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Connecting thermal physiology and latitudinal niche partitioning in marine *Synechococcus*

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Running title: Temperature adaptation in marine *Synechococcus*

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25 **Abstract**

Marine *Synechococcus* cyanobacteria constitute a monophyletic group that displays a wide latitudinal distribution, ranging from the equator to the polar fronts. Whether these organisms are all physiologically adapted to stand a large temperature gradient or stenotherms with narrow growth temperature ranges has so far remained unexplored. We submitted a panel of six strains, isolated along a gradient of latitude in the North Atlantic Ocean, to long- and short-term variations of temperature. Upon a downward shift of temperature, the strains showed strikingly distinct resistance, seemingly related to their latitude of isolation. This behaviour was associated to differential photosynthetic performances, with tropical strains collapsing while northern strains were capable of growing. In the tropical strains, the rapid photosystem II inactivation and the decrease of the antioxidant β -carotene relative to chl *a* suggested a strong induction of oxidative stress. These different responses were related to the thermal *preferenda* of the strains. The northern strains could grow at 10°C whilst the other strains preferred higher temperatures. In addition, we pointed out a correspondence between strain isolation temperatures and phylogeny. In particular, clade I and IV laboratory strains were all collected in the coldest waters of the distribution area of marine *Synechococcus*. We however show that clade I *Synechococcus* exhibit different levels of adaptation, which apparently reflect their location on the latitudinal temperature gradient. This study reveals the existence of lineages of marine *Synechococcus* physiologically specialised in different thermal niches, therefore suggesting the existence of temperature ecotypes within the marine *Synechococcus* radiation.

Keywords: Adaptation / Ecotype / Marine cyanobacteria / *Synechococcus* / Temperature

Subject category: Microbial ecology and functional diversity of natural habitats

Introduction

50 Marine picocyanobacteria belonging to the *Prochlorococcus* and *Synechococcus* genera are major contributors to carbon biomass and global oceanic primary production and may contribute up to half of the fixed carbon in some oceanic regions (Li 1994, Liu *et al.* 1997, Buitenhuis *et al.* 2012). Although phylogenetically closely related, these two cyanobacteria exhibit distinct traits of ecology, physiology and evolution (Partensky *et al.* 55 1999, Partensky and Garczarek 2010). While *Prochlorococcus* thrives in warm waters of the latitudinal 45°N-40°S band, *Synechococcus* cells prefer coastal and mesotrophic open ocean waters, with a much wider latitudinal distribution ranging from the equator to the polar fronts (Not *et al.* 2005, Zwirgmaier *et al.* 2008, Huang *et al.* 2011). In addition, *Synechococcus* has no obvious depth preference, the highest cell densities being often 60 observed in the upper mixed layer, whereas *Prochlorococcus* shows strong depth partitioning, with two main ecotypes that are both physiologically and genetically distinct: a high light-adapted (HL) ecotype, occupying the upper part of the euphotic zone and a low light-adapted ecotype (LL), dominating the bottom of the euphotic layer (Moore *et al.* 1998). More recently, the HL ecotype has been further subdivided into HLI and HLII, which exhibit 65 distinct latitudinal distributions, HLII dominating between 28°S to 33°N and HLI above 32°S or 38°N, a difference seemingly linked to the different temperature growth optima of their cultured representatives (Johnson *et al.* 2006, Zinser *et al.* 2007).

Using the 16S rRNA and ITS genetic markers, respectively ten and fifteen different clades (Rocap *et al.* 2002, Fuller *et al.* 2003) have been delineated within the main group of 70 the marine *Synechococcus* radiation (cluster 5.1; *sensu* Herdman *et al.* 2001). However, a recent study by Mazard and colleagues (2012a) has highlighted a greater genetic microdiversity than previously thought in this subcluster. Indeed, using the cytochrome *b₆* gene *petB*, they could define more than thirty different subclades of marine *Synechococcus*.

Because of this large number of lineages, the environmental factors that have directed the
75 diversification of marine *Synechococcus* appear much more complicated to understand than
in the case of *Prochlorococcus*. Through a vast phylogeographic study, Zwirgmaier and co-
workers (2007, 2008) have however shown that, out of the dozen of marine *Synechococcus*
16S rRNA clades, only four (I to IV) predominate in the oceans. While clades I and IV
generally co-occur at latitudes above 30°N/S and at depth, clade II seems to prevail in warm,
80 coastal or shelf areas (Zwirgmaier *et al.* 2007, 2008, Huang *et al.* 2011, Mella-Flores *et al.*
2011, Ahlgren and Rocap 2012). The latitudinal distribution of clade III appears to be
broader, but with an apparent predominance in oligotrophic, offshore waters (Fuller *et al.*
2005, Zwirgmaier *et al.* 2008, Mella-Flores *et al.* 2011, Post *et al.* 2011). The other clades are
usually detected at low concentrations and their distribution patterns are less clearly defined
85 (Zwirgmaier *et al.* 2008, Huang *et al.* 2011).

To understand whether some *Synechococcus* lineages are adapted to specific
ecological niches, both phylogeography and comparative physiology studies are necessary.
These two approaches indeed allow pointing out possible correspondences between cell
performances, phylogeny and ecological niches. So far, most of the comparative studies of
90 marine *Synechococcus* physiology have dealt with adaptation and acclimation capacities to
light (Six *et al.* 2004, 2007b, 2007c) and nutrients variations (e.g. Liu *et al.* 2012, Mazard *et al.*
2012b). These works suggest that marine *Synechococcus* strains can grow over large
ranges of irradiance, explaining that cells of this organism are detected from surface down to
150 m (Kana and Glibert 1987, Olson *et al.* 1990, Moore *et al.* 1995, Six *et al.* 2004, 2007b).
95 Light quality also influences marine *Synechococcus* distribution (Olson *et al.* 1990, Lantoiné
and Neveux 1997, Wood *et al.* 1998, Sherry and Wood 2001). The pigmentation of the light-
harvesting complex, the phycobilisome (PBS), is indeed highly variable among strains (Six *et al.*
2007b, 2007c, 2009) and optimized to collect the most abundant wavelengths in a given

light niche. These different pigment types, however, most often are not restricted to specific
100 *Synechococcus* lineages (Palenik 2001, Six *et al.* 2007c), and therefore do not constitute
ecotypes *sensu stricto*. Similarly, although nutrient availability contribute to some of the
variability of the *Synechococcus* community structure (Zwirgmaier *et al.* 2008, Mazard *et al.*
2012a), a clear delineation of nutrient ecotypes (equivalent to *Prochlorococcus* light
ecotypes) within the *Synechococcus* radiation remains globally elusive (Palenik *et al.* 2006,
105 Scanlan *et al.* 2009, Stuart *et al.* 2009). A notable exception concerns the response to
phosphorus limitation (including cell size change and the ability to accumulate
polyphosphate) that appears somewhat consistent with *Synechococcus* phylogeny and their
inferred ecology of the different clades (Mazard *et al.* 2012a). However, a large part of the
variation observed in the *Synechococcus* community structure and the hierarchy of
110 environmental factors shaping ecotype genomes remains largely unexplained.

Adaptation to temperature variations among marine *Synechococcus* has been so far
poorly explored. This environmental factor can significantly constrain growth, as the activity
of most enzymes and biomembranes directly depends on it, thus impacting major metabolic
processes. Among them, photosynthesis is known to be particularly affected by temperature
115 variations, notably because of the resulting changes in thylakoidal fluidity that eventually
lead to photosystem (PS) II inactivation (Murata and Los 1997, Takahashi and Murata 2008).
Like for *Prochlorococcus* (Johnson *et al.* 2006, Zinser *et al.* 2007), temperature might have
thus played an important role in the differentiation of the *Synechococcus* lineages, possibly
influencing significantly their genome shaping. However, given the scarcity of physiological
120 studies of the response of open ocean *Synechococcus* isolates to temperature (Moore *et al.*
1995, Fu *et al.* 2007), it is difficult to assert whether the large latitudinal distribution of these
picocyanobacteria is rather the result of broad acclimation capacities to temperature, or of

adaptation processes underlying the existence of different 'thermotypes' that would display distinct temperature optima for growth.

125 To explore these questions, we describe in this paper the short and long-term responses to temperature of a panel of six *Synechococcus* strains, isolated at various latitudes, in mesotrophic waters of the North Atlantic Ocean. We aimed at pointing out different resistance and acclimation capacities among the strains, with a focus on their capability to tune light utilisation in response to temperature variations. We furthermore
130 highlight ecophylogenetic features of marine *Synechococcus* and discuss the importance of temperature in the diversification of this radiation.

Material and Methods

Growth conditions and experimental design

135 In order to minimize the differences regarding other physico-chemical parameters than temperature among isolation sites, we selected six marine phycoerythrobilin-rich (pigment type 3a, Six *et al.* 2007c; Humily *et al.* in press) *Synechococcus* strains that were isolated in the North Atlantic Ocean, at similar distance from both the West or East coast, but at very different latitudes, from the polar circles to intertropical zone (Fig. 1). Clonal
140 *Synechococcus* spp. strains A15-37, M16.1, WH7803, ROS8604, MVIR-16-2 and MVIR-18-1 were retrieved from the Roscoff culture collection (Table 1; <http://www.sb-roscoff.fr/Phyto/RCC/>; Vaultot *et al.* 2004) and grown in polystyrene flasks (Nalgene, Rochester, NY, USA; or Sarsted, Nümbrecht, Germany) in PCR-S11 culture medium (Rippka *et al.* 2000) supplemented with 1 mM sodium nitrate. The seawater was reconstituted using
145 Red Sea Salt (Houston, Texas, USA) and distilled water. Continuous white light was provided by fluorescent tubes (Sylvania Daylight F18W/54-765 T8) at 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiance. Cultures of the six strains were long-term acclimated to a range of temperatures,
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from 10°C to 35°C, within temperature-controlled chambers (Liebherr-Hausgeräte, Lienz, Austria) in order to measure growth rates as a function of temperature.

150 For cold stress experiments, early exponentially growing cultures (1.5 L) of the six *Synechococcus* strains grown at $22 \pm 1^\circ\text{C}$ were transferred to $13 \pm 1^\circ\text{C}$ under $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of continuous white light. This temperature amplitude was chosen to be large enough to observe physiological readjustments in the cold resistant strains, while not causing too abrupt culture collapsing of the sensitive ones. After the transfer, about five
155 hours were necessary for the cultures to reach 13°C . The cultures were daily sampled for cell counting, fluorescence measurements and pigment analyses as described below. These experiments were repeated at least four times and compared to control cultures, *i.e.* grown routinely at 22°C . Following the cold period of six days, cultures were shifted back to 22°C , in order to estimate their recovery capacities.

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Flow cytometry

For cell density measurements, aliquots of cultures were preserved within 0.25% glutaraldehyde grade II (Sigma) and stored at -80°C until analysis (Marie *et al.* 1999). Cell concentrations were determined using a flow cytometer (FACSCanto II, Becton Dickinson,
165 San Jose, CA, USA) for which laser emission was set at 488 nm and using distilled water as sheath fluid. For steady state acclimated cultures, growth rates (μ , in day^{-1}) were computed as the slope of a $\text{Ln}(Nt)$ vs. time plot, where Nt is the cell concentration at time t . Orange and red fluorescence levels (emission at $585 \pm 21 \text{ nm}$) were normalised with standard $0.95 \mu\text{m}$ YG beads (Polysciences Warrington, PA, USA) after analysing list mode files with the custom-
170 designed freeware CYTOWIN (Vaulot 1989) or the FCS Express 4 Flow Research Edition software (De Novo Software, Los Angeles, CA, USA).

In vivo fluorescence measurements

PSII quantum yield (F_V/F_M) and non photochemical quenching associated to state transition (NPQ_{ST}) were measured upon excitation at 520 nm using a Pulse Amplitude Modulation fluorometer (Phyto-PAM, Walz, Effeltrich, Germany) equipped with a temperature controlled cuvette holder and connected to a chart recorder (Vernier, LabPro, Beaverton, OR, USA). After 5 min acclimation to dark, the maximal fluorescence levels were measured in the dark (F_{M0}) and under bright red light (655 nm, 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; F_M) in the presence of 100 μM of the PSII blocker 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), by triggering saturating light pulses (655 nm; 5000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The PSII quantum yield was calculated as:

$$F_V/F_M = (F_M - F_0)/F_M$$

where F_0 is the basal fluorescence level and F_V is the variable fluorescence (Campbell *et al.* 1998, Six *et al.* 2007a, 2009). The NPQ_{ST} factor was calculated as:

$$\text{NPQ}_{\text{ST}} = (F_M - F_{M0})/F_M$$

In addition, fluorescence emission spectra were recorded at experimental temperature (22°C or 13°C) upon excitation at 530 nm, with a LS-50B spectrofluorometer (Perkin-Elmer, Waltham, MA, USA), as described by Six *et al.* (2004, 2007b). In order to study the phycobiliprotein coupling in the PBS during the cold period, the phycoerythrin (PE) to phycocyanin (PC) fluorescence emission ratio was calculated by dividing values measured at the PE maximum (565-575 nm) to the PC maximum (645-655 nm).

D1 protein and β -chain phycoerythrin immunoblotting

Cell pellets were resuspended in extraction buffer (140 mM Tris base, 105 mM Tris-HCl, 0.5 mM ethylenediaminetetraacetic acid, 2% lithium dodecyl sulphate, 10% glycerol and 0.1 mg mL⁻¹ PefaBloc protease inhibitor; Roche, Basel, Switzerland) and lysed applying

freezing/thawing cycles and sonication. After centrifugation, total protein concentration was determined using a Lowry protein assay kit and bovine serum albumin as protein standard (Bio-Rad, Hercules, CA). Samples were then denatured by heating for 2 min at 80 °C in the presence of 50 mM dithiotreitol and an amount of 4 (PsbA protein) or 8 (β -chain PE) μ g total protein was loaded on a 4–12% acrylamide gradient precast NuPAGE Bis-Tris mini-gel (Invitrogen, Carlsbad, CA, USA). Gels were electrophoresed and the proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane, then immediately immersed into Tris Buffer Saline-Tween (TBS-T) buffer, pH 7.6 (0.1% Tween 20, 350 mM sodium chloride, 20 mM Trizma base) containing 2% (w:v) blocking agent (Amersham Biosciences, Piscataway, NJ) for 45 min. Aliquots of rabbit primary antibodies against β -subunit PE proteins (directed against the β -subunit PE of *Prochlorococcus* sp. SS120; courtesy of Wolfgang R. Hess, University of Freiburg, Germany; Six *et al.* 2007b, Hess *et al.* 1999) or PsbA (D1 protein; Agrisera) were diluted at 1:50,000 in TBS-T in the presence of 0.5% blocking agent and membranes were soaked into this solution for 1 h with agitation. After extensive washing of the membrane with TBS-T buffer, anti-rabbit secondary antibodies (Biorad) were applied with the same procedure as for primary antibodies. Membranes were developed by chemoluminescence using the ECL Advance reagent kit (Amersham Biosciences) and visualized with a LAS4000 imager equipped with a CCD camera (GE Healthcare).

Pigment analyses

After extraction in 100% methanol, pigment extracts were supplemented with 10% water in order to avoid peak distortion. Hydrophobic pigments were then measured by high pressure liquid chromatography using an HPLC 1100 Series System (Hewlett-Packard, St Palo Alto, CA, USA), equipped with a Kromasil C₈ column (150 x 4.6 mm, 3.5 μ m particles size) according to a procedure published elsewhere (Six *et al.* 2005).

Phylogenetic analyses

225 *petB* sequences of *Synechococcus* strains obtained from the Roscoff Culture
Collection (RCC, <http://www.sb-roscoff.fr/Phyto/RCC/>) were amplified as previously
described (Mazard *et al.* 2012a). Briefly, 550 bp *petB* fragments from MVIR-16-1 (RCC2570),
MVIR-11-1 (RCC1695), MVIR-7-1 (RCC1648), MVIR-18-1 (RCC2385), MVIR-16-2 (RCC3010),
MVIR-10-1 (RCC1688), MVIR-1-1 (RCC1708) and A15-44 (RCC2527) were amplified directly
230 from cultures at an annealing temperature of 55°C. Sequences were deposited in the
GenBank nucleotide sequence database under the accession numbers mentioned in Table
S2. A multiple alignment of 74 sequences from cultured isolates was generated with MAFFT
v6.818 using the G-INS-I option (Kato and Toh 2008) and trimAL 1.4 to remove poorly
aligned regions and gaps (Capella-Gutiérrez *et al.* 2009). Phylogenetic reconstructions were
235 performed on 559 aligned amino acids using three different methods: maximum likelihood
(ML), Bayesian inference and neighbor-joining (NJ). NJ analyses were performed using Phylip
3.69 (Felsenstein 1989) as previously described (Mella-Flores *et al.* 2011). The phylogenetic
inference by ML was performed with the Message Passing Interface (MPI) version of PhyML
v3.0 (Guindon and Gascuel 2003) using the TrN+I+G, a submodel of the general time-
240 reversible (GTR), with invariant sites and gamma distribution. This model was selected using
JModeltest 2.1.3 according the Akaike Information Criterion (Darriba *et al.* 2012). Bayesian
inference was conducted using MrBayes 3.1.2 after partitioning according to the position in
the codon (Huelsenbeck and Ronquist 2001). Four Markov Chain Monte Carlo (MCMC)
simulations were run for five millions generations that were sampled every 100 generations,
245 the first 12500 trees being discarded. The topology of the tree was obtained after ML
analyses and the robustness of inferred topologies was supported by 1000 non parametric
bootstrap samplings for ML and NJ.

Field temperature data collection

250 Average sea surface temperature at the strain isolation sites (resolution of 5° squares) and at the month and year of collection were determined using the satellite data available from the National Oceanic and Atmospheric Administration (NOAA, http://iridl.ldeo.columbia.edu/SOURCES/.NOAA/.NCEP/.EMC/.CMB/.GLOBAL/.Reyn_SmithOIV2/.monthly/.sst/). Regarding strains isolated before 1981 (Table S2) for which satellite data
255 are not available, we used the seasonal average temperature at the isolation site (grid of 5° squares) over 10 years (2002-2012). For the strain for which isolation date is not available, *Synechococcus* sp. SYN20, we used the annual average surface seawater temperature over the 10 years. Seawater thermal amplitude at the isolation sites were estimated through the difference between summer and winter average temperature over the 10 years.

260

Results

Photophysiological differences among the Synechococcus strains

When grown in identical conditions at 22°C, the six strains exhibited PSII quantum yields close to 0.6, indicating efficient photosynthetic activity (Table 2). The parameters
265 related to the light-harvesting system showed nevertheless significant differences. The emission spectra indeed displayed a number of strain specificities, including the wavelengths of the maxima, the relative width of the emission bands and the PE to PC ratio (hereafter PE:PC) that ranged from 2.0 to 3.5 (Table 2 and Fig. S1). Furthermore, the amplitude of the state transition process, as estimated by the NPQ_{ST} parameter (see methods), varied
270 significantly among strains from 0.2 to 0.5. *Synechococcus* sp. M16.1 showed weak state transitions with a fairly high fluorescence PE:PC, while *Synechococcus* spp. WH7803 and MVIR-18-1 displayed strong state transitions and a low PE:PC. The most striking differences

among the six strains lied in their pigment cell contents. The tropical *Synechococcus* spp. A15-37 and M16.1 displayed the lowest membrane pigment (Chl α , β -carotene and zeaxanthin) contents while it was significantly higher in the mid-latitude strains WH7803 and ROS8604 (Table 2). Similar trends were observed for flow cytometric fluorescence signals with *Synechococcus* sp. A15-37 emitting weak orange fluorescence in contrast to WH7803 and ROS8604 cells, which were brightly fluorescent.

280 ***Variations of cell density and fluorescence upon cold stress***

In order to highlight possible differences in temperature stress response, cultures fully acclimated to 22°C were shifted to 13°C. The time-course variations of cell abundances differed significantly between the six strains (Fig. 2A). For the tropical strain *Synechococcus* sp. M16.1, the cell density slightly increased after one day, then dramatically dropped down to 2% of the initial cell density after six days of cold stress. In the cultures of strains A15-37 and WH7803, the cell density decreased by half after six days. For *Synechococcus* sp. ROS8604, cell density increased during the first three days, reaching 140% of the initial cell density and then started to decrease. For these four strains, cell density kept decreasing if the cold stress was prolonged over six days (data not shown). By contrast, the two high latitude strains, *Synechococcus* spp. MVIR-16-2 and MVIR-18-1, exhibited significant growth ($0.40 \pm 0.04 \text{ d}^{-1}$ and $0.16 \pm 0.03 \text{ d}^{-1}$, respectively), which was comparable to acclimated cultures at 13°C. The former strain reached stationary phase after four to five days at 13°C. As classically observed in stationary phase cultures, drastic physiological changes occurred in the culture, including a drop of the F_M fluorescence level and an increase of the PE fluorescence yield (see below). As this downregulation of photosynthesis is under no circumstances related to a temperature effect and could lead to misinterpretation, data

subsequent to the fourth day are not shown in the following results for *Synechococcus* sp. MVIR-16-2.

Red fluorescence per cell, a proxy for chl *a* cell content, was measured by flow cytometry (Fig. S2A). This parameter decreased down to about 30% of the initial value in the strains isolated at low latitude. In *Synechococcus* sp. ROS8604, the red fluorescence per cell kept fairly stable until the third day and then dropped sharply. In the northern strains, this parameter slightly decreased and stabilised to a level corresponding at about 80% of the initial values. After the temperature shift, all strains reduced their orange fluorescence by about 50% (Fig S2B).

Phycobiliprotein coupling upon cold stress

Fluorescence emission spectra were recorded for each strain at each time point, and used to calculate the PE to PC fluorescence emission ratio (Fig. 2B). This parameter rapidly increased for the low latitude strains *Synechococcus* spp. A15-37 and M16.1, most likely due to an increase of leaking energy between PE and PC, therefore indicating a lower coupling of the PBS rod phycobiliproteins (Six *et al.* 2007b). This process was particularly notable for the tropical *Synechococcus* sp. M16.1, which exhibited a 5.5 fold increase after four days of cold stress. The PE:PC decrease during the last days of the cold period indicates PE degradation, which was also suggested by the bleaching of the cultures at this stage of the experiment. The PE:PC increase was moderate and immediate for strain WH7803, and delayed for ROS8604, starting at the fourth day. The high latitude strains did not show any variation of the PE to PC ratio. Very similar trends were observed among the six strains for the PE to PBS terminal acceptor ratio (data not shown; Fig. S1).

320

State transition amplitude upon cold stress

We used the non photochemical quenching of fluorescence associated to state transitions, NPQ_{ST} , as an index of the amplitude of this process, which balances the distribution of light energy between the two PS. This mechanism was dramatically affected
325 by low temperature in all *Synechococcus* strains considered, except for the high latitude strains MVIR-16-2 and MVIR-18-1, which showed a lesser decrease of this parameter (45% and 20% of initial condition, respectively; Fig. 3A). State transitions were totally inhibited after one to two days of cold stress in the low latitude strains. In *Synechococcus* spp. WH7803 and ROS8604, NPQ_{ST} decreased progressively to reach a minimal level after four
330 days of cold stress (Fig. 3A).

The recovery capacities of temperate and tropical strains were estimated by placing the cultures back to the initial temperature (22°C, Table S1). The tropical strain A15-37 could never recover from the cold stress and its western counterpart, M16.1, needed more than thirteen days to recover its photosynthetic parameters, and thereafter growth.
335 *Synechococcus* spp. WH7803 and ROS8604 recovered more than twice faster than M16.1.

Photosystem II quantum yield upon cold stress

Upon a 9°C downward temperature shift, we observed a gradient of photosynthetic responses among the six *Synechococcus* strains (Fig. 3B). The PSII quantum yield (F_v/F_m) of
340 the strains isolated at low and medium latitude was dramatically affected by low temperature. *Synechococcus* spp. A15-37 and M16.1 reached minimal yield values after two to three days of cold stress, with very low fluorescence signal after a couple of days. The fluorescence signal of the intermediate latitude strains reached minimum values more slowly, after four to five days. By contrast, strains isolated at high latitude, MVIR-16-2 and
345 MVIR-18-1, suffered only a moderate decrease of the PSII quantum yield, reaching a steady state of *ca* 0.4 after two days. Similarly reduced F_v/F_m (and NPQ_{ST}) values were observed for

both strains once acclimated for several weeks to 13°C (not shown), suggesting that these cultures had already reached an acclimated level for these parameters after four to five days at 13°C.

350 ***Time-course relative variations of β -subunit phycoerythrins and D1 protein***

Immunoreactions on whole cell proteins using antibodies directed against PE β -subunits and D1 allowed analyzing the variations of the relative quantity of these proteins during the cold stress experiment (Fig. 4). Both antibodies gave clean immunoblots with no parasite bands. The antibody raised against PE β -subunits cross-reacted with both CpeB (PEI) and MpeB (PEII), as previously tested on purified PE (Six *et al.* 2007). In all marine *Synechococcus* genomes, CpeB (~19.1 kDa) is systematically heavier than MpeB (~18.5 kDa). Therefore, the immunoblot profiles often showed two bands whose intensity reflected the quantity of CpeB or MpeB proteins relative to total proteins (Fig. 4A). The intensity of the bands cannot be compared across strains or between subunits because the affinity of the antibody differs depending on aminoacid sequences.

In the tropical strains *Synechococcus* A15-37 and M16.1, cold stress primarily induced MpeB degradation, as indicated by the disappearance of the lower band. CpeB variations were more variable but there was no such strong degradation. In *Synechococcus* spp. WH7803 and ROS8604, the two PE bands were less well separated but a general decreasing trend was clearly observed. In the northern strains, no PE degradation was observed.

We also investigated the relative quantity variations of the D1 protein (~32 kDa), encoded by the *psba* gene family, during the cold period. The cellular pool of this reaction centre II protein decreased rapidly in tropical strains, the signal being hardly detectable after day 3. The D1 band was progressively reduced in mid-latitudes strains *Synechococcus* WH7803 and ROS8604 until quasi disappearance at day 5. In the high latitude strains, MVIR-

16-2 and MVIR-18-1, the D1 relative quantity remains fairly constant along the stress experiment.

Time-course variations of pigment cell content upon cold stress

375 HPLC pigment analyses revealed the occurrence of three major pigments identified as chl *a*, β -carotene and zeaxanthin, as classically observed in marine *Synechococcus* (see e.g. Kana *et al.* 1988, Six *et al.* 2004), along with minor xanthophylls such as β -cryptoxanthin. The low latitude strains showed a pronounced decrease of the β -carotene to chl *a* ratio, which reached zero at about the fifth day of stress (Fig.5). The mid-latitude strains exhibited
380 different pigment responses to thermal stress. While *Synechococcus* sp. WH7803 underwent a decrease of about 50% of its initial β -carotene to chl *a* ratio, low temperature induced a notable increase of this ratio in *Synechococcus* sp. ROS8604 by a factor two at the fourth day. The large increase of this ratio while the chl *a* fluorescence per cell (reflecting chl *a* cell content) remains unchanged indicates a synthesis of β -carotene. In contrast, the two
385 northern strains did not show any clear trends in the variations of the β -carotene to chl *a* ratio, which remained fairly constant.

We also measured the variations of the zeaxanthin to chl *a* ratio (not shown). While this ratio moderately increased in northern strains due to a decrease in chl *a* cell content (see whole cell chl *a* fluorescence; Fig. S2A), no obvious variation was observed in the three
390 lower latitude strains, indicating a similar degradation rate of the two pigments.

Growth rates vs. temperature

In order to define their temperature optima for growth and compare their thermal growth range in our culture conditions, strains were acclimated to a wide range of
395 temperatures (Fig. 6). The strains showed quite different acclimation capabilities to

temperature. We were able to acclimate *Synechococcus* spp. A15-37 and M16.1, both isolated at latitude lower than 30°N, at temperatures ranging from 18°C to 35°C, with a maximum growth rate about 32°C. These strains exhibited different maximal growth rates, with *Synechococcus* sp. A15-37 growing more slowly than M16.1 ($1.70 \pm 0.03 \text{ d}^{-1}$ and $2.02 \pm 0.08 \text{ d}^{-1}$ at 32°C, respectively). Despite a similar pattern to M16.1 with a high maximal growth rate value of $2.50 \pm 0.08 \text{ d}^{-1}$ at 34°C, *Synechococcus* sp. WH7803 could however grow at lower temperatures than the two tropical strains. For the three above-mentioned strains, growth rate sharply decreased at 35°C. We managed to acclimate the strain ROS8604 within a thermal range from 18°C to 30°C, with an optimal temperature at 26°C, corresponding to a growth rate value of $0.83 \pm 0.02 \text{ day}^{-1}$. At last, strains MVIR-16-2 and MVIR-18-1, isolated near the polar circle, showed thermal niches shifted toward lower temperatures, MVIR-18-1 being able to cope with temperature down to 10°C (and probably less) and 12°C for MVIR-16-2, but none of these two strains were able to acclimate at temperature higher than 25°C. Furthermore, similar optimal temperatures were observed for the two northern strains, with *Synechococcus* sp. MVIR-16-2 and MVIR-18-1 growing optimally at about 22°C ($1.00 \pm 0.04 \text{ day}^{-1}$ and $0.82 \pm 0.07 \text{ day}^{-1}$, respectively).

Phylogeography of marine Synechococcus isolates

In order to unveil potential relationships between *Synechococcus* phylogeny and the temperature of strain isolation site, a phylogenetic analysis was conducted on the *petB* sequence of 74 cultured strains isolated from a wide range of latitudes, across different oceans and seas (Fig. 7, Table S2). This marker was chosen because it provides a better phylogenetic resolution of *Synechococcus* strains (at the subclade level) than more typical markers, such as 16S rRNA or ITS (Mazard *et al.* 2012a). Our data set includes a number of strains from the Roscoff Culture Collection, whose phylogenetic position was so far

unknown, in particular strains isolated during the MICROVIR cruise (July 2007, North Sea ;
Table S2). The data set mainly included representatives of subcluster 5.1 and the
phylogenetic tree was rooted with one representative of each of the two other subclusters
(5.2 and 5.3; *sensu* Dufresne et al., 2008). As in a previous study using the *petB* gene (Mazard
425 *et al.* (2012a), members of subcluster 5.1 were divided into eleven clades and a number of
these clades were further subdivided into 14 sub-clades (Fig. 7). It is worth noting that we
found a slight difference with regard to Mazard and coworkers' study concerning sub-clade
Ib, which does not constitute a monophyletic group in our analysis. This disparity is probably
imputable to the differences in the phylogenetic methods used between the two studies.
430 This tree allowed us to assign the tropical *Synechococcus* spp. A15-37 and M16.1 to subclade
IIa, *Synechococcus* spp. ROS8604, MVIR-16-2 and MVIR-18-1 to sub-clade Ia, while
Synechococcus sp. WH7803 is one of the rare representatives of clade V available in culture.

In parallel to the phylogenetic analyses, the average seawater temperatures at the
isolation sites were retrieved for the 74 strains. Comparison between the phylogeny and the
435 average isolation temperatures underlined that clades I and IV strains were isolated in
significantly colder waters ($15.03 \pm 2.53^{\circ}\text{C}$ and $15.05 \pm 2.13^{\circ}\text{C}$ respectively) than the isolates
from clades II and III ($23.93 \pm 3.03^{\circ}\text{C}$ and $24.86 \pm 2.84^{\circ}\text{C}$ respectively), as shown by one-way
ANOVA ($F = 36.26$, $p\text{-value} < 0.01$).

440 Discussion

In this study, we compare the physiology of six marine *Synechococcus* strains isolated
at different latitudes (from 23°N to 61°N; Table 1; Fig.1) in near coastal areas (*i.e.*
mesotrophic waters) of the North Atlantic Ocean. All of them belong to pigment type 3a (Six
et al. 2007c, Humily *et al.* in press), but in spite of this similar PBS composition, the six strains

445 exhibited large differences in pigment cell content (chl α , carotenoids and PE; Table 2), with
the mid-latitude strains being much more pigmented than their northern and tropical
counterparts. These sharp differences are probably associated with different thylakoidal
surfaces per cell and/or different cell biovolumes among strains. Moreover, whereas
fluorescence excitation spectra were quite comparable, fluorescence emission spectra
450 showed differences in wavelength maxima, maxima ratios and apparent width of the PE
emission band (Fig. S1), revealing the occurrence of distinct levels of PBP coupling. These six
strains, which display very similar fluorescence PUB to PEB ratio (ca 0.4), could thus be
differentiated by other photosynthetic features (Table 2). These observations highlight the
functional microdiversity that exists within the marine *Synechococcus* pigment types defined
455 in previous studies (Six *et al.* 2007c, Humily *et al.* in press).

Marine Synechococcus show differential resistance to cold stress

Cold stress experiments revealed large differences in thermal flexibility among the six
marine *Synechococcus* strains used in this study. Whereas tropical strains were not able to
460 stand the temperature drop challenge, the temperate strains maintained a nearly constant
cell density (Fig. 2A). By contrast, the northern strains kept dividing, at similar growth rates
than cells fully acclimated to 13°C (Fig. 2A). In these photosynthetic organisms, being able to
sustain efficient light absorption capacities and utilization is crucial for growth. In order to
better understand the differences among the strains, we first followed the response of their
465 photosynthetic antenna, the PBS, during the cold period (Fig. 2B, 4A). In contrast to the
northern strains, for which PBS fluorescence spectra and PE cell content remained fairly
constant, the strong increase in PE:PC fluorescence ratio observed in *Synechococcus* spp.
A15-37, M16.1, WH7803 and ROS8604 indicates an impairment of the phycobiliprotein
coupling, which likely led to the physical disconnection of PE molecules from the rest of the

470 PBS structure (Six *et al.* 2007b). PBS impairment was especially severe for *Synechococcus* sp.
M16.1, for which fast specific degradation of the distal PBS hexamers (PEII) was evidenced
by MpeB immunoblotting (Fig. 4A). Our results are comparable to previous studies led in
other cyanobacteria that pointed out serious PBS perturbations in response to UV (Lao and
Glazer 1996, Rajagopal and Murthy 1996, Rinalducci *et al.* 2006, Six *et al.* 2007b), high light
475 (Tamary *et al.* 2012) and temperature stress (Li *et al.* 2001, Stoitchkova *et al.* 2007), and thus
suggests that PBS uncoupling and dismantling is a usual consequence of environmental
stress in cyanobacteria. It is however worth noting that the highly fluorescent free PE may
also constitute a way of energy dissipation through fluorescence during a stress period. In
any case, the cold-induced destruction of the antenna considerably decreased cell
480 absorption capacities and must have had a strong impact on the growth capacities of the
temperate and tropical strains studied here.

Cyanobacteria balance excitation energy notably through PBS state transitions, which
regulate the energy transfer from PBS to PSI and PSII in response to environmental factors or
metabolism demands (Joshua and Mullineaux 2004, Mullineaux and Emlyn-Jones 2004). The
485 drastic decrease observed in state transitions amplitude during cold shock for the tropical
and mid-latitude strains generally arose before the PBS uncoupling in these strains,
indicating that the decline in state transition during the first few days was not directly
related to PBS dismantling (Fig. 2B, 3A). These early perturbations of the state transition
process might originate in the cold-induced changes in the fluidity of the thylakoidal
490 membranes, as low temperature is well known to induce membrane stiffening (Wada and
Murata 1990, Murata and Los 1997, Mikami and Murata 2003). Later during the cold period,
PBS dismantling likely contributes to the complete inhibition of state transitions. The high
latitude *Synechococcus* strains, spp. MVIR-16-2 and MVIR-18-1 showed much better

capacities to regulate state transitions, possibly through active regulation of thylakoid
495 fluidity (Murata and Wada 1995, Murata and Los 1997).

The PSII quantum yield (F_V/F_M) is a good proxy for the efficiency with which the
reaction center II converts the energy funneled by the PBS into electron flux. The most likely
explanation for the major non-reversible decrease of the PSII quantum yield during cold
stress in tropical and temperate strains is the photoinactivation of the reaction center II (for
500 review see e.g. Campbell and Tyystjärvi 2012). This phenomenon is unavoidably induced by
light in any oxygenic photosynthetic organism but is further accelerated by environmental
stresses that disturb the balance between light absorption and utilization, such as
temperature variations (Takahashi and Murata 2008). Cold stress is indeed thought to
decrease the repair rate of the D1 protein, the key protein of the PSII reaction center, thus
505 inducing the concomitant drop in D1 cell content and PSII quantum yield reported in the
sensitive strains (Fig. 3B and 4B). The observation that high-latitude strains did not suffer
from PSII inactivation during the cold period raise questions about the physiological
processes that allow such tolerance, and calls for further research.

510 ***Cold stress, protection and acclimation***

To counteract the photoinhibition induced by reduced metabolic rates at low
temperature (Burns *et al.* 2005), reversible physiological adjustments are induced by
photosynthetic organisms, referred as photoacclimation. Acclimation of the photosynthetic
apparatus to low temperature usually shows strong similarities to high light acclimation
515 processes (Oquist 1983, Maxwell *et al.* 1994, Huner *et al.* 1998), as it is also based on an
imbalance between light energy absorption and its downstream utilization. We indeed
observed in the northern strains hints of high light-like acclimation (see e.g. Six *et al.* 2004,
2005), such as a reduction in pigment fluorescence per cell (Fig. S2) and chl *a* (data not

shown), likely associated with the reduction of the thylakoïdal surface (Kana and Glibert
520 1987).

The PSII quantum yield of the northern strains decreased until a level close to that
measured in cultures fully acclimated to 13°C (not shown), while the D1 cell content kept
stable (Fig 4B). This suggests the induction of non photochemical quenching associated with
light energy dissipation, characterised by a reduction of the amount of energy processed by
525 the reaction center (for review see Campbell *et al.* 1998; Kirilowski and Kerfeld 2012, 2013).
The results thus suggest that, in contrast to the tropical and mid-latitude strains, the
northern ones managed to adjust their light utilization capacities to the cold-induced slowing
down of their metabolism, an ability which likely limits the formation of reactive oxygen
species (Asada 1994, Latifi *et al.* 2009). In cyanobacteria, the best known non photochemical
530 quenching mechanism associated with light energy dissipation involves the so-called orange
carotenoid protein, encoded by the *ocp* gene, which seemingly acts as a photoreceptor and
intercepts light energy at the PBS core, before it reaches reaction center II (Wilson *et al.*
2006, Kirilovsky and Kerfeld 2012). The effects of cold stress on the activity of this protein,
whose gene is present in most marine *Synechococcus* strains (Scanlan *et al.* 2009), remain to
535 be studied.

A number of β -carotene molecules bound to the reaction center II act as quenchers
of singlet oxygen and under light, are regenerated through a repair cycle (Telfer 2005,
Ishikita *et al.* 2007, Mella-Flores *et al.* 2012). This carotenoid is essential for the protection of
reaction centers (Cazzaniga *et al.* 2012) and thus may be used as an indicator of oxidative
540 stress intensity. The β -carotene measurements revealed that tropical strains suffered from
strong oxidative stress during the cold period (Fig. 5). The sharp degradation of β -carotene,
probably due to an impairment of the turnover of this antioxidant, observed for
Synechococcus spp. A15-37, M16.1 and to a lesser extent WH7803, implies that the PSII of

these strains were subjected to direct oxidative damages (Blot *et al.* 2011). Furthermore, oxidative stress is also thought to prevent the repair of the PSII key protein, D1 (Nishiyama *et al.* 2006), thus greatly enhancing PSII inactivation. Interestingly, *Synechococcus* sp. ROS8604 responded differently, as it transiently increased its β -carotene cell content until the fourth day of stress, probably by boosting the β -carotene cycle. This rapid synthesis likely allowed a better protection against oxidative stress and may partially explain the moderate growth of this strain at the beginning of the cold period (Fig. 2A). However, cultures of *Synechococcus* sp. ROS8604 eventually did not manage to cope with oxidative stress, as seen by the rising of PBS uncoupling (Fig. 2B) and the cell density decrease (Fig. 2A), starting from the fourth day of stress. Variability in carotenoid response to temperature has also been observed among cyanobacterial strains isolated from high latitude freshwaters (Tang *et al.* 1997).

Differences in cold resistance capacities were also noticeable when considering the recovery capacities (Table S1). *Synechococcus* sp. M16.1 needed much longer time to recover and the cold period turned out to be lethal for *Synechococcus* sp. A15-37. The WH7803 and ROS8604 strains were not able to maintain growth and photosynthetic activity during the cold period, but they both recovered quite quickly when replaced to initial temperature, indicating significant thermal flexibility. These strains thus appear quite well adapted to temperate environments, in which temperature fluctuate depending on the seasons.

Marine Synechococcus strains have different thermal preferenda

The different physiological responses of the six marine *Synechococcus* strains to cold stress are associated with distinct long-term acclimation capacities to different temperatures. Our study thus demonstrates for the first time that marine *Synechococcus* strains exhibit different thermal *preferenda* and, therefore, strongly suggests the existence

of strains adapted to different local temperature ranges. These preferences appear to be
570 related to the latitude of isolation of each strain, which is directly linked to the latitudinal
gradient of seawater temperature. The temperature vs. growth curve of the WH8103 strain,
isolated in the Sargasso Sea at 28.5°N, exhibits a maximum at 28°C (Moore *et al.* 1995) and
thus supports this conclusion. Our observations support recent surveys which stated that
575 optimum temperature for growth of marine phytoplankton strains is related to the latitude
and temperature of their isolation site (Thomas *et al.* 2012, Boyd *et al.* 2013), and support
field observations that the abundances of the *Synechococcus* of the 5.1 cluster fall to very
low values in the polar oceans, of about hundred cells per mL (Gradinger and Lenz 1995,
Vincent 2000, Not *et al.* 2004, Cottrell and Kirchman 2009, Vincent and Kesada 2012).

The two northern *Synechococcus* strains we studied cannot be considered as true
580 psychrophilic organisms but rather as psychrotolerant ones (Tang *et al.* 1997). Indeed,
although strains such as *Synechococcus* sp. MVIR-18-1 (one of the culture isolates which, to
our knowledge, shows the northernmost isolation latitude) can probably survive at
temperature below 10°C, true psychrophilic phytoplankton exhibit optimal temperature
lower than 15°C and can generally not grow over 20°C (Vincent 2000, Lovejoy *et al.* 2007).
585 Although we cannot exclude the existence of true psychrophilic strains within the
Synechococcus 5.1 group, as it might be the case for natural *Synechococcus* subcluster 5.2
populations (Cottrell and Kirchman 2009, Huang *et al.* 2011), it appears that northern 5.1
strains might have only moderately decreased their optimal temperature during the
evolution and acquired the capacity to stand low temperatures.

590 Our results suggest that the capacity to grow at low temperature may only be
possible to the detriment of the ability to develop high growth rates, as observed for the low
latitude strains A15-37, M16.1 and WH7803 (Fig. 6). This apparent trade-off between growth
performances and the capacity to grow over a given temperature range, which has also been

observed for *Prochlorococcus* picocyanobacteria (Johnson *et al.* 2006), might constitute a
595 general adaptative trait of marine picocyanobacteria to different thermal niches. In addition,
it is worth noting that *Synechococcus* strains display shapes of the growth vs. temperature
curves similar to *Prochlorococcus* ecotypes (Johnson *et al.* 2006, Moore *et al.* 1995). The
typical shoulder in the first part of the curve (Fig. 6) may reflect the triggering of a
thermophysiological mechanism that allows efficient acclimation to higher temperature, a
600 mechanism that was possibly lost by the northern strains.

Marine Synechococcus display temperature specialized clades

The combination of phylogenetic analyses of a large number of *Synechococcus*
culture isolates (Fig. 7; Table S2) with their isolation temperatures provides an interesting
605 complement to the field studies of Zwirgmaier and coworkers (2007, 2008). This approach
allowed us to highlight differences in the thermal niches of some of the *Synechococcus*
clades. Laboratory strains belonging to clades I and IV were isolated in colder waters than
those from clade II and III (Fig. 7). This result is coherent with the latitudinal distribution of
the *Synechococcus* clades, as field studies reported that clades I and IV are generally
610 confined to latitudes higher than 30° N/S. The two other dominant clades II and III seem to
prevail in the warmer waters of the warm temperate and tropical/sub-tropical regions of the
ocean (Zwirgmaier *et al.* 2007, 2008, Huang *et al.* 2011). It should be noted that strains
CC9605 (clade III) and A15-11 (clade II), for which temperature isolation was below 19°C,
were collected in upwelling areas (California current and Mauritania coast, respectively)
615 where water temperature is usually colder and more variable. Strains from clade VI seem to
be easily brought to culture, as shown by the relatively high number of isolates available in
culture collections (data not shown), but are not very abundant in the field (Huang *et al.*
2011). The wide spectrum of temperature at which these strains have been isolated (Fig. 7)

suggests that clade VI might gather strains with high physiological flexibility regarding
620 temperature.

The temperature adaptation capacities of the six strains considered in this study are
in line with their phylogenetic position. The tropical strains A15-37 and M16.1, which
showed high optimal temperature for growth and could not grow below c.a. 18°C, belong to
clade II. Similar observations were made for the clade III strain *Synechococcus* sp. WH8103
625 (Moore *et al.* 1995). Among the strains we studied, *Synechococcus* spp. ROS8604, MVIR-16-2
and MVIR-18-1 belong to clade I and appear to be the best adapted to cold waters. It is
however worth noting that the three strains show differential responses to cold stress (Fig.
2, 3, 4, and 5) and different temperature *preferenda* (Fig. 6), with a good correspondence to
their isolation latitude. Thus, clade I *Synechococcus* strains, living at the northernmost limit
630 of distribution (near the polar circle), show better capacities to cope with low temperature
than those isolated from cold temperate waters. This highlights the existence of significant
functional microdiversity regarding adaptative capacities to temperature within marine
Synechococcus clade I.

635 **Conclusion**

This study demonstrates for the first time that marine *Synechococcus* strains exhibit
considerably different physiological capacities to cope with temperature variations and
display different thermal *preferenda*. These abilities might rely on the capacities to maintain
the photosynthetic apparatus in a state that limits the induction of oxidative stress.
640 Furthermore, our study suggests a correlation between the ability to cope with temperature
variations and specific clades of the marine *Synechococcus* radiation. Our results suggest
that clade I and probably clade IV *Synechococcus* have a physiology preferentially adapted to
cold thermal niches. These observations, which are quite in line with phylogeographic field
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studies (Zwirgmaier *et al.* 2007, 2008), suggest the existence of low temperature ecotypes,
645 *i.e.* cold thermotypes, among marine *Synechococcus* and underline an important role of
temperature in the diversification of marine *Synechococcus* at least at high latitudes. The
deep understanding of the differentiation of cold *Synechococcus* thermotypes at high
latitudes will however not be easy, given the functional microdiversity occurring within
Synechococcus clade I, a diversity which likely reflects adaptation the continuous latitudinal
650 gradient of temperature. Although clade II (and III; Moore *et al.* 1995) cells seem more
adapted to warm waters, more work is necessary to evaluate the importance of
temperature relative to other environmental factors in the *Synechococcus* diversification at
lower latitudes.

The absence of comparative data on the thermophysiology of marine *Synechococcus*
655 has so far made it impossible to build predictive models of picocyanobacteria distribution
taking in account the thermal flexibility of these organisms (Flombaum *et al.* 2013). As our
results suggest that the composition of the *Synechococcus* natural communities may
experience major changes in the context of possible climatic changes due to the occurrence
of temperature ecotypes, future modeling efforts should incorporate growth rate data in
660 order to better predict the global response of marine *Synechococcus* to environmental
changes.

Supplementary information is available at ISMEJ's website.

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Supplementary information is available at ISMEJ's website.

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Legends to Figures

Figure 1: Location of isolation sites of the six marine *Synechococcus* spp. used in this study.

1015 **Figure 2:** Variations of relative cell density (cell mL⁻¹) expressed as % of initial cell density at the start of the experiment (**A**) and phycoerythrin to phycocyanin fluorescence emission ratio (**B**) in the tropical (triangles), mid-latitude (squares) and high latitude (circles) marine *Synechococcus* strains during six days of cold stress ($n \geq 4$).

1020 **Figure 3:** Variations of state transition amplitude as measured by non photochemical quenching of fluorescence associated to state transition (NPQ_{ST}; **A**) and photosystem II quantum yield (F_V/F_M ; **B**) in the tropical (triangles), mid-latitude (squares) and high latitude (circles) marine *Synechococcus* strains during six days of cold stress ($n \geq 4$).

1025 **Figure 4:** Relative variations of the β -subunit of phycoerythrins I and II (**A**) and of the D1 protein (**B**) estimated by immunoreactions in tropical, mid-latitude and high latitude marine *Synechococcus* strains during five days of cold stress.

1030 **Figure 5:** Variations of the β -carotene to chl *a* ratio (g/g) in the tropical (triangles), mid-latitude (squares) and high latitude (circles) marine *Synechococcus* strains during six days of cold stress ($n \geq 4$).

Figure 6: Growth rate as a function of temperature in the tropical (triangles), mid-latitude (squares) and high latitude (circles) marine *Synechococcus* strains. Error bars are standard deviation from the mean based on 4 replicates ($n \geq 4$).

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Figure 7: Maximum likelihood (ML) analysis of the *petB* gene (based on 559 aligned aminoacids) retrieved from 74 marine cultured isolates of *Synechococcus*. For each strain, sea surface temperatures at the isolation site are indicated according to the color scale. Numbers at nodes correspond to bootstrap values from ML, Posterior probability of Bayesian inference (BI; ranging between 0 and 1) and bootstraps for neighbor-joining (NJ) method. Bootstraps, represented as a percentage, were obtained through 1000 repetitions for each method, and only values higher than 70% are shown on the phylogenetic tree. Filled circles correspond to nodes supported by values higher than 70/0.8/80 for ML/BI/NJ methods, respectively. Empty circles correspond to nodes fully supported by the three methods. The six *Synechococcus* strains used in this study are in bold. *Synechococcus* sp. WH5701, affiliated to the subcluster 5.2, was used as an outgroup. The nomenclature described by Mazard et al. (2012a) was retained for the clade/ecotype numbers.

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Titles and legends to tables

Table 1: Information regarding the *Synechococcus* strains used in this study. The pigment type nomenclature is described in Six *et al.* 2007c and Humily *et al.* in press.

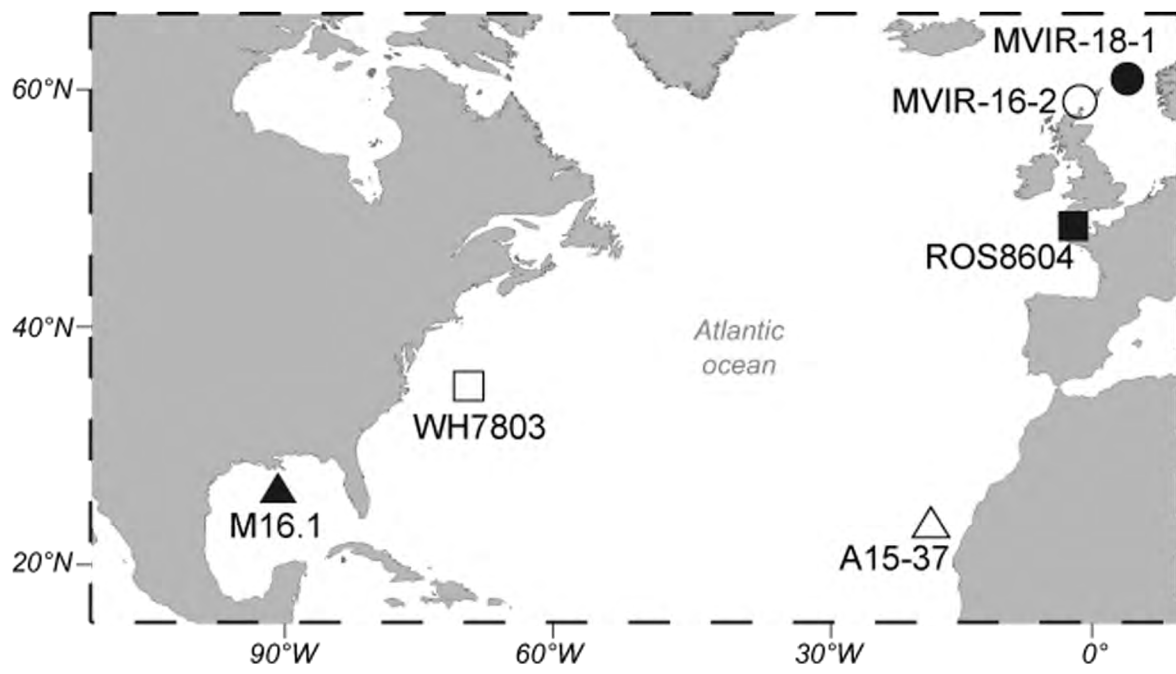
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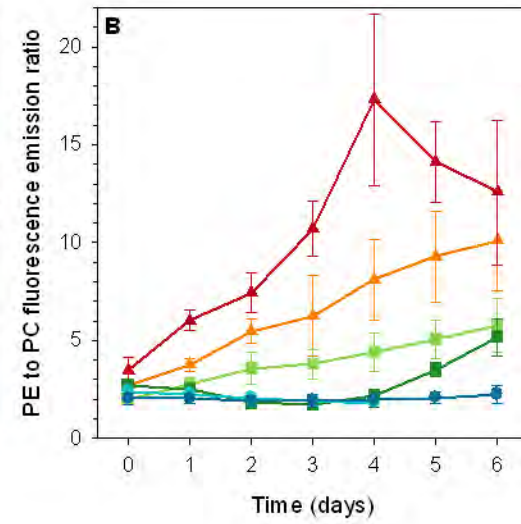
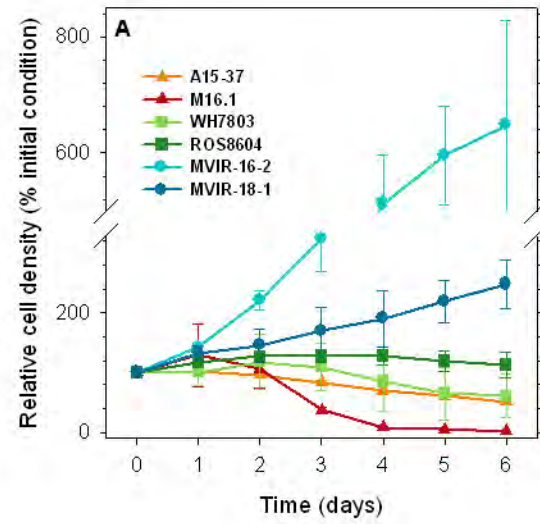
| Strain name | A15-37 | M16.1 | WH7803 | ROS8604 | MVIR-16-2 | MVIR-18-1 |
|-------------------------------|------------------------|-------------------|--------------|--------------------|---------------------------|---------------------------|
| RCC # | 2526 | 791 | 752 | 2380 | 1594 | 2385 |
| Pigment type | 3a | 3a | 3a | 3a | 3a | 3a |
| Isolation site | Offshore Mauritania | Gulf of Mexico | Sargasso Sea | English Channel | Southern Norwegian Sea | Southern Norwegian Sea |
| Isolation latitude | 23°33' N | 27°42' N | 33° 45' N | 48° 43' N | 60° 19' N | 61° 00' N |
| Isolation longitude | 19°59' W | 91°18' W | 67° 30' W | 3° 59' W | 3° 29' W | 1° 59' E |
| Isolation date | 29/09/2004 | 09/02/2004 | 03/07/1978 | 24/11/1986 | 21/07/2007 | 23/07/2007 |
| Isolation Depth (m) | 10 | 275 | 25 | 1 | 10 | 25 |
| Isolation temperature (°C) | 24.53 | 24.15 | 25.85 | 12.81 | 11.99 | 13.98 |

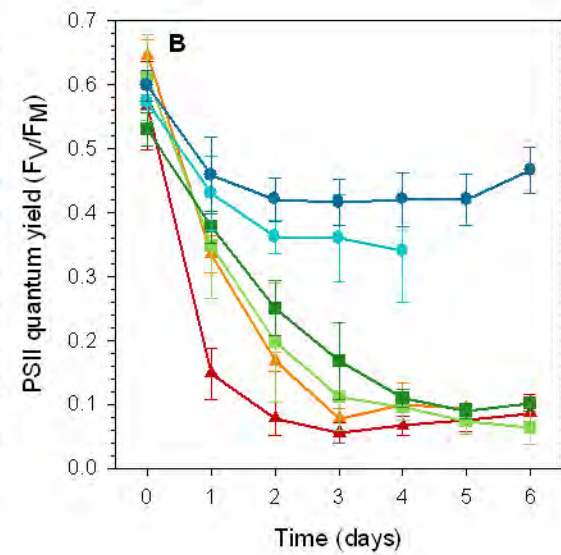
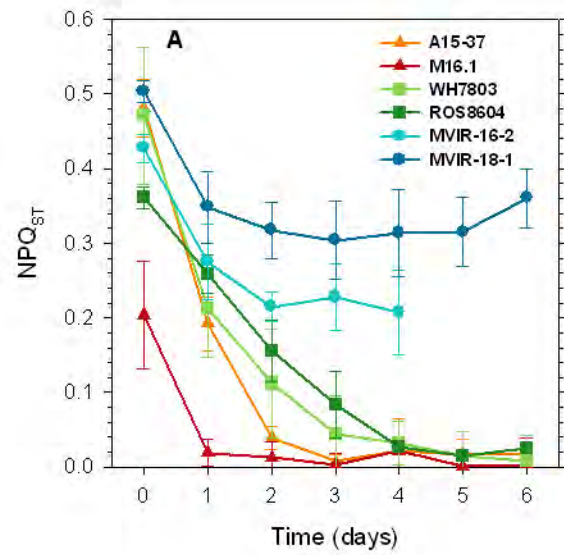
Table 2: Photophysiological features of the six marine *Synechococcus* strains, acclimated at 22°C under 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

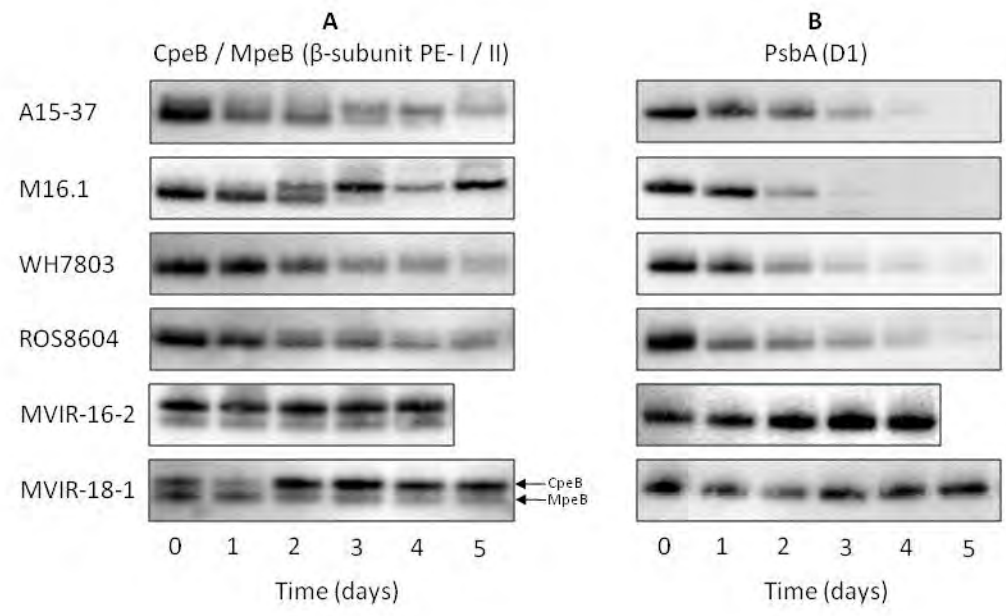
| Strain | A15-37 | M16.1 | WH7803 | ROS8604 | MVIR-16-2 | MVIR-18-1 |
|--|-------------------|-------------------|-------------------|-------------------|-------------------|------------------|
| Orange fluorescence | 0.05 \pm 0.01 | 0.18 \pm 0.01 | 0.80 \pm 0.06 | 1.10 \pm 0.22 | 0.45 \pm 0.04 | 0.21 \pm 0.07 |
| PE:PC | 2.70 \pm 0.10 | 3.50 \pm 0.70 | 2.00 \pm 0.20 | 2.70 \pm 0.20 | 2.40 \pm 0.10 | 2.00 \pm 0.30 |
| F _v :F _M | 0.64 \pm 0.03 | 0.57 \pm 0.07 | 0.61 \pm 0.07 | 0.53 \pm 0.03 | 0.57 \pm 0.01 | 0.60 \pm 0.02 |
| NPQ _{ST} | 0.48 \pm 0.04 | 0.20 \pm 0.07 | 0.47 \pm 0.09 | 0.36 \pm 0.01 | 0.43 \pm 0.02 | 0.50 \pm 0.01 |
| Chl α (fg cell ⁻¹) | 2.21 \pm 0.81 | 2.62 \pm 0.81 | 17.5 \pm 1.41 | 9.52 \pm 1.61 | 2.75 \pm 0.39 | 5.57 \pm 0.26 |
| Zeaxanthin (fg cell ⁻¹) | 1.47 \pm 0.54 | 2.06 \pm 0.59 | 5.20 \pm 0.62 | 6.54 \pm 1.71 | 2.05 \pm 0.25 | 3.05 \pm 0.07 |
| β -carotene (fg cell ⁻¹) | 0.08 \pm 0.01 | 0.15 \pm 0.05 | 1.16 \pm 0.16 | 0.88 \pm 0.09 | 0.22 \pm 0.02 | 0.40 \pm 0.02 |
| Zeaxanthin : Chl α (g/g) | 0.67 \pm 0.07 | 0.80 \pm 0.06 | 0.30 \pm 0.03 | 0.69 \pm 0.08 | 0.75 \pm 0.03 | 0.53 \pm 0.04 |
| β -carotene : Chl α (g/g) | 0.059 \pm 0.028 | 0.085 \pm 0.010 | 0.070 \pm 0.001 | 0.094 \pm 0.018 | 0.081 \pm 0.004 | 0.071 \pm 0.02 |

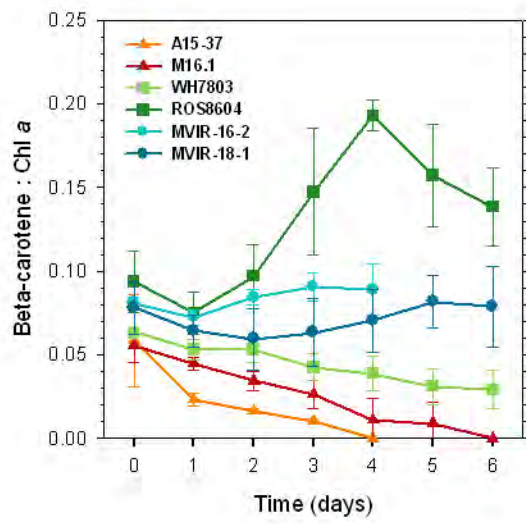
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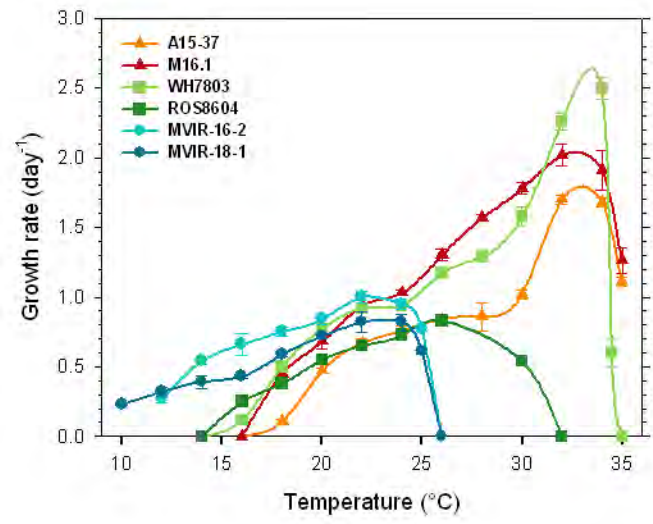






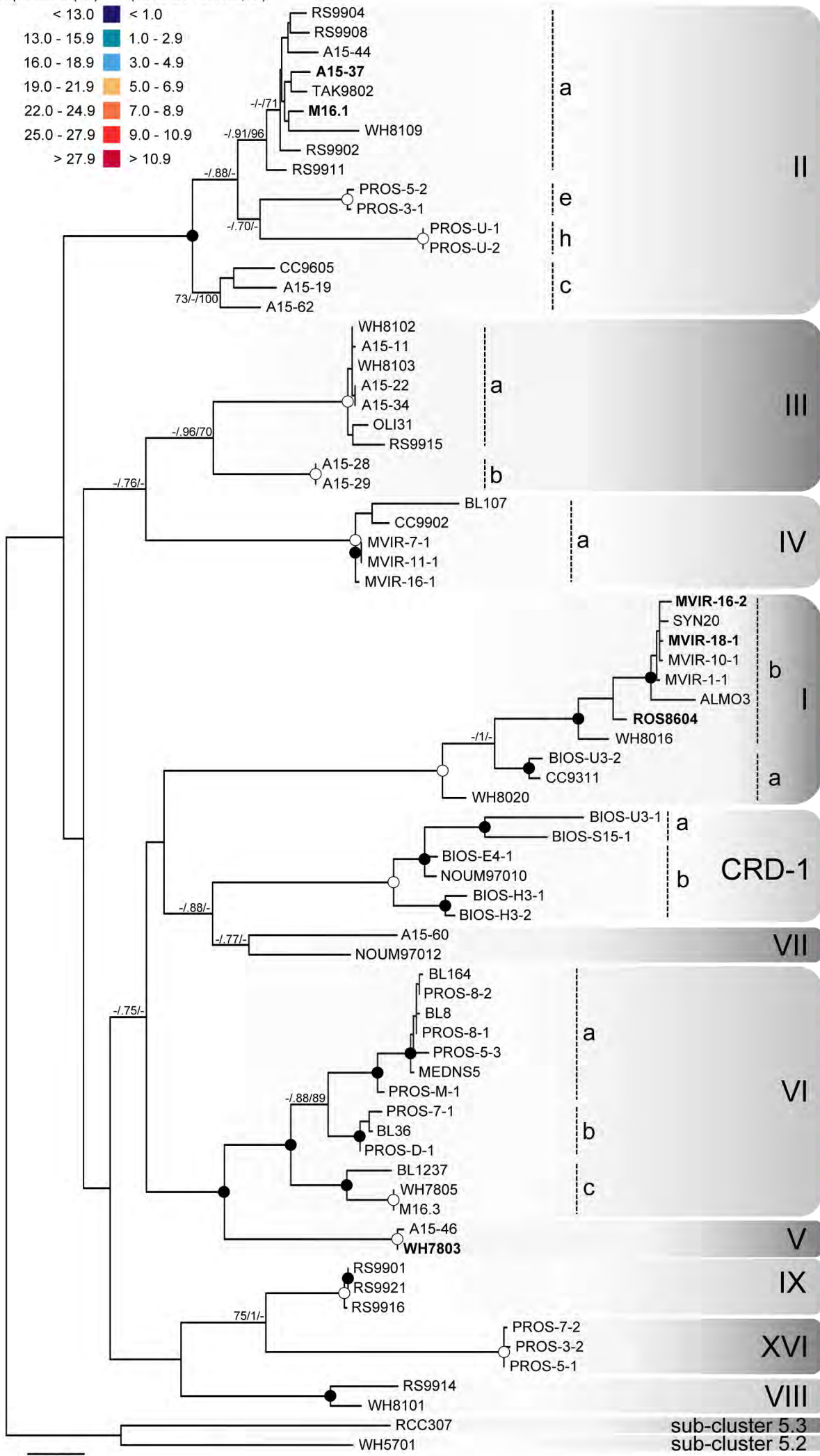






Isolation temperature (°C) Thermal amplitude (summer - winter, °C)

| | |
|-------------|------------|
| < 13.0 | < 1.0 |
| 13.0 - 15.9 | 1.0 - 2.9 |
| 16.0 - 18.9 | 3.0 - 4.9 |
| 19.0 - 21.9 | 5.0 - 6.9 |
| 22.0 - 24.9 | 7.0 - 8.9 |
| 25.0 - 27.9 | 9.0 - 10.9 |
| > 27.9 | > 10.9 |



0.08

Supplementary information

Supplementary tables

Table S1: Time (days) needed for recovery of initial values of photosynthetic parameters (photosystem II quantum yield, F_V/F_M ; non photochemical quenching associated to state transitions, NPQ_{ST} ; and phycoerythrin to phycocyanin fluorescence emission ratio, PE:PC) and to reach stationary phase at 22°C, after 6 days of cold stress at 13°C, for *Synechococcus* spp. M16.1, WH7803 and ROS8604. *Synechococcus* sp. A15-37 was not able to recover from cold stress when replaced under initial conditions.

| Strain | F_V/F_M | NPQ_{ST} | PE:PC |
|----------------|------------|------------|------------|
| M16.1 | 13.0 ± 1.4 | 14 ± 2.0 | 13.0 ± 1.7 |
| WH7803 | 5.0 ± 1.8 | 5.5 ± 1.9 | 5.0 ± 0.8 |
| ROS8604 | 5.7 ± 1.1 | 5.3 ± 0.6 | 4.3 ± 0.6 |

Table S2: Characteristics of marine *Synechococcus* strain isolation sites and accession numbers of the 74 *petB* sequences used in the phylogenetic study. Isolation temperatures were determined at the isolation site and date using satellite data and thermal amplitude was calculated using the average summer and winter temperature over 10 years (2002-2012; see methods).

| Strain name | RCC number | Isolation latitude (°N) | Isolation longitude (°E) | Isolation ocean | Isolation region | Isolation date | Isolation depth (m) | Isolation temperature (°C) | Seawater thermal amplitude (°C) | <i>petB</i> Accession number |
|------------------|------------|-------------------------|--------------------------|-------------------|---------------------------------|----------------|---------------------|----------------------------|---------------------------------|------------------------------|
| A15-11 | 2569 | 45.58 | -18.23 | North Atlantic | Eastern to the MAR ¹ | 06/06/1981 | 58 | 18.25 | 4.03 | JF307472 |
| A15-19 | 2525 | 38.53 | -20.21 | North Atlantic | Eastern to the MAR ¹ | 09/24/2004 | 75 | 22.02 | 4.48 | JF307478 |
| A15-22 | 1097 | 35.50 | -20.51 | North Atlantic | Northern gyre (edge) | 09/25/2004 | 15 | 23.78 | 4.07 | JF307480 |
| A15-28 | 2556 | 31.15 | -20.43 | North Atlantic | Northern gyre (edge) | 09/25/2004 | 10 | 25.15 | 3.08 | JF307482 |
| A15-29 | 2536 | 31.15 | -20.43 | North Atlantic | Northern gyre (edge) | 09/26/2004 | 15 | 25.15 | 3.08 | JF307483 |
| A15-34 | 1101 | 29.70 | -16.58 | North Atlantic | Morocco upwelling | 09/27/2004 | 40 | 24.61 | 2.50 | JF307485 |
| A15-37 | 2526 | 23.33 | -19.59 | North Atlantic | Morocco upwelling | 09/29/2004 | 10 | 24.53 | 1.64 | JF307486 |
| A15-44 | 2527 | 21.41 | -17.50 | North Atlantic | Morocco upwelling | 10/01/2004 | 20 | 22.61 | 2.09 | KF443066 |
| A15-46 | 2375 | 21.22 | -18.49 | North Atlantic | Morocco upwelling | 09/30/2004 | 70 | 23.30 | 1.78 | JF307490 |
| A15-60 | 2554 | 17.37 | -20.57 | North Atlantic | Cabo Verde islands | 10/04/2004 | 10 | 26.02 | 1.32 | JF307495 |
| A15-62 | 2374 | 17.37 | -20.57 | North Atlantic | Cabo Verde islands | 10/04/2004 | 15 | 26.02 | 1.27 | JF307497 |
| ALMO3 | 2432 | 36.11 | -1.51 | Mediterranean sea | Alboran sea | 05/01/1991 | 0 | 17.51 | 7.64 | JF307500 |
| BIOS-E4-1 | 2533 | -33.60 | -73.22 | South Pacific | Chile upwelling | 12/06/2004 | 40 | 16.56 | 3.32 | JF307519 |
| BIOS-H3-1 | 1018 | -9.40 | -136.59 | South Pacific | Central HNLC area | 11/02/2004 | 100 | 28.08 | 0.32 | JF307514 |

| | | | | | | | | | | |
|-------------------|------|--------|---------|-------------------|--------------------|------------|------|-------|------|--------------|
| BIOS-H3-2 | 1030 | -9.40 | -136.59 | South Pacific | Central HNLC area | 11/04/2004 | 30 | 28.08 | 0.32 | JF307521 |
| BIOS-S15-1 | 1023 | -30.47 | -95.26 | South Pacific | Gyre | 11/24/2004 | 100 | 18.66 | 4.07 | JF307516 |
| BIOS-U3-1 | 2534 | -31.52 | -91.25 | South Pacific | Gyre | 11/28/2004 | 40 | 16.56 | 3.32 | JF307517 |
| BIOS-U3-2 | 2532 | -33.00 | -73.20 | South Pacific | Chile upwelling | 12/08/2004 | 30 | 16.56 | 3.32 | JF307511 |
| BL107 | 515 | 41.43 | 3.33 | Mediterranean sea | Balearic sea | 03/15/2001 | 1800 | 13.89 | 9.00 | AATZ00000000 |
| BL1237 | - | 41.40 | 2.48 | Mediterranean sea | Balearic sea | 12/22/2000 | - | 15.25 | 9.45 | JF307501 |
| BL164 | - | 41.40 | 2.48 | Mediterranean sea | Balearic sea | 24/12/2000 | 3 | 15.25 | 9.45 | JF307503 |
| BL36 | 508 | 41.40 | 2.48 | Mediterranean sea | Balearic sea | 12/24/2000 | 0 | 15.25 | 9.45 | JF307531 |
| BL8 | | 41.40 | 2.48 | Mediterranean sea | Balearic sea | 12/21/2000 | - | 15.25 | 9.45 | JF307506 |
| CC9311 | 1086 | 31.90 | -124.16 | North Pacific | California current | 12/26/2000 | 95 | 16.59 | 2.29 | CP000435 |
| CC9605 | 753 | 30.41 | -123.98 | North pacific | California current | 01/01/1993 | - | 18.02 | 2.03 | CP000110 |
| CC9902 | 2673 | 32.90 | -117.25 | North pacific | California current | 10/01/1996 | 5 | 15.56 | 4.38 | CP000097 |
| M16.1 | 791 | 27.42 | -91.18 | North Altantic | Gulf of Mexico | 02/09/2004 | 275 | 24.15 | 5.45 | JF307548 |
| M16.3 | 792 | 27.42 | -91.18 | North Altantic | Gulf of Mexico | 02/09/2004 | 275 | 24.15 | 5.45 | JF307549 |
| MEDNS5 | 2368 | 41.00 | 6.00 | Mediterranean sea | Balearic sea | 07/01/1993 | 80 | 21.75 | 8.80 | JF307539 |
| MinSyn016 | 307 | 39.10 | 6.10 | Mediterranean sea | Balearic sea | 06/20/1996 | 15 | 21.08 | 8.80 | CT978603 |
| MVIR-16-1 | 2570 | 60.19 | -3.29 | North Atlantic | North sea | 07/21/2007 | 10 | 11.99 | 3.06 | KF443070 |
| MVIR-16-2 | 3010 | 60.19 | -3.29 | North Atlantic | North sea | 07/21/2007 | 10 | 11.99 | 3.06 | KF443071 |
| MVIR-7-1 | 1648 | 53.90 | 2.47 | North Atlantic | North sea | 07/10/2007 | 10 | 15.69 | 8.06 | KF443073 |
| MVIR-1-1 | 1708 | 48.46 | -3.56 | North Atlantic | North sea | 07/04/2007 | 10 | 16.14 | 5.54 | KF443067 |
| MVIR-11-1 | 1695 | 56.56 | 3.59 | North Atlantic | North sea | 07/14/2007 | 10 | 15.09 | 5.24 | KF443069 |
| MVIR-10-1 | 1688 | 55.40 | 2.16 | North Atlantic | North sea | 07/13/2007 | 55 | 15.02 | 7.44 | KF443068 |
| MVIR-18-1 | 2385 | 61.00 | 1.59 | North Atlantic | North sea | 07/23/2007 | 25 | 13.98 | 6.01 | KF443072 |

| | | | | | | | | | | |
|------------------|------|--------|---------|-------------------|---------------------|------------|-----|-------|-------|----------|
| NOUM97010 | 66 | 0.00 | -180.00 | South pacific | Eastern HNLC area | 11/04/1996 | 80 | 28.65 | .70 | JF307546 |
| NOUM97012 | 67 | -22.20 | 166.20 | South pacific | New Caledonia coast | 11/19/1996 | 70 | 24.42 | 3.31 | JF307547 |
| OLI31 | 44 | -5.60 | -150.00 | South pacific | Central HNLC area | 11/11/1994 | 70 | 28.56 | 0.33 | JF307510 |
| PROS-3-1 | 321 | 38.00 | 3.50 | Mediterranean sea | Balearic sea | 09/16/1999 | 5 | 25.79 | 8.84 | JF307529 |
| PROS-3-2 | 316 | 38.00 | 3.50 | Mediterranean sea | Balearic sea | 09/16/1999 | 110 | 25.79 | 8.84 | JF307524 |
| PROS-5-1 | 318 | 36.29 | 13.19 | Mediterranean sea | Thyrrhenian sea | 09/18/1999 | 65 | 26.41 | 8.32 | JF307526 |
| PROS-5-2 | 374 | 36.29 | 13.19 | Mediterranean sea | Thyrrhenian sea | 09/18/1999 | 25 | 26.41 | 8.32 | JF307537 |
| PROS-5-3 | 528 | 33.60 | 22.10 | Mediterranean sea | Ionian sea | 09/20/1999 | 50 | 26.59 | 7.36 | JF307545 |
| PROS-7-1 | 2381 | 37.24 | 15.37 | Mediterranean sea | Thyrrhenian sea | 09/26/1999 | 5 | 25.99 | 9.33 | JF307531 |
| PROS-7-2 | 317 | 37.24 | 15.37 | Mediterranean sea | Thyrrhenian sea | 09/26/1999 | 90 | 25.99 | 9.33 | JF307525 |
| PROS-8-1 | 527 | 39.70 | 14.50 | Mediterranean sea | Thyrrhenian sea | 09/27/1999 | 110 | 25.41 | 10.08 | JF307544 |
| PROS-8-2 | 523 | 39.70 | 14.50 | Mediterranean sea | Thyrrhenian sea | 09/27/1999 | 70 | 25.41 | 10.08 | JF307541 |
| PROS-D-1 | 319 | 43.24 | 7.49 | Mediterranean sea | Gulf of lion | 09/30/1999 | 15 | 23.72 | 8.81 | JF307469 |
| PROS-M-1 | 324 | 36.29 | 13.19 | Mediterranean sea | Ionian sea | 09/18/1999 | 5 | 26.41 | 8.32 | JF307470 |
| PROS-U-1 | 2369 | 30.80 | -10.30 | North Atlantic | Maroccan upwelling | 09/12/1999 | 5 | 21.51 | 2.80 | JF307538 |
| PROS-U-2 | 442 | 30.80 | -10.30 | North Atlantic | Maroccan upwelling | 09/12/1999 | 5 | 21.51 | 2.80 | JF307540 |
| ROS8604 | 2380 | 48.43 | -3.59 | North Atlantic | English Channel | 11/24/1986 | 0 | 12.81 | 5.10 | JF307527 |
| RS9901 | 2529 | 29.28 | 34.55 | Indian ocean | Red Sea | 03/29/1999 | 1 | 21.07 | 5.64 | JF307552 |
| RS9902 | 2376 | 29.28 | 34.55 | Indian ocean | Red Sea | 03/23/1999 | 1 | 21.07 | 5.64 | JF307553 |
| RS9904 | 543 | 29.28 | 34.55 | Indian ocean | Red Sea | 06/14/1999 | 10 | 25.69 | 5.64 | JF307554 |
| RS9908 | 2530 | 31.28 | 34.55 | Indian ocean | Red Sea | 09/07/1999 | - | 28.37 | 5.64 | JF307558 |
| RS9911 | 550 | 29.28 | 34.55 | Indian ocean | Red Sea | 05/11/1999 | 10 | 23.77 | 5.64 | JF307561 |
| RS9914 | 553 | 29.28 | 34.55 | Indian ocean | Red Sea | 10/18/1999 | 10 | 26.98 | 5.64 | JF307564 |

| | | | | | | | | | | |
|----------------|------|--------|---------|----------------|----------------|------------|-----|-------------------|-------|--------------|
| RS9915 | 2553 | 29.28 | 34.55 | Indian ocean | Red Sea | 10/18/1999 | 10 | 23.98 | 5.64 | JF307565 |
| RS9916 | 555 | 29.28 | 34.55 | Indian ocean | Red Sea | 11/22/1999 | 10 | 25.67 | 5.64 | AAUA00000000 |
| RS9921 | 559 | 29.28 | 34.55 | Indian ocean | Red Sea | 11/22/1999 | 150 | 25.67 | 5.64 | JF307567 |
| SYN20 | 2035 | 60.37 | 5.41 | North Atlantic | North Sea | - | 0 | 9.99 ² | 6.97 | JF307568 |
| TAK9802 | 2528 | -14.30 | -145.20 | South Pacific | Takapoto atoll | 02/06/1998 | 7 | 29.81 | 1.10 | JF307523 |
| WH5701 | 1084 | 41.05 | -72.75 | North Atlantic | Sargasso sea | 01/01/1957 | - | 6.03 | 13.31 | AANO00000000 |
| WH7803 | 752 | 33.45 | -67.30 | North Atlantic | Sargasso sea | 07/03/1978 | 25 | 25.85 | 5.88 | CT971583 |
| WH7805 | 1085 | 33.44 | -67.30 | North Altantic | Sargasso Sea | 06/30/1978 | - | 25.85 | 5.88 | AAOK00000000 |
| WH8016 | 2535 | 41.53 | -70.67 | North Altantic | Sargasso Sea | 06/01/1980 | - | 17.83 | 12.06 | JF307569 |
| WH8020 | 2437 | 38.41 | -69.19 | North Altantic | Sargasso Sea | 06/16/1980 | 50 | 16.89 | 8.37 | JF307571 |
| WH8101 | 2555 | 41.53 | -70.67 | North Altantic | Sargasso Sea | 01/01/1981 | - | 5.77 | 11.90 | JF307572 |
| WH8102 | 539 | 22.29 | -65.36 | North Altantic | Carribean Sea | 03/15/1981 | - | 25.78 | 2.55 | BX548020 |
| WH8103 | 29 | 28.30 | -67.23 | North Altantic | Sargasso Sea | 03/17/1981 | - | 25.45 | 2.90 | JF307573 |
| WH8109 | 2033 | 39.29 | -70.28 | North Altantic | Sargasso sea | 06/01/1981 | - | 21.60 | 10.19 | ACNY00000000 |

¹ MAR : Mid Atlantic Ridge

² Annual mean of sea surface temperature at the isolation site over the past 10 years (see methods).

Supplementary Figures

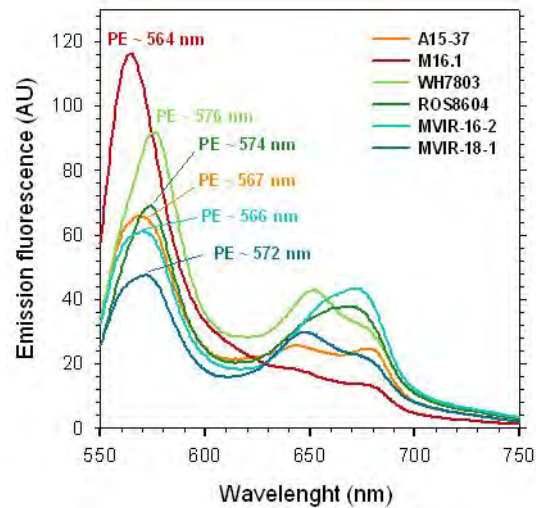


Figure S1: Fluorescence emission spectra (excitation at 530 nm) of the six *Synechococcus* strains used in this study. Wavelengths of phycoerythrin (PE) maxima are indicated on the figure, while phycocyanin emission maxima are 650, 644, 652, 655, 652 and 656 nm respectively for *Synechococcus* spp. A15-37, M16.1, WH7803, ROS8604, MVIR-16-2 and MVIR-18-1.

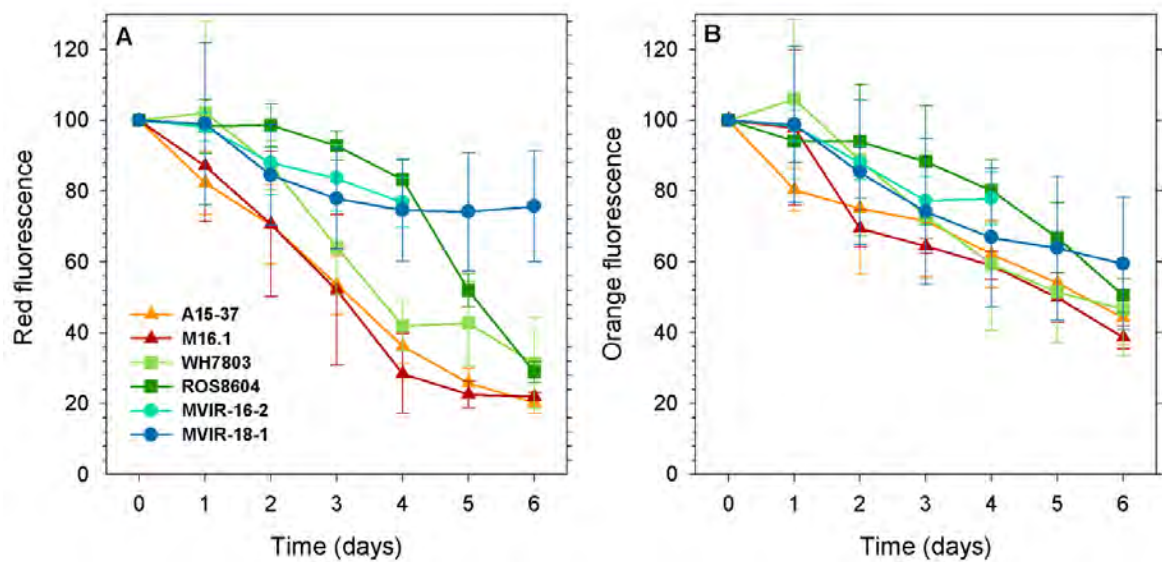


Figure S2: Variations of red (A) and orange (B) fluorescence in the tropical (triangles), mid-latitude (squares) and high latitude (circles) marine *Synechococcus* strains during six days of cold stress.