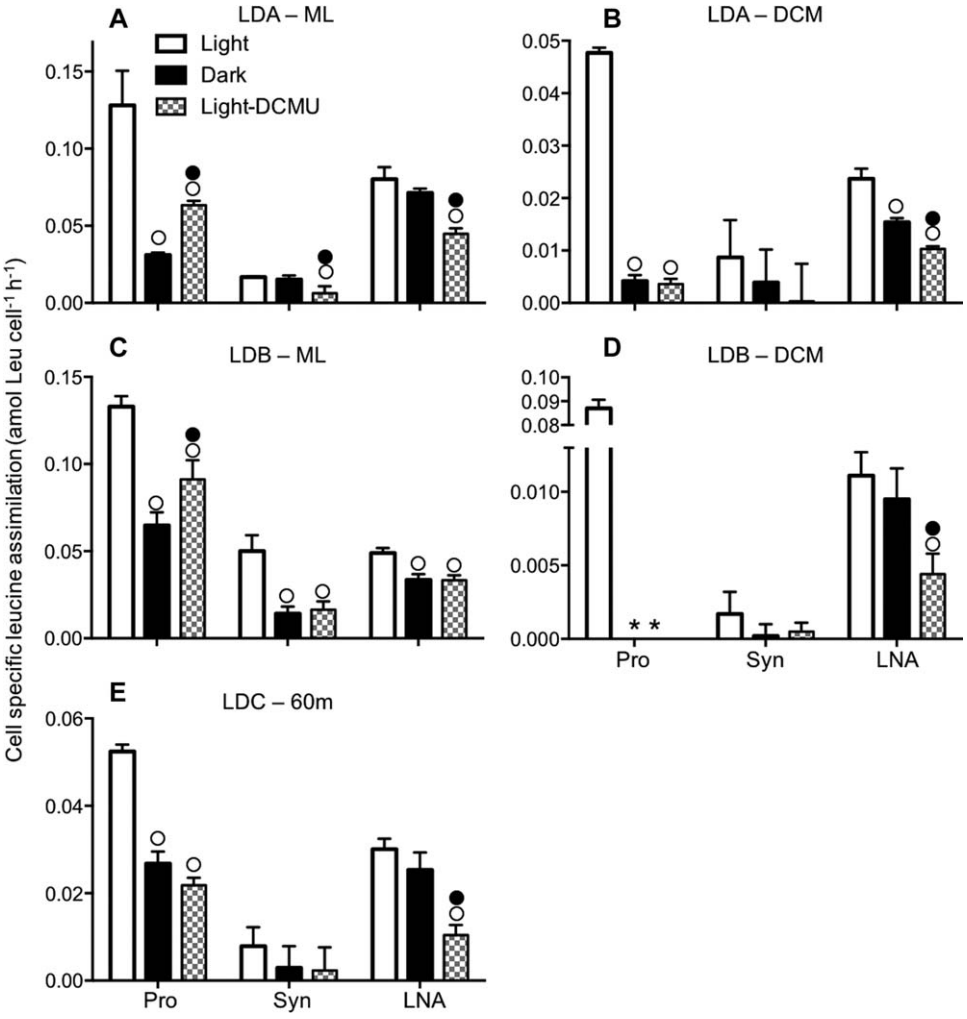

Fig. 4. Cell specific leucine assimilation rate (amol Leu $cell^{-1}$ h^{-1}) at LDA – ML (A), LDA – DCM (B), LDB – ML (C), LDB – DCM (D) and LDC – 60 m (E), in incubations in the light (white bars), in the dark (black bars) and in the light with DCMU (checker board pattern) for *Prochlorococcus* (Pro), *Synechococcus* (Syn) and LNA bacteria (LNA). Error bars represent standard deviation on triplicate samples. One-way ANOVA multiple treatment comparison results are represented by white or black circles when values are significantly ($p < 0.05$) different from the light or the dark treatments respectively. * indicate nonmeasurable rates (killed control corrected rates $\leq$ killed control).

We also explored the participation of photosynthetic electron transport in the regulation of organic molecules assimilation using the photosynthesis inhibitor DCMU (Rippka, 1972; Stanier, 1973; Neilson and Lewin, 1974; Paerl, 1991; Moore, 2013). In chlorophyll and bacteriochlorophyll containing organisms, DCMU blocks electron flow between photosystem II and plastoquinone, resulting in no O_2 and NADPH production, but allowing ATP synthesis through cyclic electron flow around photosystem I. Our results show that DCMU completely inhibited CO_2 fixation by *Prochlorococcus* and *Synechococcus* but only partially inhibited their assimilation of glucose, leucine and ATP. For most samples and molecules examined, the assimilation rates were significantly higher or not statistically different in the light with DCMU compared to the dark, suggesting that organic molecules incorporation is partially tied to photosynthetic production of energy in the light. The light harvested by *Prochlorococcus* and *Synechococcus* photosynthetic apparatus may thus transfer energy into ATP that can be used in the active transport of organic molecules. Similarly, Muñoz-Marín *et al.* (2017) found different inhibitory effects on *Prochlorococcus* SS120 glucose uptake using the quinone analogue inhibitor of cytochrome b6f complex in photosystem I –DBMIB (~100%), or the inhibitor of photosystem II –DCMU (~50%), and argued that the ATP generated by photosystem I could maintain up to 50% of the glucose uptake. Seawater used in our experiments was sampled in the early morning and thus cells were likely light energy depleted, assuring that continuing uptake in our dark incubations was not a result of stored energy during the light phase. Therefore, our results demonstrate that natural populations of *Prochlorococcus* and *Synechococcus* are nutritionally and metabolically flexible. We confirm that *in situ*, picocyanobacteria can assimilate selected organic molecules including glucose, a molecule devoid of heteroatoms and show that assimilation rates are reduced but continue even in the dark or when photosynthesis is impaired. This mixotrophic metabolism could explain the recent findings of the substantial presence of *Prochlorococcus* in the aphotic ocean (Jiao *et al.*, 2014) and the survival of specific strains of *Prochlorococcus* in extended darkness (Coe *et al.*, 2016).

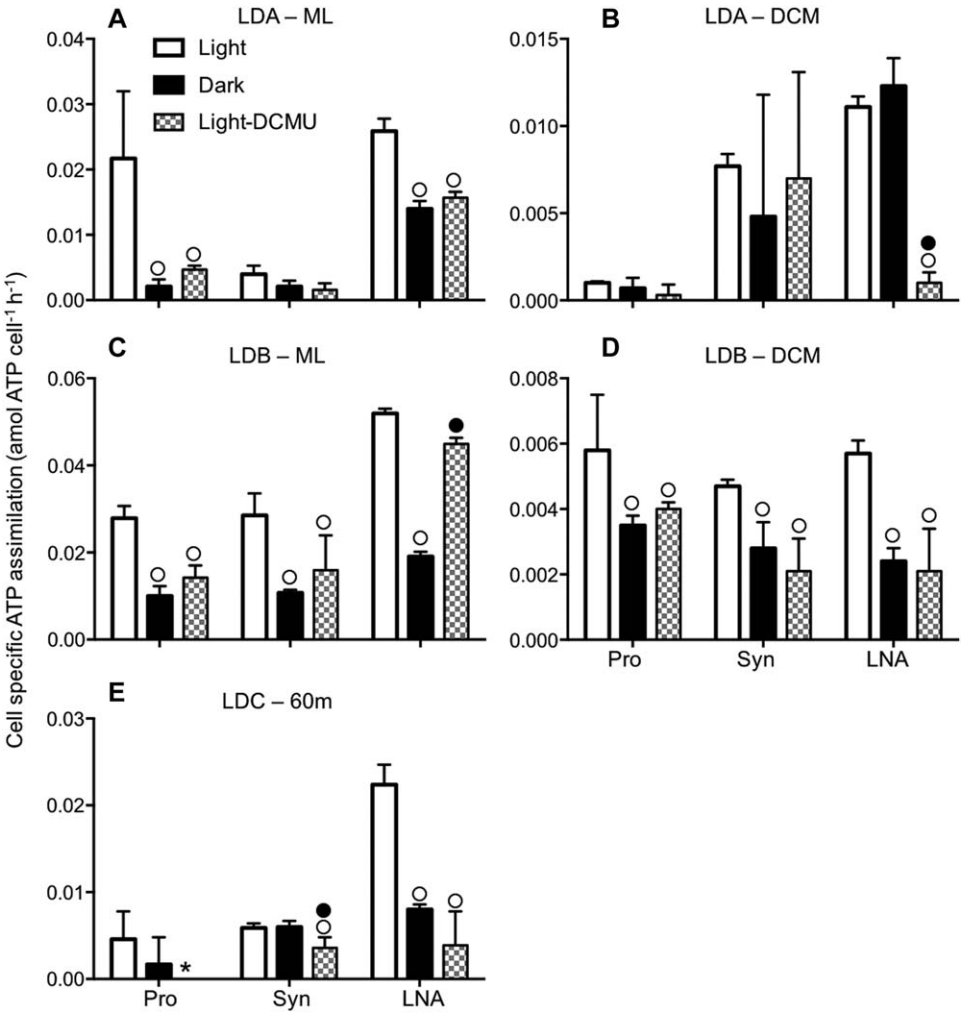

Fig. 5. Cell specific ATP assimilation rate (amol ATP cell^{-1} h^{-1}) at LDA – ML (A), LDA – DCM (B), LDB – ML (C), LDB – DCM (D) and LDC – 60 m (E), in incubations in the light (white bars), in the dark (black bars) and in the light with DCMU (checker board pattern) for *Prochlorococcus* (Pro), *Synechococcus* (Syn) and LNA bacteria (LNA). Error bars represent standard deviation on triplicate samples. One-way ANOVA multiple treatment comparison results are represented by white or black circles when values are significantly ($p < 0.05$) different from the light or the dark treatments respectively. * indicate nonmeasurable rates (killed control corrected rates $\leq$ killed control).

Relative importance of mixotrophy for picocyanobacterial C assimilation

Cell-specific glucose uptake in natural *Prochlorococcus* was on average 0.00021 ± 0.00011 fg C cell^{-1} h^{-1}, similar to results by Muñoz-Marín *et al*. (2013) in the Atlantic Ocean (0.00010 ± 0.00008 fg C cell^{-1} h^{-1}, where added and ambient glucose concentrations were comparable to those in our study). *Synechococcus* had higher cell specific glucose assimilation rates than *Prochlorococcus*, but using an average biovolume of 0.17 and 0.33 μm^3 for *Prochlorococcus* and *Synechococcus*, respectively (Grob *et al*., 2007), we calculated that glucose uptake by *Prochlorococcus* and *Synechococcus* was similar (0.0013 ± 0.0007 and 0.0009 ± 0.0005 fg C μm^{-3} h^{-1} respectively). Compared to carbon uptake from ^{14}C-sodium bicarbonate (4.4 ± 1.7 and 44 ± 36 fg C cell^{-1} d^{-1} for *Prochlorococcus* and *Synechococcus* respectively), glucose uptake represented a small fraction ($< 1\%$) of total (inorganic + organic) C uptake, similar to the values calculated using results in Muñoz-Marín *et al*. (2013). This implies that mixotrophy may represent a marginal fraction of *Prochlorococcus* and *Synechococcus* C uptake in the Atlantic and Pacific Oceans, and confirms culture-based studies where *Prochlorococcus* actively takes up glucose when available, but remains primarily autotrophic (Muñoz-Marín *et al*., 2017). Yet considering that glucose is only one of the greatly diverse dissolved organic C molecules present in the ocean (Moran *et al*., 2016), the low assimilation rates may be the result of glucose uptake competition with other sugars. Thus, other organic C compounds need to be tested as substrates for mixotrophic growth by marine cyanobacteria. However, mixotrophy by marine picocyanobacteria may represent a greater advantage in response to inorganic nutrient limitation than to access an alternative form of C. Indeed, we found that cell specific uptake of leucine and ATP by *Prochlorococcus* were on average 35.5 ± 16.5 and 3.4 ± 2.3 times greater than cell specific uptake rates of glucose (mol:mol). Still, Muñoz-Marín *et al*. (2013) argued that the bioenergetic advantage of glucose uptake versus glucose synthesis de novo may save *Prochlorococcus* significant energy for other metabolic uses.

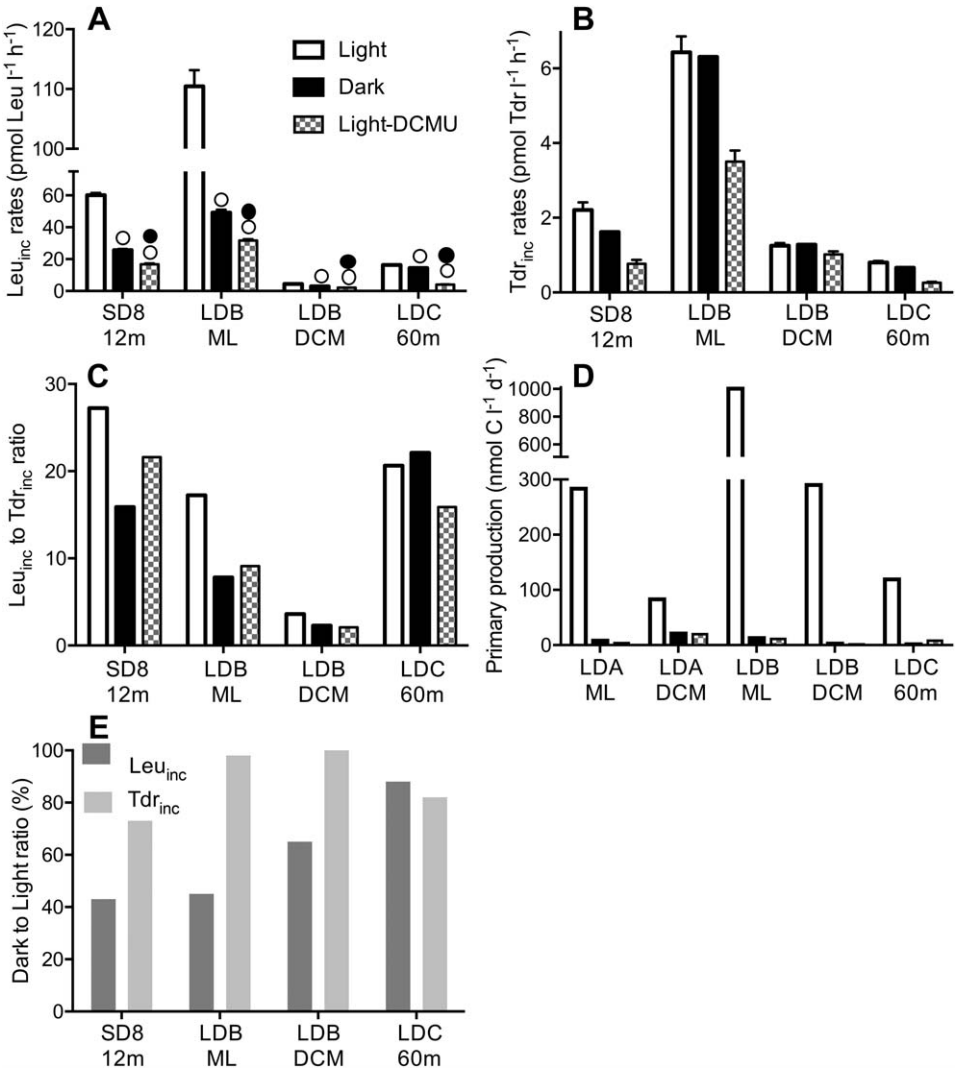

Fig. 6. Bacterial production rates measured using leucine (A, Leu_{inc}, pmol Leu l^{-1} h^{-1}) or thymidine (B, Tdr_{inc}, pmol Tdr l^{-1} h^{-1}) incorporation into TCA insoluble material; leucine to thymidine incorporation ratio (C, Leu_{inc} to Tdr_{inc} ratio); primary production rates (D, nmol C l^{-1} d^{-1}) in incubations in the light (white bars), in the dark (black bars) and in the light with DCMU (white and grey checker board pattern); dark to light ratio (E, %) for Leu_{inc} (grey bars, Leu) and Tdr_{inc} (light grey bars, Tdr). Error bars represent standard deviation on triplicate samples (A) or absolute difference between duplicate samples (B, thymidine in the light and light with DCMU). One-way ANOVA multiple treatment comparison results are represented by white or black circles when values are significantly ($p < 0.05$) different from the light or the dark treatments respectively.

Photoheterotrophy by LNA bacteria

The LNA group has been previously characterized using molecular tools and results consistently show that it is largely dominated by SAR11 (Mary *et al.*, 2006; 2008b; Gómez-Pereira *et al.*, 2013; Morán *et al.*, 2015), a highly abundant group in the subtropical Pacific (West *et al.*, 2016). SAR11 is a clade of proteorhodopsin-based photoheterotrophic bacteria, which are characterized by light-controlled growth and proteorhodopsin expression (Lami *et al.*, 2009). Like in other studies (Mary *et al.*, 2008b, Zubkov, 2009; Gómez-Pereira *et al.*, 2013; Evans *et al.*, 2015) we showed that light enhances the assimilation of leucine and ATP by the LNA group. In our experiments, Tdr_{inc} was an excellent proxy of strict heterotrophic bacterial production as the Tdr_{inc} rates were similar in light and dark incubations, unlike Leu_{inc} rates (Fig. 6). This confirms that increased Leu_{inc} rates in the light were mostly due to photoheterotrophy and mixotrophic capacities of *Prochlorococcus*, but not to an indirect effect related to enhanced phytoplankton excretion/exudation in the light. Light-enhanced cell-specific glucose uptake by LNA bacteria was large (~35%), albeit lower than for *Prochlorococcus* and *Synechococcus*, implying that photons can supply a significant part of the energy demand during daytime and the requirement for organic molecules as energy sources is significantly decreased. Surprisingly, the addition of DCMU reduced organic molecules incorporation by LNA. DCMU has been widely used to study phytoplankton metabolism (Jeanjean, 1976; Lewis *et al.*, 1984; Garrigue *et al.*, 1992; Ikeya *et al.*, 1997; DeLorenzo *et al.*, 2001; Laurent *et al.*, 2013; Halsey *et al.*, 2014), including photoheterotrophy (Estep and Hoering, 1981; Paerl, 1991; Johnson and Alric, 2012; Knoop *et al.*, 2013; You *et al.*, 2015; Oren *et al.*, 2016; Muñoz-Marín *et al.*, 2017). These studies indicate that DMCU is not lethal, that its inhibitory effect is reversible and that it does not affect heterotrophic processes, even in autotroph-heterotroph symbiotic

associations (Vandermeulen *et al*., 1972; Mühlbauer and Eichacker, 1998; Francoeur *et al*., 2007). Combined with good efficiency against algal photosynthesis (Fig. 6D), and because it was also chosen to study photoheterotrophic metabolism in culture isolates of *Prochlorococcus* (Muñoz-Marín *et al*., 2017), we used DCMU as selective inhibitor of the photosystem II in cyanobacteria. However, we observed a reduction in organic molecules incorporation in LNA which suggests that DCMU affected LNA directly or indirectly. A direct effect could be either toxicity on heterotrophic metabolism of strict heterotrophs or an action on the light-driven proton pump, proteorhodopsin. Yet, at the end of incubation, LNA cell abundances were similar between treatments, indicating that DCMU may not be lethal to LNA. Moreover, DCMU did not inhibit the light-driven proton pump of the cyanobacteria *Gloeobacter violaceus* which has two types of light-driven proton pumps, chlorophyll-based photosystems and rhodopsin (Choi *et al*., 2014). Alternatively, the reduction of activity by LNA in DCMU samples may be indirect, resulting from the inhibition of photosynthate production by phytoplankton (photosynthesis was inhibited, Fig. 6D) on which bacteria rely greatly as a source of labile organic substrates for growth (Church *et al*., 2004; Ruiz-Gonzalez *et al*., 2012a, 2013).

Implication of picocyanobacterial uptake of leucine for bacterial production measurements in the ocean

Despite being important for our understanding of biological productivity in the ocean, light enhanced bacterial production and uptake of leucine by the most abundant marine microbes, *Prochlorococcus*, *Synechococcus* and LNA, have been the subject of a limited number of studies (Church *et al*., 2004; Michelou *et al*., 2007; Mary *et al*., 2008b; Ruiz-Gonzalez *et al*., 2013; Björkman *et al*., 2015). In incubations with saturating concentrations of leucine (20 nM), previous reports found that *Prochlorococcus* contributes significantly to bacterial production estimates in the North Pacific (Björkman *et al*., 2015), North Atlantic (Michelou *et al*., 2007) and Mediterranean Sea (Talarmin *et al*., 2011a), both in dark or in the light. Here, we show that light enhanced bulk Leu_{inc} rates (incorporation rates of leucine into TCA insoluble material), as well as cell-specific leucine uptake by *Prochlorococcus*, *Synechococcus* and LNA in the tropical Southwest Pacific Ocean. However, light significantly enhanced bulk Tdr_{inc} rates to a much lesser extent (dark to light ratio was 73%–100%, mean 89%). This may be due to thymidine being preferentially used by heterotrophic bacteria than leucine which can be used by mixotrophic phytoplankton (Michelou *et al*., 2007; Björkman *et al*., 2015). Indeed, in our samples, over a third of the total leucine uptake was attributable to the *Prochlorococcus* group. Unfortunately, due to low thymidine specific activity, we were not able to measure its group-specific uptake. This should be verified in future studies as cyanobacteria tested so far do not incorporate Tdr in culture, which was related to a probable lack of thymidine kinase (Pollard and Moriarty, 1984). The contribution of picocyanobacteria to bacterial production estimates, particularly using ^{3}H-leucine, should, thus, be considered when measuring bacterial production in marine environments, even in dark incubations (Talarmin *et al*., 2011a; Björkman *et al*., 2015). Longnecker *et al*. (2006) found higher Leu_{inc}/Tdr_{inc} ratio in dark incubated HNA than in the LNA group, and also higher in surface samples than below, particularly in the open-sea station. Besides the general hypothesis of higher rates of protein synthesis relative to DNA synthesis in larger cells, this result could also be partially due to the difficulty to separate *Prochlorococcus* from HNA during cell sorting after SYBR green DNA staining, particularly in subsurface waters. As seen from seawater cultures, the Leu_{inc}/Tdr_{inc} ratio can be representative of decoupling between cell division and biomass production (Chin-Leo and Kirchman, 1990). *In situ*, Leu_{inc} rates has been shown also to vary more than Tdr_{inc} rates along diel cycles (Riemann and Bell, 1990). Consequently, the use of Leu_{inc}/Tdr_{inc} ratio as a proxy of unbalanced growth should be misleading when samples are incubated in the light as we demonstrated that it was also affected by photoheterotrophic processes (higher in the light than in the dark or with DCMU).

Conclusion

We present several lines of evidence that natural *Prochlorococcus* and *Synechococcus* can assimilate organic molecules with variable C:N:P composition, as well as organic molecules devoid of heteroatoms (i.e., glucose). *Prochlorococcus* and *Synechococcus* assimilated organic molecules in the light but also in the dark or when photosynthesis was altered by DCMU, albeit at significantly reduced rates, verifying previous findings in culture indicating that cyanobacteria are nutritionally versatile. Yet *Prochlorococcus* and *Synechococcus* C uptake from glucose was small compared to CO_2 uptake, indicating that they obtain carbon primarily through an autotrophic metabolism. Nevertheless, mixotrophy by these unicellular cyanobacteria was widespread in biogeochemically distinct regions of the WTSP Ocean and cell and group-specific assimilation rates were generally higher in surface than at the DCM. However, cell-specific assimilation rates of the N- and P- containing molecules (leucine and ATP) were significantly higher than that of glucose. Thus, mixotrophy by marine cyanobacteria is more likely to be an adaptation to low inorganic nutrient availability. Many details of marine cyanobacteria mixotrophic metabolism remain to be elucidated. In particular, additional experiments will be necessary to evaluate the global importance of organic versus inorganic C uptake by marine cyanobacteria and

assess the diel variability in these processes, in order to improve C fluxes models (Gasol *et al.*, 2008; Zubkov, 2009). Further study of mixotrophic metabolism is likely to contribute not only to our understanding of microbial adaptations to light and nutrient availability but also to our knowledge of marine DOM cycling, and the role of light in modulating bacteria and cyanobacteria biogeochemical functions in the ocean.

Experimental procedures

Field sampling

This study was conducted in the WTSP along trophic gradients during the OUTPACE cruise (DOI: https://doi.org/10.17600/15000900, RV *L'Atalante*, February–April 2015) between New Caledonia and Tahiti (Moutin *et al.*, 2017). Three incubation experiments were carried out at the long duration stations LDA, LDB and LDC, selected for their contrasted biogeochemical conditions (Table 1). At LDA and LDB, seawater was sampled within the well-lit top mixed layer (ML at 54% PAR, 7 and 9 m depths respectively) and at the DCM (0.3% PAR, 70 and 90 m respectively). LDC was sampled at 60 m depth (10% PAR).

Bacterioplankton enumeration

Bacterioplankton groups were enumerated from untreated samples using a BD Influx flow cytometer (BD Biosciences, San Jose, CA, USA). *Prochlorococcus* and *Synechococcus* were enumerated in unstained samples while the low-DNA-content (LNA) and high-DNA-content (HNA) bacteria groups were discriminated in a sample aliquot stained with SYBR Green I DNA dye (0.01% final), following published protocols (Gasol *et al.*, 1999; Duhamel *et al.*, 2014). Using a forward scatter detector with small particle option and focusing a 488 plus a 457 nm (200 and 300 mW solid state respectively) laser into the same pinhole greatly improved the resolution of dim surface *Prochlorococcus* population from background noise in unstained samples. However, in stained samples from the ML, *Prochlorococcus* overlapped with HNA bacteria and HNA abundances were calculated by subtracting *Prochlorococcus* enumerated from unstained samples. Calibration and alignment were done using 1-μm yellow–green microspheres (Polysciences, USA).

Incubation experiments

Seawater collected at the LD stations was distributed into acid-washed and sample rinsed transparent polycarbonate bottles for separate incubations with different radioactive-labelled molecules and under different treatment conditions. For each radioactive molecule tested, a killed control was prepared by adding paraformaldehyde (0.5% final w/v) for 30 min before adding the radioisotope. Bottles were incubated in on-deck blue-shielded incubators to mimic the amount of transmitted light at the corresponding sampled depth and cooled with surface seawater. Samples were treated with or without addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, at 20 μM final, for 15 min before adding the radioisotope). For dark incubations, the bottles were masked using multilayers of matte black aluminum foil (Rosco Matte Black Cinefoil).

For each treatment, D-[6-3H(N)]-glucose (45.7 Ci mmol^{-1}), L-[3,4,5-3H(N)]-Leucine (112 Ci mmol^{-1}) and [2,5′,8-3H]-Adenosine-5′-triphosphate (52 Ci mmol^{-1}), were used in separate incubations to measure assimilation rates of organic C alone (glucose, Glc), and N- (leucine, Leu) and P- (Adenosine-5′-triphosphate, ATP) – enriched organic compounds respectively (Perkin Elmer, Waltham, MA, USA). Isotope additions were kept as low as possible considering the specific activity and sensitivity of the cell sorting procedure described below: ^{3}H-glucose, ^{3}H-leucine and ^{3}H-ATP were added at a final concentration of 2, 7 and 1 nmol l^{-1} respectively. Samples were incubated for 4 to 6 h and uptake linearity was checked before each experiment. Additional incubations were done using [^{14}C]-sodium bicarbonate (43.3 mCi mmol^{-1}) at 3.3 μCi ml^{-1} final concentration from dawn to dusk (~8 h). At the end of incubation, samples were fixed with paraformaldehyde (0.5% final, for 15-min in the dark), 20 μl were sampled to measure total activity (dpm l^{-1}, with β-phenylethylamine for ^{14}C samples) and determine the concentration of added molecules (S^*, nmol l^{-1}), 4 ml were filtered onto 0.2-μm polycarbonate membranes to monitor incorporation by the total microbial community (total activity –dpm l^{-1}, and total microbial assimilation rate –nmol l^{-1} h^{-1}) and 30 to 50 ml was gently concentrated to 4 ml and preserved at −80°C for flow cytometry cell sorting. Radioactivity was measured using scintillation cocktail with low background and high ^{3}H counting efficiency (Ultima Gold LLT, Perkin Elmer) and a Packard Tri-Carb 3110 TR liquid scintillation counter with ultra-low-level option kit. The turnover time (h) was calculated by dividing the total activity (dpm l^{-1}) by the activity on the 0.2-μm filter (dpm l^{-1} h^{-1}). The total microbial assimilation rate (nmol l^{-1} h^{-1}) was calculated by dividing the substrate concentration (ambient concentration (S_a) plus S^*) by the turnover time (h). The ambient concentrations of glucose, leucine and ATP were estimated using a concentration series bioassay of untreated live samples as described by Wright and Hobbie (1966) and modified by Zubkov and Tarran (2005), which represents an upper estimate of ambient concentrations. Thus, calculated assimilation rates represent an upper estimate of assimilation rates at *in situ* concentrations.

Flow cytometry cell sorting

Bacterioplankton groups were characterized as described above. Note that because of the overlap in *Prochlorococcus* with HNA in stained ML samples, we did not systematically sort the HNA population for which results were biased by the contribution of *Prochlorococcus* activity. The Influx flow cytometer was set at the highest sorting purity (1.0 drop single mode) and potential attached cells were discarded using a pulse width versus forward scatter plot. The drop delay was calibrated using Accudrop Beads (BD Biosciences, USA) and sorting efficiency was verified manually by sorting a specified number of 1-μm yellow–green microspheres (Polysciences, USA) onto a glass slide and counting the beads under an epifluorescence microscope. Using 1.0 drop single mode we systematically recovered 100% of the targeted beads. Performance was validated by sorting *Prochlorococcus* and *Synechococcus* from natural samples and reanalysing the

sorted cells flow cytometrically to confirm sort purity, which exceeded 96%, a result similar to Baer *et al*. (2017). Increasing numbers of cells from the same incubation sample were sorted (20,000–600,000 LNA and 10,000–300,000 *Prochlorococcus* and *Synechococcus*). Sorted cells were assessed by liquid scintillation following Talarmin *et al*. (2011b). The ^{14}C-labelled samples were acidified with 0.5 ml of 1N HCl for 24 h to remove any unincorporated ^{14}C-sodium bicarbonate. For each group, at least three samples were sorted and regression analysis between the number of cells sorted and the radioactivity taken up by the sorted cells was used to calculate the per cell activity (dpm cell^{-1}). Radioactivity in the killed control sorted samples (dpm cell^{-1}) was deduced from radiaoactivity in the respective sorted samples. The cell-specific assimilation rate (nmol cell^{-1} h^{-1}) was calculated by dividing the radioactivity per cell (dpm cell^{-1}) by the total microbial activity (dpm l^{-1}) measured in the same treatment, and then multiplied by the total microbial assimilation rate at ambient plus added organic substrate concentration ($S_a + S^*$, nmol l^{-1} h^{-1}). Statistical tests (one-way ANOVA, Tukey's multiple comparisons test) were carried out to assess significant differences between treatments ($p < 0.05$) using Prism 6 (GraphPad software, La Jolla, CA, USA).

Bulk bacterial production

Samples were incubated with [methyl-^{3}H]-thymidine (Tdr, 20 nM, 48.8 Ci mmol^{-1}) or with L-[3,4,5-^{3}H(N)]- leucine (Leu, 6 nM, 112 Ci mmol^{-1}) for 5 to 6 h. Killed controls and incubation terminations were fixed with formalin 1% final concentration. Tdr samples were treated by the filtration technique (Bell, 1993, ice cold- trichloroacetic acid (TCA) extract) and Leu samples by the centrifuge technique (Smith and Azam, 1992). In both methods, an ethanol rinse was included. Note that bulk bacterial production experiments could not be done at LDA but were done at the short duration station 8 instead (hereafter SD 8, in the ML, 12 m). We occasionally checked that we were working at saturating concentration of Tdr and Leu by testing activities using a range of concentrations of Tdr varying from 5 to 60 nM and of Leu varying from 2 to 45 nM. We confirmed that the Tdr concentration was saturating at all stations and that Leu concentration was saturating at all stations, excepted at SD 8 where measured rates were about half the maximum velocities.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We thank T. Moutin, chief scientist of the OUTPACE cruise, O.R. Anderson, A. Martiny and an anonymous reviewer for their insightful comments to previous versions of this manuscript. We are indebted to O. Grosso and S. Helias-Nunige for nutrient measurements and to G. Rougier and M. Picheral for their help in CTD rosette management and data processing. We are grateful to the crew of the R/V *L'Atalante* for outstanding shipboard operation. SD was funded by the National Science Foundation (OCE-1434916). MB was funded by the People Program (Marie Skłodowska-Curie Actions) of the European Union's Seventh Framework Program (FP7/2007–2013) under REA grant agreement number 625185. This is a contribution of the OUTPACE project (https://outpace.mio.univ-amu.fr/) funded by the French research national agency (ANR-14-CE01–0007-01), the LEFE-CyBER program (CNRS-INSU), the GOPS program (IRD) and the CNES.

References

Baer, S.E., Lomas, M.W., Terpis, K.X., Mouginot, C., and Martiny, A.C. (2017) Stoichiometry of Prochlorococcus, Synechococcus, and small eukaryotic populations in the western North Atlantic Ocean. *Environmental Microbiology* **19:** 1568–1583.

Béjà, O., and Suzuki, M. (2008) Photoheterotrophic marine prokaryotes. In *Microbial Ecology of the Oceans*. Kirchman, D.L. (ed.). Hoboken, NJ: Wiley.

Bell, R.T. (1993) Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine. In *Handbook of Methods in Aquatic Microbial Ecology*. Kemp, P.F., Sherr, B.F., Sherr, E.B., and Cole, J.J. (eds). Boca Raton, FL: Lewis Publishers.

Benavides, M., Berthelot, H., Duhamel, S., Raimbault, P., and Bonnet, S. (2017) Dissolved organic matter uptake by *Trichodesmium* in the Southwest Pacific. *Sci Rep* **7:** 41315.

Björkman, K.M., Church, M.J., Doggett, J.K., and Karl, D.M. (2015) Differential assimilation of inorganic carbon and leucine by *Prochlorococcus* in the oligotrophic North Pacific Subtropical Gyre. *Front Microbiol* **6:** 1401.

Chin-Leo, G., and Kirchman, D.L. (1990) Unbalanced growth in natural assemblages of marine bacterioplankton. *Mar Ecol Progr Ser* **63:** 1–8.

Choi, A.R., Shi, L., Brown, L.S., and Jung, K.-H. (2014) Cyanobacterial light-driven proton pump, gloeobacter rhodopsin: complementarity between rhodopsin-based energy production and photosynthesis. *PLoS One* **9:** e110643.

Church, M.J., Ducklow, H.W., and Karl, D.M. (2004) Light dependence of [^{3}H]Leucine incorporation in the oligotrophic North Pacific Ocean. *Appl Environ Microbiol* **70:** 4079–4087.

Claustre, H., Sciandra, A., and Vaulot, D. (2008) Introduction to the special section bio-optical and biogeochemical conditions in the South East Pacific in late 2004: the BIOSOPE program. *Biogeosciences* **5:** 679–691.

Coe, A., Ghizzoni, J., LeGault, K., Biller, S., Roggensack, S.E., and Chisholm, S.W. (2016) Survival of *Prochlorococcus* in extended darkness. *Limnol Oceanogr* **61:** 1375–1388.

DeLorenzo, M.E., Lewitus, A.J., Scott, G.I., and Ross, P.E. (2001) Use of metabolic inhibitors to characterize ecological interactions in an estuarine microbial food web. *Microb Ecol* **42:** 317–327.

Duhamel, S., Björkman, K.M., and Karl, D.M. (2012) Light dependence of phosphorus uptake by microorganisms in the North and South Pacific subtropical gyres. *Aquat Microb Ecol* **67:** 225–238.

Duhamel, S., Björkman, K.M., Doggett, J.K., and Karl, D.M. (2014) Microbial response to enhanced phosphorus cycling in the North Pacific Subtropical Gyre. *Mar Ecol Progr Ser* **504:** 43–58.

Estep, M.F., and Hoering, T.C. (1981) Stable hydrogen isotope fractionations during autotrophic and mixotrophic growth of microalgae. *Plant Physiol* **67:** 474–477.

Evans, C., Gómez-Pereira, P.R., Martin, A.P., Scanlan, D.J., and Zubkov, M.V. (2015) Photoheterotrophy of bacterioplankton is ubiquitous in the surface oligotrophic ocean. *Progr Oceanogr* **135:** 139–145.

Francoeur, S.N., Johnson, A.C., Kuehn, K.A., and Neely, R.K. (2007) Evaluation of the efficacy of the photosystem II inhibitor DCMU in periphyton and its effects on nontarget microorganisms and extracellular enzymatic reactions. *J North Am Benthol Soc* **26:** 633–641.

Gao, H., and Xu, X.D. (2012) The cyanobacterial NAD kinase gene sll1415 is required for photoheterotrophic growth and cellular redox homeostasis in *Synechocystis* sp strain PCC 6803. *J Bacteriol* **194:** 218–224.

Garrigue, C., Clavier, J., and Boucher, G. (1992) The use of photosynthesis inhibitor (DCMU) for in situ metabolic and primary production studies on soft bottom benthos. *Hydrobiologia* **246:** 141–145.

Gasol, J.M., Zweifel, U.L., Peters, F., Fuhrman, J.A., and Hagstrom, A. (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. *Appl Environ Microbiol* **65:** 4475–4483.

Gasol, J.M., Pinhassi, J., Alonso-Sáez, L., Ducklow, H., Herndl, G.J., Koblízek, M., *et al.* (2008) Towards a better understanding of microbial carbon flux in the sea. *Aquat Microb Ecol* **53:** 21–38.

Gomez-Baena, G., Lopez-Lozano, A., Gil-Martinez, J., Lucena, J.M., Diez, J., and Candau, P. (2008) Glucose uptake and its effect on gene expression in *Prochlorococcus*. *PLoS One* **3:** e3416.

Gómez-Pereira, P.R., Hartmann, M., Grob, C., Tarran, G.A., Martin, A.P., and Fuchs, B.M. (2013) Comparable light stimulation of organic nutrient uptake by SAR11 and *Prochlorococcus* in the North Atlantic subtropical gyre. *ISME J* **7:** 603–614.

Grob, C., Ulloa, O., Claustre, H., Huot, Y., Alarcon, G., and Marie, D. (2007) Contribution of picoplankton to the total particulate organic carbon concentration in the eastern South Pacific. *Biogeosciences* **4:** 837–852.

Halsey, K.H., Milligan, A.J., and Behrenfeld, M.J. (2014) Contrasting strategies of photosynthetic energy utilization drive lifestyle strategies in ecologically important picoeukaryotes. *Metabolites* **4:** 260–280.

Hill, P.G., Zubkov, M.V., and Purdie, D.A. (2010) Differential responses of *Prochlorococcus* and SAR11-dominated bacterioplankton groups to atmospheric dust inputs in the tropical Northeast Atlantic Ocean. *FEMS Microbiol Lett* **306:** 82–89.

Ikeya, T., Ohki, K., Takahashi, M., and Fujita, Y. (1997) Study on phosphate uptake of the marine cyanophyte *Synechococcus* sp. NIBB 1071 in relation to oligotrophic environments in the open ocean. *Mar Biol* **129:** 195–202.

Jeanjean, R. (1976) The effect of metabolic poisons on ATP level and on active phosphate uptake in *Chlorella pyrenoidosa*. *Physiol Plant* **37:** 107–110.

Jiao, N., Luo, T., Zhang, R., Yan, W., Lin, Y., Johnson, Z.I., *et al.* (2014) Presence of *Prochlorococcus* in the aphotic waters of the western Pacific Ocean. *Biogeosciences* **11:** 2391–2400.

Johnson, X., and Alric, J. (2012) Interaction between starch breakdown, acetate assimilation, and photosynthetic cyclic electron flow in *Chlamydomonas reinhardtii*. *J Biol Chem* **287:** 26445–26452.

Johnson, Z.I., and Lin, Y. (2009) *Prochlorococcus*: approved for export. *Proc Natl Acad Sci USA* **106:** 10400–10401.

Karl, D.M. (2007) Microbial oceanography: paradigms, processes and promise. *Nat Rev Microbiol* **5:** 759–769.

Knoop, H., Gründel, M., Zilliges, Y., Lehmann, R., Hoffmann, S., Lockau, W., *et al.* (2013) Flux balance analysis of cyanobacterial metabolism: the metabolic network of *Synechocystis* sp. PCC 6803. *PLOS Comput Biol* **9:** e1003081.

Lami, R., Cottrell, M.T., Campbell, B.J., and Kirchman, D.L. (2009) Light-dependent growth and proteorhodopsin expression by Flavobacteria and SAR11 in experiments with Delaware coastal waters. *Environ Microbiol* **11:** 3201–3209.

Laurent, J., Tambutté, S., Tambutté, É., Allemand, D., and Venn, A. (2013) The influence of photosynthesis on host intracellular pH in scleractinian corals. *J Exp Biol* **216:** 1398–1404.

Lewis, M.R., Cullen, J.J., and Piatt, T. (1984) Relationships between vertical mixing and photoadaptation of phytoplankton: similarity criteria. *Mar Ecol Progr Ser* **15:** 141–149.

Li, W.K.W., Rao, D.V.S., Harrison, W.G., Smith, J.C., Cullen, J.J., Irwin, B., and Platt, T. (1983) Autotrophic picoplankton in the tropical ocean. *Science* **219:** 292–295.

Longnecker, K., Sherr, B.F., and Sherr, E.B. (2006) Variation in cell-specific rates of leucine and thymidine incorporation by marine bacteria with high and with low nucleic acid content off the Oregon coast. *Aquat Microb Ecol* **43:** 113–125.

Martiny, A.C., Kathuria, S., and Berube, P.M. (2009) Widespread metabolic potential for nitrite and nitrate assimilation among *Prochlorococcus* ecotypes. *Proc Natl Acad Sci USA* **106:** 10787–10792.

Mary, I., Heywood, J.L., Fuchs, B.M., Amann, R., Tarran, G.A., Burkill, P.H., *et al.* (2006) SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. *Aquat Microb Ecol* **45:** 107–113.

Mary, I., Garczarek, L., Tarran, G.A., Kolowrat, C., Terry, M.J., and Scanlan, D.J. (2008a) Diel rhythmicity in amino acid uptake by *Prochlorococcus*. *Environ Microbiol* **10:** 2124–2131.

Mary, I., Tarran, G.A., Warwick, P.E., Terry, M.J., Scanlan, D.J., Burkill, P.H., *et al.* (2008b) Light enhanced amino acid uptake by dominant bacterioplankton groups in surface waters of the Atlantic Ocean. *FEMS Microbiol Ecol* **63:** 36–45.

Michelou, V.K., Cottrell, M.T., and Kirchman, D.L. (2007) Light-stimulated bacterial production and amino acid assimilation by cyanobacteria and other microbes in the North Atlantic Ocean. *Appl Environ Microbiol* **73:** 5539–5546.

Moore, L.R. (2013) More mixotrophy in the marine microbial mix. *Proc Natl Acad Sci USA* **110:** 8323–8324.

Moran, M.A., Kujawinski, E.B., Stubbins, A., Fatland, R., Aluwihare, L.I., Buchan, A., *et al.* (2016) Deciphering ocean carbon in a changing world. *Proc Natl Acad Sci USA* **113:** 3143–3151.

Morán, X.A.G., Alonso-Sáez, L., Nogueira, E., Ducklow, H.W., González, N., López-Urrutia, Á., *et al.* (2015) More, smaller bacteria in response to ocean's warming? *Proc R Soc Lond [Biol]* **282:** 20150371.

Moutin, T., Doglioli, A.M., de Verneil, A., and Bonnet, S. (2017) Preface: the oligotrophy to the UlTra-oligotrophy PACific Experiment (OUTPACE cruise, 18 February to 3 April 2015). *Biogeosciences* **14:** 3207–3220.

Moutin, T., Wagener, T., Caffin, M., Fumenia, A., Gimenez, A., Baklouti, M., Bouruet-Aubertot, P., Pujo-Pay, M., Leblanc, K., Lefevre, D., Helias Nunige, S., Leblond, N., Grosso, O., and de Verneil, A. (2018) Nutrient availability and the ultimate control of the biological carbon pump in the Western Tropical South Pacific Ocean, Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-565, in review.

Mühlbauer, S.K., and Eichacker, L.A. (1998) Light-dependent formation of the photosynthetic proton gradient regulates translation elongation in chloroplasts. *J Biol Chem* **273:** 20935–20940.

Muñoz-Marín, M.D., Luque, I., Zubkov, M.V., Hill, P.G., Diez, J., and Garcia-Fernandez, J.M. (2013) *Prochlorococcus* can use the Pro1404 transporter to take up glucose at nanomolar concentrations in the Atlantic Ocean. *Proc Natl Acad Sci USA* **110:** 8597–8602.

Muñoz-Marín, M.D., Gómez-Baena, G., Díez, J., Beynon, R.J., González-Ballester, D., Zubkov, M.V., and García-Fernández, J.M. (2017) Glucose uptake in *Prochlorococcus*: diversity of kinetics and effects on the metabolism. *Front Microbiol* **8:** 327.

Neilson, A.H., and Lewin, R.A. (1974) The uptake and utilization of organic carbon by algae: an essay in comparative biochemistry. *Phycologia* **13:** 227–264.

Oren, A., Abu-Ghosh, S., Argov, T., Kara-Ivanov, E., Shitrit, D., Volpert, A., *et al*. (2016) Expression and functioning of retinal-based proton pumps in a saltern crystallizer brine. *Extremophiles* **20:** 69–77.

Paerl, H.W. (1991) Ecophysiological and trophic implications of light-stimulated amino acid utilization in marine picoplankton. *Appl Environ Microbiol* **57:** 473–479.

Paoli, A., Celussi, M., Del Negro, P., Umani, S.F., and Talarico, L. (2008) Ecological advantages from light adaptation and heterotrophic-like behavior in *Synechococcus* harvested from the Gulf of Trieste (Northern Adriatic Sea). *FEMS Microbiol Ecol* **64:** 219–229.

Partensky, F., and Garczarek, L. (2010) *Prochlorococcus*: advantages and limits of minimalism. *Ann Rev Mar Sci* **2:** 305–331.

Pollard, P.C., and Moriarty, D.J. (1984) Validity of the tritiated thymidine method for estimating bacterial growth rates: measurement of isotope dilution during DNA synthesis. *App Env Microbiol* **48:** 1076–1083.

Richardson, T.L., and Jackson, G.A. (2007) Small phytoplankton and carbon export from the surface ocean. *Science* **315:** 838–840.

Riemann, B., and Bell, R.T. (1990) Advances in estimating bacterial production and growth in aquatic systems. *Arch Hydrobiol* **118:** 385–402.

Rippka, R. (1972) Photoheterotrophy and chemoheterotrophy among unicellular blue-green algae. *Arch Mikrobiol* **87:** 93–98.

Ruiz-Gonzalez, C., Gali, M., Lefort, T., Cardelus, C., Simo, R., and Gasol, J.M. (2012a) Annual variability in light modulation of bacterial heterotrophic activity in surface northwestern Mediterranean waters. *Limnol Oceanogr* **57:** 1376–1388.

Ruiz-Gonzalez, C., Simo, R., Vila-Costa, M., Sommaruga, R., and Gasol, J.M. (2012b) Sunlight modulates the relative importance of heterotrophic bacteria and picophytoplankton in DMSP-sulphur uptake. *ISME J* **6:** 650–659.

Ruiz-Gonzalez, C., Simo, R., Sommaruga, R., and Gasol, J.M. (2013) Away from darkness: a review on the effects of solar radiation on heterotrophic bacterioplankton activity. *Front Microbiol* **4:** 131.

Smith, D.C., and Azam, F. (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. *Mar Microb Food Webs* **6:** 107–114.

Stanier, R.Y. (1973) Autotrophy and heterotrophy in unicellular blue-green algae. In *Biology of Blue-Green Algae*. Carr Bawe, N.G. (ed.). Oxford: Blackwell.

Talarmin, A., Van Wambeke, F., Catala, P., Courties, C., and Lebaron, P. (2011a) Flow cytometric assessment of specific leucine incorporation in the open Mediterranean. *Biogeosciences* **8:** 253–265.

Talarmin, A., Van Wambeke, F., Duhamel, S., Catala, P., Moutin, T., and Lebaron, P. (2011b) Improved methodology to measure taxon-specific phosphate uptake in live and unfiltered samples. *Limnol Oceanogr Methods* **9:** 443–453.

Vandermeulen, J.H., Davis, N.D., and Muscatine, L. (1972) The effect of inhibitors of photosynthesis on zooxanthellae in corals and other marine invertebrates. *Mar Biol* **16:** 185–191.

de Verneil, A., Rousselet, L., Doglioli, A.M., Petrenko, A.A., Maes, C., Bouruet-Aubertot, P., and Moutin, T. (2017) OUTPACE long duration stations: physical variability, context of biogeochemical sampling, and evaluation of sampling strategy. *Biogeosciences Discuss.*, https://doi.org/10.5194/bg-2017-455, in review.

Waterbury, J.B., Watson, S.W., Valois, F.W., and Franks, D.G. (1986) Biological and ecological characterization of the marine unicellular cyanobacterium *synechococcus*. *Can J Fish Aquat Sci* **214:** 71–120.

West, N.J., Lepère, C., Manes, C.L.D., Catala, P., Scanlan, D.J., and Lebaron, P. (2016) Distinct spatial patterns of SAR11, SAR86, and Actinobacteria diversity along a transect in the ultra-oligotrophic south Pacific Ocean. *Front Microbiol* **7:**234. doi:10.3389/fmicb.2016.00234.

Wright, R.R., and Hobbie, J.E. (1966) Use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology* **47:** 447–464.

Yelton, A.P., Acinas, S.G., Sunagawa, S., Bork, P., Pedros-Alio, C., and Chisholm, S.W. (2016) Global genetic capacity for mixotrophy in marine picocyanobacteria. *ISME J* **10:** 2946–2957.

You, L., He, L., and Tang, Y.J. (2015) Photoheterotrophic fluxome in *Synechocystis* sp. strain PCC 6803 and its implications for cyanobacterial bioenergetics. *J Bacteriol* **197:** 943–950.

Zubkov, M.V. (2009) Photoheterotrophy in marine prokaryotes. *J Plankton Res* **31:** 933–938.

Zubkov, M.V., Fuchs, B.M., Tarran, G.A., Burkill, P.H., and Amann, R. (2003) High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Applied and Environmental Microbiology* **69:** 1299–1304.

Zubkov, M.V., and Tarran, G.A. (2005) Amino acid uptake of *Prochlorococcus* spp. in surface waters across the South Atlantic Subtropical Front. *Aquat.Microb Ecol* **40:** 241–249.

Zubkov, M.V., Martin, A.P., Hartmann, M., Grob, C., and Scanlan, D.J. (2015) Dominant oceanic bacteria secure phosphate using a large extracellular buffer. *Nat Commun* **6:** 7878.