Ecophysiological characteristics of red, green and brown strains of the Baltic picocyanobacterium *Synechococcus* sp. – a laboratory study

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Abstract. The contribution of picocyanobacteria (PCY) to summer phytoplankton blooms, accompanied by an ecological crisis is a new phenomenon in Europe. This issue requires careful investigation. The present study examined the response of *Synechococcus* sp. physiology to different environmental conditions. Three strains of *Synechococcus* sp. (red BA-120, green BA-124, and brown BA-132) were cultivated in a laboratory under previously determined environmental conditions. These conditions were as follows: temperature (T) from 10 by 5 to 25°C, salinity from 3 by 5 to 18 PSU and Photosynthetically Active Radiation (PAR) from 10 by 90 to 280 μmol photons m^{-2} s^{-1}, which gave 64 combinations of synthetic, though realistic environmental scenarios. Scenarios reflecting all possible combinations were applied in the laboratory experiments. Results pointed to differences in final numbers of cells among strains. However, there was also a similar tendency for BA-124 and BA-132, which demonstrated the highest concentrations of PCY cells at elevated T and PAR. This was also the case for BA-120, but only to a certain degree as the number of cells started to decrease above 190 μmol photons m^{-2} s^{-1} PAR. Pigmentation, chlorophyll *a* (Chl *a*), fluorescence and rate of photosynthesis presented both similarities and differences among strains. In this context, more consistent features were observed between brown and red strains when compared to the green. In this paper the ecophysiological responses of PCY are defined.

1 Introduction

The presence of picoplankton and its contribution to marine biomass were ignored in environmental studies conducted before 1970. This was related to the poor accuracy of research equipment, which did not enable the recording and recognition of such small organisms. Before the discovery of picocyanobacteria (PCY) in the oceans by Johnson and Sieburth (1979) and Waterbury et al. (1979) there only existed incidental reports of this fraction of cyanobacteria occurrence in aquatic ecosystems. Since then, the number of PCY observations has rapidly increased, and currently they are known to be present in many marine, brackish and freshwater ecosystems of the world (e.g., Callieri, 2010; Sorokin and Zakuskina, 2010; Flombaum et al., 2013; Jodłowska and Śliwińska, 2014; Jasser and Callieri, 2017). Additionally, recent works showed that many aquatic ecosystems have been experiencing super-dense, long-term blooms of picocyanobacteria (Sorokin et al., 2004; Sorokin and Zakuskina, 2010), whilst in the past PCY were often described as a non-blooming group (Stockner et al., 1988). Sorokin and Zakuskina (2010) found that the picocyanobacteria blooms were accompanied by great changes in the benthic habitats.

Picocyanobacteria of the *Synechococcus* genus are extremely important organisms in the world's oceans. This is the smallest fraction of plankton ranked by the size of cells, which ranges from 0.2 to 2.0 μm (Sieburth et al., 1978). Chrococcoid genus of the *Synechococcus* is an ubiquitous component of the natural plankton communities in aquatic environments. Picocyanobacteria of the *Synechococcus* group span a range of different colors, depending on their pigments

composition (Stomp et al., 2007; Haverkamp et al., 2008). *Synechococcus* sp. ranged by the pigment content are divided into two main groups: strains rich in the pigment phycoerythrin (PE), rendering the representatives a variety of orange, brown, reddish, pink and purple colors, and strains rich in phycocyanin (PC), coloring the organism in various shades of blue-green (Haverkamp et al., 2009). Baltic strains of *Synechococcus* sp. are classified as three main groups: red and brown strains rich in PE and green strains rich in PC (Mazur-Marzec et al., 2013; Jodłowska and Śliwińska, 2014). The difference between red and brown strains is a proportion of two different bilin pigments known as phycoerythrobilin (PEB) and phycourobilin (PUB), which both bind to the PE apoprotein (Everroad and Wood, 2006; Stomp et al., 2007; Six et al., 2007a, b; Haverkamp et al., 2008; 2009). The three strains of *Synechococcus* sp.: BA-120 (red), BA-124 (green), and BA-132 (brown) examined in this work (Fig. 1) are different phenotype representatives. Coexistence of PE and PC-rich picocyanobacteria can be found in waters of intermediate turbidity, such as many freshwater lakes and coastal seas including Baltic Sea (Andersson et al., 1996; Hajdu et al., 2007; Stomp et al., 2007; Haverkamp et al., 2008; Haverkamp et al., 2009; Mazur-Marzec et al., 2013; Larsson et al., 2014; Paczkowska et al., 2017).

Picocyanobacterial species are phylogenetically divided into several major clusters. These clusters have been identified, based on photosynthetic pigmentation, nitrogen requirements, motility and salinity preferences (Herdman et al., 2001). Picocyanobacteria that are often found and isolated from marine, brackish and freshwater environments are related to *Synechococcus* cluster 5 (Herdman et al., 2001). *Synechococcus* cluster 5 is divided into two sub-clusters: 5.1 and 5.2. The members of cluster 5.1 typically produce PE as their main photosynthetic pigment. In contrast, members of cluster 5.2 have a green coloration because they produce PC (Herdman et al., 2001; Larsson et al., 2014). The diversity of picocyanobacteria has been investigated mainly by analysis of the 16S rRNA gene. However, the phylogenetic tree of *Synechococcus* sp. is not always consistent with their phenotype (Haverkamp et al., 2008). Thus, the actual taxonomic position may be incorrectly defined due to the morphological plasticity of these organisms (Callieri, 2010).

Despite its association with open ocean systems, it has become increasingly evident in recent years that *Synechococcus* sp. is a significant contributor to cyanobacterial blooms (Beardall, 2008). Surprisingly, this species may also comprise 80% and more of the total cyanobacterial biomass during cyanobacterial blooms in the Baltic Sea (Stal et al., 2003; Mazur-Marzec et al., 2013).

Recently, it has been confirmed that PCY are able to excrete harmful and allelopathic substances (e.g., Jakubowska and Szeląg-Wasilewska, 2015; Jasser and Callieri, 2017; Śliwińska-Wilczewska et al., 2017; Barreiro Felpeto et al., 2018). Many different factors, including physical parameters, availability and competition for resources, selective grazing and allelopathic interactions can affect the occurrence of harmful blooms in aquatic ecosystems. The development of massive algal blooming is a consequence of the interaction between many favorable factors. *Synechococcus* sp. greatly contributes to these massive blooms, but so far the characteristics of the life cycle of Baltic PCY has not been sufficiently studied. This knowledge needs to be expanded and improved, especially because of bloom toxicity and negative impacts on ecosystems (Jasser and Callieri, 2017; Śliwińska-Wilczewska et al., 2018a).

According to the above all, phytoplankton is of great interest to scientists in terms of understanding its life cycles and impact on the ecosystem in different parts of the world's oceans and within diverse environmental conditions. In order to investigate it, scientists use various types of research methodology: in-situ measurements, laboratory experiments, and numerical estimations. All of these approaches are necessary and essential in marine phytoplankton research. Some laboratory and field studies of ecophysiological responses of picocyanobacteria to different growth conditions have already been completed for typical oceanic mediums, semi-closed seas and lakes (e.g., Glover et al., 1986; Kuosa, 1988; Stal et al., 1999; Agawin et al., 2000; Callieri and Stockner, 2002; Hajdu et al., 2007; Sánchez-Baracaldo et al., 2008; Cai and Kong, 2013; Motwani et al., 2013; Jodłowska and Śliwińska, 2014, Stawiarski et al., 2016). However, there is still a need to provide more systematic information about these organisms. What is more, the need is amplified by the fact that there are only a few research papers on the brown strain of Baltic *Synechococcus* sp. (Stal et al., 2003; Haverkamp et al., 2008; 2009; Jodłowska and Śliwińska, 2014). This gives limited knowledge of PCY and their life cycle in the Baltic Sea, as brown form

also contributes to total pico- and phytoplankton biomass in the area of interest (Stal et al., 2003). The above strengthens the motivation to conduct studies on the brown strain of *Synechococcus* sp.

The overall goal of this paper is to determine the most favorable and unfavorable environmental conditions for PCY to grow on the basis of three different strains of *Synechococcus* sp. ecophysiological analysis. What is more, this study aims at describing pigmentation, Chl *a* fluorescence parameters and photosynthesis performance of PCY cells grown in different environmental conditions. The goal is also to demonstrate how the increasing abundance of PCY in the Baltic Sea may impact the marine ecosystem functioning. The initial step of these works was to carry out laboratory experiments with *Synechococcus* sp. cultures. In order to create different environmental conditions in the Baltic Sea range, combinations of physical quantities were determined. In total, 64 combinations (environmental scenarios) were generated. The second step was to plot and analyze all results after seven days of incubations. For the results, the number of cells, pigmentation, Chl *a* fluorescence parameters, and the rate of photosynthesis were collected. The third step was to extract any significant relations between the results and specific physical factors by using a statistical analysis, which included the variance method analysis (two-way ANOVA) and Tukey's HSD post-hoc test. Derived laboratory results will help to develop the knowledge of the picocyanobacteria life cycle. Moreover, the PCY experiments underlie the improved numerical approach to phytoplankton modeling development. On the basis of derived results, the algorithm for picocyanobacterium growth will be created in a separate study.

2 Material and methods

2.1 Material and culture conditions

Three different phenotypes of picocyanobacteria strains from the genus *Synechococcus* were examined: BA-120 (red), BA-124 (green), and BA-132 (brown). The *Synechococcus* sp. strains were isolated from the coastal zone of the Gulf of Gdansk (southern Baltic Sea) and maintained as unialgal cultures in the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdańsk, Poland (Latała et al., 2006).

The experiments on the 'batch cultures' were carried out in 25-mL glass Erlenmeyer flasks containing sterilized f/2 medium (Guillard, 1975). Culture media was prepared with artificial seawater filtered through a 0.45-µm filter (Macherey-Nagel MN GF-5) using a vacuum pump (600 mbar) and autoclaved. The cultures were incubated in 35 mL Erlenmeyer glass flasks. The salinity of the media was prepared by dissolving Tropic Marine Synthetic Sea Salt in distilled water. The major nutrients, microelements and vitamin concentrations were added according to a method proposed by Guillard (1975) (any of the components in f/2 media were not replaced by Tropic Marine Synthetic Sea Salt).

The picocyanobacteria cultures were adapted to the various synthetic environmental conditions for two days. The conditions were the combinations of different values of: scalar irradiance in Photosynthetically Active Radiation (PAR) spectrum (10, 100, 190 and 280 µmol photons m^{-2} s^{-1}), temperature (T) (10, 15, 20 and 25°C), and salinity (3, 8, 13 and 18 PSU). The strains were incubated under a 16:8 h light:dark cycle. The measurements of all strains were taken when the experiment incubations completed (after full 7 days) at the same time during the light:dark cycle (in the light phase). The salinity was verified by salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany). The intensity of PAR was measured using a LI-COR spherical quantum-meter (LI-189, LI-COR Inc., Nebraska, USA). Fluorescent lamps (Cool White 40W, Sylvania, USA) were used as a source of irradiance and were combined with halogen lamps (100W, Sylvania, USA) to obtain more intensive light. Both light sources give PAR spectrum. This was proved by Jodłowska and Latała (2010) and Jodłowska and Śliwińska (2014). What is more, LI-COR manual with technical specification therein, says that the sensor first checks the light spectrum and if it responds PAR spectrum, the intensity of radiation is measured. This implies, all the results given by LI-COR refers to PAR. Values of quantities representing each environmental condition were applied at the fixed intervals, i.e.: PAR, interval 90; T, interval 5; salinity, interval 5.

131   The synthetic environmental conditions of salinity and T applied in the laboratory are representative for the Baltic Sea
132 area (Feistel et al., 2008; 2009; Siegel and Gerth, 2017). Moreover, the values of environmental conditions variables
133 (salinity, temperature, PAR) were also specified in certain ranges to make this study comparable with other laboratory
134 cultures experiments available in the literature. The combination of the quantities of environmental variables is called a
135 scenario in the present paper. After acclimation time (2 d), the picocyanobacteria cells served as inoculum for the right test
136 cultures with the initial number of cells equal to 10^6 cells mL^{-1}. During the acclimation time, cell division rate for the strains
137 was about 1 day^{-1}, averagely. It was enough to enable the cells to acclimate to environmental conditions without the risk of
138 stress severity. The acclimation cultures used for inoculation were isolated from the logarithmic growth phase. The flasks
139 with picocyanobacteria were shaken (once a day) during the experiment. In order to achieve the most reliable results, test
140 cultures were grown in three replicas and were incubated for one week at each combination of light, temperature and
141 salinity. The initial cells number, i.e. the culture density just after inoculation, was the same for all replicas. On the last day
142 of incubation the number of cells, pigment content, Chl a fluorescence, and the rate of photosynthesis were measured in each
143 replica. Results were reported as mean values ± standard deviation (SD). Additionally, to broaden the understanding and
144 comparison possibilities, the number of generations (number of generations = elapsed time (t) /doubling time (d)) for each
145 strain were demonstrated in supplementary materials (Fig. S1).

146

147 **2.2 Determination of the number of cells**

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149 The flow cytometry was used to establish the initial number of picocyanobacteria cells and to measure the final cells
150 concentration after the incubation period. The number of cells (N) in cultures was counted with flow cytometer BD Accuri™
151 C6 Plus (BD Biosciences, San Jose, CA, USA) according to the procedure proposed by Śliwińska-Wilczewska et al.
152 (2018b). Events were recorded in list form. Samples were run at a flow rate of approximately 14 µL min^{-1}. Selection of this
153 flow rate was based on previous introductory experiments to determine the most relevant effectiveness. Choosing an
154 adequate discriminator and thresholds plays a key role in recording the cells correctly. The most reasonable solution to
155 record chlorophyll fluorescing cyanobacteria and microalgae is to choose the red fluorescence as the discriminator (Fig. S1)
156 and to select a high threshold, enough to eliminate optical and electronic noise (Marie et al., 2005). Concerning this, the
157 discriminator was set on the red (chlorophyll) fluorescence with a standard threshold of 80,000 on FSC-H. The flow was
158 daily calibrated with Spherotech 6- and 8- Peak Validation Beads (BD, San Jose, USA). This ensures that the cytometer is
159 working properly before running experimental samples. FITC, PE, and PE-Cy5 detectors were daily calibrated with
160 SPHERO™ Rainbow Calibration Particles (BD, San Jose, USA), and the APC channel was calibrated with SPHERO 6-
161 peaks Allophycocyanin Calibration Particles (APC). Detectors FL1, FL2, and FL3 read fluorescence emissions excited by
162 the blue laser (480 nm), while detector FL4 reads emissions excited by the red laser (640 nm).

163

164 **2.3 Determination of the pigments content**

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166 The concentration of photosynthetic pigments of analyzed picocyanobacteria was measured by the spectrophotometric
167 method (Strickland and Parsons, 1972). The analysis of mL-specific (pigment content per mL) and cell-specific (pigment
168 content per cell) pigmentation was conducted. Note that mL-specific means volume-specific, whereas the volume is fixed to
169 1 mL. After seven days of incubation, 4 mL of culture was filtered in order to separate the picocyanobacteria cells from the
170 medium. Chl a and carotenoids (Car) were extracted from the picocyanobacteria cells with cold 90% acetone (5 mL). To
171 improve extraction, the cells were disintegrated for two minutes by ultrasonication. Then, the test-tube with the extract was
172 held in the dark for three hours at -60°C. To remove cell debris and filter out the particles, the extracts were centrifuged at
173 10,000 rpm (8496 × g) for 5 min (Sigma 2-16P, Osterode am Harz, Germany). The absorbance of pigments was estimated on
174 the basis of Beckman spectrophotometer UV-VIS DU 530 measurements at specific wavelengths (750, 665 and 480 nm),

using 1 cm quartz cuvette. Pigment concentration was calculated according to Strickland and Parsons (1972). The following formulas have been used: Chl a (μg mL^{-1}) = 11.236(A_{665}-A_{750})V_a/V_b, Car (μg mL^{-1}) = 4(A_{480}-A_{750})V_a/V_b, where: V_a - extract volume (in this study 5 mL), V_b - sample volume (in this study 4 mL), and A_x - absorbance estimated at wavelength x in a 1-cm cuvette.

2.4 Chlorophyll fluorescence analyses

Chl a fluorescence was measured with a Pulse Amplitude Modulation (PAM) fluorometer (FMS1, Hansatech, King's Lynn, Norfolk, UK). The FMS1 uses a 594 nm amber modulating beam with 4-step frequency control as a measuring light and is equipped with a dual-purpose halogen light source providing actinic light (0 – 3000 μmol photons m^{-2} s^{-1} in 50 steps) and a saturating pulse (0 – 20000 μmol photons m^{-2} s^{-1} in 100 steps). FMS1 also has a 735 nm far-red LED source for preferential PSI excitation allowing accurate determination of the F_o' parameter. Samples were filtered onto 13-mm glass fiber filters (Whatman GF/C, pore size = 1.2 μm). Before measurement, the filtered sample was kept in the dark for 10 min. The maximum photochemical efficiency of photosystem II (PSII) at the dark-adapted state (F_v/F_m) and the photochemical efficiency of PSII under actinic light intensity (ΦPSII) were estimated. The actinic light was different for different cultures, the same as the PAR level was for each incubation. The above is similar to the method used by Campbell et al. (1998).

2.5 Measurements of photosynthesis rate

The measurements of oxygen evolution were carried out on the day seventh of the experiment using a Clark-type oxygen electrode (Chlorolab 2, Hansatech). The temperature was controlled with a cooling system LAUDA (E100, Germany). Illumination was provided by a high-intensity probe-type light array with 11 red LED's centered on 650 nm. Irradiance was measured with a quantum sensor (Quantitherm, Hansatech, King's Lynn, Norfolk, UK). Dark respiration was estimated from O_2 uptake by cells incubated in the dark. Experimental data were fitted to the photosynthesis irradiance response (P-E) curves using equation of Jassby and Platt (1976) and photosynthetic parameters, i.e., the photosynthetic capacity (P_m) and the initial slope of P-E curve (α) (Sakshaug et al., 1997). The calculations were done in Statistica® 13.1 software.

2.6 Statistical analyses

The effect of light and temperature separately and then their combinations impact on growth, pigments content, fluorescence and photosynthesis performance of examined strains were analyzed using two-way variance analysis (ANOVA). A post hoc test (Tukey's HSD) was used to show which results differed under varied conditions over the experimental period (Sheskin 2000). The confident levels in the statistical analysis were: 95% (*$p < 0.05$), 99% (**$p < 0.01$), 99.9% (***$p < 0.001$). The statistical analyses were performed using Statistica® 13.1 and Matlab 2012b software. According to the literature, light and temperature are major factors controlling the growth and distribution of picocyanobacteria (e.g.: Jasser and Arvola, 2003), and they may have considerable significance on the abundance of the *Synechococcus* community (Glover, 1985; Glover et al., 1985; 1986, Joint and Pomroy, 1986; Jasser and Arvola, 2003; Jasser, 2006; Jodłowska and Śliwińska, 2014), as a result it was decided that light and temperature would be the independent variables in ANOVA and post-hoc test analysis. The dependent variable was always the parameter, which had been measured.

3 Results

3.1 Number of cells

219 For all three picoplankton strains, ANOVA analysis indicated that in each scenario the independent variable (temperature or

220 PAR) significantly influenced the dependent variable. What is more, post-hoc tests indicated that multiple factors (T and

221 PAR together) had an impact on the PCY growth.

222 According to post-hoc tests, 2008 multiple comparisons (70%) out of all 2880 completed for three strains, indicated the

223 highest statistical significance (Tukey HSD, *** $p < 0.001$), 160 multiple comparisons (6%) pointed to the statistical

224 significance of $0.001 < ** p < 0.01$, and 114 (4%) stated for the significance of $0.01 < * p < 0.05$. The rest of the multiple

225 comparisons (598, 20%) indicated no statistically significance differences (Tukey HSD, $p \geq 0.05$).

226 Both PAR and T affected the number of *Synechococcus* sp. BA-120 cells significantly (Table S1 in Supplement). For

227 BA-120, the number of cells increased with T in each medium (salinities 3, 8, 13, 18 PSU) (Fig. 2A, a-d). The minimum

228 number of cells was estimated in salinity 3 PSU, T 10°C and PAR 10 μmol photons $m^{-2} s^{-1}$ (1.6×10^6 cell mL^{-1}, Fig. 2A, a),

229 whilst the maximum in salinity 18 PSU, T 25°C, PAR 190 μmol photons $m^{-2} s^{-1}$ (11.5×10^6 cell mL^{-1}, Fig. 2A, d). The

230 decrease in the number of cells was observed from PAR 190 μmol photons $m^{-2} s^{-1}$ onwards. This can likely be related to the

231 photo-inhibition of photosystem II (PSII). The above was the case in each salinity (Figs. 2A, a-d). Additionally, the results

232 analysis (Fig 2A, a-d) showed that the most important environmental factor influencing BA-120 number of cells was T, with

233 PAR playing an additional role, for instance in the context of photo-inhibition. This was pronounced the most within lower

234 temperatures (10 and 15°C), where the change in BA-120 abundance along with PAR increase was barely observed being

235 plainly visible along with T increase at once. Multiple comparisons tests pointed to the strong significance of PAR and T

236 combined in influencing the number of *Synechococcus* sp. BA-120 cells. According to the statistics, 82% of multiple

237 comparisons were statistically significant (Tukey HSD, * $p < 0.05$) with 91% of them having the highest significance level

238 (Tukey HSD, *** $p < 0.001$).

239 Both PAR and T also significantly affected the number of *Synechococcus* sp. BA-124 cells (Table S1). For BA-124, the

240 number of cells increased with T and PAR in all salinities (Figs. 2B, a-d). The lowest number of cells was calculated in

241 salinity 3 PSU, T 10°C and PAR 10 μmol photons $m^{-2} s^{-1}$ (2.0×10^6 cell mL^{-1}, Fig. 2B, a) and the highest number of cells was

242 reached in salinity 18 PSU, T 25°C, PAR 280 μmol photons $m^{-2} s^{-1}$ (43.6×10^6 cell mL^{-1}, Fig. 2B, d). High abundances were

243 estimated also under the highest T and PAR conditions in salinity 13 PSU, where a number of cells equalled 41.1×10^6 cell

244 mL^{-1} (Fig. 2B, c). Generally, the number of cells was the highest in BA-124 cultures when compared to BA-120 and BA-132

245 cultures in respective scenarios. One of the observations was the difference in BA-124 number of cells between lower and

246 higher PAR and T conditions (scenarios with lower PAR and T and scenarios with higher PAR and T). BA-124 seemed to be

247 more sensitive to changes in PAR and T in their lower rather than in higher ranges. Regarding salinity, the highest number of

248 BA-124 cells were noted in moderate- and high-salinity mediums. Optimum salinities for strain BA-124 were 8 and 13 PSU.

249 Due to post-hoc analysis, salinity 13 PSU differentiated the conditions for cell abundances under different PAR and T at a

250 lower degree when compared to other salinities under respective PAR and T (the least statistically significant differences

251 observed in medium 13 PSU), which is also noticeable in Fig. 2B, c. Another feature of BA-124 was the number of cells in

252 low T and high PAR scenarios were nearly equal to cell abundances in high T and low PAR scenarios. This was not the case

253 for BA-120 and BA-132 strains. The observation was supported by Tukey's tests, where only few statistically significant

254 differences in number of cells were observed between scenarios with elevated PAR (280 μmol photons $m^{-2} s^{-1}$), low T (10,

255 15°C) and those with high T (25°C) and low PAR (10 μmol photons $m^{-2} s^{-1}$). These differences were observed between 15°C

256 and 280 μmol photons $m^{-2} s^{-1}$ and 25°C and 10 μmol photons $m^{-2} s^{-1}$ in salinities 3 and 8 PSU (Tukey HSD, ** $p < 0.05$ in

257 both cases, Figs. 2B, a-b). Multiple comparisons tests showed the high significance of PAR and T combinations in affecting

258 the number of cells. According to Tukey HSD tests, 72% of multiple comparisons were statistically significant (* $p < 0.05$)

259 with 82% of them with the highest significance level (*** $p < 0.001$).

260 Similarly to BA-120 and BA-124, it was found that PAR and T significantly affected the number of *Synechococcus* sp.

261 BA-132 cells (Table S1). For BA-132, the positive impact of T and PAR on the number of cells (Figs. 2C, a-d) was observed

262 in each medium. Note that positive impact means the increasing (positive) dependency, whilst negative impact means

263 decreasing (negative) dependency between the independent and dependent variable, e.g.: between T and abundance. Salinity

264 played a more significant role here than when compared to BA-124. It was found that the higher the salinity, the higher the

265 number of cells of BA-132. What is more, according to the statistical analysis, salinity 18 PSU differentiated the number of

266 cells the most (Fig. 2C, d). In salinity 18 PSU, the cell abundances could be described as an increasing linear function of

267 ambient T and PAR. This was also observed in other salinities but not as intensively pronounced as in the highest-saline

268 medium. Moreover, in high salinity, the sensitivity of the number of cells to T changes was much lower than in low

269 salinities. PAR did not determine the number of cells as strongly as T, which was quite consistent with the observation noted

270 for BA-120. The minimum number of cells was observed in 3 PSU, 10°C and 10 µmol photons $m^{-2}\,s^{-1}$ (1.4×10^6 cell mL^{-1},

271 Fig. 2C, a), whilst the maximum in 18 PSU, 25°C, 280 µmol photons $m^{-2}\,s^{-1}$ (16.1×10^6 cell mL^{-1}, Fig. 2C, d). In addition,

272 the lowest values of BA-132 number of cells were calculated for the lowest T and PAR condition in each salinity. Tukey

273 HSD post hoc tests indicated the high significance of PAR and T combinations in affecting the cell abundances. According

274 to those tests, 84% of multiple comparisons were statistically significant (* $p < 0.05$) with 90% of them with the highest

275 significance (*** $p < 0.001$).

276   Regarding all three strains, high salinity generally had a positive impact on the number of *Synechococcus* sp. cells. What

277 is more, the relations between salinity and the number of cells for all strains, especially red and brown, were increasing

278 almost linearly with the highest average increase for BA-132.

279

280 **3.2 Pigment content**

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282 The results showed that for all strains, cell-specific pigment composition (pigment content per cell) was environmentally

283 driven (Figs. 3, 4). The analysis of mL-specific pigmentation (pigment content per mL) was also done (Figs. S2-S3 and

284 Tables S4-S5 in Supplement), however, the mL-specific pigment content is another way to illustrate the biomass and that is

285 why it is not described in this section in detail.

286   It was estimated, that PAR and T significantly affected the Chl *a* cell-specific content of *Synechococcus* sp. BA-120

287 (Table S2). Both PAR and T also affected the Car content in the BA-120 strain cells significantly (Table S3). It was found

288 that cell-specific Chl *a* and Car concentrations decreased with the increase of salinity (Figs. 2A, 3A). On average, the cell

289 content of pigments for BA-120 was the highest when compared to the other strains. Chl *a* concentration dominated over Car

290 concentration in each scenario. What is more, there were very high cell-specific concentrations of Chl *a* observed for the

291 whole T domain at low PAR. Maximum Chl *a* content was measured under T 25°C and PAR 10 µmol photons $m^{-2}\,s^{-1}$. This

292 was the case in each salinity. The highest Chl *a* concentration within all scenarios was reached in BA-120 cells in salinity 3

293 PSU and was equal to 0.339 pg $cell^{-1}$ (Fig. 3A, a). For other salinities these maximums were as follows: 0.233 pg $cell^{-1}$ (8

294 PSU, Fig. 3A, b), 0.164 pg $cell^{-1}$ (13 PSU, Fig. 3A, c), 0.100 pg $cell^{-1}$ (18 PSU, Fig. 3A, d). The highest Car content was

295 measured in salinity 3 PSU under T of 20°C and PAR 10 µmol photons $m^{-2}\,s^{-1}$ and reached 0.160 pg $cell^{-1}$ (Fig. 4A, a). The

296 lowest concentrations of Chl *a* (0.038 pg $cell^{-1}$) and Car (0.031 pg $cell^{-1}$) were measured in salinity 18 PSU, T 25°C, PAR

297 190 µmol photons $m^{-2}\,s^{-1}$ (Fig. 3A, d) and salinity 18 PSU, T 15°C, PAR 280 µmol photons $m^{-2}\,s^{-1}$ (Fig. 4A, d),

298 respectively. Multiple comparisons tests indicated the significance of PAR and T combined in shaping the pigmentation. Due

299 to those tests, 52% and 55% of multiple comparisons in Chl *a* and Car content analysis, respectively, were statistically

300 significant (Tukey HSD, * $p < 0.05$) with 80% (for Chl *a*) and 74% (for Car) of them with the highest significance (Tukey

301 HSD, *** $p < 0.001$).

302   Both PAR and T affected the Chl *a* cell-specific content (Table S2) and Car cell-specific content (Table S3) of

303 *Synechococcus* sp. BA-124 significantly. Generally, PAR and high T increase had a negative impact on pigmentation (Figs.

304 3B, 4B). Maximum values of cell-specific Chl *a* and Car concentrations were measured under 10°C and 10 µmol photons $m^{-2}\,s^{-1}$ in each salinity medium. These values, concerning salinities from the lowest to the highest, were as follows: 0.095,

306 0.102. 0.176, 0.148 pg $cell^{-1}$ for Chl *a* (Figs. 3B, a-d) and 0.051, 0.067, 0.087, 0.079 pg $cell^{-1}$ for Car (Figs. 4B, a-d).

307 Nonetheless, there were also some exceptions. In salinity 3 PSU, high Car contents were calculated under 280 μmol photons
308 $m^{-2}\,s^{-1}$ and T: 15, 20°C and equaled to 0.042 pg $cell^{-1}$ and 0.041 pg $cell^{-1}$, respectively (Fig. 4B, a). On average, salinity
309 increase had a negative impact on pigmentation. The lowest cell-specific concentrations of Chl a and Car in BA-124 cells
310 were estimated in the same scenario: salinity 18 PSU, T 10°C, PAR 280 μmol photons $m^{-2}\,s^{-1}$ and were equal to 0.013 pg
311 $cell^{-1}$ (Fig. 3B, d) and 0.009 pg $cell^{-1}$ (Fig. 4B, d), for Chl a and Car, respectively. Multiple comparisons tests pointed to the
312 significance of PAR and T combined in influencing the pigmentation. According to the statistics, 47% and 54% of multiple
313 comparisons in Chl a and Car content analysis, were statistically significant (Tukey HSD, * $p < 0.05$) with 83% (for Chl a)
314 and 79% (for Car) of them with the highest significance level (Tukey HSD, *** $p < 0.001$).

315   It was also examined that PAR and T affected the Chl a cell-specific content (Table S2) and Car cell-specific content
316 (Table S3) of *Synechococcus* sp. BA-132 significantly. It was found that salinity increase had a negative impact on cell-
317 specific Chl a and Car concentrations. BA-132 was richer in cell-specific pigments than BA-124 (Figs. 3C, 4C). Along with
318 PAR increase, the Chl a concentration decreased significantly. The maximum Chl a cell-specific content was measured in
319 moderate or high T (20°C in salinity 13 PSU and 25°C in salinity 3, 8, 18 PSU) under the lowest PAR (10 μmol photons m^{-2}
320 s^{-1}). These maximums were 0.299 pg $cell^{-1}$ in salinity 3 PSU (Fig. 3C, a), 0.248 pg $cell^{-1}$ in salinity 8 PSU (Fig. 3C, b),
321 0.151 pg $cell^{-1}$ in salinity 13 PSU (Fig. 3C, c) and 0.073 pg $cell^{-1}$ in salinity 18 PSU (Fig. 3C, d). Consistently with Chl a,
322 Car cell-specific content maximums were also measured under the lowest PAR (10 μmol photons $m^{-2}\,s^{-1}$) but contrary to Chl
323 a, at the lowest T (10°C). These maximums were: 0.194 pg $cell^{-1}$ in salinity 3 PSU (Fig. 4C, a), 0.131 pg $cell^{-1}$ in salinity 8
324 PSU (Fig. 4C, b), 0.097 pg $cell^{-1}$ in salinity 13 PSU (Fig. 4C, c), 0.062 pg $cell^{-1}$ in salinity 18 PSU (Fig. 4C, d). Minimums of
325 Chl a and Car cell-specific contents within all scenarios were estimated in salinity 18 PSU, T 15°C and PAR 280 μmol
326 photons $m^{-2}\,s^{-1}$ and equaled to 0.020 pg $cell^{-1}$ (Fig. 3C, d) and 0.19 pg $cell^{-1}$ (Fig. 4C, d), for Chl a and Car, respectively.
327 Regarding Chl a for minimum content per cell the same concentration as above mentioned (0.020 pg $cell^{-1}$) was also
328 estimated in salinity 13 PSU for the same conditions of T and PAR (Fig. 3C, c). Tukey HSD tests pointed to the significance
329 of PAR and T combined in impacting the pigmentation. According to those tests, 66% and 61% of multiple comparisons in
330 Chl a and Car content analysis, respectively, were statistically significant (Tukey HSD, * $p < 0.05$), with 81% (for Chl a) and
331 75% (for Car) of them with the highest significance (Tukey HSD, *** $p < 0.001$).

332

3.3 Chl a fluorescence

334

335 The parameters of Chl a fluorescence were depicted as two-factor-dependent graphs, where the values in between the
336 specific measurements were interpolated (Figs. 5, 6). For all strains, Chl a fluorescence parameters were measured and
337 examined. These parameters were: the maximum photochemical efficiency of photosystem II (PSII) at the dark-adapted state
338 (F_v/F_m) and the photochemical efficiency of PSII under actinic light intensity (ΦPSII).

339   The results showed that PAR and T affected F_v/F_m (Table S6) and ΦPSII (Table S7) of *Synechococcus* sp. BA-120
340 significantly. For this strain, especially in low T scenarios and in all scenarios with the lowest salinity, higher F_v/F_m was
341 observed for 280 μmol photons $m^{-2}\,s^{-1}$ when compared to 190 μmol photons $m^{-2}\,s^{-1}$ (Fig. 5A, a). Generally, strong
342 fluctuations were noticeable in F_v/F_m values, which disabled the fixed environmentally driven pattern determination.
343 However, there was a constant relation noted between T and PAR and ΦPSII. PAR and T increase had a negative impact on
344 ΦPSII. The impact was the strongest in low salinity (Figs. 6A, a-b). Nonetheless, in each salinity, the lowest ΦPSII were
345 observed under the highest T and elevated PAR (190 or 280 μmol photons $m^{-2}\,s^{-1}$). On the contrary, the highest ΦPSII
346 values were calculated in the lowest T and PAR conditions in every salinity. The highest F_v/F_m, for all BA-120 experiments,
347 equaled 0.804 and was estimated for the scenario: salinity 18 PSU, T 10°C, PAR 280 μmol photons $m^{-2}\,s^{-1}$ (Fig. 5A, d).
348 Generally, maximum values of F_v/F_m in each medium were associated with the lowest temperature. Minimum F_v/F_m within
349 all scenarios was estimated for salinity 3 PSU, T 25°C and PAR 190 μmol photons $m^{-2}\,s^{-1}$ (0.409, Fig. 5A, a). Concerning
350 ΦPSII, the greatest value was 0.768 estimated in salinity 18 PSU, T 10°C and PAR 10 μmol photons $m^{-2}\,s^{-1}$ (Fig. 6A, d).

351 Minimum ΦPSII was measured in salinity 3 PSU, T 25°C and PAR 280 µmol photons m^{-2} s^{-1} (0.241, Fig. 6A, a). Multiple

352 comparisons tests pointed to a strong environmental influence on Chl a fluorescence parameters. Regarding F_v/F_m, 65% of

353 all comparisons were statistically significant (Tukey HSD, * $p < 0.05$) with 78% of them having the highest significance

354 (Tukey, HSD, *** $p < 0.001$). For ΦPSII the percentages were as follows: 80% of all comparisons were statistically

355 significant (Tukey HSD, * $p < 0.05$) and 87% of them had the highest significance (*** $p < 0.001$).

356   Both PAR and T significantly affected F_v/F_m (Table S6) and ΦPSII (Table S7) of *Synechococcus* sp. BA-124. For this

357 strain, F_v/F_m reached the lowest values when compared to the respective incubations of other strains. The values of F_v/F_m

358 generally decreased along with PAR and T increases but with some exceptions. Generally, ΦPSII environmentally driven

359 characteristics were similar to F_v/F_m characteristics. The F_v/F_m minimums were measured under the lowest T and highest

360 PAR in each salinity (Figs. 5B, a-d). The lowest value within all scenarios was 0.124 and was observed in salinity 3 PSU, T

361 10°C and PAR 280 µmol photons m^{-2} s^{-1} (Fig. 5B, a). The F_v/F_m maximums were estimated for the highest T and the lowest

362 PAR in each salinity. The highest F_v/F_m equaled 0.560 for salinity 3 PSU, T 25°C and PAR 10 µmol photons m^{-2} s^{-1} (Fig.

363 5B, a). Minimums of ΦPSII, consistently with F_v/F_m, were noted under the lowest T and highest PAR. The lowest ΦPSII

364 within all BA-124 experiments was 0.114 (followed by the minimum in salinity 3 PSU equaled to 0.116, Fig. 6B, a) and was

365 measured in salinity 13 PSU (Fig. 6B, c). Maximums of ΦPSII were observed in the highest T and lowest PAR in each

366 medium, similarly to F_v/F_m. The greatest value of ΦPSII was 0.542 and was measured in salinity 3 PSU, T 25°C and PAR 10

367 µmol photons m^{-2} s^{-1} (Fig. 6B, a). Tukey HSD post hoc test showed that PAR and T combined influenced Chl a fluorescence

368 parameters significantly. Concerning F_v/F_m, 77% of all comparisons were statistically significant (* $p < 0.05$) with 88% of

369 them having the highest significance (*** $p < 0.001$). For ΦPSII the percentages were as follows: 79% of all comparisons

370 were statistically significant (* $p < 0.05$) and 89% of them had the highest significance (*** $p < 0.001$).

371   It was found that both PAR and T affected F_v/F_m (Table S6) and ΦPSII (Table S7) of *Synechococcus* sp. BA-132,

372 significantly. For this strain, F_v/F_m decreased along with the PAR increase but was positively affected by T in each salinity

373 (Figs. 5C, a-d). Minimum values of F_v/F_m were measured in the highest PAR and the lowest T in each salinity. The lowest

374 F_v/F_m within all experiments on BA-132 stated for salinity 13 PSU (F_v/F_m = 0.155, Fig. 5C, c). In salinity 3 PSU, under

375 aforementioned conditions of T and PAR, the F_v/F_m value was also low compared to the others and equaled 0.160 (Fig. 5C,

376 a). The maximums of F_v/F_m were measured in T 25°C and PAR 10 µmol photons m^{-2} s^{-1}. This was the case for all mediums.

377 The highest F_v/F_m values were noted in salinities 13 and 18 PSU and equaled 0.742 (Fig. 5C, c) and 0.733 (Fig. 5C, d),

378 respectively. The lowest ΦPSII were noted under the highest PAR and T conditions in every salinity (Figs. 6C, a-d). The

379 minimum ΦPSII, within all gathered results, was obtained in salinity 3 PSU and equaled 0.281 (Fig. 6C, a). Maximums of

380 ΦPSII were measured under completely opposite conditions to the ones stating for minimums, i.e. the lowest PAR and T.

381 The highest ΦPSII, 0.786, was noted in salinity 8 PSU, T 10°C and PAR 10 µmol photons m^{-2} s^{-1} (Fig. 6C, b). The ΦPSII

382 reached generally higher values than F_v/F_m in BA-132 experiments. ΦPSII reached lower values than ΦPSII measured under

383 respective conditions for two other strains. Multiple comparisons tests point to a strong environmental influence on Chl a

384 fluorescence parameters. For F_v/F_m, 78% of all comparisons were statistically significant (Tukey HSD, * $p < 0.05$) with 89%

385 of them with the highest significance (Tukey, HSD, *** $p < 0.001$). For ΦPSII, 82% of all comparisons were statistically

386 significant (Tukey HSD, * $p < 0.05$), with 89% of them having the highest significance level (Tukey, HSD, *** $p < 0.001$).

387   Generally, for the BA-120 strain, F_v/F_m was affected negatively by T increase, while BA-124 and BA-132 strains were

388 affected positively. T increase had a positive impact on ΦPSII for BA-124 and a negative impact for BA-120 and BA-132.

389 On average, ΦPSII decreased along with PAR increase in all cultures.

390

3.4 Photosynthesis

392

393   Net photosynthetic light-response curves for three PCY strains were analyzed. For all cultures, the photosynthesis

394   parameters were: maximum of photosynthesis and photosynthesis efficiency at low irradiance (P_m and α, respectively) and

395   these were estimated for Chl a-specific and cell-specific domains (Figs. S4-S5 in Supplement).

396   For BA-120, the statistical study showed a significant dependence of PAR and T on Chl a-specific P_m in salinities 3, 8

397   and 18 PSU and pointed to no statistically significant dependence of ecological conditions on P_m in salinity 13 PSU (Table

398   S8). Regarding cell-specific P_m, there was no statistically significant influence of PAR and T on this parameter in salinity 18

399   PSU but was in salinity 3, 8, and 13 PSU (Table S9). For Chl a-specific α, the statistical study indicated no environmental

400   impacts in salinities 3, 8 and 13 PSU but an impact of PAR and T in salinity 18 PSU (Table S10), while for cell-specific α

401   statistical significance of PAR and T influence was obtained for all salinities (Table S11). Tukey HSD tests pointed to some

402   statistically significant multiple comparisons but showed a weak influence of PAR and T combined on Chl a-specific

403   parameters. Regarding α, only 3% of all multiple comparisons were statistically significant (* $p < 0.05$) with 7% of them at

404   the highest statistical significance level (*** $p < 0.001$). For P_m, 36% of all multiple comparisons were statistically

405   significant (* $p < 0.05$) with 64% of them with the highest significance (*** $p < 0.001$). Note that in this section, to make it

406   more concise, the notation for all statistically significance multiple comparisons percentage (* $p < 0.05$) and the percentage

407   of the multiple comparisons of the highest significance within the significant ones *** $p < 0.001 \times$ (* $p < 0.05)^{-1}$ were

408   written in parenthesis, one by one, separated with comma. Similarly to Chl a-specific calculations, Tukey HSD test pointed

409   to a selective influence of PAR and T combined on cell-specific parameters. However, this dependence was stronger when

410   compared to Chl a-specific estimations (P_m (16%, 52%), α (19%, 43%)). Nonetheless, there were also some fixed relations

411   noted for both calculation domains. For Chl a-specific photosynthesis, P_m increased along with PAR up to PAR of 190 µmol

412   photons m^{-2} s^{-1} (Figs. S4, a, c). Above this level P_m value started to decrease slightly. This was the case in all salinities.

413   Minimum P_m was measured for cells grown in scenario: salinity 3 PSU, T 15°C, PAR 10 µmol photons m^{-2} s^{-1} and it was

414   0.12 µmol O$_2$ (µg Chl a)$^{-1}$ h^{-1} (Fig. S4, a), whilst the maximum equalled 1.31 µmol O$_2$ (µg Chl a)$^{-1}$ h^{-1} and was reached in

415   salinity 18 PSU, T 25°C, 190 µmol photons m^{-2} s^{-1} (Fig. S4, c). On the contrary, it was more difficult to determine a fixed

416   pattern of α changes unequivocally. The most fixed tendency of α changes was observed in all temperature-differenced

417   scenarios in 18 PSU salinity medium (Figs. S5, a, c). Under those conditions, it was noticeable that α decreased with PAR

418   and T increase till it reached PAR level of 190 µmol photons m^{-2} s^{-1}. Then, α started to rise slowly. Regarding all gathered

419   results (all mediums together), α minimum was measured in salinity 3 PSU, T 25°C, PAR 10 µmol photons m^{-2} s^{-1} and

420   equalled 0.002 µmol O$_2$ (µg Chl a)$^{-1}$ h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$ (Fig. S5, a), whilst maximum was 0.013 µmol O$_2$ (µg Chl

421   a)$^{-1}$ h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$ in salinity 13 PSU, T 10°C, PAR 10 µmol photons m^{-2} s^{-1}. On the other hand, for the cell-

422   specific domain, P_m increased along with T and it was more pronounced in higher salinities. Concerning all results, minimum

423   P_m was 28.58 µmol O$_2$ cell 10^{-9} h^{-1} and, similarly to Chl a-specific P_m was measured in scenario: salinity 13 PSU, T 10°C,

424   PAR 10 µmol photons m^{-2} s^{-1}, whilst maximum P_m equalled 55.16 µmol O$_2$ cell 10^{-9} h^{-1} and was reached in salinity 8 PSU,

425   T 25°C, 190 µmol photons m^{-2} s^{-1} (data not shown). Regarding α, this parameter was generally negatively affected by PAR

426   and T up to PAR of 190 µmol photons m^{-2} s^{-1}. However minimum value was obtained for cells growing in moderate T

427   (salinity 8 PSU, T 20°C, PAR 10 µmol photons m^{-2} s^{-1}) and equalled 0.81 µmol O$_2$ cell 10^{-9} h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$.

428   Maximum α equalled 1.57 µmol O$_2$ cell 10^{-9} h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$ and was measured in salinity 18 PSU, T 10°C, PAR

429   10 µmol photons m^{-2} s^{-1} (Fig. S5, d).

430   For BA-124, the statistical study showed the significant dependence of ecological conditions on photosynthesis

431   parameters, excluding Chl a-specific α and cell-specific P_m (Table S8-S11). Post-hoc tests showed there must have been

432   other factors, which affected the whole process of photosynthesis as there were many not statistically significant multiple

433   comparisons defined. Generally, Tukey HSD tests pointed to only few statistically significant multiple comparisons, in both

434   Chl a-specific, especially for P_m (P_m (60%, 76%), α (9%, 29%) and cell-specific (P_m (22%, 56%), α (34%, 63%))

435   estimations. Nonetheless, for P_m, there was a tendency noted, which suggested that on average, the maximum of

436   photosynthesis was higher at elevated PAR. This was the case in both estimations, Chl a-specific and cell-specific.

437 Maximum Chl a-specific P_m was 3.0 and minimum 0.16 µmol O_2 (µg Chl a)$^{-1}$ h^{-1}. These values were measured in salinity 18

438 PSU in T 25°C, PAR 280 µmol photons m^{-2} s^{-1} and T 10°C, PAR 10 µmol photons m^{-2} s^{-1}, respectively (Fig. S4, g).

439 Maximum cell-specific P_m was obtained in salinity 8 PSU, T 25°C, PAR 280 µmol photons m^{-2} s^{-1} and minimum in salinity

440 13 PSU, T 20°C, PAR 10 µmol photons m^{-2} s^{-1} (data not shown here). These extreme values were 53.41 and 19.17 µmol O_2

441 cell·10^{-9} h^{-1}, respectively. It was difficult to determine a fixed relation between ecological state and α changes in both

442 domains, which was supported by the post-hoc test (more than 91% of multiple comparisons were not statistically significant

443 ($p \geq 0.05$) in Chl a-specific and more than 35% in cell-specific estimations). Maximum Chl a-specific α was 0.02 µmol O_2

444 (µg Chl a)$^{-1}$ h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$ and was measured in salinity 3 PSU, T 15°C, PAR 100 µmol photons m^{-2} s^{-1} (Fig.

445 S5, e), while maximum cell-specific α (1.77 µmol O_2 cell 10^{-9} h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$) was obtained in salinity 13 PSU,

446 T 10°C, PAR 10 µmol photons m^{-2} s^{-1}. Minimum Chl a-specific α was 0.003 µmol O_2 (µg Chl a)$^{-1}$ h^{-1} [µmol photons m^{-2} s^{-}

447 ^{1}]$^{-1}$ and was measured in two scenarios: salinity 3 PSU, T 10°C, PAR 280 µmol photons m^{-2} s^{-1} (Fig. S5, e) and salinity 18

448 PSU, T 15°C, PAR 10 µmol photons m^{-2} s^{-1} (Fig. S5, g). Minimum cell-specific α equalled 0.08 µmol O_2 cell 10^{-9} h^{-1} [µmol

449 photons m^{-2} s^{-1}]$^{-1}$ and was measured in salinity 18 PSU, T 15°C, PAR 190 µmol photons m^{-2} s^{-1} (Fig. S5, h).

450 Generally, for BA-132, the statistical study showed the significant dependence of PAR and T on Chl a- and cell-specific

451 parameters (Tables S8-S11). It was observed, that in cell-specific estimations, P_m increased along with PAR increase, while

452 α decreased at elevated PAR. Maximum cell-specific P_m was 158.94 µmol O_2 cell 10^{-9} h^{-1} and was reached in salinity 8

453 PSU, T 25°C, PAR 280 µmol photons m^{-2} s^{-1}, whilst minimum equalled 28.04 µmol O_2 cell 10^{-9} h^{-1} in salinity 18 PSU, T

454 15°C, PAR 10 µmol photons m^{-2} s^{-1} (Fig. S4, l). Maximum cell-specific α was 1.78 µmol O_2 cell 10^{-9} h^{-1} [µmol photons m^{-2}

455 s^{-1}]$^{-1}$ and was measured in salinity 13 PSU, T 20°C, PAR 10 µmol photons m^{-2} s^{-1}, while minimum was reached in salinity

456 18 PSU, T 20°C, PAR 100 µmol photons m^{-2} s^{-1} and equalled 0.19 µmol O_2 cell 10^{-9} h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$ (Fig. S5, l).

457 For Chl a-specific P_m, the increases along with T and salinity was observed, whilst α presented strong changing

458 characteristics between scenarios. The fixed influence of PAR and T on α values was difficult to determine, which was

459 supported by statistics (Table S10-S11). Maximum Chl a-specific P_m was 6.22 µmol O_2 (µg Chl a)$^{-1}$ h^{-1} and was reached in

460 salinity 18 PSU, T 25°C, PAR 280 µmol photons m^{-2} s^{-1} (Fig. S4, k), whilst minimum equalled 0.12 µmol O_2 (µg Chl a)$^{-1}$ h^{-}

461 ^{1} in salinity 3 PSU, T 25°C, PAR 10 µmol photons m^{-2} s^{-1} (Fig. S4, i). Maximum Chl a-specific α was 0.02 µmol O_2 (µg Chl

462 a)$^{-1}$ h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$ and was measured in salinity 18 PSU, T 15°C, PAR 10 µmol photons m^{-2} s^{-1} (Fig. S5, k),

463 while minimum was reached in salinity 3 PSU, T 15°C, PAR 10 µmol photons m^{-2} s^{-1} and equalled 0.003 µmol O_2 (µg Chl

464 a)$^{-1}$ h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$ (Fig. S5, i). Generally, in both domains, photosynthesis parameters were the highest for BA-

465 132 when compared to other strains.

466 The analysis of photosynthesis characteristics enabled examining and defining the photoacclimation process of all three

467 strains of *Synechococcus* sp. This was done on the basis of the photosynthetic parameters (Figs. S4-S5) and Photosynthesis-

468 Irradiance (*P-E*) curves (exemplification was shown in Fig. 7). The curves were plotted on the basis of laboratory results

469 (Clark oxygen electrode measurements) using the equation of Jassby and Platt (1976). According to a photoacclimation

470 model description (Prezelin, 1981; Prezelin and Sweeney, 1979; Ramus, 1981; Richardson et al., 1983; Pniewski et al.,

471 2016), the results of the present study indicated changes in Photosynthetic Units (PSU) sizes as the photoacclimation

472 mechanism, which occurred most frequently (Table 1). There were also *P-E* curves pointing to some changes in enzymatic

473 reactions and the altering of accessory pigments activity. Changes in PSU numbers were noted as well, but these

474 observations were episodic. In this paper the term 'OTHER' states for changes in enzymatic reactions and the altering of

475 accessory pigments activity and concerns photoacclimation mechanisms other than changes in PSU sizes (PSUsize) or

476 changes in PSU number (PSUno.). In general, photoacclimation did not occur in low-saline medium (salinity 3). According

477 to our results, the process was observed in only four cases in low salinity: BA-120 25°C salinity 3 PSU, BA-124 25°C

478 salinity 3 PSU, BA-132 10°C salinity 3 PSU, and BA-132 25°C salinity 3 PSU. For BA-120, photoacclimation occurred

479 more frequently at higher T (20 and 25°C) than at lower T (10 and 15°C). However, if it had been observed in low T

480 conditions, it usually stated for OTHER, not for PSUsize or PSUno. For BA-124 and BA-132 photoacclimation was noted in
481 the whole T range. All photoacclimation mechanisms observed for different strains are listed in Table 1.
482
483 **4 Discussion**
484
485 Picoplanktonic organisms show a lot of adaptations, which enable them to spread in aquatic environments (e.g., Stomp et al.,
486 2007; Jodłowska and Śliwińska, 2014; Larsson et al., 2014; Jasser and Callieri, 2017). What is more, picocyanobacteria
487 often dominate and occupy the niches, which are inaccessible to other photoautotrophs. Owing to the fact that PCY are
488 small-sized cells and consequently possess an advantageous surface area to volume ratio, they can assimilate trace amount of
489 nutrients and effectively absorb light. Therefore, in oligotrophic regions of seas and oceans PCY compete with other
490 cyanobacteria and microalgae and they can determine the primary production of the whole marine ecosystem (Six et al.,
491 2007a; Richardson and Jackson, 2007; Worden and Wilken, 2016). This is also true for eutrophic basins (Stal et al., 2003;
492 Haverkamp et al., 2008; 2009; Callieri, 2010; Mazur-Marzec et al., 2013).

493   The distribution of PCY is determined by their optimal ecological requirements for light and temperature. Due to the
494 presented results, PAR and T had positive effects on the number of cells for two out of the three studied strains of
495 *Synechococcus* sp. The highest cell concentrations were noted at the highest T (25°C) and the highest PAR level (280 µmol
496 photons m^{-2} s^{-1}) for BA-124 and BA-132. The BA-120 strain behaved differently when compared to the other two in high
497 PAR conditions. The decrease in the number of cells appeared then, i.e. cell abundances for cultures grown under the most
498 elevated PAR were lower than the number of cells measured for *Synechococcus* sp. BA-120 cells grown under 190 µmol
499 photons m^{-2} s^{-1}. According to the results derived from pigmentation, Chl *a* fluorescence and photosynthesis sections of the
500 present study, the decrease in the number of cells under the elevated PAR could have likely been associated with
501 Photosystem II photo-inhibition. This was a result of a few observations, which are described as follows. Firstly, there was a
502 higher cell-specific Car content observed for 280 µmol photons m^{-2} s^{-1} when compared to 190 µmol photons m^{-2} s^{-1}.
503 Secondly, higher $F_\mathrm{v}/F_\mathrm{m}$ values were observed for 280 µmol photons m^{-2} s^{-1} when compared to 190 µmol photons m^{-2} s^{-1},
504 especially for low T scenarios and for all scenarios in the lowest salinity medium. Thirdly, for Chl *a*-specific photosynthesis,
505 P_m increased along with PAR until 190 µmol photons m^{-2} s^{-1}, above which the values started to decrease slightly in all
506 salinity mediums. According to the above, a PAR level of 190 µmol photons m^{-2} s^{-1} could be defined as the PSII photo-
507 inhibition point for the red strain. This implies BA-120 did not lead as effective photosynthesis being grown in PAR of more
508 than 190 µmol photons m^{-2} s^{-1} as the cells grown in PAR levels equal or are beneath the above-mentioned value. The results
509 showed that in all synthetically created environmental scenarios, BA-124 was the strain of the highest cell abundance. This is
510 consistent with the Baltic Sea field studies (Mazur-Marzec et al., 2013).

511   Cyanobacteria are generally recognized to prefer low light intensity for growth (Fogg and Thake, 1987; Ibelings, 1996).
512 Some picoplanktonic organisms demonstrated the ability to survive and resume growth after periods of total darkness. Such
513 a pronounced capacity for survival in the dark would enable these organisms to outlive the seasonal rhythm of winter
514 darkness and sinking into the aphotic zone (Antia, 1976). The investigated strains of *Synechococcus* sp. were found to be
515 well adapted to relatively low and high PAR levels. The latter was especially evident at the high treatment T. This
516 conclusion is consistent with the observations of picocyanobacteria maximum abundance at the euphotic zone in coastal and
517 offshore marine waters (Stal et al., 2003; Callieri, 2010). Moreover, Kana and Gilbert (1987a,b) showed that *Synechococcus*
518 sp. could grow at irradiance as high as 2000 µmol photon m^{-2} s^{-1}.

519   Surface and near-surface populations experience extremely variable light and temperature conditions (Millie et al.,
520 1990), and these factors are the ones that affect the composition of photosynthetic pigments and photosynthesis performance
521 of picocyanobacteria (Jodłowska and Śliwińska, 2014). Picocyanobacteria with a high concentration of PC are chromatically
522 better adapted to harvest longer wavelengths of PAR than those with PE as a dominating pigment. Therefore, such
523 picocyanobacteria, such as the BA-124 strain, usually dominate in surface euphotic waters (Stal et al., 2003; Haverkamp et

al., 2008; 2009). On the other hand, the strains rich in PE (BA-120 and BA-132), usually occurred deeper (Fahnenstiel et al., 1991; Hauschild et al., 1991; Vörös et al., 1991). Nonetheless, generally PCY, thanks to their high concentration of photosynthetic pigments, may occur in waters under low light intensity (Stal et al., 2003). Furthermore, carotenoids play an important role in the cells as they provide protection against photooxidation (Siefermann-Harms, 1987; Kana and Glibert, 1988; Jodłowska and Latała, 2010). This feature additionally explains why picoplanktonic *Synechococcus* is able to grow successfully both in the surface layer of the sea and also in deeper waters (Stal and Walsby, 2000; Stal et al., 2003). This research showed that for BA-120 cell-specific pigments content, there were very high concentrations of Chl *a* observed in the whole T domain under low PAR. This could have implied the photoacclimation type, which was the change in PSU number. This mechanism was also observed in *P-E* curves for the scenario with salinity 8 PSU and 20°C temperature.

In this study, the pigment content was generally the highest under the low PAR treatment for all strains. This was a striking observation, however, with some exceptions. For instance, concerning Cars, BA-120 cell-specific Car reached high concentrations in the whole light range for high T. This was pronounced in mediums of moderate and high salinity (8, 13, 18). Moreover, in medium 3, BA-124 demonstrated high cell-specific Car concentration under the highest analyzed light level. The cell-specific Car peaking in the lowest light was pronounced the most in BA-132 cultures. This is consistent with the literature (Jodłowska and Latała, 2010). Regarding cell-specific Chl *a* peaks, they were noticeable in the low PAR range for all strains with no exceptions. The difference between the strains were various effects of temperature. For BA-120 and BA-132 the highest cell-specific Chl *a* concentrations were estimated in the highest T, while for BA-124 oppositely, i.e. for the lowest T. This has not been observed before (according to Authors' best knowledge, not reported in the literature yet). Moreover, the Car/Chl *a* ratio increase along the PAR increase was observed. This, together with low pigment contents in under high PAR, is a very interesting observation, which makes the motivation for further studies on *Synechococcus* sp. stronger. The Authors plan to extend their research on picocyanobacteria in the future (the pigment content composition analysis, proportion of Chl *a* and carotenoids – Zeaxanthin, *β*-carotene – and Phycobilins).

PAR and T were the main factors also in terms of influencing the changes in Chl *a* fluorescence in three strains of *Synechococcus* sp. This may likely be linked with a great importance of PCY domination in many aquatic ecosystems during the summer period. Due to Chl *a* fluorescence parameters results, it should be noted that PAR increase always had a negative impact on ΦPSII, which implied that cells, previously acclimated to high light conditions, had lower PSII photosystem efficiency under actinic light.

The results showed that T, PAR, and salinity influenced the photosynthesis parameters only to a certain degree. There were many not statistically significant multiple comparisons pointed by post hoc tests. However, it was found that generally, in cell-specific estimations, elevated PAR had a negative effect on α and PAR increase influenced the respiration negatively. For each of the studied strains of *Synechococcus* sp., the highest α was noted for the cells grown under the lowest PAR (10 μmol photons m^{-2} s^{-1}). On the other hand, the highest values of P_m were noted at the highest PAR. It pointed to the inability for the cells incubated in low PAR conditions to be as effective in photosynthesis as the cells grown under high irradiances. Due to our results, on the basis of *P-E* curves, three types of photoacclimation mechanisms of *Synechococcus* sp. were observed: change in PSU size, change in PSU number and altering accessory pigments activity and changes in enzymatic reactions. This was a striking observation because in the literature results predominantly derive the two first aforementioned types of recognition (Stal et al., 2003; Jodłowska and Śliwińska, 2014). The present study showed that changes in PSU size occur most frequently (Table 1). The second, ranked by frequency of occurrence, was the altering of accessory pigment activity. PSU number changes in *Synechococcus* sp. rarely occurred, which is consistent with the literature (Jodłowska and Śliwińska, 2014). Moreover, in our study, photoacclimation mechanisms occurred less frequently in the scenarios with salinity 3 PSU. The photosynthesis parameters (P_m, α) changes under different environmental conditions explain the occurrence of different photoacclimation mechanisms. According to our results, *Synechococcus* strains present different ecophysiological characteristics, however, they all demonstrate their tolerance to elevated PAR (for BA-120 to a certain degree) and T levels and could have effectively acclimated to varied water conditions. These strains were able to change the

composition of photosynthetic pigments in order to use light quanta better. The ability of *Synechococcus* to sustain their growth in low light conditions and their low photoinhibition in exposure to high light intensities could give picocyanobacteria an advantage in optically changeable waters (Jasser, 2006).

Due to the occurrence of extremes in salinity and other environmental conditions in the Baltic Sea area, the Baltic inhabitants are highly adapted to different regions and often reach their physiological limits (Snoeijs-Leijonmalm and Andrén, 2017). The changing environmental conditions the cultures were grown in during the experiments were salinity, T, and PAR. Daily mean sea surface temperature (Leppäranta and Myrberg, 2009) presents strongly pronounced annual cycles in the Baltic Sea area. Sea surface temperature (SST) range between about 10 and 20ºC may be timed in the Baltic between June and September with some inter-annual changes (Siegel and Gerth, 2017). SSTs reaching and exceeding 20ºC are also observed in the Baltic basin. For instance, according to Siegel and Gerth (2017), SSTs higher than 20ºC were recorded in almost whole Baltic area beyond Danish Straits, Bothnian Bay and northern Bothnian Basin in the warmest week of 2016, in July. According to the above, the temperatures, under which the picocyanobacterium cultures were grown in the present study (10 – 25ºC) can be defined as representative for the Baltic Sea. Furthermore, the salinity ranges applied in the experiment are also Baltic's representatives. The Baltic Sea horizontal salinity gradient is high and different sub-basins are characterized by different mean salinity values. The gradient decreases North towards. The highest salinity is observed in the Baltic Sea boundary to the North Sea (Skagerrak, mean salinity ranges between 28.34 and 32.71), while the lowest mean salinity is observed in the Baltic northernmost regions (around 2.35 – 3.96 in Bothnian Basin). These numbers were determined on the basis of climatological data from the Baltic Atlas of Long-Term Inventory and Climatology (Feistel et al., 2008; 2010). Thus, the presented analysis may derive accurate assumptions regarding the regional distribution of *Synechococcus* sp. strains in the Baltic Sea. For instance, a salinity horizontal gradient can be one of the factors determining the abundance of a certain strain in the basin. More saline waters are most preferred by BA-132. On that basis, one can assume the concentration of this strain will be higher near the Baltic Sea entrance (Danish Straits) than in Bothnian Bay. Additionally, it was observed that despite elevated PAR conditions being more suitable for BA-124 and BA-132 to grow intensively, all analyzed strains were able to survive and grow in low PAR conditions. This is consistent with other previously published Baltic studies (Stal et al., 2003; Jodłowska and Śliwińska, 2014) stating that this is caused by phycobilisomes, which are structural components of picocyanobacteria PSII photosystem. The presence of PCY cells throughout the whole euphotic water column was also reported in limnological studies (Becker et al., 2004, Callieri, 2007).

The discrepancies between the strains ecophysiology derived in this study amplified the need for in-depth investigation of three strains separately. What is more, according to the author's best knowledge, Baltic brown strain (BA-132) is the least recognized strain out of three analyzed *Synechococcus* sp. strains, so far. Stal et al. (2003) and Haverkamp et al. (2008) pointed to its inhabitation in the Baltic Sea but did not give its characteristics in detail. In more recent research new information has appeared, which has provided a more detailed examination of BA-132 (Jodłowska and Śliwińska, 2014). Nonetheless, this strain still required careful studies. The present paper derives the new knowledge on the BA-132 responses to changing ecological conditions. What is more, the study places BA-132 among the other *Synechococcus* sp. strains and compares their ecophysiology pointing to significant differences between these organisms.

The study of Baltic picoplankton ecophysiology is also of a great importance in the context of climate change. According to Belkin (2009), the Baltic Sea is among the Large Marine Ecosystems (LME), where the most rapid warming is being observed (the increase in SST between 1982 and 2006 > 0.9°C). Moreover, there are studies pointing to an increase of average winter temperatures in northern Europe by several degrees by the year 2100 (Meier, 2002). These along with the presented results, which suggest that all analyzed strains of *Synechococcus* sp. were positively affected by T can be a strong argument for further numerical research on examining the effect of long-term positive temperature trend on the abundance of PCY in the Baltic Sea (the need for picoplankton model representation). What is more, the feedback relation, which is the surface most layer being warmed up by irradiance trapped in the cells of phytoplankton may derive interesting conclusions on the functioning of the ecosystem and the living organisms being the internal source of heat in the marine medium.

612 The observed feature that T increase had a positive impact on all strains' number of cells is also consistent with field
613 studies, which indicate the seasonal cycle of PCY maximal abundances (Flombaum et al., 2013; Dutkiewicz et al., 2015;
614 Worden and Wilken, 2016). Hajdu et al. (2007) showed that during the decline phase of Baltic cyanobacterial blooms in late
615 summer, unicellular and colony-forming picocyanobacteria increased in abundance. Mazur-Marzec et al. (2013) indicated
616 that in summer cyanobacterial biomass was usually high and ranged from 20% at the beginning of July to 97% in late July
617 and August. Moreover, Paczkowska et al. (2017) pointed to the abundance of 40-90% in the summertime in the Baltic Sea
618 and to PCY being a dominant size group in all Baltic basins. Stal et al. (1999) reported that 65% of the phytoplankton-
619 associated Chl *a* concentration in the Baltic Proper during late summer belonged to picoplankton, while the second most
620 dominant group was nitrogen-fixing cyanobacteria (*Aphanizomenon* sp., *Dolichospermum* sp. and *Nodularia* sp.). Contrary
621 to that, there were also some reports regarding high PCY abundance in the wintertime. For instance, during the winter–
622 spring period, picocyanobacteria was the second most dominant fraction in the Baltic Sea (Paczkowska et al., 2017). The
623 present study showed that PCY can survive and grow also in low T and PAR conditions, which is consistent with the above-
624 cited field research of Paczkowska et al. (2017).

625 The studies of autecology of the PCY community and an understanding of its response to main environmental factors
626 could be an important step in recognizing the phenomenon of PCY blooms in marine environments. Additionally, the
627 laboratory experiments became a foundation in developing a new approach to Baltic Sea phytoplankton modeling -
628 development of pico-bioalgorithm describing PCY growth, which will enable long-term numerical studies on the response of
629 PCY to changes in environmental conditions.

5 Conclusions

633 Discrepancies in the number of cells, pigmentation changes, Chl *a* fluorescence and photosynthesis characteristics implied
634 that BA-120, BA-124, and BA-132 should be studied and examined separately.

635 Nonetheless, there were also fixed features referring to all analyzed strains. This reasoned the association of these
636 features with *Synechococcus* sp. in general, as a species. For instance, according to the derived results, PAR and T played a
637 key role in the life cycle of all three strains. Additionally, the positive impact of salinity on the number of cells was observed
638 in each culture. Another similarity was the prevalence of the change in PSU size among the mechanisms of
639 photoacclimation. The second most dominant was altering of accessory pigments, whilst the least frequent was the change in
640 PSU number.

641 Contrary to that, the main differences were: different responses of the number of cells to different environmental
642 conditions in different cultures; various photoacclimation mechanisms observed; and changes in pigmentation. According to
643 the latest research, PCY are a great contributor to total primary production in the Baltic Sea and may contribute to summer
644 cyanobacteria bloom to a high degree. This explains the authors' motivation to lead an in-depth investigation on Baltic PCY
645 response to a changing environment. The present research is a first step on the way to deriving new knowledge on
646 *Synechococcus* sp. ecophysiology and is a foundation for further studies.

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660   **References**

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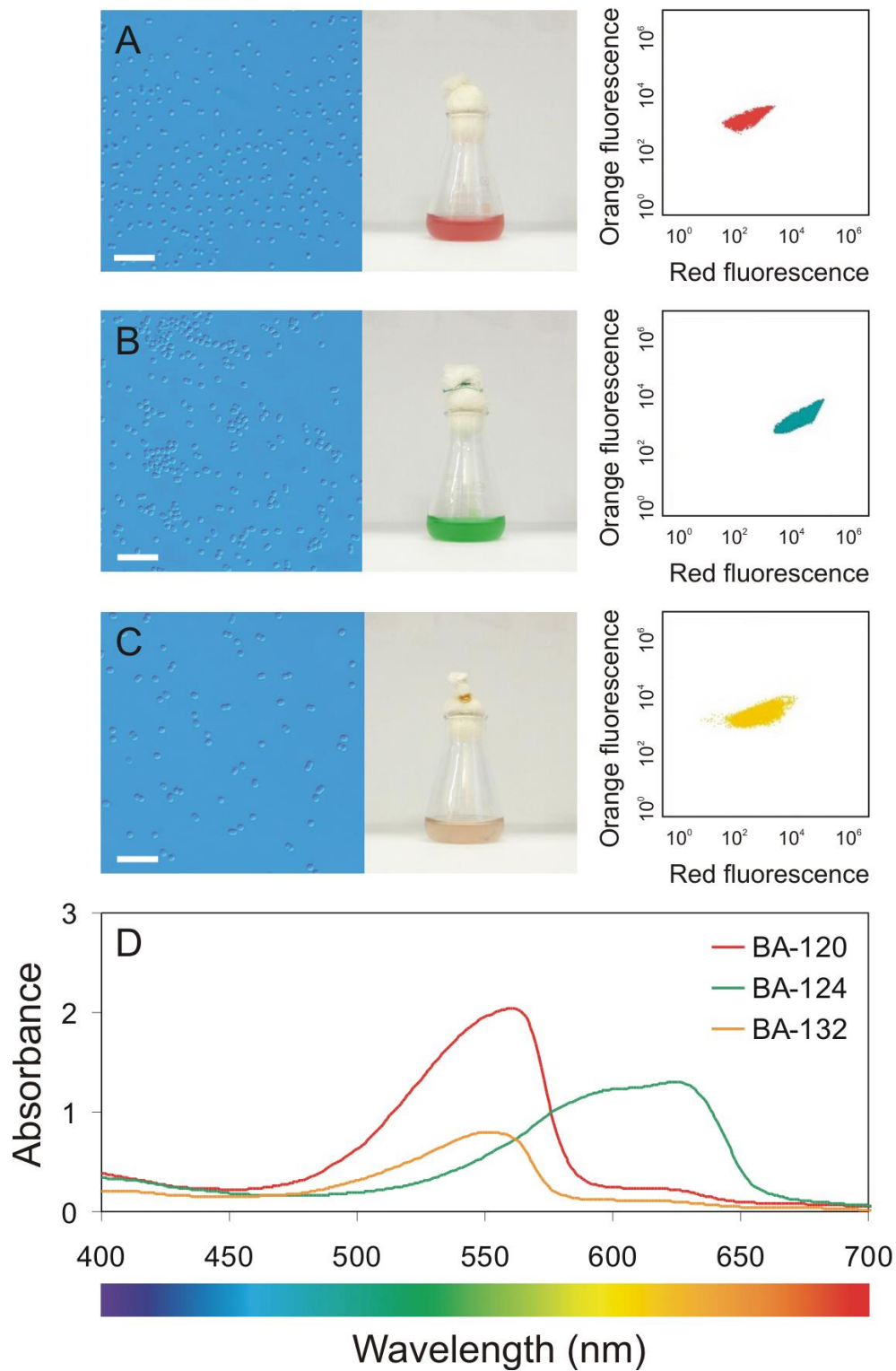
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863 **Figure 1**: Left-side top panel (A, B, C) – light microscope photographs of three *Synechococcus* sp. strains (scale bar = 10
864 μm) along with the photographs of the cultures in 25-mL glass Erlenmeyer flasks; right-side top panel – scatter plots of
865 orange fluorescence vs. red fluorescence analyzed using a BD Accuri™ C6 Plus flow cytometer and bottom panel (D) –
866 PAR absorption spectra determined for the mixture of phycobilin pigments for each *Synechococcus* sp. strain.
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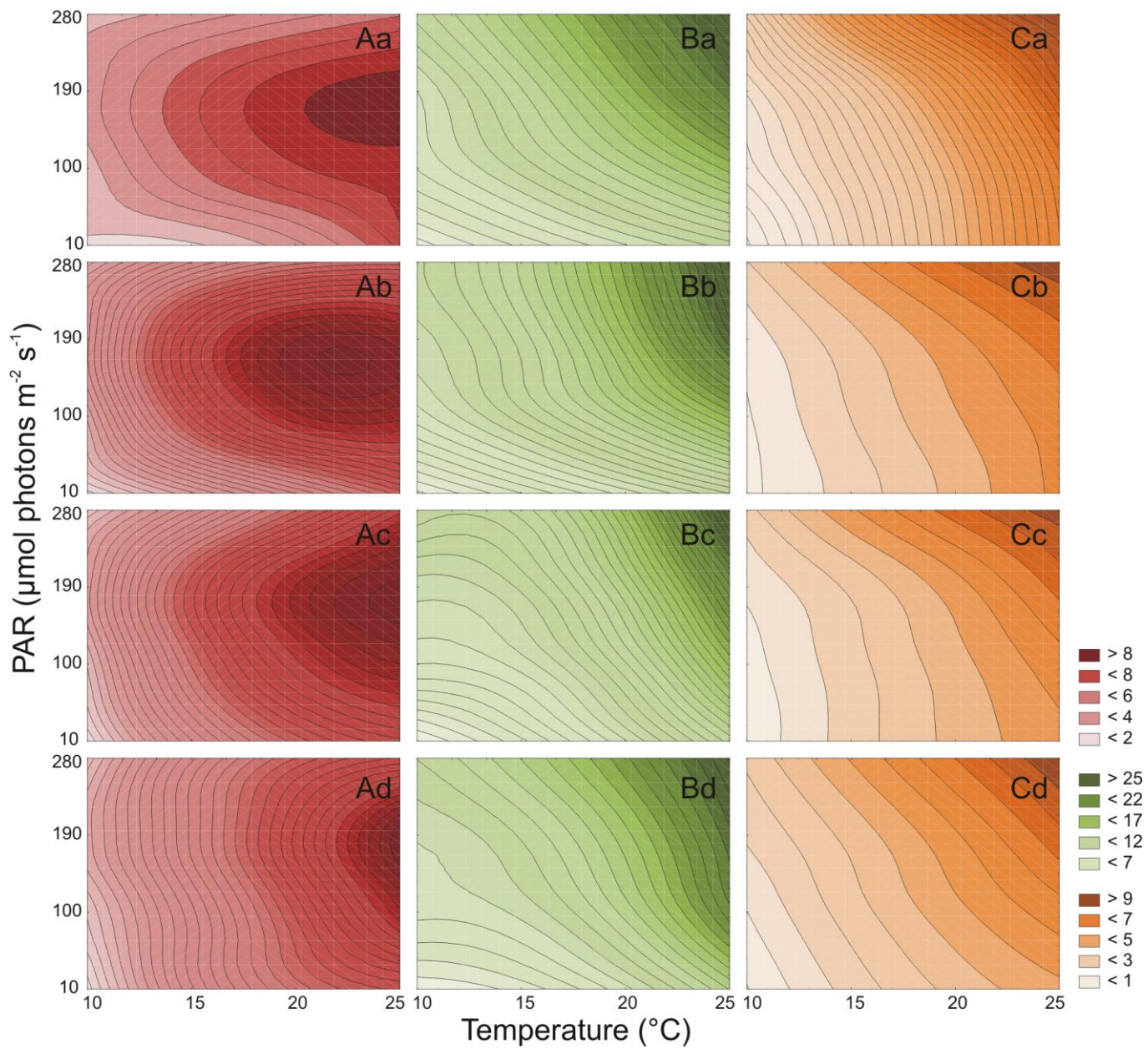
869 **Figure 2.** Cell number (10^6 cell mL^{-1}) for three *Synechococcus* sp. strains: BA-120 (A), BA-124 (B) and BA-132 (C) under

870 different PAR and temperature conditions in 4 salinity mediums: 3 PSU (a), 8 PSU (b), 13 PSU (c) and 18 PSU (d).

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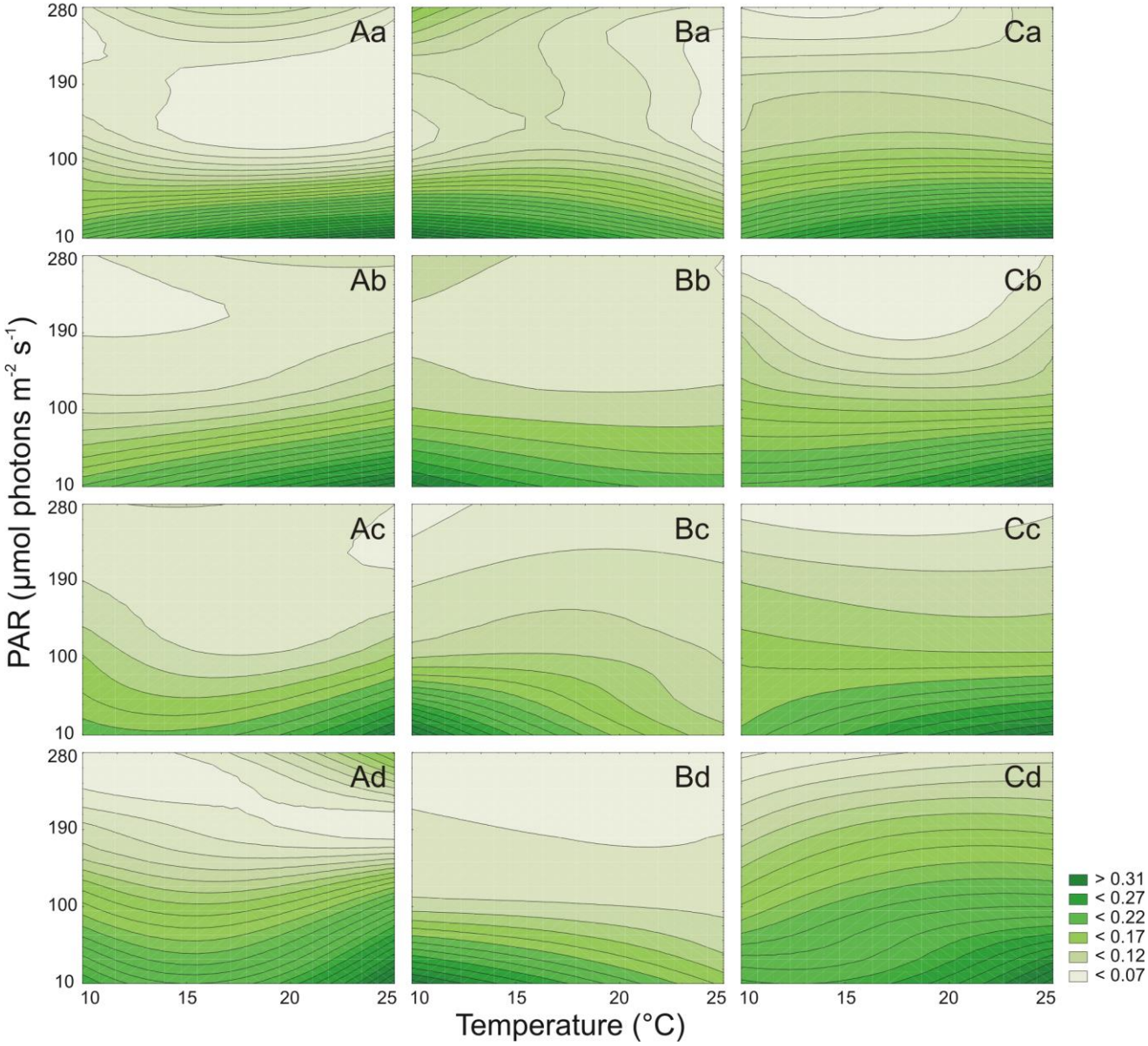
Figure 3. Cell-specific Chl a (pg cell^{-1}) changes for three *Synechococcus* sp. strains: BA-120 (A), BA-124 (B) and BA-132 (C) under different PAR and temperature conditions in 4 salinity mediums : 3 PSU (a), 8 PSU (b), 13 PSU (c) and 18 PSU (d).
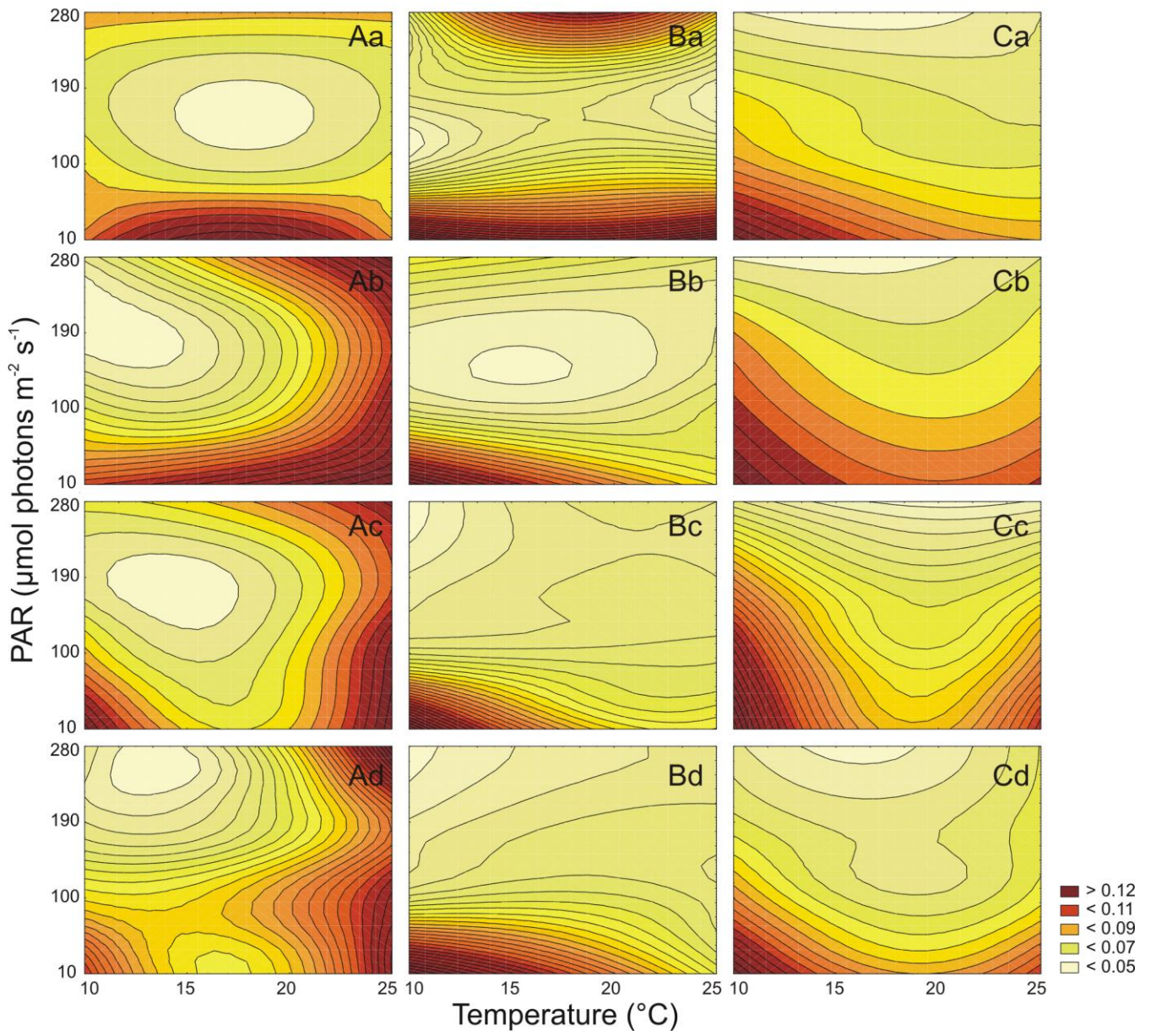
Figure 4. Cell-specific Car (pg cell^{-1}) changes for three *Synechococcus* sp. strains: BA-120 (A), BA-124 (B) and BA-132 (C) under different PAR and temperature conditions in 4 salinity mediums: 3 PSU (a), 8 PSU (b), 13 PSU (c) and 18 PSU (d).

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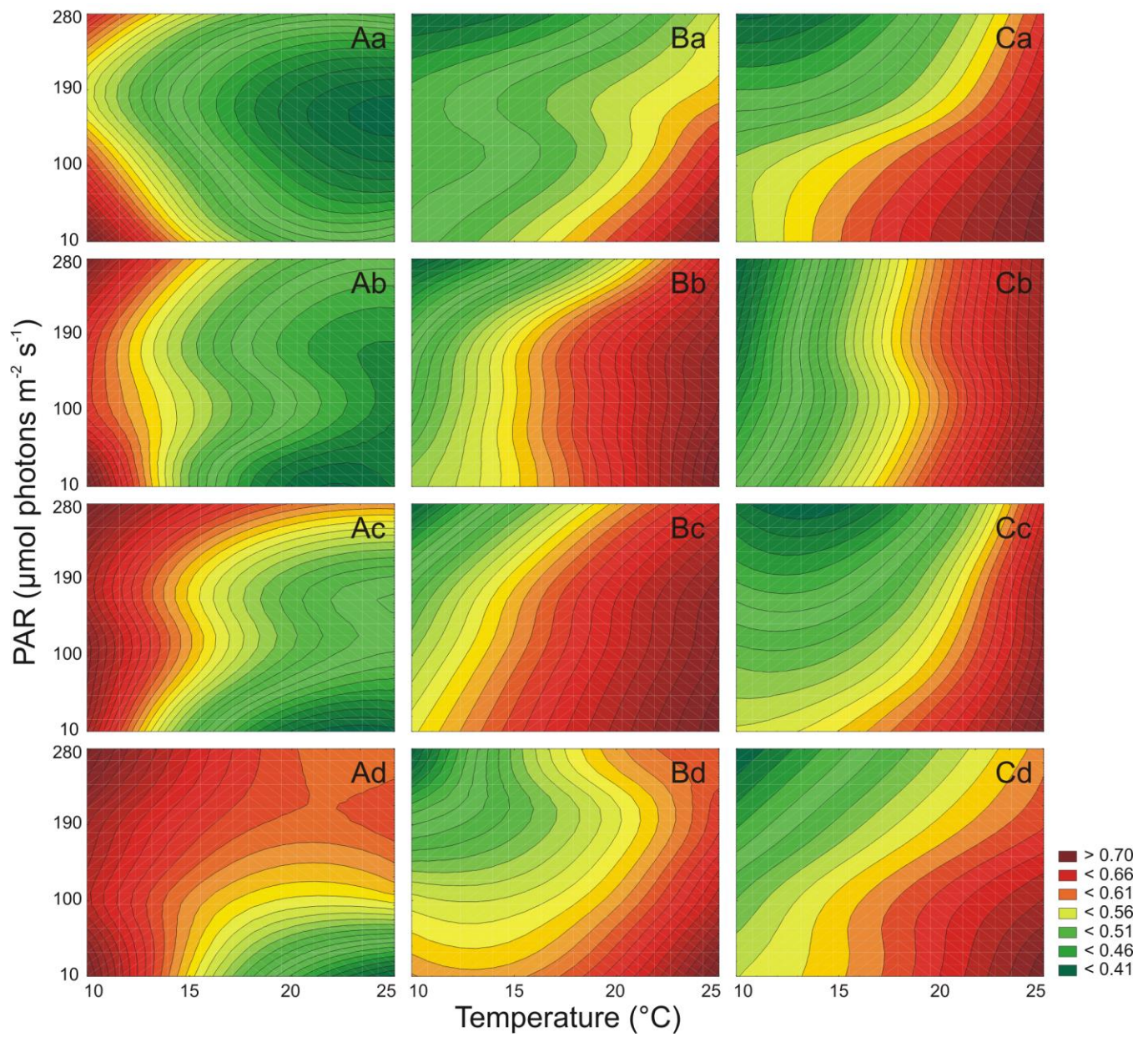
Figure 5. The maximum photochemical efficiency of PSII in the dark-adapted state (F_v/F_m) for three *Synechococcus* sp.
strains: BA-120 (A), BA-124 (B) and BA-132 (C) under different PAR and temperature conditions in 4 salinity mediums: 3
PSU (a), 8 PSU (b), 13 PSU (c) and 18 PSU (d).

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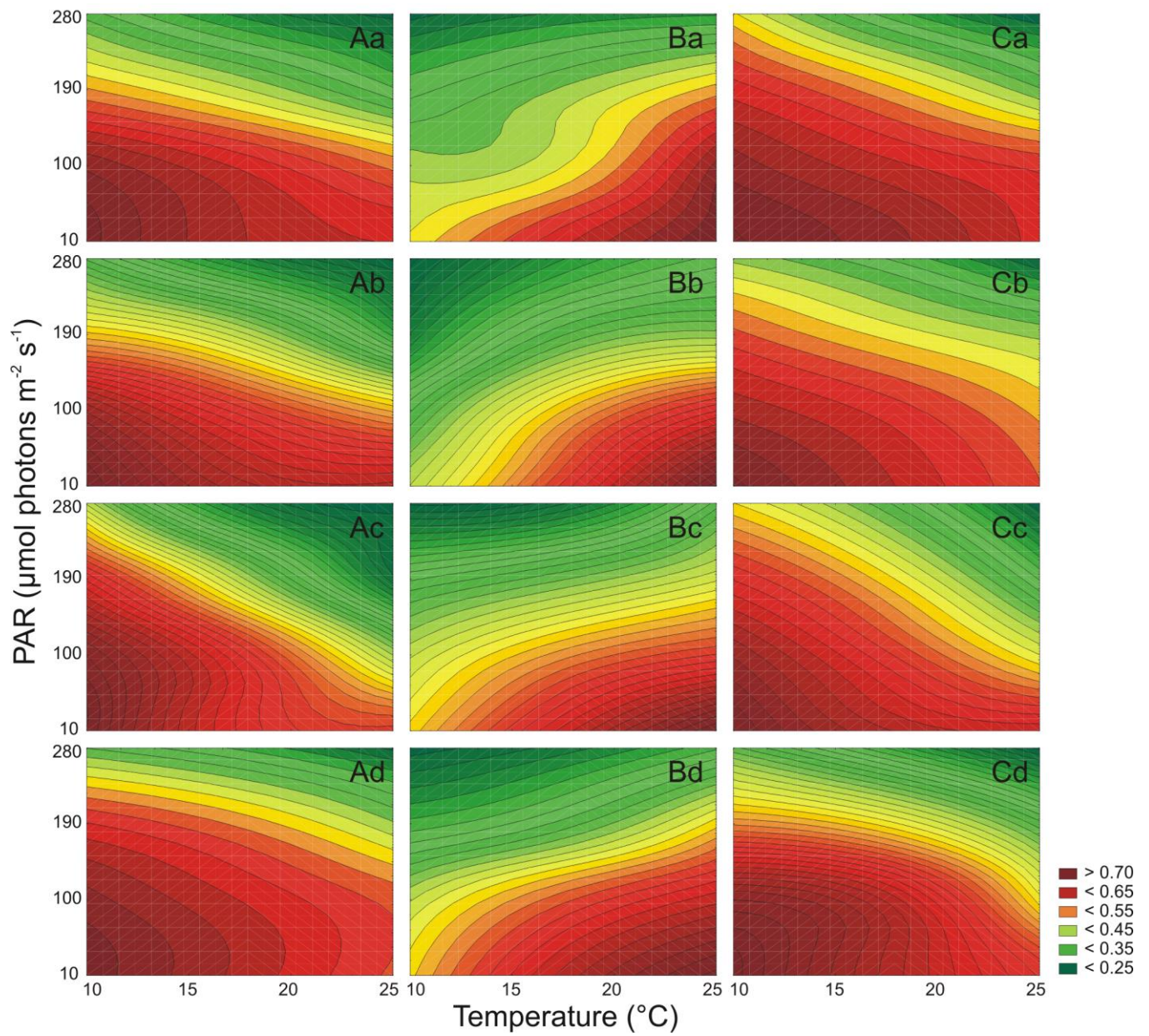
Figure 6. The photochemical efficiency of PSII under actinic light intensity (ΦPSII) for three *Synechococcus* sp. strains:
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BA-120 (A), BA-124 (B) and BA-132 (C) under different PAR and temperature conditions in 4 salinity mediums: 3 PSU (a),
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8 PSU (b), 13 PSU (c) and 18 PSU (d).
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Figure 7. Selected Chl *a* - specific and cell-specific (right side and left side panel, respectively) net photosynthetic–light
response curves for three *Synechococcus* sp. strains: BA-120 (a, b), BA-124 (c, d) and BA-132 (e, f) strains. Curves present
examples of three types of photoacclimation observed for *Synechococcus* sp. and these are as follows: change in number of
photosynthesis units (PSU) (a, b), change in size of PSU (c, d) and change in accessory pigments activity (e, f).

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Table 1. Photoacclimation types (mechanisms) for three *Synechococcus* sp. strains: BA-120, BA-124 and BA-132 at different ecological conditions. OTHER states for altering of accessory pigments activity or changes in enzymatic reactions; PSUsize states for the change in PSU sizes; PSUno. states for the change in PSU number. The symbols of labels indicate the strain for which the mechanism is observed and are as follows: [red] for BA-120, [green] for BA-124 and [brown] for BA-132.

CONDITIONS	Salinity 3 PSU	Salinity 8 PSU	Salinity 13 PSU	Salinity 18 PSU
10°C	PSUsize [brown]	OTHER [red] PSUsize [green] PSUsize [brown]	PSUsize [red] OTHER [red] OTHER [green]	OTHER [red] PSUsize [green] PSUsize [brown]
15°C	-	PSUsize [green]	OTHER [red] PSUsize [green] OTHER [brown]	PSUsize [brown]
20°C	-	PSUno. [red] OTHER [green] PSUsize	PSUsize (or OTHER) [red] OTHER [green] PSUsize	PSUsize [green]
25°C	OTHER [red] PSUsize [brown]	PSUsize [red] PSUsize [green] OTHER [brown]	PSUsize [red] PSUsize [green] PSUsize [brown]	PSUsize [green] PSUsize [brown]