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► **To cite this version:**

Mathilde Ferrieux, Louison Dufour, Hugo Doré, Morgane Ratin, Audrey Guéneuguès, et al.. Comparative Thermophysiology of Marine Synechococcus CRD1 Strains Isolated From Different Thermal Niches in Iron-Depleted Areas. *Frontiers in Microbiology*, 2022, 13, pp.893413. 10.3389/fmicb.2022.893413 . hal-03768045

HAL Id: hal-03768045

<https://hal.sorbonne-universite.fr/hal-03768045v1>

Submitted on 5 Oct 2022

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Comparative thermophysiology of marine *Synechococcus* CRD1 strains isolated from different thermal niches in iron-depleted areas

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22 **Keywords:** Marine picocyanobacteria, *Synechococcus*, CRD1, thermotype, temperature
23 adaptation

24

25 **Abstract**

26 Marine *Synechococcus* cyanobacteria are ubiquitous in the ocean, a feature likely related to their
27 extensive genetic diversity. Amongst the major lineages, clades I and IV preferentially thrive in
28 temperate and cold, nutrient-rich waters, whilst clades II and III prefer warm, nitrogen or phosphorus-
29 depleted waters. The existence of such cold (I/IV) and warm (II/III) thermotypes is corroborated by
30 physiological characterization of representative strains. A fifth clade, CRD1, was recently shown to
31 dominate the *Synechococcus* community in iron-depleted areas of the world ocean and to encompass
32 three distinct ecologically significant taxonomic units (ESTUs CRD1A-C) occupying different thermal
33 niches, suggesting that distinct thermotypes could also occur within this clade.

34 Here, using comparative thermophysiology of strains representative of these three CRD1 ESTUs we
35 show that the CRD1A strain MITS9220 is a warm thermotype, the CRD1B strain BIOS-U3-1 a cold
36 temperate thermotype, and the CRD1C strain BIOS-E4-1 a warm temperate stenotherm. Curiously, the
37 CRD1B thermotype lacks traits and/or genomic features typical of cold thermotypes. In contrast, we
38 found specific physiological traits of the CRD1 strains compared to their clade I, II, III and IV
39 counterparts, including a lower growth rate and photosystem II maximal quantum yield at most
40 temperatures and a higher turnover rate of the D1 protein. Together, our data suggests that the CRD1
41 clade prioritizes adaptation to low-iron conditions over temperature adaptation, even though the
42 occurrence of several CRD1 thermotypes likely explains why the CRD1 clade as a whole occupies
43 most iron-limited waters.

44

45 **INTRODUCTION**

46 Marine picocyanobacteria contribute to the biogeochemical cycling of various elements, most notably
47 carbon, contributing ~25% of ocean net primary productivity, of which the *Synechococcus* genus alone
48 is responsible for about 16% (Flombaum et al., 2013). The large geographic distribution of these
49 organisms, extending from the equator to subpolar waters, is largely attributable to their extensive
50 genetic and functional diversity (Zwirgmaier et al., 2008; Farrant et al., 2016; Doré et al., 2020).
51 Amongst the nearly 20 clades within subcluster (SC) 5.1, the most abundant and diversified
52 *Synechococcus* lineage in oceanic ecosystems (Dufresne et al., 2008; Scanlan et al., 2009; Ahlgren and
53 Rocap, 2012), only four (clades I, II, III and IV) were thought to largely dominate *in situ*. Clades I and
54 IV mainly thrive in temperate and cold, nutrient-rich waters, while clades II and III reside in warm,
55 oligotrophic or mesotrophic areas (Zwirgmaier et al., 2008; Mella-Flores et al., 2011), suggesting the

56 existence of cold (I/IV) and warm (II/III) *Synechococcus* ‘thermotypes’. This hypothesis was
57 subsequently confirmed by work demonstrating that strains representative of these different clades
58 exhibit distinct thermal *preferenda* (Mackey et al., 2013; Pittera et al., 2014; Breton et al., 2020; Six et
59 al., 2021), a feature notably linked to differences in the thermostability of light-harvesting complexes
60 (Pittera et al., 2017), lipid desaturase gene content (Pittera et al., 2018) and the ability of some strains
61 to induce photoprotective light dissipation at colder temperatures using the orange carotenoid protein
62 (OCP; Six et al., 2021). Field studies using global ocean datasets have allowed to refine the respective
63 ecological niches of the different thermotypes, with clade I extending further north than clade IV
64 (Paulsen et al., 2016; Doré et al., 2022) and clades II and III predominating in N- and P-depleted waters,
65 respectively, but also to highlight the importance of a fifth clade within SC 5.1, the CRD1 clade
66 (Farrant et al., 2016; Sohm et al., 2016; Kent et al., 2019). Initially thought to be limited to the Costa
67 Rica dome area (Saito et al., 2005; Gutiérrez-Rodríguez et al., 2014), the latter clade was recently found
68 to be a major component of *Synechococcus* communities in Fe-depleted areas (Farrant et al., 2016;
69 Sohm et al., 2016; Ahlgren et al., 2020). Furthermore, analysis of the global distribution of these
70 organisms using high-resolution marker genes has highlighted large within-clade microdiversity
71 associated with niche differentiation in marine *Synechococcus* (Farrant et al., 2016; Larkin and
72 Martiny, 2017; Xia et al., 2019), as also observed in *Prochlorococcus* (Kashtan et al., 2014; Larkin et
73 al., 2016). Using the *petB* gene encoding cytochrome *b₆*, Farrant et al. (2016) showed that most major
74 clades encompassed several Ecologically Significant Taxonomic Units (ESTUs), i.e. genetically
75 related subgroups within clades occupying distinct oceanic niches. This is notably the case for ESTU
76 IIB that occupies a cold thermal niche in sharp contrast with IIA, the dominant ESTU within clade II
77 that occupies warm, mesotrophic, and oligotrophic iron (Fe)-replete waters. Similarly, three distinct
78 ESTUs with distinct thermal niches were identified within the CRD1 clade and the co-occurring clade
79 EnvB (a.k.a. CRD2; Ahlgren et al., 2020): i) CRD1B/EnvBB are found in cold mixed waters in co-
80 occurrence with ESTUs IA, IVA and IVC, ii) CRD1C/EnvBC dominate in warm, high-nutrient low-
81 chlorophyll (HNLC) regions such as the central Pacific Ocean, and iii) CRD1A/EnvBA are present in
82 both environments and thus span a much wider range of temperatures than CRD1B and C (Farrant et
83 al., 2016). This suggests that these three CRD1 ESTUs could correspond to different thermotypes.

84 In order to test this hypothesis, we used strains representative of each of the three CRD1 ESTUs to
85 determine the fundamental thermal niches of these organisms as compared to typical cold (clades I and
86 IV) and warm (clades II and III) thermotypes. Furthermore, given the strong influence of temperature

87 on optimal functioning of the photosynthetic apparatus in marine *Synechococcus* (Pittera et al., 2014,
88 2017; Guyet et al., 2020), we also examined the effect of temperature acclimation on the
89 photophysiology of CRD1 ESTUs compared to their clade I and IV counterparts and show that CRD1
90 thermotypes actually differ more strongly in this respect to members of clades I-IV than from each
91 other.

92 **MATERIALS AND METHODS**

93 **Strains and growth conditions**

94 The eight *Synechococcus* spp. strains used in this study were retrieved from the Roscoff Culture
95 Collection (RCC; <https://roscoff-culture-collection.org/>), including representative strains of the three
96 known CRD1 ESTUs (CRD1A – C) and one or two of each of the four dominant clades in the field
97 (clades I – IV) used as controls (Table 1 and Supplementary Fig. 1). Cells were grown in 50 mL flasks
98 (Sarstedt, Germany) in PCR-S11 culture medium (Rippka et al., 2000) supplemented with 1 mM
99 sodium nitrate. Cultures were acclimated for at least two weeks in temperature-controlled chambers
100 across a range of temperatures dependent on the thermal tolerance of each strain and under a continuous
101 light of 75 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (hereafter $\mu\text{E m}^{-2} \text{s}^{-1}$) provided by a white-blue-green LED system
102 (Alpheus, France). For each experiment cultures were grown in triplicate, inoculated at an initial cell
103 density of $\sim 3 \times 10^6$ cells mL^{-1} , and samples harvested daily to measure growth rate and fluorescence
104 parameters as described below.

105 In order to compare the capacity of strains to repair the D1 subunit of photosystem II (PSII; see
106 ‘Measurement of PSII repair rate’ section), cultures grown in 250 ml flasks at 75 $\mu\text{E m}^{-2} \text{s}^{-1}$ were
107 acclimated at 18, 22 and 25°C, temperatures at which all strains were able to grow, were subjected to
108 high light stress (375 $\mu\text{E m}^{-2} \text{s}^{-1}$). Exponentially growing cultures were sampled at T0 and after 15, 30,
109 60, and 90 min of stress, before shifting cultures back to the initial light conditions and then sampling
110 again after 15, 30, 60 min and 24h of recovery (R). While D1 repair measurements were performed at
111 all time points, cell concentrations were measured by flow cytometry only at T0, T30min, T90min,
112 R30min and R24h and liposoluble pigment content was determined only at T0.

113 **Flow cytometry**

114 Culture aliquots (200 μl) sampled for flow cytometry were fixed using 0.25% (v/v) glutaraldehyde
115 (grade II, Sigma Aldrich, USA) and stored at -80°C until analysis (Marie et al., 1999). Cell
116 concentrations were estimated using a Guava easyCyte flow cytometer (Luminex Corporation, USA)

117 and maximum growth rates (μ_{\max}) were calculated as the slope of the linear regression of ln(cell
118 density) vs. time during the exponential growth phase. *Synechococcus* cells were identified based on
119 their red (695 nm) and orange (583 nm) fluorescence, proxies for their chlorophyll *a* and phycoerythrin
120 content, respectively. Fluorescence, forward scatter and side scatter values were normalized to that of
121 standard 0.95 μm beads using Guavasoft software (Luminex Corporation, USA).

122 **Fluorescence measurements**

123 The maximum PSII quantum yield (F_V/F_M) was estimated using a Pulse Amplitude Modulation
124 fluorimeter (Phyto-PAM II, Walz, Germany) during the exponential growth phase after 10 min dark
125 acclimation followed by addition of 100 μM of the PSII blocker 3-(3,4-dichlorophenyl)-1,1-
126 dimethylurea (DCMU, Sigma-Aldrich, USA; Campbell et al., 1998).

127 The PSII quantum yield was calculated as:

$$128 \quad F_V/F_M = (F_M - F_0)/F_M$$

129 where F_0 is basal fluorescence, F_M maximal fluorescence level and F_V variable fluorescence (Campbell
130 et al., 1998; Six et al., 2007).

131 Fluorescence excitation (with emission set at 580 nm) and emission (with excitation set at 530 nm)
132 spectra were generated using a LS-50B spectrofluorometer (Perkin-Elmer, USA) as described in Six
133 et al. (2004). The fluorescence excitation ratio ($\text{Exc}_{495:550\text{nm}}$) was used as a proxy for the PUB:PEB
134 ratio. Phycobilisome (PBS) rod length and the degree of coupling of the PBS to PSII reaction center
135 chlorophylls was then assessed using fluorescence emission spectra by calculating the phycoerythrin
136 (PE, $F_{\max} = 565\text{--}575$ nm) to phycocyanin (PC, $F_{\max} = 645\text{--}655$ nm) ratio as well as the PC to PBS
137 terminal acceptor (TA; $F_{\max} = 680$ nm) ratio, respectively (Pittera et al., 2017).

138 **Pigment analyses**

139 Triplicate cultures were harvested during the exponential phase when F_V/F_M was maximum for each
140 temperature condition. Cultures (50 mL) were subjected to centrifugation in the presence of 0.01%
141 (v/v) pluronic acid (Sigma-Aldrich, Germany) at 4°C, 14,000 x g for 7 min, using an Eppendorf 5804R
142 (Eppendorf, France). Pellets were resuspended and transferred to 1.5 ml Eppendorf tubes and
143 centrifuged at 4°C, 17,000 x g for 2 min using an Eppendorf 5417R centrifuge (Eppendorf, France).
144 Once the supernatant was removed samples were stored at -80 °C until further analysis. Pigment

145 content was subsequently assessed using calibrated high-performance liquid chromatography (HPLC
146 1100 Series System, Hewlett Packard, St Palo Alto, CA), as previously described (Six et al., 2005).

147 **Measurement of the photosystem II repair rate**

148 Each culture acclimated to $75 \mu\text{E m}^{-2} \text{s}^{-1}$ and 18, 22 or 25 °C was split into two new 50 mL flasks
149 (Sarstedt Germany) with one used as a control and the other flask supplemented with lincomycin (0.5
150 mg mL⁻¹ final concentration, Sigma-Aldrich, USA) in order to inhibit protein synthesis and thus D1
151 repair (Guyet et al., 2020). Both sub-cultures were then subjected to light stress by exposing cultures
152 to $375 \mu\text{E m}^{-2} \text{s}^{-1}$ continuous light (at the same temperature), and F_V/F_M measured at different time
153 points as described above. The PSII repair rate for each strain at each temperature was determined from
154 the coefficient differences between the exponential curves fitted over the 90 min time course of F_V/F_M
155 measurements for control and +lincomycin samples. This light stress experiment was replicated on
156 four independent cultures.

157 **Determination of the realized environmental niches of major *Synechococcus* ESTUs**

158 The realized niches of CRD1 and clades I-IV ESTUs were determined using *petB* reads extracted from
159 metagenomic data from the *Tara* Oceans and *Tara* Polar circle expeditions, the Ocean Sampling Day
160 (OSD; June 21st 2014) campaign, and *petB* metabarcodes from i) various oceanographic cruises
161 (CEFAS, BOUM, Micropolar, RRS Discovery cruise 368 and several in the northwestern Pacific
162 Ocean as detailed in Xia et al., 2017), ii) two individual sampling sites in the Mediterranean Sea
163 (Boussole, Point B) as well as iii) a bi-monthly sampling at the long-term observatory site SOMLIT
164 (“Service d'Observation en Milieu Littoral”)-Astan located 2.8 miles off Roscoff between July 2009
165 and December 2011 (Supplementary Table 1).

166 *petB* metagenomic recruitment using the *Tara* Oceans and OSD datasets was performed as described
167 previously (Farrant et al., 2016). *Synechococcus petB* sequences from both metagenomes and
168 metabarcodes were used to define operational taxonomic units (OTUs) at 97% identity using Mothur
169 v1.34.4 (Schloss et al., 2009) that were then taxonomically assigned using a *petB* reference database
170 (Farrant et al., 2016). OTUs encompassing more than 3% of the total *Synechococcus* reads for a given
171 sample were grouped into ESTUs and used to determine the whole temperature range occupied by each
172 of the five major *Synechococcus* ESTUs.

173

174 **Comparative genomics**

175 The Cyanorak v2.1 information system (<http://www.sb-roscoff.fr/cyanorak/>; Garczarek et al., 2021)
 176 was used to compare the phyletic pattern i.e., the presence/absence pattern of each cluster of likely
 177 orthologous genes (CLOG) in each strain, for CRD1 strains and their clades I-IV counterparts for a
 178 number of selected genes potentially involved in adaptation to low temperature based on previous
 179 literature (see results).

180

181 **RESULTS**

182 **The fundamental thermal niches of CRD1 vs. clades I to IV strains**

183 In order to determine the temperature optima and boundary limits of the different CRD1 strains and to
 184 compare them to those of typical cold and warm *Synechococcus* thermotypes, representative strains of
 185 each of the three CRD1 ESTUs and strains of clades I, II, III and IV were grown over a range of
 186 temperatures from 6 to 36°C. The growth responses of all strains to temperature followed a typical
 187 asymmetric bell-shaped curve over the selected temperature range (Fig. 1), with a progressive rise in
 188 growth rate (μ) until T_{opt} (the temperature associated with maximum μ : μ_{max}) was reached, and a sharp
 189 decline above T_{opt} . BIOS-U3-1 (CRD1-B) was able to grow between 12 and 29°C with a T_{opt} at 25°C
 190 ($\mu_{max} = 0.78 \pm 0.02 \text{ d}^{-1}$), a growth pattern most similar to that of the clade IV strain BL107, while the
 191 clade I strain MVIR-18-1 was able to grow at much lower temperatures, down to 8°C but could not
 192 grow above 25°C (Fig. 1A). MITS9220 (CRD1-A) and BIOS-E4-1 (CRD1-C) displayed thermal
 193 growth characteristics more similar to the clade II (A15-62 and M16.1) and III (WH8102) strains,
 194 representatives of warm thermotypes (Fig. 1B). While most strains in this category displayed a minimal
 195 growth temperature of 16°C, large variations between strains were observed at the highest thermal
 196 boundary limit (T_{max}). Maximum growth temperature was obtained for M16.1 (II; T_{max} : 34°C), then
 197 A15-62 (II) and WH8102 (III; both with T_{max} at 32°C), MITS9220 (CRD1-A; T_{max} : 31°C) and finally
 198 for BIOS-E4-1 (CRD1-C; T_{max} : 30°C). The latter strain also displayed the highest minimal growth
 199 temperature (T_{min} : 18°C) and thus possesses the narrowest temperature range for growth of all the
 200 strains studied (12°C vs. 15-18°C). It is also worth noting that CRD1 strains display a lower maximum
 201 growth rate and more generally lower growth rates at most temperatures than their clade I, II, III and
 202 IV counterparts.

203

204 **The environmental realized niches of CRD1 vs. clades I to IV strains**

205 We then compared the fundamental thermal niches of all studied strains, i.e. the whole temperature
 206 range over which they can grow in a laboratory setting in the absence of biotic interactions (e.g.
 207 competition or predation), with environmental realized niches (*sensu* Pearman et al., 2008) of the
 208 corresponding ESTUs. For this, we determined the distribution limits of each of these ESTUs along
 209 the *Tara* Oceans and *Tara* Polar circle transects, 203 samples from OSD2014 and additional
 210 oceanographic cruises and individual sampling sites, altogether encompassing 413 samples worldwide
 211 covering a wide range of temperature conditions (Fig. 1, Supplementary Fig. 2, Supplementary Table
 212 1). This made it possible to have much finer estimates of the limits of the thermal niches of the different
 213 ESTUs than in the study performed by Farrant et al. (2016), in particular for the cold adapted ESTUs,
 214 which were poorly represented in the initial *Tara* Oceans dataset (Supplementary Fig. 2).

215 This analysis showed that the CRD1B ESTU displayed a reduced thermal tolerance range in the
 216 environment (14 to 24.5°C) compared to the BIOS-U3-1 strain in culture (12 to 29°C), while the typical
 217 cold thermotypes colonized larger thermal niches *in situ* than their representative strains (Fig. 1A).
 218 Environmental realized niches indeed ranged from 2.5 to 24°C for ESTU IA (compared to 8 to 25°C
 219 for MVIR-18-1) and from 8.5 to 25°C for ESTU IVA (compared to 12 to 29°C for BL107).
 220 Interestingly, the median temperature of the CRD1B ESTU is 3°C higher than that observed for ESTUs
 221 IA and IVA.

222 As concerns warm thermotypes, CRD1C displayed a fairly narrow thermal tolerance range *in situ*
 223 (22 to 29.5°C), which, similar to the cold thermotype CRD1B, was even narrower than for its
 224 representative strain BIOS-E4-1 (18 to 30°C; Fig. 1B). Comparatively, the CRD1A ESTU was detected
 225 across a wider temperature range (14 to 30.5°C) than the other two CRD1 ESTUs and also slightly
 226 larger than the corresponding strain in culture (MITS9220, 16 to 31°C). Still, the most extended
 227 temperature range was observed for ESTU IIA and IIIA (12 to 32°C) that reached significantly lower
 228 temperature limits than the corresponding clade II (16 to 32-34°C) and III (16 to 32°C) strains. Of note,
 229 although both IIA and IIIA ESTUs displayed a similar temperature range, the median temperature of
 230 ESTU IIA (25°C) was about 3°C higher than that of ESTU IIIA (22°C) and the maximum median
 231 temperature was surprisingly observed for the CRD1C ESTU (26.5°C). In this context, it is also worth
 232 mentioning that although clade II strains are clearly both warm thermotypes, M16.1 displays a

233 significantly higher temperature limit for growth than A15-62 and more generally than all other strains.
 234 This suggests that ESTU IIA may encompass two distinct ESTUs, but such a high temperature niche
 235 (>32 °C) where they could be discriminated is exceptional and not available in our dataset
 236 (Supplementary Table 1).

237 **Comparative genomics**

238 In order to assess whether the cold, temperate thermotype BIOS-U3-1 (CRD1B) exhibits similar
 239 adaptation mechanisms to those previously described for the cold-adapted clades I and/or IV, we
 240 examined a number of clusters of likely orthologous genes (CLOGs) from all *Synechococcus* genomes
 241 belonging to clades I-IV and CRD1 present in the Cyanorak v2.1 information system (Garczarek et al.,
 242 2021). First, we looked for the occurrence of two amino-acid substitutions in phycocyanin α - (RpcA)
 243 and β -subunits (RpcB), which were shown to differ between cold- (Gly in clades I and IV for RpcA43;
 244 Ser in RpcB42) and warm-thermotypes (Ala in clades II and III for RpcA43; Asp in RpcB42), these
 245 substitutions being potentially responsible for the differential thermotolerance of this phycobiliprotein
 246 between thermotypes (Pittera et al., 2017). In all three CRD1 strains, both sites displayed the warm-
 247 type residue (Supplementary Fig. 3A-B), suggesting that in contrast to typical cold and warm
 248 thermotypes, the molecular flexibility of this phycobiliprotein does not differ between CRD1
 249 thermotypes. We then looked at fatty acid desaturases that are essential for regulating membrane
 250 fluidity and thus the activity of integral membrane proteins, including photosynthetic complexes
 251 (Mikami and Murata, 2003; Pittera et al., 2018; Breton et al., 2020). All three CRD1 strains surprisingly
 252 possess in addition to the core $\Delta 9$ -desaturase gene *desC3*, a second $\Delta 9$ -desaturase, *desC4*
 253 (Supplementary Table 2), previously thought to be specific to cold-adapted strains as well as the $\Delta 12$ -
 254 desaturase *desA3* found in both cold-adapted clades I and IV as well as in clade III, a warm thermotype
 255 subjected to much stronger seasonal variability than its (sub)tropical clade II counterparts (Pittera et
 256 al., 2018). Furthermore, BIOS-U3-1 also possesses *desA2*, thought to be specific to warm
 257 environments, while this gene is in contrast absent from the other two CRD1 warm-adapted strains.
 258 Thus, CRD1 strains exhibit a different desaturase gene set and potentially display a larger capacity to
 259 regulate membrane fluidity than typical cold- or warm-adapted thermotypes. Finally, while all clades
 260 I, III and IV genomes possess the *ocp* operon, involved in the protection of PSII against
 261 photoinactivation through the dissipation of excess light energy (Kirilovsky, 2007) and which was
 262 recently shown in marine *Synechococcus* to play a key role at low temperature (Six et al., 2021), none
 263 of the three CRD1 genomes possess this operon.

264

265 **Photosynthetic activity and pigment content**

266 PSII quantum yield (F_V/F_M), used as a proxy of photosynthetic activity, was measured for each strain
 267 over their whole temperature growth range. Most strains displayed a decrease in this parameter at both
 268 low and high boundary limits of their growth temperature range and this effect was particularly striking
 269 for BIOS-U3-1, reaching values down to 0.11 at 14°C and 0.32 at 28°C (Fig. 2A). Besides MVIR-18-
 270 1 that exhibited a quite constant F_V/F_M over its whole temperature range, the decrease in F_V/F_M at high
 271 temperature was stronger for cold than warm thermotypes that are able to maintain a quite high F_V/F_M
 272 in the warmest growth conditions (Fig. 2B). Finally, as for growth rate, CRD1 strains exhibited lower
 273 F_V/F_M at all temperatures than clade I to IV strains.

274 The $\text{Exc}_{495:550\text{nm}}$ fluorescence excitation ratio, used as a proxy for PUB:PEB ratio, was consistent
 275 with the pigment type of each strain (Humily et al., 2013; Table 1; Supplementary Fig. 4A). This ratio
 276 remained pretty constant over the whole temperature range for all strains except for the chromatic
 277 acclimator BL107 (pigment type 3dA), for which a sharp increase was observed at its maximal growth
 278 temperature (28°C) to reach a value (1.35) intermediate between that typically observed in green light
 279 (or white light; 0.6-0.7) and blue light (1.6-1.7). This suggests that the chromatic acclimation process
 280 could be affected by growth temperature, at least in this strain. The phycobilisome (PBS) rod lengths
 281 and the degree of coupling of PBS to PSII reaction center chlorophylls, as estimated from PE:PC and
 282 PE:TA ratios respectively, showed fairly limited variations over the temperature range, indicating that
 283 the phycobiliprotein composition of PBS is quite stable over the growth temperature range of each
 284 strain (Supplementary Fig. 4B-C). One notable exception was a rise in both ratios for strain A15-62 at
 285 its minimal growth temperature, likely attributable to the partial decoupling of individual
 286 phycobiliproteins and of the whole PBS from PSII, a phenomenon typically observed under stressful
 287 conditions (Six et al., 2007; Guyet et al., 2020). It is also worth noting that MITS9220 and to some
 288 extent BIOS-E4-1, exhibited a significantly higher PE:PC ratio than the five other strains, potentially
 289 indicating a different phycobiliprotein composition and/or length of PBS rods.

290 In terms of liposoluble pigments, the β -carotene/chlorophyll *a* (β -car/Chl *a*) ratio tended to
 291 increase with temperature in BIOS-E4-1 and MITS9220, as observed for the other warm thermotypes,
 292 whilst this ratio was more stable in the cold thermotypes BIOS-U3-1 and BL107, and seemed to slightly
 293 increase in the lower part of the thermal range for the clade I strain MVIR-18-1 (Fig. 3). For all strains,

294 these ratios result from a concomitant increase with temperature of Chl *a* and β -car content per cell
295 (Supplementary Fig. 5), indicating an enhancement of the surface of thylakoids per cell at higher
296 temperatures that was particularly marked for BIOS-E4-1 and A15-62, whilst this variation was fairly
297 limited in the other two CRD1 strains. As these two pigments are present in different proportions in
298 PSI and II (Umena et al., 2011; Xu and Wang, 2017), the higher β -car/Chl *a* ratio measured in clades
299 I and IV strains also suggests that they may have a higher PSII:PSI ratio than all other strains, including
300 BIOS-U3-1, and that this ratio might be more strongly affected by temperature in warm than cold
301 thermotypes.

302 As concerns the zeaxanthin/chlorophyll *a* (Zea/Chl *a*) ratio, although an increase in this ratio was
303 measured at low temperature for all strains, the amplitude was globally larger for cold than for warm
304 thermotypes, with BIOS-U3-1 behaving very similarly to the clade IV strain BL107 that exhibits the
305 largest variation in this ratio (Fig. 3). Changes in this ratio likely originate partially from the decrease
306 in Chl *a* content in response to cold, a strategy typically used by cells to regulate light utilization under
307 slow growth conditions (Inoue et al., 2001). However, several strains also displayed an increase in their
308 Zea content per cell at low temperature, a response particularly striking in BIOS-U3-1 and A15-62, but
309 that also seems to occur in M16.1 and in the two other CRD1 strains BIOS-E4-1 and MITS9220
310 (Supplementary Fig. 5). Thus, although Zea has been hypothesized to be involved in the
311 photoprotection of cold-adapted strains by dissipating excess light energy under low temperature
312 conditions (Kana et al., 1988; Breton et al., 2020), this process seems to be present in both cold and
313 warm-adapted CRD1 strains and in most warm thermotypes as well. In this context, it is also worth
314 noting that the two clade II strains, A15-62 and M16.1, displayed fairly distinct temperature-induced
315 variations in their Zea:Chl *a* ratios and individual pigment contents, possibly linked to their different
316 isolation temperatures (see discussion below).

317 **Photosystem II repair capacity**

318 The ability of the different strains to repair PSII in response to light stress ($375 \mu\text{E m}^{-2} \text{s}^{-1}$) was
319 determined in cultures acclimated to 18, 22 and 25°C by measuring changes in $F_v:F_M$ over time after
320 adding the protein synthesis inhibitor lincomycin, or not (Supplementary Fig. 6). While a decrease in
321 $F_v:F_M$ ratio during the 90 min light stress period was observed in both cultures supplemented with
322 lincomycin and controls, this ratio only re-increased back up to initial $F_v:F_M$ values, after shifting
323 cultures back to standard light conditions ($75 \mu\text{E m}^{-2} \text{s}^{-1}$), in the control group in most strains and

324 temperature conditions. Thus, all studied strains were able to recover from this light stress, as long as
325 the D1 repair cycle was not inactivated by inhibition of protein synthesis. Yet, a fast decrease in $F_V:F_M$
326 was observed for all three CRD1 cultures supplemented with lincomycin, while the +/- lincomycin
327 curves overlapped during the first 15-30 min of light stress in most other strains and conditions. This
328 suggests that the initial decrease in $F_V:F_M$ in clades I-IV strains was not due to D1 damage but rather
329 to dissipation of light energy as heat through non-photochemical quenching (Campbell et al., 1998),
330 whilst the damage and hence repair of D1 proteins only occurred later on.

331 The PSII repair rate (R_{PSII}), as calculated from the time course of $F_V:F_M$ with and without
332 lincomycin, increased with temperature in most strains, except for BIOS-U3-1 that displayed its highest
333 rate at 22°C (Fig. 4). Strikingly, all three CRD1 strains displayed significantly higher R_{PSII} than clade
334 I-IV strains at all three tested temperatures, a difference ranging from 3- to nearly 40-fold at the lowest
335 common temperature (18°C). Furthermore, CRD1 strains displayed fairly limited variation in R_{PSII} with
336 temperature (ranging from 1.33 to 1.87-fold) compared to the other strains, the strongest increase in
337 R_{PSII} being observed for the clade I strain MVIR-18-1 (21.5-fold) and the clade III strain WH8102 (5.5-
338 fold). This indicates that CRD1 strains exhibit a constitutively high level of PSII repair compared to
339 the other strains whatever the growth temperature and only trigger a moderate increase in R_{PSII} in
340 response to temperature variations.

341

342 **DISCUSSION**

343 Temperature constitutes one the strongest driving factors that have shaped genetic diversification and
344 niche partitioning in marine cyanobacteria (Scanlan et al., 2009; Flombaum et al., 2013; Biller et al.,
345 2015) and phytoplankton at large (Sunagawa et al., 2015; Delmont et al., 2020). While temperature has
346 caused one major diversification event in *Prochlorococcus*, resulting in the divergence of the cold-
347 adapted HLI from the warm-adapted HLII clades (Johnson et al., 2006; Kettler et al., 2007), several
348 independent temperature-related diversification events also occurred in the *Synechococcus* SC 5.1
349 radiation, leading to the emergence of clades I and IV (Dufresne et al., 2008; Zwirgmaier et al., 2008).
350 Here, determination of the temperature optimum and boundary limits (i.e., the fundamental niche) of
351 strains representative of the three CRD1 ESTUs identified in the field (Farrant et al., 2016) showed
352 that different thermotypes can also be delineated within the CRD1 clade, which dominates the
353 *Synechococcus* populations in low-Fe areas of the world Ocean. Comparison with representative strains

354 of the cold-adapted *Synechococcus* ecotypes (clades I and IV) on the one hand, and warm-adapted
 355 ecotypes (clades II and III) on the other, made it possible to classify i) the CRD1A strain MITS9220,
 356 isolated from the equatorial Pacific Ocean, as a warm thermotype, ii) the CRD1B strain BIOS-U3-1,
 357 isolated from the Chilean upwelling, as a cold temperate thermotype, and finally iii) the CRD1C strain
 358 BIOS-E4-1, isolated from the edge of the South Pacific gyre, a stable, warm, Fe-depleted oceanic
 359 region (Claustre et al., 2008), as a warm temperate stenotherm.

360 As expected from theory (Pearman et al., 2008), the realized environmental thermal niches of
 361 CRD1 ESTUs were narrower than their fundamental niches (or similar for MITS9220). In contrast, for
 362 ESTUs IA to IVA, the realized environmental niche was significantly more extended towards the low
 363 thermal limit than the fundamental niches of their representative strains, this expansion being
 364 particularly marked for ESTU IA (Fig. 1). This could be due to passive transport of *Synechococcus*
 365 populations by currents into water masses colder than their temperature limits for growth.
 366 Alternatively, these ESTUs may exhibit a greater microdiversity than previously assessed (Farrant et
 367 al., 2016) and could be subdivided into distinct ESTUs occupying slightly different thermal niches
 368 from the current ones, although representative strains to test this hypothesis remain to be isolated. In
 369 agreement with the latter hypothesis Paulsen et al. (2016) measured a positive growth rate of
 370 *Synechococcus* natural populations dominated by clade I in waters as cold as 2°C in the vicinity of the
 371 Svalbard island. Thus, CRD1 ESTUs appear to be strongly outcompeted by their ESTU IA to IVA
 372 counterparts at their lower temperature limits. Consistent with this, comparison of their gene content
 373 showed that CRD1 ESTUs, including the cold thermotype CRD1B, lack the main adaptation
 374 mechanisms reported so far for typical cold thermotypes. Indeed, all CRD1 strains examined in this
 375 study i) exhibit warm-type substitutions in their α and β -phycocyanin subunits, influencing the
 376 thermotolerance of this phycobiliprotein (Pittera et al., 2017; Supplementary Fig. 3A-B); ii) possess a
 377 different set of desaturase genes, involved in regulation of membrane fluidity (Pittera et al., 2018), than
 378 typical warm and cold thermotypes (Supplementary Table 2) and iii) lack the OCP system, involved
 379 in the protection of PSII against photoinactivation, which seemingly plays a key role at low temperature
 380 (Kirilovsky, 2007; Six et al., 2021). Still, we cannot exclude that CRD1 strains could use alternative
 381 strategies to cope with temperature variations and notably to deal with the generation of reactive
 382 oxygen species, known to be generated by a variety of factors including low and high temperature
 383 (Nishiyama et al., 2006; Latifi et al., 2009). For instance, all CRD1 strains possess the *srxA* gene
 384 encoding sulfiredoxin catalyzing the reduction of 2-Cys peroxiredoxin involved in H₂O₂ detoxification

385 (Findlay et al., 2005; Guyet et al., 2020) as well as *isiA* that, besides its role in increasing the light-
 386 harvesting efficiency of PSI under conditions of Fe-limitation, was also shown to provide
 387 photoprotection to PSII by dissipating excess light energy under oxidative stress conditions
 388 (Yeremenko et al., 2004; Ihalainen et al., 2005; Supplementary Table 2).

389 The ability of cyanobacteria to grow over a large temperature range largely relies on their capacity
 390 to optimize the functioning of their photosynthetic apparatus, notably at low temperature that induces
 391 a general slowing down of cell metabolism (Murata et al., 2007; Pittera et al., 2014, 2017). For this
 392 reason, we also compared the photophysiology of CRD1 and clades I-IV strains at three growth
 393 temperatures common to all strains. These analyses showed that all three CRD1 strains exhibit a lower
 394 growth rate at most temperatures than clade I to IV strains (Fig. 1), possibly explaining why they are
 395 easily outcompeted by other taxa when iron is no longer limiting, as observed for instance around the
 396 Marquesas Islands (Caputi et al., 2019). Moreover, CRD1 strains also display a lower PSII maximum
 397 quantum yield (Fig. 2), suggesting that PSII is partially photoinactivated, that is their D1 repair cycle
 398 does not fully compensate damage to this protein, even under optimal growth temperatures. Consistent
 399 with this, the very high turnover rate of the D1 protein measured in all CRD1 strains indicates that their
 400 PSII is much more sensitive to light stress than other strains and can only trigger a moderate increase
 401 in R_{PSII} in response to both light and temperature variations, possibly indicating that they are adapted
 402 to live deeper in the water column than clades I to IV. This sensitivity could be partially linked to the
 403 abovementioned absence of the OCP, potentially reducing their ability to dissipate excess light energy,
 404 although it must be noted that the clade II strain A15-62 also lacks the OCP system. Interestingly in
 405 this context, all cold thermotypes including the CRD1B strain BIOS-U3-1, possess more copies of the
 406 D1:2 isoform (3-6 copies, average: 3.9 ± 1.1) than warm thermotypes (2-3 copies, average: 2.2 ± 0.4),
 407 this isoform providing a lower quantum yield but higher PSII resistance to photoinhibition than D1:1
 408 (Supplementary Table 2; Clarke et al., 1993a, 1993b; Campbell et al., 1995; Garczarek et al., 2008).
 409 Moreover, A15-62 is one of the only *Synechococcus* strains to possess two complete copies of the D1:1
 410 isoform, a duplication which could partly explain why, despite the absence of OCP, this atypical clade
 411 II strain is able to maintain a high PSII quantum yield over its whole growth temperature range with
 412 fairly low D1 repair rates. Interestingly, CRD1 strains also possess a paralog of *psbN*, which was found
 413 to be required for the assembly of the PSII reaction centre in *Nicotiana tabacum* and would play an
 414 important role in the D1 repair cycle (Torabi et al., 2014).

415 Taken together, both comparative genomics and photophysiological analyses highlighted a
416 number of specificities of CRD1 strains compared to their clade I-IV counterparts, rather than them
417 possessing traits distinctive of cold or warm thermotypes. In this context, it is worth noting that
418 although strains representative of ESTUs IVA and CRD1B exhibit a similar fundamental thermal niche
419 in culture, the higher median temperature of CRD1B in the field indicates that it preferentially thrives
420 in temperate waters (about 18°C, Fig. 1), where energetically costly temperature adaptation
421 mechanisms might not be essential. This suggests that for members of the CRD1 clade, adaptation to
422 low-Fe conditions likely prevails over adaptation to temperature variations, and/or that adaptation
423 mechanisms to temperature variations might be more complex and diversified than previously thought.
424 Still, in terms of a realized environmental thermal niche, the occurrence of several CRD1 thermotypes
425 likely explains why the CRD1 clade as a whole occupies most Fe-limited areas, a vast ecosystem
426 constituting about 30% of the world Ocean (Moore et al., 2013; Bristow et al., 2017). A notable
427 exception is the Southern Ocean, for which from the little available data shows that *Synechococcus* is
428 scarce south of the polar front (Wilkins et al., 2013; Farrant et al., 2016), consistent with the fairly high
429 low-temperature limit (14°C) of the CRD1B environmental realized niche (Fig. 1), while low-Fe
430 availability likely limits the growth of clades I and IV in this area. In contrast, CRD1 growth does not
431 currently appear to be limited by warm temperatures since most oceanic waters display a temperature
432 below 30°C (Supplementary Fig. 2B; Supplementary Table 1). However, one cannot exclude that with
433 global change, some areas of the world Ocean could become warmer than the highest limits determined
434 here for representative strains of CRD1A and C, i.e. 31°C and 30°C respectively. In this context, it is
435 worth mentioning that in the dataset used for this study, several coastal stations, sampled during the
436 OSD campaign reached 31.5 °C (Supplementary Table 1). Thus, although biogeochemistry global
437 models predict that *Synechococcus* could be one of the winners of the phytoplankton community in a
438 future world Ocean (Flombaum et al., 2013; Schmidt et al., 2020; Visintini et al., 2021), it might well
439 not be able to survive in the warmest low-Fe areas, an ecological niche that is currently expanding
440 (Polovina et al., 2008). Although a few studies have started to analyze the genomic bases of adaptation
441 of *Synechococcus* cells to Fe-limitation in the field (Ahlgren et al., 2020; Garcia et al., 2020), further
442 comparative genomic and physiological studies are still needed to decipher the specific capacity of
443 CRD1 clade members to deal with Fe-limitation which should help predict the future distribution and
444 dynamics of *Synechococcus* taxa in the world Ocean.

445

446 **DATA AVAILABILITY STATEMENT**

447 Unpublished metabarcoding data supporting the conclusions of this article are available as raw data
448 (SRA accession numbers) and processed data (number of *petB* reads per ESTU) in Supplementary
449 Table 1. The latter Table also encompasses the description of all environmental samples used in this
450 study.

451

452 **CONFLICT OF INTEREST**

453 The authors declare that the research was conducted in the absence of any commercial or financial
454 relationships that could be construed as a potential conflict of interest.

455

456 **AUTHOR CONTRIBUTIONS**

457 MF, HD and LG designed the experiments. MF, LD, HD, MR, AG, FRJ, TS, LC and GM collected the
458 samples and performed the physiological measurements. MF and DM ran the flow cytometry analyses.
459 FL isolated several CRD1 strains used in this study. HD, XX, DJS, HL and LG performed sequencing
460 and bioinformatics analyses of metabarcodes. MH, EC, FP and LG developed and refined the Cyanorak
461 v2.1 database. MF, HD, FP and LG made the figures. MF, LD, HD, FP and LG interpreted results. All
462 the authors contributed to the preparation of the manuscript, read and approved the final manuscript.

463

464 **FUNDING**

465 This work was supported by the French “Agence Nationale de la Recherche” Programs CINNAMON
466 (ANR-17-CE02-0014-01) and EFFICACY (ANR-19-CE02-0019) as the European program Assemble
467 Plus (H2020-INFRAIA-1-2016-2017; grant no. 730984).

468

469 **ACKNOWLEDGEMENTS**

470 We would like to thank Thierry Cariou for providing physico-chemical parameters from the SOMLIT-
471 Astan station and Gwenn Tanguy (Biogenouest genomics core facility) and Monica Moniz for their
472 sequencing of *petB* metabarcodes. We are also most grateful to Nathalie Simon for coordinating the
473 phytoplankton time-series at the SOMLIT-Astan station, Christophe Six for technical hints on PAM
474 fluorimetry and HPLC analyses as well as Priscillia Gourvil and Martin Gachenot from the Roscoff
475 Culture Collection (<http://roscoff-culture-collection.org/>) and Florian Humily for isolating and/or
476 maintaining the *Synechococcus* strains used in this study. We also thank the support and commitment
477 of the *Tara* Oceans coordinators and consortium, Agnès b. and E. Bourgois, the Veolia Environment
478 Foundation, Région Bretagne, Lorient Agglomération, World Courier, Illumina, the EDF Foundation,
479 FRB, the Prince Albert II de Monaco Foundation, the *Tara* schooner and its captains and crew.

480

481 **SUPPLEMENTARY MATERIAL**

482 **Supplementary Table 1** | Environmental samples used in this study for the determination of the
483 realized environmental niches of the major *Synechococcus* ESTUs

484 **Supplementary Table 2** | Phyletic pattern of CRD1 and clade I-IV genes mentioned in this study
485 retrieved from the Cyanorak v2.1 database

486 **Supplementary Figure 1. Isolation sites of the *Synechococcus* strains used in this study.** Isolation
487 site of each strain is indicated on the map by a bubble arrow colored according to their corresponding
488 ESTUs indicated below the strain name. Longhurst provinces (Longhurst A., 2007, Ecological
489 Geography of the Sea, Academic Press, London) are shown as a colored background shown in the
490 insert. Only provinces from which at least one strain has been isolated are indicated on the map using
491 the following abbreviations: PEQD (Pacific equatorial divergence, Pacific, Trade wind), CHIL (Chile-
492 Peru, current coastal province, Pacific, Coastal), NAST W (Northwest Atlantic subtropical gyral,
493 Atlantic, Westerly), CARB (Caribbean, Atlantic, Trade wind), MEDI (Mediterranean Sea, Atlantic,
494 Westerly), SARC (Atlantic sub-Arctic, Atlantic, Polar).

495 **Supplementary Figure 2. Oceanwide environmental data used in this study to determine the**
 496 **environmental realized thermal niches of the main ESTUs from clades CRD1 and I to IV. (A)**
 497 **Map of the sampling sites, (B) Relative abundance of *Synechococcus* ESTUs IA to IVA and CRD1A**
 498 **to C. (C) Temperature distribution of the sampling sites. The inserts specify (A, C) the name of**
 499 **campaigns or datasets analyzed and (B) the *Synechococcus* ESTUs.**

500 **Supplementary Figure 3. Alignment of RpcA and RpcB, encoding phycocyanin α - and b-**
 501 **subunits, from CRD1 and clades I-IV *Synechococcus* strains. (A) RpcA. (B) RpcB. Substitutions**
 502 **potentially involved in thermotolerance are shown by a red rectangle in the alignment.**

503 **Supplementary Figure 4. Variation with growth temperature of phycobilins and**
 504 **phycobiliproteins fluorescence excitation and emission ratios. (A) Average PUB:PEB ratios. (B)**
 505 **Average phycoerythrin (PE) to phycocyanin (PC) ratios. (C) Average PC to terminal acceptor (TA)**
 506 **ratios. The insert indicates the strain names, their corresponding ESTU (*sensu* Farrant et al., 2016) and**
 507 **pigment type (*sensu* Humily et al., 2013) between brackets.**

508 **Supplementary Figure 5. Variation with growth temperature of the three main liposoluble**
 509 **pigments per cell for CRD1 vs. clade I and IV strains. (A, D) Chlorophyll (Chl) *a* content (fg/cell).**
 510 **(B, E) Zeaxanthin (Zea) content (fg/cell). (C, F) β -carotene (β -car) content (fg/cell). (A-C) CRD1-B**
 511 **strain BIOS-U3-1 vs. cold thermotypes. (D-F) CRD1-A strain MITS9220 and CRD1-C strain BIOS-**
 512 **E4-1 vs. warm thermotypes. Inserts indicate the strain names and their corresponding ESTU (*sensu***
 513 **Farrant et al., 2016) between brackets.**

514 **Supplementary Figure 6. Time course of photosystem II quantum yield (F_v/F_M) following light**
 515 **stress in the presence or absence of lincomycin for CRD1 and clade I-IV strains acclimated to**
 516 **different temperatures. Cultures acclimated to $75 \mu\text{E m}^{-2} \text{s}^{-1}$ were shifted to $375 \mu\text{E m}^{-2} \text{s}^{-1}$ at T0 for**
 517 **90 min, then shifted back to the initial light conditions for 24h as indicated by a vertical dashed line on**
 518 **each figure. Strain names and their corresponding ESTU between brackets (*sensu* Farrant et al., 2016)**
 519 **are indicated on the right-hand side, acclimation temperatures are indicated on the top, whilst line**
 520 **colour indicates the lincomycin treatment (i.e. -/+ linco).**

521

522

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TABLES

729

730 **TABLE 1** | Characteristics of the *Synechococcus* strains used in this study

Strains name	MVIR-18-1	A15-62	M16.1	WH8102	BL107	BIOS-E4-1	BIOS-U3-1	MITS9220
RCC # ¹	2385	2374	791	539	515	2534	2533	2571
Subcluster ²	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
Clade ²	I	II	II	III	IV	CRD1	CRD1	CRD1
ESTU ²	IA	IIA	IIA	IIIA	IVA	CRD1C	CRD1B	CRD1A
Pigment type ³	3aA	3dB	3a	3c	3dA	3cA	3dA	3dA
Ocean	Atlantic	Atlantic	Atlantic	Atlantic	Med. Sea	Pacific	Pacific	Pacific
Region	North Sea	Offshore Mauritania	Gulf of Mexico	Caribbean Sea	Balearic Sea	South East Pacific	Chile upwelling	Equatorial Pacific
Isolation latitude	61°00' N	17°37' N	27°70' N	22°48' N	41°72' N	31°52' S	34°00' S	0°00' N
Isolation longitude	1°59' E	20°57' W	91°30' W	65°36' W	3°33' E	91°25' W	73°22' W	140°00' W

731 ¹Roscoff Culture Collection, ²Farrant et al. (2016), ³Humily et al. (2013).

732

FIGURE LEGENDS

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735 **FIGURE 1 | Fundamental thermal niches of CRD1 vs. clade I, II, III and IV strains and environmental**
 736 **realized niches of the corresponding ESTUs. (A)** CRD1-B strain BIOS-U3-1 vs. cold thermotypes. **(B)**
 737 CRD1-A strain MITS9220 and the CRD1-C strain BIOS-E4-1 vs. warm thermotypes. The insert indicates
 738 the strain names and their corresponding ESTU (*sensu* Farrant et al., 2016) between brackets. Each
 739 data point is the average of at least 3 biological replicates. Environmental realized niches are indicated
 740 as horizontal boxplots for each ESTU above each graph with the whiskers corresponding to 1.5-fold
 741 the interquartile range and outliers being plotted as individual points.

742

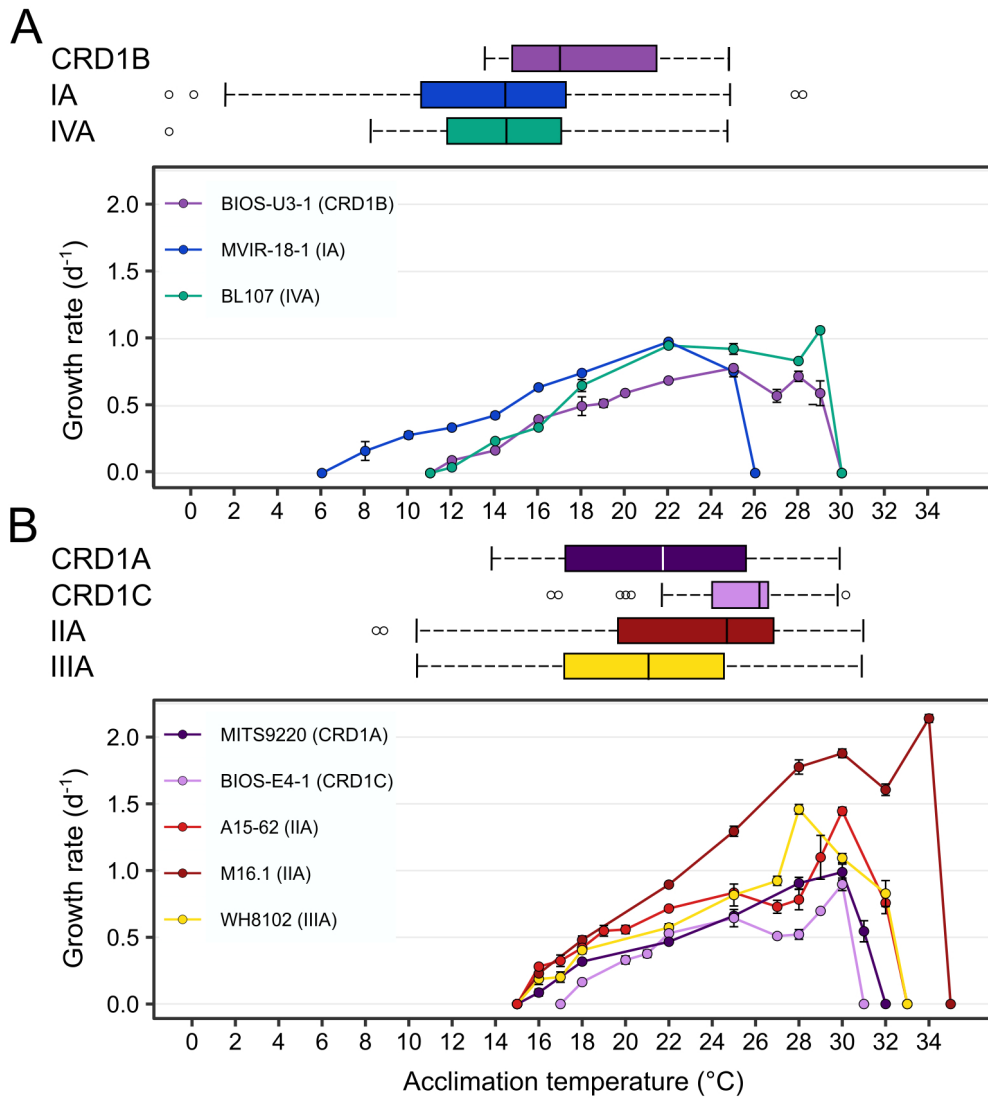
743 **FIGURE 2 | Variation with growth temperature of photosystem II quantum yield (F_v/F_M) for CRD1**
 744 **vs. clade I, II, III and IV strains. (A)** CRD1-B strain BIOS-U3-1 vs. cold thermotypes. **(B)** CRD1-A strain
 745 MITS9220 and the CRD1-C strain BIOS-E4-1 vs. warm thermotypes. The insert indicates the strain
 746 names and their corresponding ESTU (*sensu* Farrant et al., 2016) between brackets.

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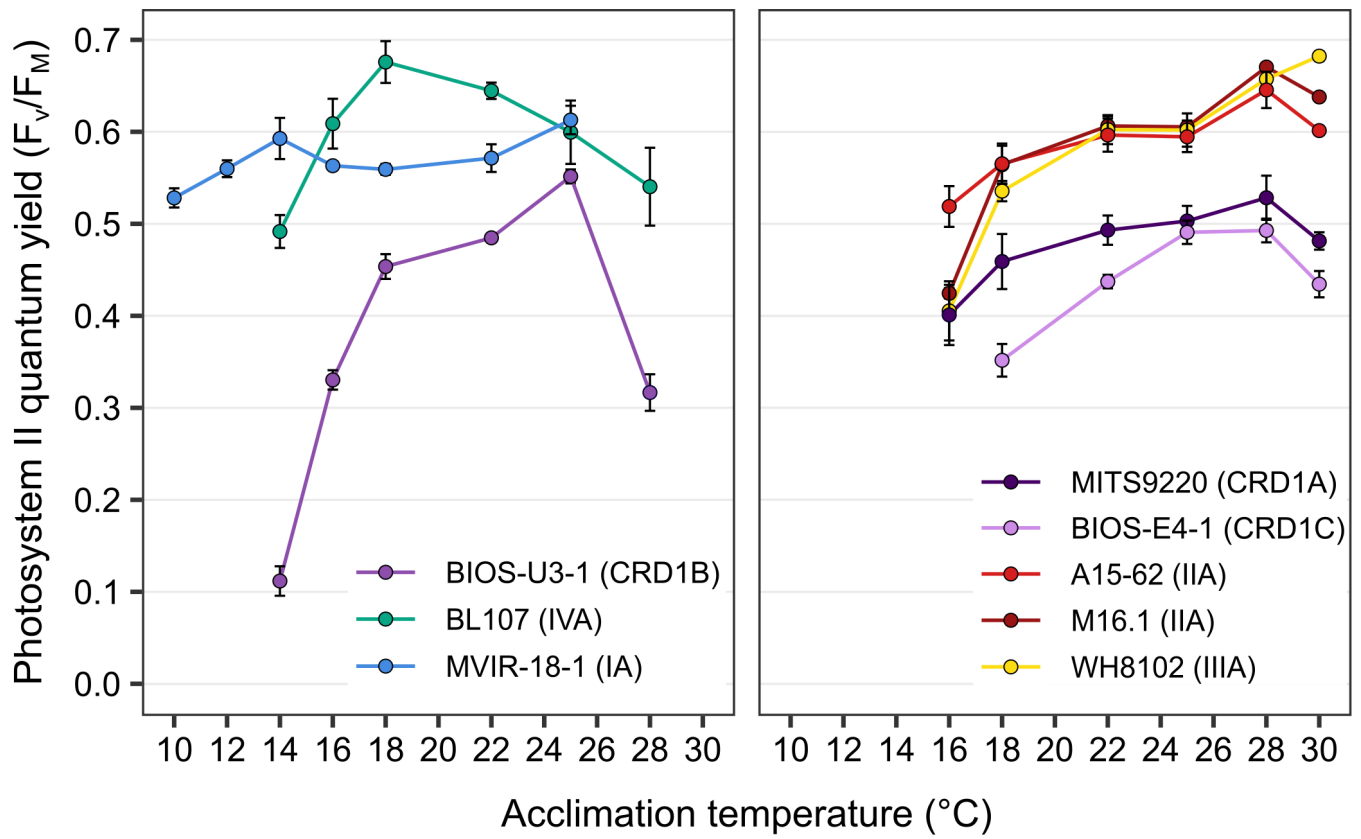
748 **FIGURE 3 | Variation with growth temperature of cellular mass pigment ratios of CRD1 vs. clade I,**
 749 **II, III and IV strains. (A, B)** β -carotene (β -car) to chlorophyll *a* (Chl*a*) ratio. **(C, D)** Zeaxanthin (Zea) to
 750 Chl*a* ratio with **(A, C)** CRD1-B strain BIOS-U3-1 vs. cold thermotypes and **(B, D)** CRD1-A strain
 751 MITS9220 and the CRD1-C strain BIOS-E4-1 vs. warm thermotypes. Inserts indicate strain names and
 752 their corresponding ESTUs (*sensu* Farrant et al., 2016) between brackets.

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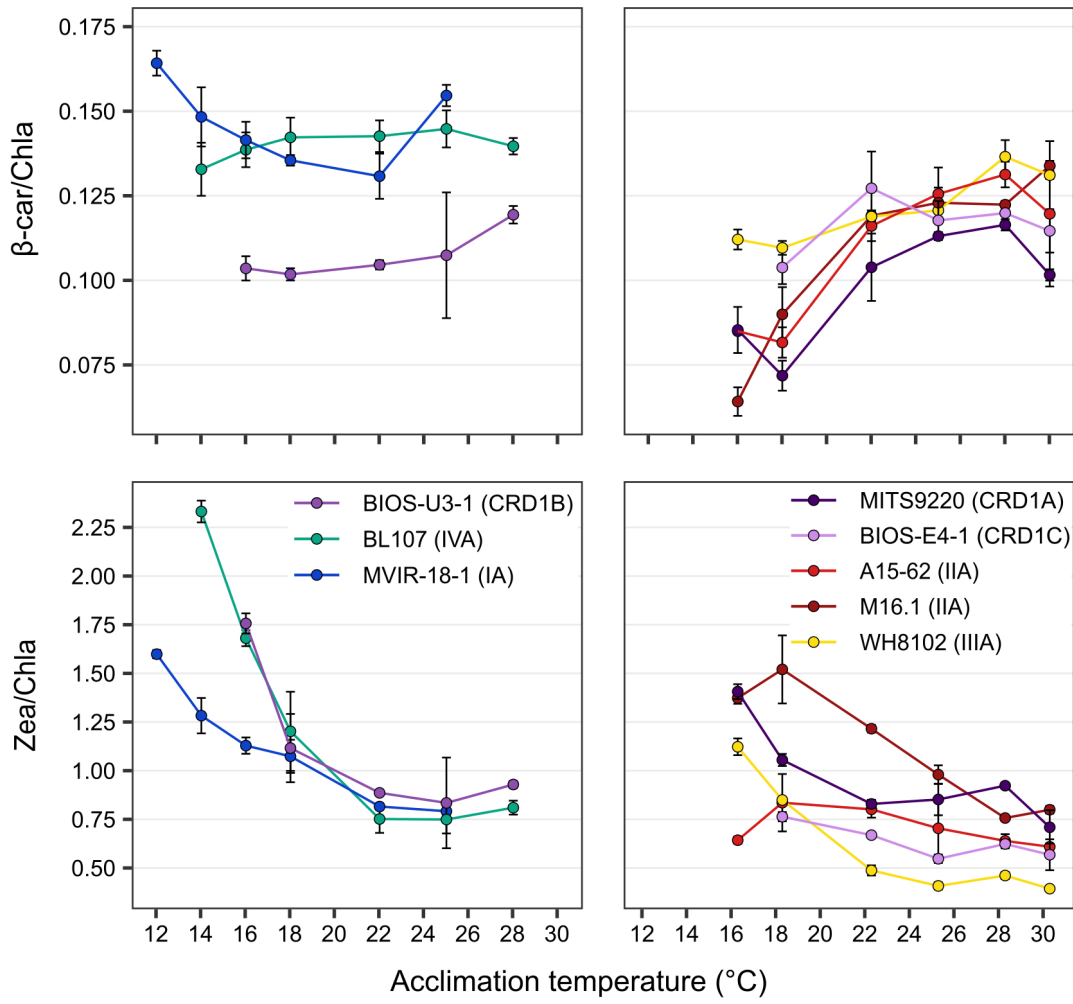
754 **FIGURE 4 | Variation with growth temperature of cumulative photosystem II repair rate (RPSII) of**
 755 **CRD1 vs. clade I, II, III and IV strains.** The insert indicates the strain names and their corresponding
 756 ESTU (*sensu* Farrant et al., 2016) between brackets.



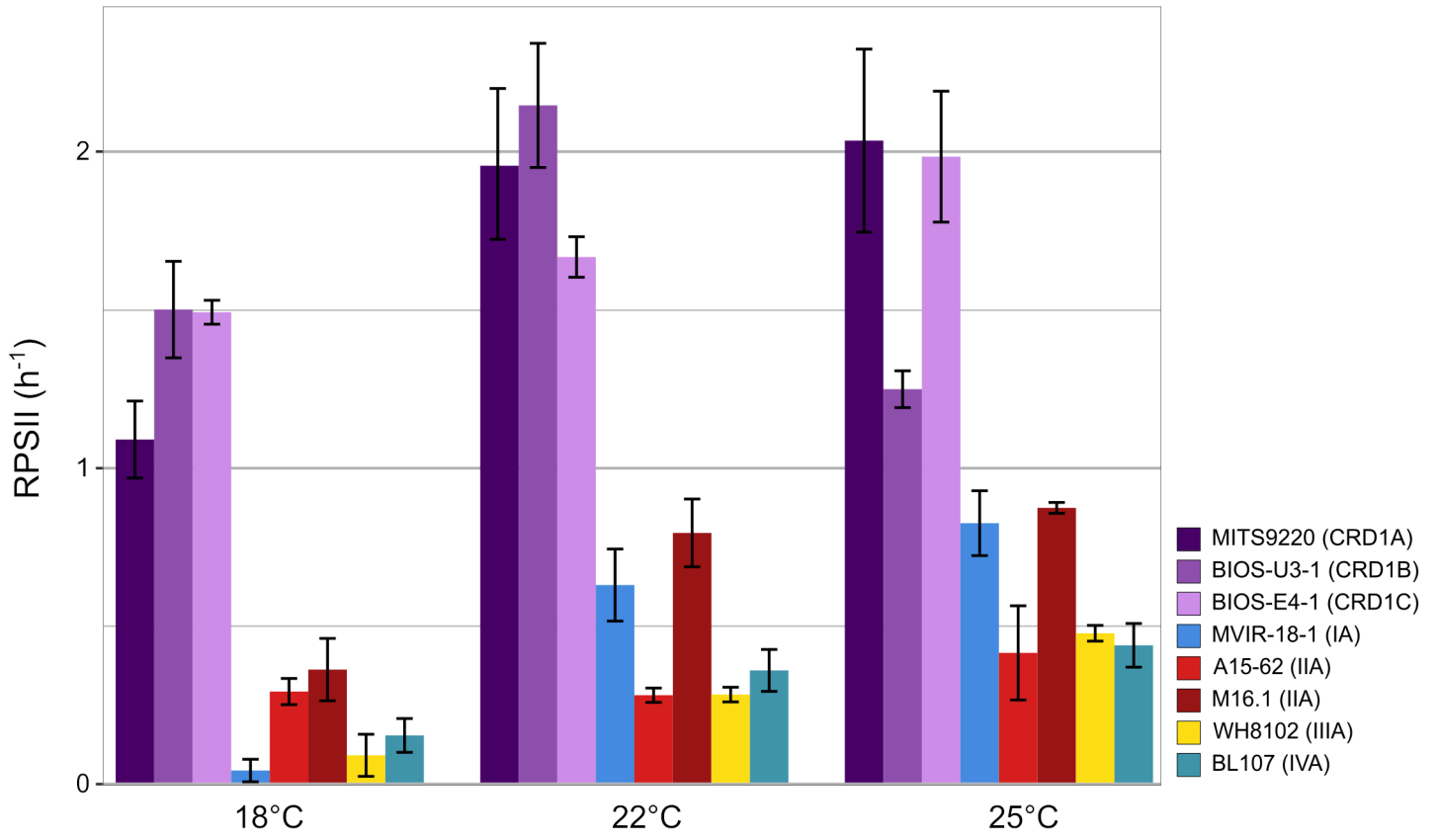
Ferrieux et al., Figure 1



Ferrieux et al., Figure 2



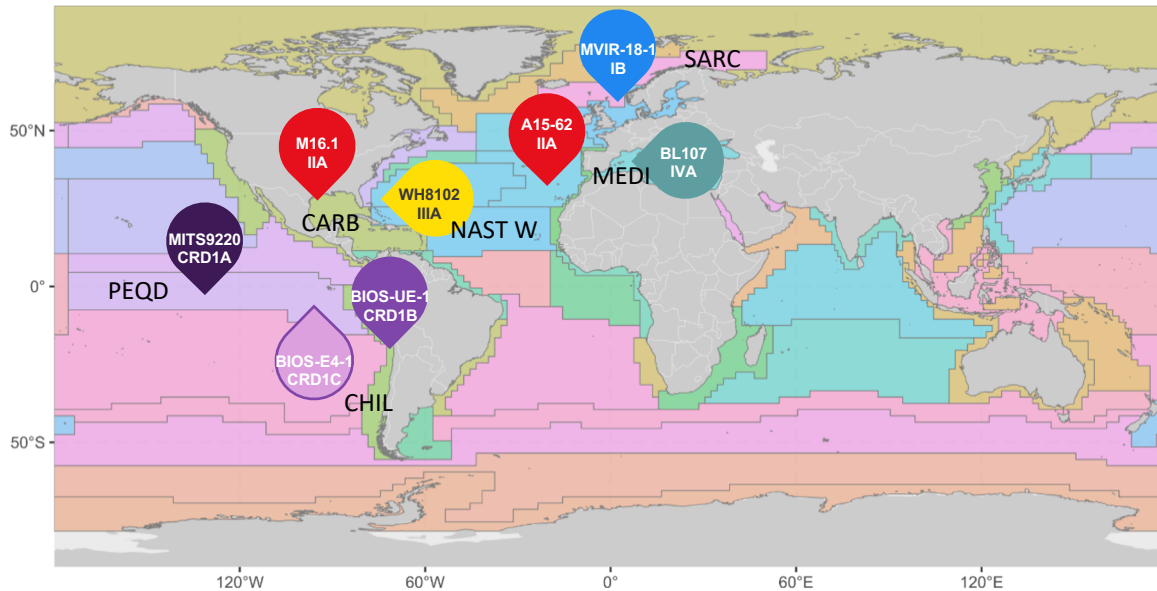
Ferrieux et al., Figure 3



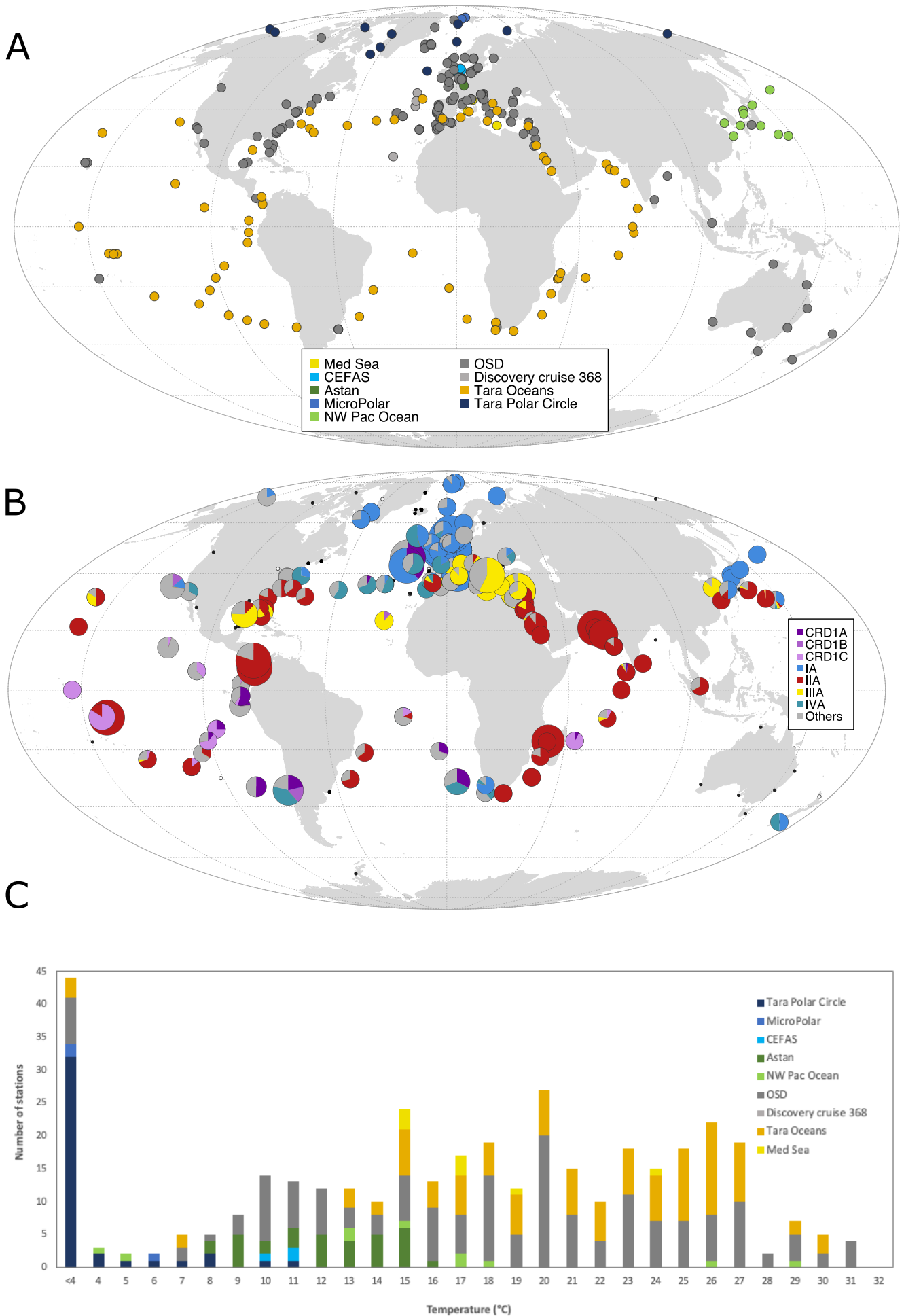
Ferrieux et al., Figure 4

Ferrieux et al.
Supplementary Figures





Supplementary Figure 1. Isolation sites of the *Synechococcus* strains used in this study. Isolation site of each strain is indicated on the map by a bubble arrow colored according to their corresponding ESTUs indicated below the strain name. Longhurst provinces (Longhurst A., 2007, *Ecological Geography of the Sea*, Academic Press, London) are shown as a colored background shown in the insert. Only provinces from which at least one strain has been isolated are indicated on the map using the following abbreviations: PEQD (Pacific equatorial divergence, Pacific, Trade wind), CHIL (Chile-Peru, current coastal province, Pacific, Coastal), NAST W (Northwest Atlantic subtropical gyral, Atlantic, Westerly), CARB (Caribbean, Atlantic, Trade wind), MEDI (Mediterranean Sea, Atlantic, Westerly), SARC (Atlantic sub-Arctic, Atlantic, Polar).

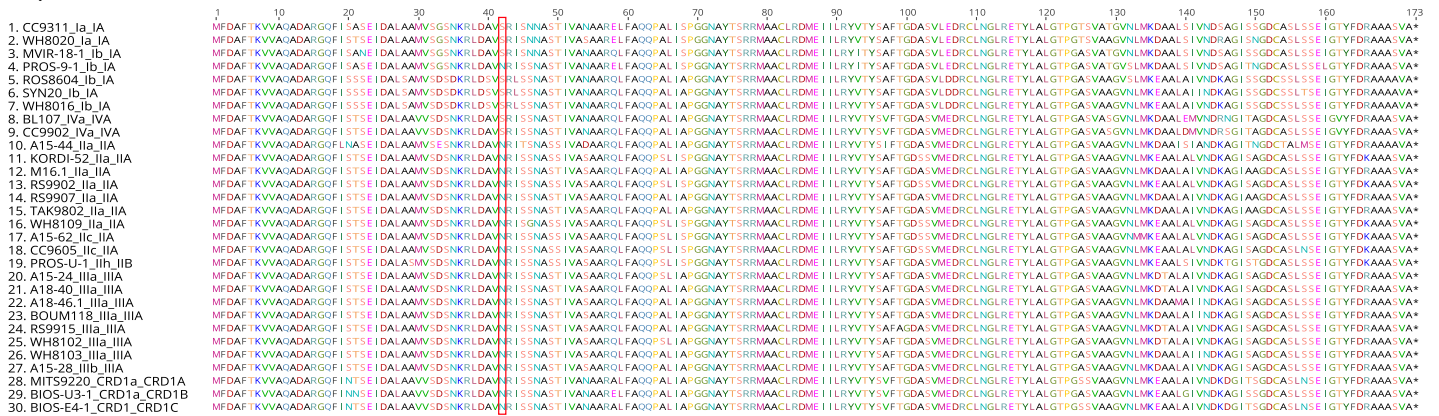


Supplementary Figure 2. Oceanwide environmental data used in this study to determine the environmental realized thermal niches of the main ESTUs from clades CRD1 and I to IV. (A) Map of the sampling sites, (B) Relative abundance of *Synechococcus* ESTUs IA to IVA and CRD1A to C. (C) Temperature distribution of the sampling sites. The inserts specify (A,C) the name of campaigns or datasets analyzed and (B) the *Synechococcus* ESTUs.

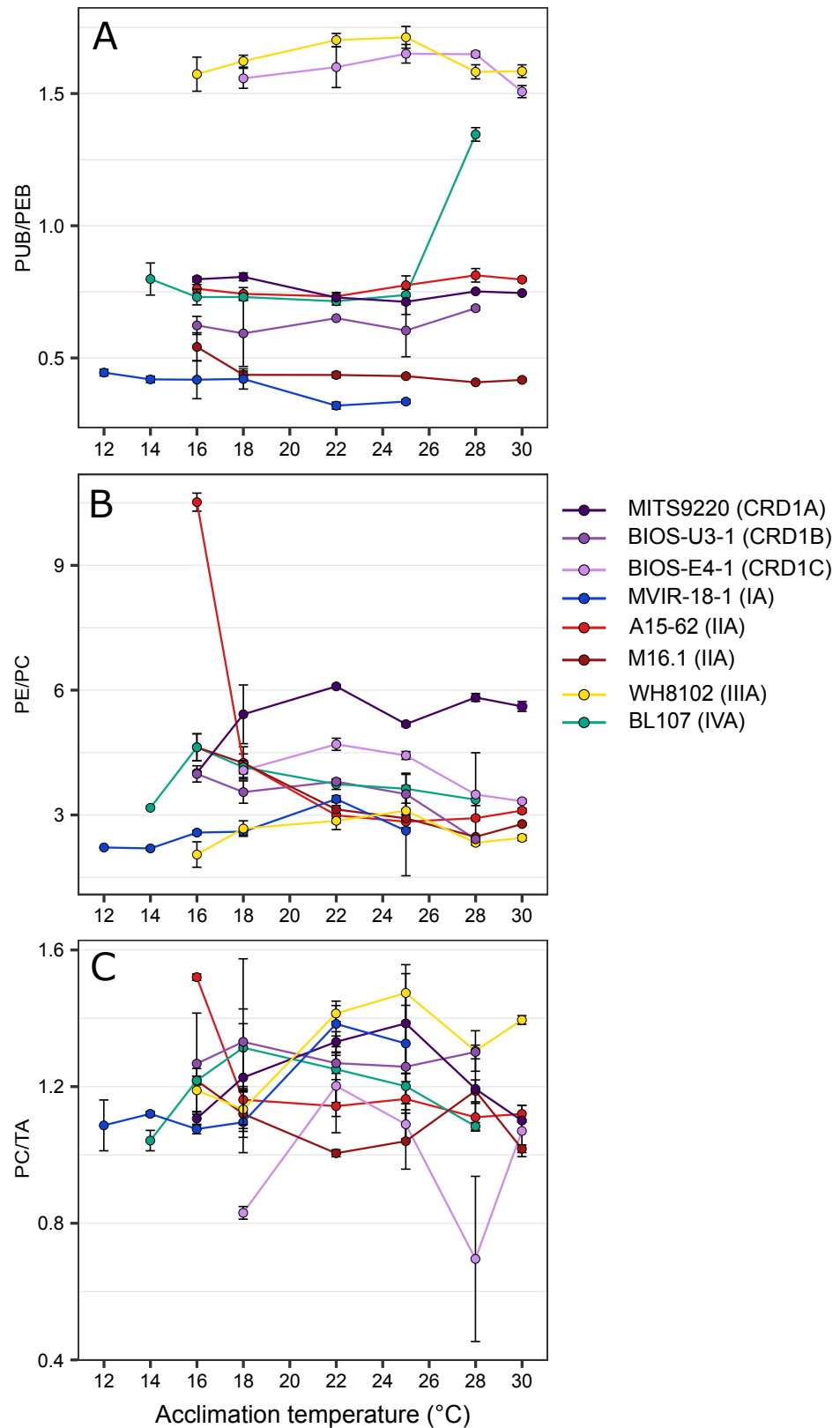
A. RpcA



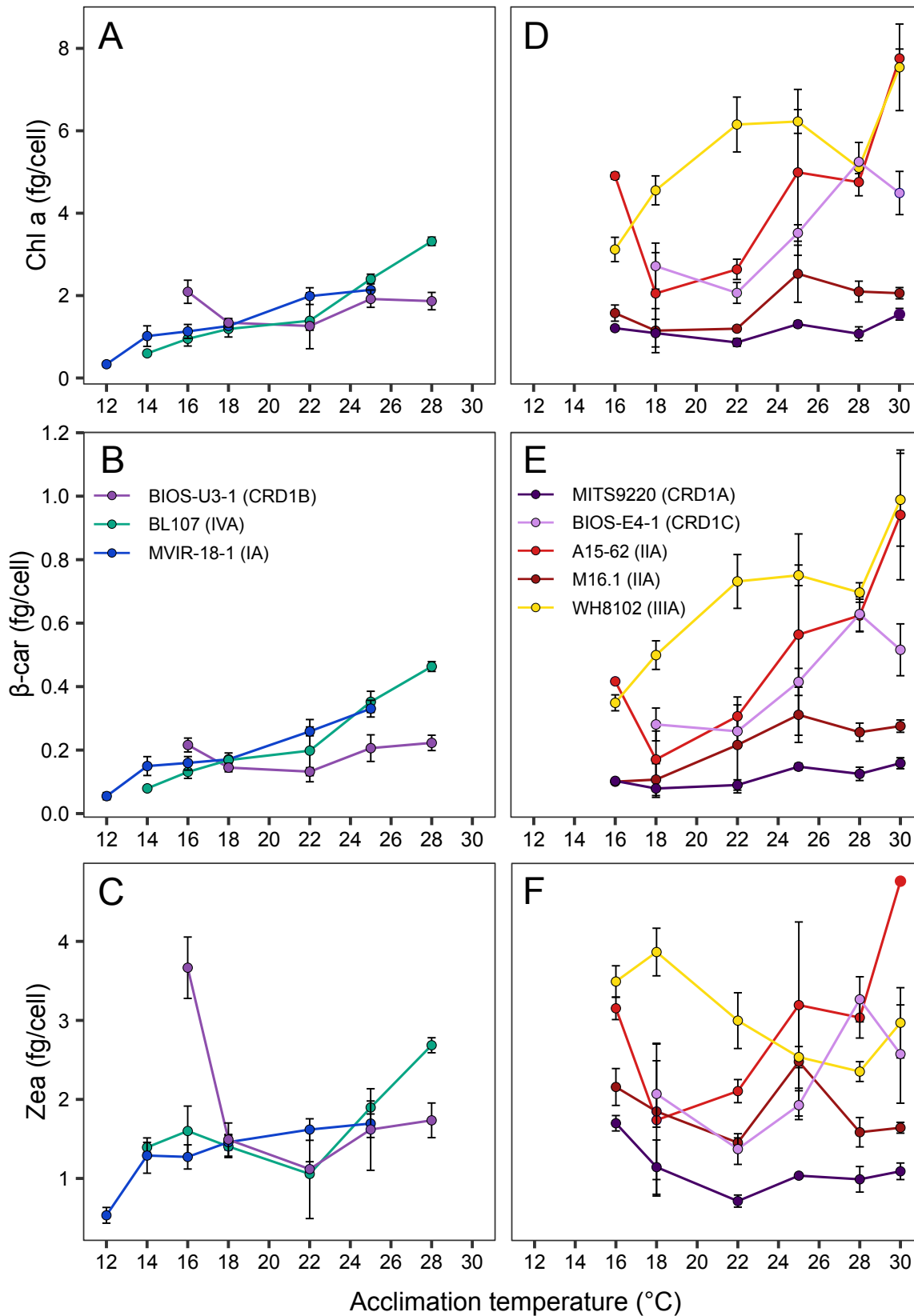
B. RpcB



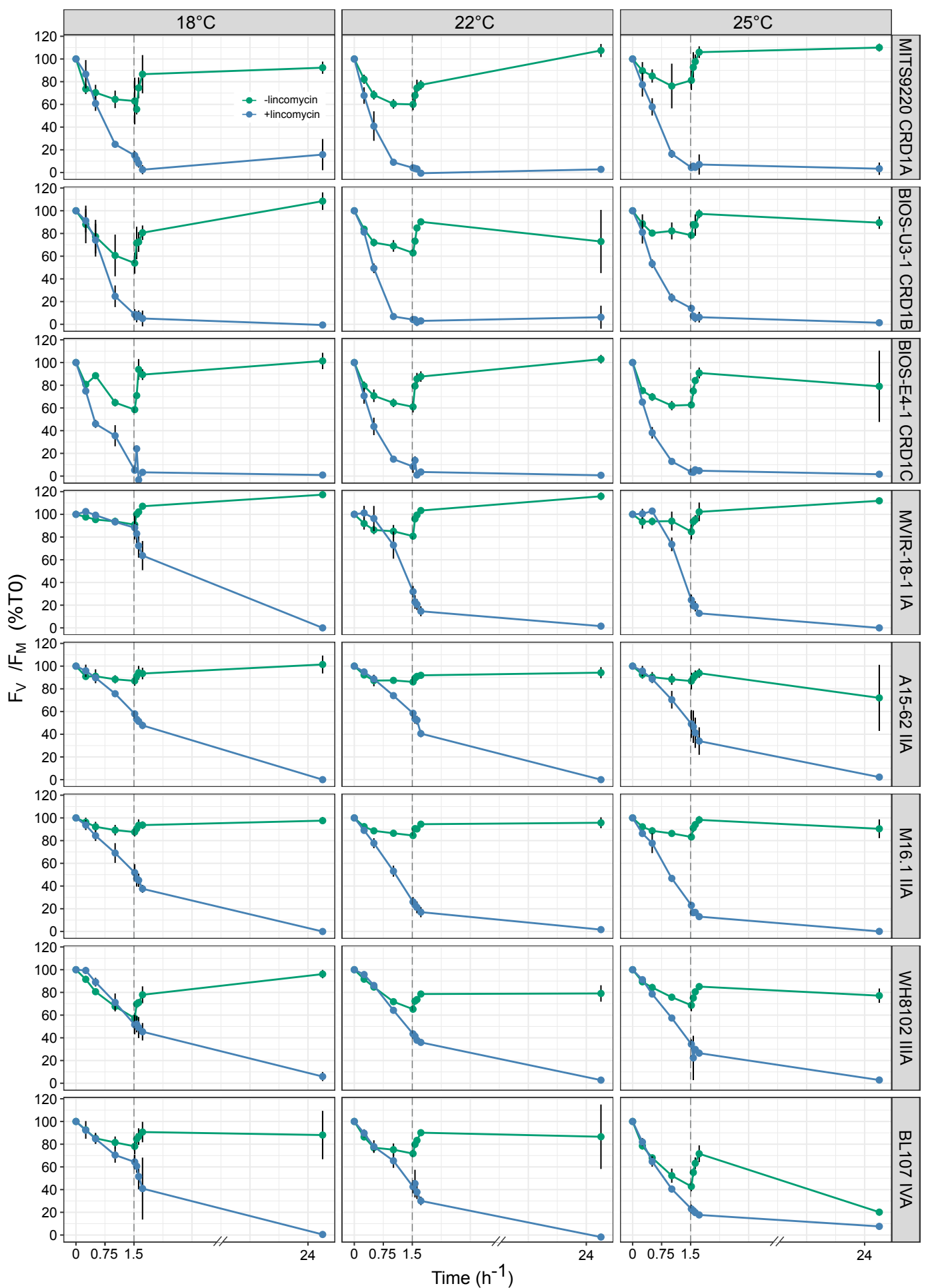
Supplementary Figure 3. Alignment of RpcA and RpcB, encoding phycocyanin α - and β -subunits, from CRD1 and clades I-IV *Synechococcus* strains. (A) RpcA. (B) RpcB. Substitutions potentially involved in thermotolerance are shown by a red rectangle in the alignment.



Supplementary Figure 4. Variation with growth temperature of phycobilins and phycobiliproteins fluorescence excitation and emission ratios. (A) Average PUB:PEB ratios. (B) Average phycoerythrin (PE) to phycocyanin (PC) ratios. (C) Average PC to terminal acceptor (TA) ratios. The insert indicates the strain names, their corresponding ESTU (sensu Farrant et al., 2016) and pigment type (sensu Humily et al., 2013) between brackets.



Supplementary Figure 5. Variation with growth temperature of the three main liposoluble pigments per cell for CRD1 vs. clade I and IV strains. (A, D) Chlorophyll (Chl) a content (fg/cell). (B, E) Zeaxanthin (Zea) content (fg/cell). (C, F) beta-carotene (beta-car) content (fg/cell). (A-C) CRD1-B strain BIOS-U3-1 vs. cold thermotypes. (D-F) CRD1-A strain MITS9220 and CRD1-C strain BIOS-E4-1 vs. warm thermotypes. Inserts indicate the strain names and their corresponding ESTU (*sensu* Farrant et al., 2016) between brackets.



Supplementary Figure 6. Time course of photosystem II quantum yield (F_v/F_m) following light stress in the presence or absence of lincomycin for CRD1 and clade I-IV strains acclimated to different temperatures. Cultures acclimated to $75 \mu\text{E m}^{-2} \text{s}^{-1}$ were shifted to $375 \mu\text{E m}^{-2} \text{s}^{-1}$ at T0 for 90 min, then shifted back to the initial light conditions for 24h as indicated by a vertical dashed line on each figure. Strain names and their corresponding ESTU between brackets (*sensu* Farrant et al., 2016) are indicated on the right hand side, acclimation temperature are indicated on the top, whilst line colour indicates the lincomycin treatment (i.e. -/+ linco).