Responses to anonymous Reviewer

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

General comments

I read the manuscript and found that the authors arranged the experiment in an elaborate way and the results reported may have some significance in biogeochemistry in the Baltic Sea. However, the authors totally failed to describe what is important and what is the ecological significance. The authors just presented list of outputs in the Result section, which made me fatigue. I believe that this is because the authors were not conscious enough on what is to be clarified in this study. In the Discussion section, the authors make some ecological discussion as if they had just come to this issue for the first time. However, such an issue should have been presented in the Introduction and the authors should have clarified what is the REAL OBJECTIVE. If the authors successfully notice what is the objective, the Result section could have been more arranged with appropriate selection of what is necessary and what is not.

Additionally, wording and phrasing in English were terrible. Actually, I could not catch what the authors meant in some sentences. I felt that many sentences are just literal translations from the authors' mother language. I STRONGLY RECOMMEND the authors to have this manuscript checked and edited by a native English speaker or an editing service.

And the authors should reduce the volume of the manuscript. It is too lengthy and redundant. Probably most of the Results section can be omitted, if the authors notice what is important.

REPLY:
The authors would like to thank anonymous Reviewer for the general comment, and hereby they want to inform that appropriate modifications have been introduced to the revised manuscript. Regarding the English writing style, please, note that before the submission, the MS was verified by the professional Proof Reading Service company. Nevertheless, the text has been checked again, following the Reviewer's advice. We hope that the present version is satisfactory. All the modifications in the manuscript are marked in blue color.

Specific comments

L44 This is confusing and I am afraid that the authors may have misunderstood the pigment of cyanobacteria. PE and PC are apoproteins, while PUB and PEB are phycobilins connected to apoproteins. These are different concepts. The readers may question "What is the phycobilin composition of red and green strains?" or "Both red and brown strains contain PE… What is the difference?"
REPLY:
Authors are aware of differences between apoproteins and phycobillins. In order to emphasize understanding the fragment pointed by the Reviewer, the Authors decided to rephrase it and provide it with more details. This issue was clarified in the revised manuscript in the way shown below (L: 42-54, in the revised MS)

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. S1 in Supplement) are different morphotypes 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 the 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).

L84 If this is your overall goal, this journal is not suitable for you. You should submit your manuscript to the journal more oriented to physiology. You should aim at a more ecological issue.
REPLY:
According to Authors' best knowledge, the objectives of the paper are suitable for the Biogeosciences' scope. However, in order to highlight the overall goal of this study, the Authors described it in more details in L: 90-93

L87 Minute figures for experimental settings should not appear in the Introduction. It appears again in Methods and redundant.
REPLY:
The Introduction section in the paper aim at giving the background information on the issue and motivation to conduct the study. It should also provide for a brief description of what the study is based on. Nevertheless, slight modifications have been introduced, i.e.: the sentence: '*These quantities were as follows: scalar irradiance in Photosynthetically Active Radiation (PAR) spectrum range (10, 100, 190, 280 µmol photons* m^{-2} s^{-1}*), salinity (3, 8, 13, 18 PSU), and temperature (T) (10, 15, 20, 25°C).'* was deleted.

L108 Information on salinity appears again in L116. It is redundant. Generally, this section is too wordy and redundant. Make it clear.
REPLY:
We changed "Material and culture conditions" section in order to address Reviewer's suggestions. However, we did not decide to shorten it significantly since the in-depth view of Material and Methods section was demanded by previous Reviewers and the detailed form of it is essential in the MS according to Authors' opinion as well.

L125 If the authors use halogen lamps only for higher irradiance, there would make difference in the spectrum of the light received among the "scenarios". As the authors know, the wavelength is an important factor for the growth of different strains of Synechococcus sp., because the different phycobilin compositions result in different absorption spectra of their photosynthetic antenna. How do the authors explain it?
REPLY:
In the study, the Authors used the light sources, which both give PAR spectrum. This was checked in other studies conducted in the Laboratory of Marine Plant Ecology of the Institute of

Oceanography University of Gdańsk. The confirmation is provided in Jodłowska and Latała (2010) and Jodłowska and Śliwińska (2014). These references were added in the text (L: 124-125). What is more, according to the LI-COR manual and technical specification therein, the sensor analyzes the spectrum and if it responds to Photosynthetically Active Radiation (PAR) spectrum, the intensity of PAR is measured. Considering the above, the Authors ensure that the scalar irradiance applied in each experiment was PAR.

L147 This sentence should appear first of the paragraph.
REPLY:
The Authors thank for drawing their attention to that. They agree with it. The sentence appears at the beginning of the paragraph in the revised MS.

L148 Do the authors mean "volume-specific" by "mL-specific"?
REPLY:
We clarified this aspect (L: 161-162).

L171 Replace ""through" with "onto", because the authors use the particle filtered onto a filter for analysis.
REPLY:
We corrected this aspect.

L197 Why did the authors ignore the salinity for independent variable and their confounding effects?
REPLY:
The Authors aimed at demonstrating the influence of temperature and PAR on picocyanobacteria physiology and examine how different are potential impacts of these variables on the organisms growing in different mediums of salinity. This is why the independent variables in the statistics were temperature and PAR. What is more, please, note that the Authors did not ignore the influence of salinity on the picocyanobacteria physiology at all. The positive influence of increasing salinity is one of the striking observation derived in this study.
Please, note also that in section *2.6 Statistical analyses* of the original MS, the Authors provided for the explanation of why temperature and light had been the independent variables in the study.

L206 Use tables to show the significance of relationships between the variables. The endless listing of the values does not interest the readers.
REPLY:
The Authors tried to rearrange the text and replace the statistics listing with tables, however they did not decide to present this information in the manuscript in this form, finally. This is because the tables are of 11 pages, which, even with the listing deleted completely within the MS text, would extend the MS size a lot. Authors claim that organizing the main statistic characteristics in the way it had been previously done is the best solution. However, the Authors agree with the Reviewer that the listing may impede reading for the reader who is not interested particularly in statistics numbers. Due to that, the Authors decided to write the statistics in italic, which noticeably reduces the impediment; see for instance L: 221-223. Now, it is much easier to omit these fragments by the potential reader who is not interested in ANOVA values.

L217 The authors just examined for different light intensities, and the setting over 190 is just one setting, 280. Then is it appropriate to use "onward"? The authors cannot tell whether the cell abundance continues to decrease "onward". One biggest concern about that is that it may be

inappropriate to use ANOVA to describe such a relationship, because it is based on the assumption of linear relationship between the two variables.

REPLY:

The Authors analyzed the influence of specific environmental conditions on the picocyanobacteria physiology, whereas the conditions were different variables ranged from-to specific limits. The 'onwards' term goes for values within these limits, i.e.: onwards within the ranges not onwards generally. However, indeed, Authors try to extrapolate their observations beyond the analyzed ranges, which is not inappropriate in scientific concluding.

Regarding ANOVA – the validity of applying this method to the present study is obvious, according to Authors' best knowledge. This was done in many ecophysiological studies before (e.g.: Defew et al., 2004; Jodłowska and Latała, 2010). Furthermore, please note that ANOVA is a statistical method, which enables one to examine the influence of independent variables on dependent one. It has nothing to do with linearity.

L219 This description is too speculative and far from quantitative. What do you mean by "important"? And on which result is this description based on? This comment points to many other sentences in this section.

REPLY:

The Authors meant that the most important environmental factor influencing BA-120 number of cells was temperature (T). This was pronounced the most within lower temperatures (10 and 15°C), where the change in BA-120 abundance along with PAR increase was barely observed (being plainly visible along with T increase at once) (see Fig 1A, a-d).

The additional explanation was added in the text (L: 228-231).

L228 Actually, I could not understand what the authors intended to say in these sentences (to L234). Please rearrange them.

REPLY:

The paragraph has been rephrased (L: 240:249).

L488 From here on, every paragraph is just a repetition of the first one, where some figures may have been replaced. It obscures what is important and which the reader should be reminded of. My question here is only "So what?".

REPLY:

This is the results section. Since the study provides for many results, the best way to describe them was to organize them in a similar way. According to that, the paragraphs 'look' similarly but it is only about the appearance itself since the content (merit) of each fragment is different. Providing for so many parameters and their values in different environmental conditions for different picocyanobacteria cultures needs a good method to present them in one paper in a concise way. The Authors believe they chose an appropriate method to do this limpidly not leading the reader to confusion. Regarding to 'So what?' question, the answer is provided in Discussion section.

L544 Cite appropriate literatures to support this description.

REPLY:

We added appropriate references (L: 558-559).

L573 "Acclimation" means the phenotypic phenomenon that one organism strengthens its ecological fitness by changing gene expression. Do you mean it here or did you intend "adapted"?

REPLY:

We changes this aspect (L: 586).

L590 Why only in this scenario? Is it a universal phenomenon?

REPLY:

Yes, this is a universal phenomenon but it does not have to occur always and in every environmental conditions. In this study it was observed that PSU number change occurred in BA-120 cultures grown under 20°C in medium of 8 PSU.

L591 You say "also", but in addition to what?

REPLY:

Authors thank for drawing their attention to that. The 'also' was replaced. Presently, the sentence sounds as follows: PAR and T were the main factors also in terms of influencing the changes in Chl *a* fluorescence in three strains of *Synechococcus* sp. (L: 606-608).

L617 "the Baltic inhabitants are highly adapted to different regions" This sentence is too abstract.

REPLY:

Authors do not consider this sentence as too abstract. What is more, they cite the appropriate literature to confirm their point of view.

L620-L625 These should have been placed in Introduction.

REPLY:

Authors do not agree to move this fragment to Introduction section. This is because the paper is not on the environmental conditions in the Baltic Sea explicitly. According to Authors' opinion, placing the fragment in Introduction could lead to confusion while reading the introductory part. Furthermore, Discussion is the section where the results are analyzed and discussed also regarding the natural conditions in the Baltic. Considering that, Authors hold that the sentences were placed appropriately within the MS. What is more, the previous Reviewer suggested to add more detail description on Baltic representative conditions while writing about the application of derived results to natural environment (which is in Discussion section). Since the Authors try to address all the comments on the manuscript, from both the present and the previous revision, and to follow their own opinions at once, they decided to leave the indicated fragment in the Discussion section, as originally (after first revision).

L640 This paragraph should have been placed in Introduction.

REPLY:

According to Authors' opinion, this fragment should not be placed in the Introduction section as this fragment involves the results derived from the study. Authors cannot recall observations before deriving them, which is in results section. Considering that, the Authors hold the place to locate this fragment is discussion.

L644 What is "new information"? How is it related with your results?

REPLY:

New information means here new (foreground) knowledge. The brown strain of picocyanobacteria has not been examined in such details so far, which is highlighted in the Introduction (L: 84-88) with appropriate literature citation given. In the present paper, the autecology of this strain was analyzed in details (not only the abundance but also photochemical processes (briefly speaking) performed by this strain). Furthermore, the results derived in this study demonstrate how BA-132 is different from the two other analyzed strains. This was possible to be done only because all the strains were grown in exactly the same synthetic environments, meticulously controlled in the laboratory. Additionally, scientific concluding on the strain distribution, living and surviving in the natural Baltic environment was also conducted (oceanic features of Baltic organism – preference of BA-132 to high salinity conditions).

L678 “This study shows differences and similarities” This sentence does not give any information. EVERY STUDY shows differences and similarities among different things. How different? Which is how? At which point different? What does it mean?

REPLY:

The sentence: *This study shows differences and similarities* stated only for an introductory sentence in Conclusion section. This was done because in next lines the Authors gave precise and concise information about the differences and similarities observed between strains. Nevertheless, in order to address the anonymous Reviewer’s suggestion, Authors decided to delete the first sentence of the Conclusion section.

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

S. Śliwińska-Wilczewska[1], A. Cieszyńska[2], and A. Latała[1]

[1]University of Gdańsk, Institute of Oceanography, Laboratory of Marine Plant Ecophysiology, Gdynia, Poland

[2]Institute of Oceanology Polish Academy of Sciences, Department of Marine Physics, Marine Biophysics Laboratory, Sopot, Poland

Correspondence to: A. Cieszyńska (acieszynska@iopan.gda.pl, cieszynska.agata@gmail.com)

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. Therefore, the work, which examines the response of *Synechococcus* sp. physiology to different environmental conditions was conducted. 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 real environmental conditions. Scenarios reflecting all possible combinations were applied in the laboratory experiments. Results pointed to differences in final number of cells between strains. However, there was also a similar pattern for BA-124 and BA-132, which showed the highest concentrations of picocyanobacteria cells at higher 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 between the strains. In this context, more consistent features were observed for brown and red strains when compared to the green. In this paper are defined the ecophysiological responses of PCY.

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 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* are ubiquitous components 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. S1 in Supplement) are different morphotypes 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 the 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 found and isolated from marine, brackish and freshwater environments are often related to *Synechococcus* cluster 5 (Herdman et al., 2001). *Synechococcus* cluster 5 is divided in 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 PCY has been investigated mainly by analysis of the 16S rRNA gene. However, the phylogenetic tree of *Synechococcus* sp. is not always consistent with their pigmentation type (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 blooms 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 their negative impacts on ecosystems (Jasser and Callieri, 2017; Śliwińska-Wilczewska et al., 2018a).

According to the all above, 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 this study was to carry out laboratory experiments on *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 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 help to develop the knowledge on the picocyanobacteria life cycle. Moreover, the PCY experiments underlie the improved numerical approach to phytoplankton modeling development. On the basis of derived results, the algorithms for picocyanobacterium growth is being 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 filters (Macherey-Nagel MN GF-5) using a vacuum pump (600 mbar) and autoclaved. The cultures were incubated in 35 mL Erlenmeyer glass flasks. 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 PCY 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 salinity was controlled 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 source of irradiance and 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.

The synthetic environmental conditions of salinity and T applied in the laboratory are representative for the Baltic Sea area (Feistelet al., 2008; 2009; Siegel and Gerth, 2017). Moreover, the values of environmental conditions variables (salinity,

temperature, PAR) were also specified in certain ranges to make this study comparable with other laboratory cultures experiments available in literature. The combination of the quantities of environmental variables is called a scenario in the present paper. After acclimation time (2 d), the PCY cells served as inoculum for the right test cultures with the initial number of cells equal to 10^6 cells mL^{-1}. The flasks with picocyanobacteria were shaken (once a day) during the experiment. In order to achieve the most reliable results, test cultures were grown in three replicas and were incubated for one week at each combination of light, temperature and salinity. On the last day of incubation the number of cells, pigment content, Chl *a* fluorescence, and rate of photosynthesis were measured in each replica. Results were reported as mean values ± standard deviation (SD).

2.2 Determination of the number of cells

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

2.3 Determination of the pigments content

The concentration of photosynthetic pigments of analyzed picocyanobacteria was measured by the spectrophotometric method (Strickland and Parsons, 1972). The analysis of mL-specific (pigment content per mL) and cell-specific (pigment content per cell) pigmentation was conducted. Note that mL-specific means volume-specific, whereas the volume is fixed to 1 mL. After seven days of incubation, 4 mL of culture was filtered in order to separate the picocyanobacteria cells from the medium. Chl *a* and carotenoids (Car) were extracted from the PCY cells with cold 90% acetone (5 mL). To improve extraction, the cells were disintegrated for two minutes by ultrasonication. Then, the test-tube with the extract was held in the dark for three hours at -60°C. To remove cell debris and filter out the particles, the extracts were centrifuged at 10,000 rpm (8496 × g) for 5 min (Sigma 2-16P, Osterode am Harz, Germany). The absorbance of pigments was estimated on 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 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 cultures grown in different environmental conditions and referred to the PAR value in respective scenarios. 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 seventh day of the experiment using a Clark-type oxygen electrode (Chlorolab 2, Hansatech). 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 (photosynthetic parameters, i.e., the photosynthetic capacity (P_m), the initial slope of *P-E* curve (α) and the dark respiration (R_d)) was fitted to the photosynthetesis irradiance response (*P-E*) curves using equation (Jassby and Platt, 1976) and Statistica® 13.1 software (Sakshaug et al., 1997).

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). Due to that, 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

For all three picoplankton strains, ANOVA analysis indicated that in each scenario the independent variable (temperature or PAR) significantly influenced the dependent variable. What is more, post-hoc tests indicated that multiple factors (T and PAR together) had an impact on the PCY growth.

According to post-hoc tests, 2008 multiple comparisons (70%) out of all 2880 completed for three strains, indicated the highest statistical significance (Tukey HSD, *** $p < 0.001$), 160 multiple comparisons (6%) pointed to the statistical

significance of 0.001 < ** p < 0.01, and 114 (4%) showed the significance of 0.01 < * p < 0.05. The rest of the multiple comparisons (598, 20%) indicated no statistically significance differences (Tukey HSD, $p \geq 0.05$).

Both PAR and T affected the number of *Synechococcus* sp. BA-120 cells significantly *(ANOVA, $F_{9,32}$ = 42.3, *** p < 0.001, ANOVA, $F_{9,32}$ = 22.7, *** p < 0.001, ANOVA, $F_{9,32}$ = 9.6, *** p < 0.001 and ANOVA, $F_{9,32}$ = 12.2, *** p < 0.001, for salinity 3, 8, 13, 18 PSU, respectively)*. For BA-120, the number of cells increased with T in each medium (salinities 3, 8, 13, 18 PSU) (Fig. 1A, a-d). The minimum 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. 1A, a), 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. 1A, d). The decrease in number of cells was observed from PAR 190 µmol photons m^{-2} s^{-1} onwards. This can likely be related to the photo-inhibition of photosystem II (PSII). The above was the case in each salinity (Figs. 1A, a-d). Additionally, the results analysis (Fig 1A, a-d) showed that the most important environmental factor influencing BA-120 number of cells was T, with PAR playing an additional role, for instance in the context of photo-inhibition. This was pronounced the most within lower temperatures (10 and 15°C), where the change in BA-120 abundance along with PAR increase was barely observed being plainly visible along with T increase at once. Multiple comparisons tests pointed to the strong significance of PAR and T combined in influencing the number of *Synechococcus* sp. BA-120 cells. According to the statistics, 82% of multiple comparisons were statistically significant (Tukey HSD, * p < 0.05) with 91% of them having the highest significance level (Tukey HSD, *** p < 0.001).

Both PAR and T also significantly affected the number of *Synechococcus* sp. BA-124 cells *(ANOVA, $F_{9,32}$ = 7.9, *** p < 0.001, ANOVA, $F_{9,32}$ = 13.6, *** p < 0.001, ANOVA, $F_{9,32}$ = 8.4, *** p < 0.001 and ANOVA, $F_{9,32}$ = 2.8, ** p < 0.01, for salinity 3, 8, 13, 18 PSU, respectively)*. For BA-124, number of cells increased with T and PAR in all salinities (Figs. 1B, a-d). The lowest number of cells was calculated in salinity 3 PSU, T 10°C and PAR 10 µmol photons m^{-2} s^{-1} (2.0×10^6 cell mL^{-1}, Fig. 1B, a) and the highest number of cells was reached in salinity 18 PSU, T 25°C, PAR 280 µmol photons m^{-2} s^{-1} (43.6×10^6 cell mL^{-1}, Fig. 1B, d). High abundances were estimated also under the highest T and PAR conditions in salinity 13 PSU, where a number of cells equalled 41.1×10^6 cell mL^{-1} (Fig. 1B, c). Generally, the number of cells was the highest in BA-124 cultures when compared to BA-120 and BA-132 cultures in respective scenarios. One of the observations was the difference in BA-124 number of cells between lower and higher PAR and T conditions (scenarios with lower PAR and T and scenarios with higher PAR and T). BA-124 seemed to be more sensitive to changes in PAR and T in their lower rather than in higher ranges. Regarding salinity, the highest number of BA-124 cells were noted in moderate- and high-salinity mediums. Optimum salinities for strain BA-124 were 8 and 13 PSU. Due to post-hoc analysis, salinity 13 PSU differentiated the conditions for cell abundances under different PAR and T at a lower degree when compared to other salinities under respective PAR and T (the least statistically significant differences observed in medium 13 PSU), which is also noticeable in Fig. 1B, c. Another feature of BA-124 was the number of cells in low T and high PAR scenarios were nearly equal to cell abundances in high T and low PAR scenarios. This was not the case for BA-120 and BA-132 strains. The observation was supported by Tukey's tests, where only few statistically significant differences in number of cells were observed between scenarios with elevated PAR (280 µmol photons m^{-2} s^{-1}), low T (10, 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 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 both cases, Figs. 1B, a-b). Multiple comparisons tests showed high significance of combinations of PAR and T in affecting the number of cells. According to Tukey HSD tests, 72% of multiple comparisons were statistically significant (* p < 0.05) with 82% of them with the highest significance level (*** p < 0.001).

Similarly to BA-120 and BA-124, it was found that PAR and T significantly affected the number of *Synechococcus* sp. BA-132 cells *(ANOVA, $F_{9,32}$ = 6.8, *** p < 0.001, ANOVA, $F_{9,32}$ = 5.4, *** p < 0.001, ANOVA, $F_{9,32}$ = 5.6, *** p < 0.001 and ANOVA, $F_{9,32}$ = 12.5, ** p < 0.01, for salinity 3, 8, 13, 18 PSU, respectively)*. For BA-132, the positive impact of T and PAR on number of cells (Figs. 1C, a-d) was observed in each medium. Note that positive impact means the increasing (positive) dependency, whilst negative impact means decreasing (negative) dependency between the independent and

dependent variable, e.g.: between T and abundance. Salinity played a more significant role here than when compared to BA-124. It was found that the higher the salinity, the higher the number of cells of BA-132. What is more, according to the statistical analysis, salinity 18 PSU differentiated the number of cells the most (Fig. 1C, d). In salinity 18 PSU, the cell abundances could be described as a linear increasing function of ambient T and PAR. This was also observed in other salinities but not as intensively pronounced as in the highest-saline medium. Moreover, in high salinity, the sensitivity of number of cells to T changes was much lower than in low salinities. PAR did not determine the number of cells as strongly as T, which was quite consistent to the observation noted 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}, Fig. 1C, a), whilst the maximum in 18 PSU, 25°C, 280 µmol photons m^{-2} s^{-1} (16.1×10^6 cell mL^{-1}, Fig. 1C, d). In addition, the lowest values of BA-132 number of cells were calculated for the lowest T and PAR condition in each salinity. Tukey HSD post hoc tests indicated high significance of the combination of PAR and T in affecting the cell abundances. Regarding those tests, 84% of multiple comparisons were statistically significant (* $p < 0.05$) with 90% of them with the highest significance (*** $p < 0.001$).

Concerning all three strains, high salinity generally had a positive impact on number of *Synechococcus* sp. cells. What is more, the relations between salinity and number of cells for all strains, especially red and brown were almost increasing linearly with the highest average increase for BA-132.

3.2 Pigment content

The results showed that for all strains, cell-specific pigment composition (pigment content per cell) was environmentally driven (Figs. 2, 3). The analysis of mL-specific pigmentation (pigment content per mL) was also done (Figs. S2 and S3 in Supplement), however, the mL-specific pigment content is another way to illustrate the biomass and that is why it is not described in this section in detail.

It was estimated, that PAR and T significantly affected the Chl *a* cell-specific content of *Synechococcus* sp. BA-120 *(ANOVA, $F_{9,32}$ = 33.7, *** $p < 0.001$, ANOVA, $F_{9,32}$ = 5.3, *** $p < 0.001$, ANOVA, $F_{9,32}$ = 15.6, *** $p < 0.001$ and ANOVA, $F_{9,32}$ = 5.7, *** $p < 0.001$, for salinity 3, 8, 13, 18 PSU, respectively)*. Both PAR and T also affected the Car content in the BA-120 strain cells significantly *(ANOVA, $F_{9,32}$ = 25.8, *** $p < 0.001$, ANOVA, $F_{9,32}$ = 7.5, *** $p < 0.001$, ANOVA, $F_{9,32}$ = 7.3, *** $p < 0.001$, and ANOVA, $F_{9,32}$ = 12.0, *** $p < 0.001$, for salinity 3, 8, 13, 18 PSU, respectively)*. It was found that cell-specific Chl *a* and Car concentrations decreased with the increase of salinity (Figs. 2A, 3A). On average, the cell content of pigments for BA-120 was the highest when compared to the other strains. Chl *a* concentration dominated over Car concentration in each scenario. What is more, there were very high cell-specific concentrations of Chl *a* observed for the whole T range at low PAR. Maximum Chl *a* content was measured under T 25°C and PAR 10 µmol photons m^{-2} s^{-1}. This was the case in each salinity. The highest Chl *a* concentration within all scenarios was reached in BA-120 cells in salinity 3 PSU and was equal to 0.339 pg $cell^{-1}$ (Fig. 2A, a). For other salinities these maximums were as follows: 0.233 pg $cell^{-1}$ (8 PSU, Fig. 2A, b), 0.164 pg $cell^{-1}$ (13 PSU, Fig. 2A, c), 0.100 pg $cell^{-1}$ (18 PSU, Fig. 2A, d). The highest Car content was 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. 3A, a). The 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 190 µmol photons m^{-2} s^{-1} (Fig. 2A, d) and salinity 18 PSU, T 15°C, PAR 280 µmol photons m^{-2} s^{-1} (Fig. 3A, d), respectively. Multiple comparisons tests indicated the significance of PAR and T combined in shaping the pigmentation. Due to those tests, 52% and 55% of multiple comparisons in Chl *a* and Car content analysis, respectively, were statistically significant (Tukey HSD, * $p < 0.05$) with 80% (for Chl *a*) and 74% (for Car) of them with the highest significance (Tukey HSD, *** $p < 0.001$).

Both PAR and T affected the Chl *a* cell-specific content *(ANOVA, $F_{9,32}$ = 3.3, ** $p < 0.01$, ANOVA, $F_{9,32}$ = 8.3, *** $p < 0.001$, ANOVA, $F_{9,32}$ = 69.8, *** $p < 0.001$ and ANOVA, $F_{9,32}$ = 17.5, *** $p < 0.001$, for salinity 3, 8, 13, 18 PSU, respectively)* and Car cell-specific content *(ANOVA, $F_{9,32}$ = 4.6, *** $p < 0.001$, ANOVA, $F_{9,32}$ = 65.5, *** $p < 0.001$,*

*ANOVA, $F_{9,32}$ = 83.1, *** $p < 0.001$ and ANOVA, $F_{9,32}$ = 43.2, *** $p < 0.001$, for salinity 3, 8, 13, 18 PSU, respectively)* of *Synechococcus* sp. BA-124 significantly. Generally, PAR and high T increase had a negative impact on pigmentation (Figs. 2B, 3B). 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, 0.102. 0.176, 0.148 pg $cell^{-1}$ for Chl *a* (Figs. 2B, a-d) and 0.051, 0.067, 0.087, 0.079 pg $cell^{-1}$ for Car (Figs. 3B, a-d). Nonetheless, there were also some exceptions. In salinity 3 PSU, high Car contents were calculated under 280 μmol photons 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. 3B, a). On average, salinity increase had a negative impact on pigmentation. The lowest cell-specific concentrations of Chl *a* and Car in BA-124 cells 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 $cell^{-1}$ (Fig. 2B, d) and 0.009 pg $cell^{-1}$ (Fig. 3B, d), for Chl *a* and Car, respectively. Multiple comparisons tests pointed to the significance of PAR and T combined in influencing the pigmentation. According to the statistics, 47% and 54% of multiple comparisons in Chl *a* and Car content analysis, were statistically significant (Tukey HSD, * $p < 0.05$) with 83% (for Chl *a*) and 79% (for Car) of them with the highest significance level (Tukey HSD, *** $p < 0.001$).

It was also examined that PAR and T affected the Chl *a* cell-specific content *(ANOVA, $F_{9,32}$ = 6.5, $p < 0.001$, ANOVA, $F_{9,32}$ = 11.1, $p < 0.001$, ANOVA, $F_{9,32}$ = 21.5, $p < 0.001$ and ANOVA, $F_{9,32}$ = 6.5, $p < 0.001$, for salinity 3, 8, 13, 18 PSU, respectively)* and Car cell-specific content *(ANOVA, $F_{9,32}$ = 8.6, $p < 0.001$, ANOVA, $F_{9,32}$ = 9.6, $p < 0.001$, ANOVA, $F_{9,32}$ = 4.6, $p < 0.001$ and ANOVA, $F_{9,32}$ = 26.8, $p < 0.001$, for salinity 3, 8, 13, 18 PSU, respectively)* of *Synechococcus* sp. BA-132 significantly. It was found that salinity increase had a negative impact on cell-specific Chl *a* and Car concentrations. BA-132 was richer in cell-specific pigments than BA-124 (Figs. 2C, 3C). Along with PAR increase, the Chl *a* concentration decreased significantly. The maximum Chl *a* cell-specific content was measured in 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} s^{-1}). These maximums were 0.299 pg $cell^{-1}$ in salinity 3 PSU (Fig. 2C, a), 0.248 pg $cell^{-1}$ in salinity 8 PSU (Fig. 2C, b), 0.151 pg $cell^{-1}$ in salinity 13 PSU (Fig. 2C, c) and 0.073 pg $cell^{-1}$ in salinity 18 PSU (Fig. 2C, d). Consistently with Chl *a*, Car cell-specific content maximums were also measured under the lowest PAR (10 μmol photons m^{-2} s^{-1}) but contrary to Chl *a*, at the lowest T (10°C). These maximums were: 0.194 pg $cell^{-1}$ in salinity 3 PSU (Fig. 3C, a), 0.131 pg $cell^{-1}$ in salinity 8 PSU (Fig. 3C, b), 0.097 pg $cell^{-1}$ in salinity 13 PSU (Fig. 3C, c), 0.062 pg $cell^{-1}$ in salinity 18 PSU (Fig. 3C, d). Minimums of Chl *a* and Car cell-specific contents within all scenarios were estimated in salinity 18 PSU, T 15°C and PAR 280 μmol photons m^{-2} s^{-1} being equal to 0.020 pg $cell^{-1}$ (Fig. 2C, d) and 0.19 pg $cell^{-1}$ (Fig. 3C, d), for Chl *a* and Car, respectively. Regarding Chl *a* for minimum content per cell the same concentration as above mentioned (0.020 pg $cell^{-1}$) was also estimated in salinity 13 PSU for the same conditions of T and PAR (Fig. 2C, c). Tukey HSD tests pointed to the significance of PAR and T combined in impacting the pigmentation. According to those tests, 66% and 61% of multiple comparisons in Chl *a* and Car content analysis, respectively, were statistically significant (Tukey HSD, * $p < 0.05$), with 81% (for Chl *a*) and 75% (for Car) of them with the highest significance (Tukey HSD, *** $p < 0.001$).

3.3 Chl *a* fluorescence

The parameters of Chl *a* fluorescence were depicted as two-factor-dependent graphs, where the values in between the specific measurements were interpolated (Figs. 4, 5). For all strains, Chl *a* fluorescence parameters were measured and examined. These parameters were: the maximum photochemical efficiency of photosystem II (PSII) at dark-adapted state (F_v/F_m) and the photochemical efficiency of PSII under actinic light intensity (ΦPSII).

The results showed that PAR and T affected F_v/F_m *(ANOVA, $F_{9,32}$ = 5.2, $p < 0.001$, ANOVA, $F_{9,32}$ = 5.7, $p < 0.001$, ANOVA, $F_{9,32}$ = 4.8, $p < 0.001$ and ANOVA, $F_{9,32}$ = 33.9, $p < 0.001$, for salinity 3, 8, 13, 18 PSU, respectively)* and ΦPSII *(ANOVA, $F_{9,32}$ = 4.5, $p < 0.001$, ANOVA, $F_{9,32}$ = 5.7, $p < 0.001$, ANOVA, $F_{9,32}$ = 6.3, $p < 0.001$ and ANOVA, $F_{9,32}$ = 2.3, $p < 0.05$, for salinity 3, 8, 13, 18 PSU, respectively)* of *Synechococcus* sp. BA-120 significantly. For this strain, especially in low

T scenarios and in all scenarios with the lowest salinity, higher F_v/F_m was observed for 280 μmol photons m^{-2} s^{-1} when compared to 190 μmol photons m^{-2} s^{-1} (Fig. 4A, a). Generally, strong fluctuations were noticeable in F_v/F_m values, which disabled the fixed environmentally driven pattern determination. However, there was a constant relation noted between T and PAR and ΦPSII. PAR and T increase had a negative impact on ΦPSII. The impact was the strongest in low salinity (Figs. 5A, a-b). Nonetheless, in each salinity, the lowest ΦPSII were observed under the highest T and elevated PAR (190 or 280 μmol photons m^{-2} s^{-1}). On the contrary, the highest ΦPSII values were calculated in the lowest T and PAR conditions in every salinity. The highest F_v/F_m, for all BA-120 experiments equaled 0.804 and was estimated for scenario: salinity 18 PSU, T 10°C, PAR 280 μmol photons m^{-2} s^{-1} (Fig. 4A, d). Generally, maximum values of F_v/F_m in each medium were associated with the lowest temperature. Minimum F_v/F_m within all scenarios was estimated for salinity 3 PSU, T 25°C and PAR 190 μmol photons m^{-2} s^{-1} (0.409, Fig. 4A, a). Concerning 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. 5A, d). Minimum ΦPSII was measured in salinity 3 PSU, T 25°C and PAR 280 μmol photons m^{-2} s^{-1} (0.241, Fig. 5A, a). Multiple comparisons tests pointed to a strong environmental influence on Chl *a* fluorescence parameters. Regarding F_v/F_m, 65% of all comparisons were statistically significant (Tukey HSD, * $p < 0.05$) with 78% of them having the highest significance (Tukey, HSD, *** $p < 0.001$). For ΦPSII the percentages were as follows: 80% of all comparisons were statistically significant (Tukey HSD, * $p < 0.05$) and 87% of them had the highest significance (*** $p < 0.001$).

Both PAR and T significantly affected F_v/F_m *(ANOVA, $F_{9,32} = 46.2$, *** $p < 0.001$, ANOVA, $F_{9,32} = 5.1$, *** $p < 0.001$, ANOVA, $F_{9,32} = 5.0$, *** $p < 0.001$ and ANOVA, $F_{9,32} = 20.6$, *** $p < 0.001$, for 3, 8, 13, 18 PSU, respectively)* and ΦPSII *(ANOVA, $F_{9,32} = 25.0$, *** $p < 0.001$, ANOVA, $F_{9,32} = 11.6$, *** $p < 0.001$, ANOVA, $F_{9,32} = 15.4$, $p < 0.001$ and ANOVA, $F_{9,32} = 5.2$, $p < 0.001$, for 3, 8, 13, 18 PSU, respectively)* of *Synechococcus* sp. BA-124. For this 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 generally decreased along with PAR and T increases but with some exceptions. Generally, ΦPSII environmentally driven characteristics were similar to F_v/F_m characteristics. The F_v/F_m minimums were measured under the lowest T and highest PAR in each salinity (Figs. 4B, a-d). The lowest value within all scenarios was 0.124 and was observed in salinity 3 PSU, T 10°C and PAR 280 μmol photons m^{-2} s^{-1} (Fig. 4B, a). The F_v/F_m maximums were estimated for the highest T and the lowest 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. 4B, a). Minimums of ΦPSII, consistently with F_v/F_m, were noted under the lowest T and highest PAR. The lowest ΦPSII within all BA-124 experiments was 0.114 (followed by the minimum in salinity 3 PSU being equal to 0.116, Fig. 5B, a) and was measured in salinity 13 PSU (Fig. 5B, c). Maximums of ΦPSII were observed in the highest T and lowest PAR in each 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 μmol photons m^{-2} s^{-1} (Fig. 5B, a). Tukey HSD post hoc test showed that PAR and T combined influenced Chl *a* fluorescence parameters significantly. Concerning F_v/F_m, 77% of all comparisons were statistically significant (* $p < 0.05$) with 88% of them having the highest significance (*** $p < 0.001$). For ΦPSII the percentages were as follows: 79% of all comparisons were statistically significant (* $p < 0.05$) and 89% of them had the highest significance (*** $p < 0.001$).

It was found that both PAR and T affected F_v/F_m *(ANOVA, $F_{9,32} = 4.3$, $p < 0.001$, ANOVA, $F_{9,32} = 4.8$, $p < 0.001$, ANOVA, $F_{9,32} = 4.5$, $p < 0.001$ and ANOVA, $F_{9,32} = 5.7$, $p < 0.001$, for salinity 3, 8, 13, 18 PSU, respectively)* and ΦPSII *(ANOVA, $F_{9,32} = 10.1$, $p < 0.001$, ANOVA, $F_{9,32} = 7.7$, $p < 0.001$, ANOVA, $F_{9,32} = 4.7$, $p < 0.001$ and ANOVA, $F_{9,32} = 7.0$, $p < 0.001$, for salinity 3, 8, 13, 18 PSU, respectively)* of *Synechococcus* sp. BA-132, significantly. For this strain, F_v/F_m decreased along with the PAR increase but was positively affected by T in each salinity (Figs. 4C, a-d). Minimum values of F_v/F_m were measured in the highest PAR and the lowest T in each salinity. The lowest F_v/F_m within all experiments on BA-132 was estimated in salinity 13 PSU (F_v/F_m = 0.155, Fig. 4C, c). In salinity 3 PSU, under aforementioned conditions of T and PAR, the F_v/F_m value was also low compared to the others and equaled 0.160 (Fig. 4C, 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. The highest F_v/F_m were noted in salinities 13 and 18 PSU and equaled 0.742 (Fig. 4C, c) and 0.733 (Fig. 4C, d), respectively. The lowest ΦPSII were

noted under the highest PAR and T conditions in every salinity (Figs. 5C, a-d). The minimum ΦPSII, within all gathered results, was obtained in salinity 3 PSU and equaled 0.281 (Fig. 5C, a). Maximums of ΦPSII were measured under completely opposite conditions to the ones stating for minimums, i.e. the lowest PAR and T. The highest ΦPSII, 0.786, was noted in salinity 8 PSU, T 10°C and PAR 10 µmol photons m^{-2} s^{-1} (Fig. 5C, b). The ΦPSII reached generally higher values than F_v/F_m in BA-132 experiments. ΦPSII reached lower values than ΦPSII measured under respective conditions for two other strains. Multiple comparisons tests point to a strong environmental influence on Chl *a* fluorescence parameters. For F_v/F_m, 78% of all comparisons were statistically significant (Tukey HSD, * $p < 0.05$) with 89% of them with the highest significance (Tukey, HSD, *** $p < 0.001$). For ΦPSII, 82% of all comparisons were statistically significant (Tukey HSD, * $p < 0.05$), with 89% of them having the highest significance level (Tukey, HSD, *** $p < 0.001$).

Generally, for the BA-120 strain, F_v/F_m was affected negatively by T increase, while BA-124 and BA-132 strains were affected positively. T increase had a positive impact on ΦPSII for BA-124 and a negative impact for BA-120 and BA-132. On average, ΦPSII decreased along with PAR increase in all cultures.

3.4 Photosynthesis

Net photosynthetic light-response curves for three PCY strains were analyzed. For all cultures, the photosynthesis parameters were: maximum of photosynthesis, photosynthesis efficiency at low irradiance, and dark respiration (P_m , α, R_d, respectively) and these were estimated for Chl *a*-specific and cell-specific domains (Figs. S4-S6 in Supplement). It should be noted that dark respiration values were negative (less oxygen than carbon dioxide (CO_2)), which meant the lower R_d, the less net oxygen concentration was. This, in turn, indicated higher respiration rate.

For BA-120 statistical study showed significant dependence of PAR and T on Chl *a*-specific P_m in salinities 3, 8 and 18 PSU *(ANOVA, $F_{9,32} = 2.4$, $p < 0.05$, $F_{9,32} = 3.2$, $p < 0.05$ and $F_{9,32} = 5.2$, $p < 0.001$, respectively)* and pointed to no statistically significant dependence of ecological conditions on P_m in salinity 13 PSU *(ANOVA, $p \geq 0.05$)*. Regarding cell-specific P_m there was no statistically significant influence of PAR and T on this parameter in salinity 18 PSU *(ANOVA, $p \geq 0.05$)* but was in salinity 3 PSU *(ANOVA, $F_{9,32} = 3.5$, $p < 0.05$)*, 8 PSU *(ANOVA, $F_{9,32} = 2.6$, $p < 0.05$)*, and 13 PSU *(ANOVA, $F_{9,32} = 3.0$, $p < 0.05$)*. For Chl *a*-specific α, statistical study indicated no environmental impacts in salinities 3, 8 and 13 PSU but an impact of PAR and T in salinity 18 PSU *(ANOVA, $F_{9,32} = 2.7$, $p < 0.05$)*, while for cell-specific α statistical significance of PAR and T influence was obtained for all salinities *(ANOVA, $F_{9,32} = 5.1$, $p < 0.001$, ANOVA, $F_{9,32} = 2.9$, $p < 0.05$, ANOVA, $F_{9,32} = 2.5$, $p < 0.05$ and ANOVA, $F_{9,32} = 4.8$, $p < 0.001$, for salinity 3, 8, 13 and 18 PSU, respectively)*. Regarding R_d, two-way ANOVA pointed to no environmental determination of Chl *a*-specific R_d values *(ANOVA, $p > 0.05$)* but it showed the influence of PAR and T on cell-specific R_d *(ANOVA, $F_{9,32} = 9.2$, $p < 0.001$, ANOVA, $F_{9,32} = 3.8$, $p < 0.01$, ANOVA, $F_{9,32} = 3.8$, $p < 0.01$, ANOVA, $F_{9,32} = 4.5$, $p < 0.001$, in salinities 3, 8, 13, 18 PSU, respectively)*. Tukey HSD tests pointed to some statistically significant multiple comparisons but showed a weak influence of PAR and T combined on Chl *a*-specific parameters. Regarding α, only 3% of all multiple comparisons were statistically significant (* $p < 0.05$) with 7% of them at the highest statistical significance level (*** $p < 0.001$). For P_m, 36% of all multiple comparisons were statistically significant (* $p < 0.05$) with 64% of them with the highest significance (*** $p < 0.001$). Regarding R_d, as mentioned above, no statistically significant analysis of variance was indicated. Due to that, no post hoc tests were proceeded. Note that in order to shorten the text and emphasize reading, in this section the notation for the percentage of all statistically significant multiple comparisons (* $p < 0.05$) and the percentage of the multiple comparisons of the highest significance within the all significant comparisons (*** $p < 0.001 \times$ (* $p < 0.05$)–1) were written in parenthesis, one by one, separated with comma. For instance: X (15%, 20%) would mean that there were 15% of statistically significant multiple comparisons for parameter X in the post hoc tests results, whereas 20% of them were statistically the most significant. Similarly to Chl *a*-specific calculations, Tukey HSD test pointed to a selective influence of PAR and T combined on cell-specific parameters. However, this dependence was stronger when compared to Chl *a*-specific estimations (P_m (16%,

52%), α (19%, 43%), R_d (28%, 56%)). Nonetheless, there were also some fixed relations noted for both calculation domains. For Chl *a*-specific photosynthesis, P_m increased along with PAR up to PAR of 190 µmol 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. 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 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 salinity 18 PSU, T 25°C, 190 µmol photons m^{-2} s^{-1} (Fig. S4, c). Dark respiration rate (R_d) increased with T increase and decreased with PAR increase (Figs. S5, a, c). Minimum R_d (-0.31 µmol O_2 (µg Chl *a*)$^{-1}$ h^{-1}) was measured in salinity 18 PSU, T 10°C, PAR 280 µmol photons m^{-2} s^{-1} (Fig. S5, c), while maximum (-0.02 µmol O_2 (µg Chl *a*)$^{-1}$ h^{-1}) was estimated in salinity 3 PSU, T 25°C, PAR 10 µmol photons m^{-2} s^{-1} (Fig. S5, a). On the contrary, it was more difficult to determine a fixed pattern of α changes unequivocally. The most fixed tendency of α changes was observed between all temperature-differenced scenarios in 18 PSU salinity medium (Figs. S6, a, c). Under those conditions, it was noticeable that α decreased with PAR 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 results (all mediums together), minimum α was measured in salinity 3 PSU, T 25°C, PAR 10 µmol photons m^{-2} s^{-1} and equalled 0.002 µmol O_2 (µg Chl *a*)$^{-1}$ h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$ (Fig. S6, a), whilst maximum was 0.013 µmol O_2 (µg Chl *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 cell-specific domain, P_m increased along with T and it was more pronounced in higher salinities. Concerning all results, minimum 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, 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, T 25°C, 190 µmol photons m^{-2} s^{-1} (data not shown). Regarding α, this parameter was generally negatively affected by PAR and T up to PAR of 190 µmol photons m^{-2} s^{-1}. However minimum value was obtained for cells growing in moderate T (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}]$^{-1}$. 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 10 µmol photons m^{-2} s^{-1} (Fig. S6, d). Generally, T and PAR had a positive impact on R_d for cultures grown in PAR range up to 190 µmol photons m^{-2} s^{-1}. For cultures grown under elevated PAR conditions, R_d was lower (more intensive respiration) when compared to low PAR scenarios. The lowest R_d within all BA-120 experiments results was -16.97 µmol O_2 cell 10^{-9} h^{-1} and noted in salinity 3 PSU, T 10°C, PAR 10 µmol photons m^{-2} s^{-1} (Fig. S5, b), whilst the highest R_d was measured in salinity 18 PSU, T 25°C, PAR 100 µmol photons m^{-2} s^{-1} and equalled -2.06 µmol O_2 cell 10^{-9} h^{-1} (Fig. S5, d).

For BA-124, statistical study showed significant dependence of ecological conditions on photosynthesis parameters, excluding Chl *a*-specific α *(ANOVA, $p \geq 0.05$)* and cell-specific P_m *(ANOVA, $p \geq 0.05$)*. For the rest parameters ANOVA results were as follows: Chl *a*-specific P_m *(ANOVA, $F_{9,32} = 4.8$, $p < 0.001$, ANOVA, $F_{9,32} = 19.7$, $p < 0.001$, ANOVA, $F_{9,32} = 9.14$, $p < 0.001$, ANOVA, $F_{9,32} = 6.5$, $p < 0.001$ in salinity 3, 8, 13, 18 PSU, respectively)*; cell-specific P_m *(ANOVA, $F_{9,32} = 7.5$, $p < 0.001$, ANOVA, $F_{9,32} = 6.1$, $p < 0.001$, ANOVA, $F_{9,32} = 4.3$, $p < 0.001$ in salinity 8, 13 and 18 PSU, respectively)*; Chl *a*-specific α *(ANOVA, $F_{9,32} = 5.0$, $p < 0.001$, ANOVA, $F_{9,32} = 3.3$, $p < 0.01$, ANOVA, $F_{9,32} = 3.8$, $p < 0.01$ in salinity 3, 8 and 18 PSU, respectively)*; cell-specific α *(ANOVA, $F_{9,32} = 6.6$, $p < 0.001$, ANOVA, $F_{9,32} = 17.9$, $p < 0.001$, ANOVA, $F_{9,32} = 18.9$, $p < 0.001$, ANOVA, $F_{9,32} = 3.1$, $p < 0.01$, in salinity 3, 8, 13, 18 PSU, respectively)*; Chl *a*-specific R_d *(ANOVA, $F_{9,32} = 10.0$, $p < 0.001$, ANOVA, $F_{9,32} = 4.9$, $p < 0.001$, ANOVA, $F_{9,32} = 3.8$, $p < 0.01$, ANOVA, $F_{9,32} = 2.6$, $p < 0.05$, in salinity 3, 8, 13, 18 PSU, respectively)*; cell-specific R_d *(ANOVA, $F_{9,32} = 13.0$, $p < 0.001$, ANOVA, $F_{9,32} = 2.2$, $p < 0.05$, ANOVA, $F_{9,32} = 40.4$, $p < 0.001$, ANOVA, $F_{9,32} = 3.1$, $p < 0.01$)*. Post-hoc tests showed there must have been other factors, which affected the whole process of photosynthesis as there were many not statistically significant multiple comparisons defined. Generally, Tukey HSD tests pointed to only few statistically significant multiple comparisons, in both Chl *a*-specific, especially for P_m, (P_m (60%, 76%), α (9%, 29%), R_d (30%, 47%) and cell-specific (P_m (22%, 56%), α (34%, 63%), R_d (30%, 74%)) estimations. Nonetheless, for P_m there was a tendency noted, which suggested that on average, the maximum of photosynthesis was higher at elevated PAR. This was the case in both estimations, Chl *a*-specific and cell-specific. 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 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). 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 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 cell·10^{-9} h^{-1}, respectively. It was difficult to determine a fixed relation between ecological state and α changes in both domains, which was supported by the post-hoc test (more than 91% of multiple comparisons were not statistically significant ($p \geq 0.05$) in Chl *a*-specific and more than 35% in cell-specific estimations). Maximum Chl *a*-specific α was 0.02 μmol O_2 (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. S6, 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, 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^{-1}]$^{-1}$ and was measured in two scenarios: salinity 3 PSU, T 10°C, PAR 280 μmol photons m^{-2} s^{-1} (Fig. S6, e) and salinity 18 PSU, T 15°C, PAR 10 μmol photons m^{-2} s^{-1} (Fig. S6, g). Minimum cell-specific α equalled 0.08 μmol O_2 cell 10^{-9} h^{-1} [μmol 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. S6, h). Similarly to α, it was difficult to determine fixed relations between PAR and T and R_d, which was supported by statistics (about 70% of multiple comparisons for both Chl *a*-specific and cell-specific R_d were not statistically significant (Tukey HSD, $p \geq 0.05$)). Nonetheless, it was observed that, generally, R_d decreased along with PAR increase in cell-specific estimations. Maximum Chl *a*-specific and cell-specific R_d was -0.03 μmol O_2 (μg Chl *a*)$^{-1}$ h^{-1} and -1.52 μmol O_2 cell 10^{-9}, respectively. These values were obtained in salinity 13 PSU, T 20°C, PAR 10 μmol photons m^{-2} s^{-1} and salinity 18 PSU, T 20°C, PAR 190 μmol photons m^{-2} s^{-1}, respectively for Chl *a*- and cell-specific calculations. Minimum Chl *a*-specific R_d was measured in salinity 13 PSU, T 10°C, PAR 280 μmol photons m^{-2} s^{-1} and was -0.27 μmol O_2 (μg Chl *a*)$^{-1}$ h^{-1}, whilst minimum cell-specific R_d was measured in salinity 13 PSU, T 10°C, PAR 10 μmol photons m^{-2} s^{-1} and equalled -12.19 μmol O_2 cell 10^{-9} h^{-1} (data not shown here).

For BA-132, statistical study showed significant dependence of PAR and T on Chl *a*- and cell-specific P_m *(for Chl a-specific: ANOVA, $F_{9,32} = 6.2$, $p < 0.001$, ANOVA, $F_{9,32} = 23.1$, $p < 0.001$, ANOVA, $F_{9,32} = 25.2$, $p < 0.001$, ANOVA, $F_{9,32} = 16.0$, $p < 0.001$; for cell-specific: ANOVA, $F_{9,32} = 4.8$, $p < 0.001$, ANOVA, $F_{9,32} = 24.3$, $p < 0.001$, ANOVA, $F_{9,32} = 24.3$, $p < 0.001$, ANOVA, $F_{9,32} = 21.2$, $p < 0.001$; all numbers given for salinities 3, 8, 13, 18 PSU, respectively)*. Regarding other Chl *a*-specific parameters, there were no statistically significant impacts of PAR and T on α in salinities 3, 13, 18 PSU *(ANOVA, $p \geq 0.05$)* but were in salinity 8 PSU *(ANOVA, $F_{9,32} = 2.7$, $p < 0.05$)* and no impacts on Chl *a*-specific R_d in salinities 3, 8, 18 PSU *(ANOVA, $p \geq 0.05$)* but were in salinity 13 PSU *(ANOVA, $F_{9,32} = 2.8$, $p < 0.05$)*. Regarding other than P_m cell-specific parameters, there was no ecological determination of α noted in salinities 3 and 8 PSU and of R_d in salinity 13 PSU *(ANOVA, $p \geq 0.05$),* while there were statistically significant environmental impacts calculated for α in salinity 13 PSU *(ANOVA, $F_{9,32} = 3.2$, $p < 0.01$)* and 18 PSU *(ANOVA, $F_{9,32} = 2.9$, $p < 0.05$)* and for R_d in salinities 3, 8 and 18 PSU *(ANOVA, $F_{9,32} = 3.2$, $p < 0.05$, ANOVA, $F_{9,32} = 3.1$, $p < 0.01$, ANOVA, $F_{9,32} = 2.4$, $p < 0.05$, respectively)*. Tukey HSD tests pointed to statistically significant multiple comparisons, in both Chl *a*-specific and cell-specific maximum of photosynthesis (P_m (68%, 85%), P_m (62%, 76%), respectively). Post hoc tests indicated no significant multiple comparisons for Chl *a*-specific α (>1%, >1%), a few significant multiple comparisons for Chl *a*-specific R_d (8%, 38%), cell-specific α (18%, 67%) and cell-specific R_d (6%, 20%)). It was observed, that in cell-specific estimations, P_m increased along with PAR increase, while α decreased at elevated PAR. It was the most difficult to determine a fixed tendency for the R_d response to changing environmental conditions. This was supported by statistical tests (Tukey HSD, more than 93% of multiple comparisons were not statistically significant ($p \geq 0.05$)). Maximum cell-specific P_m was 158.94 μmol O_2 cell 10^{-9} h^{-1} and was reached in salinity 8 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 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} 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 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. S6, l). Regarding cell-specific R_d, maximum was measured in salinity 18 PSU, T 15°C, PAR 100 μmol photons m^{-2} s^{-1} and equalled -3.17 μmol O_2 cell 10^{-9} h^{-1} (Fig. S5, l), whilst minimum was -15.55 μmol O_2 cell 10^{-9} h^{-1} and was obtained in salinity 3

PSU, T 10°C, PAR 10 µmol photons m^{-2} s^{-1} (Fig. S5, j). For Chl *a*-specific P_m, the increases along with T and salinity was observed, whilst α presented strong changing characteristics between scenarios. The fixed influence of PAR and T on α values was difficult to determine, which was supported by statistics *(ANOVA, $p \geq 0.05$)*. Contrary to the above, it was plainly evident that PAR increase had a negative impact on Chl *a*-specific R_d. Maximum Chl *a*-specific P_m was 6.22 µmol O_2 (µg Chl *a*)$^{-1}$ h^{-1} and was reached in 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^{-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 *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. S6, k), 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 *a*)$^{-1}$ h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$ (Fig. S6, i). Concerning Chl *a*-specific R_d, maximum was measured in salinity 3 PSU, T 20°C, PAR 10 µmol photons m^{-2} s^{-1} and equalled -0.02 µmol O_2 cell 10^{-9} h^{-1} (Fig. S5, i), whilst minimum was -0.39 µmol O_2 cell 10^{-9} h^{-1} and was obtained in salinity 13 PSU, T 25°C, PAR 280 µmol photons m^{-2} s^{-1}. Generally, in both domains, photosynthesis parameters were the highest for BA-132 when compared to other strains.

The analysis of photosynthesis characteristics enabled examining and defining the photoacclimation process of all three strains of *Synechococcus* sp. This was done on the basis of the photosynthetic parameters (Figs. S4-S6) and Photosynthesis-Irradiance (*P-E*) curves (exemplification shown in Fig. 6). The curves were plotted on the basis of laboratory results (Clark oxygen electrode measurements) using the equation of Jassby and Platt (1976). According to a photoacclimation model description (Prezelin, 1981; Prezelin and Sweeney, 1979; Ramus, 1981; Richardson et al., 1983; Pniewski et al., 2016), the results of the present study indicated changes in Photosynthetic Units (PSU) sizes as the photoacclimation mechanism, which occurred most frequently (Table 1). There were also *P-E* curves pointing to some changes in enzymatic reactions and the altering of accessory pigments activity. Changes in PSU numbers were noted as well, but these observations were episodic. In this paper the term 'OTHER' stands for changes in enzymatic reactions and the altering of accessory pigments activity and concerns photoacclimation mechanisms other than changes in PSU sizes (PSUsize) or changes in PSU number (PSUno.). In general, photoacclimation did not occur in low-saline medium (salinity 3). According to the results, photoacclimation mechanisms were observed in only four scenarios with low salinity: BA-120 25°C salinity 3 PSU, BA-124 25°C salinity 3 PSU, BA-132 10°C salinity 3 PSU, and BA-132 25°C salinity 3 PSU. For BA-120, photoacclimation occurred more frequently at higher T (20 and 25°C) than lower T (10 and 15°C). However, if it had been observed in low T conditions, it usually stood for OTHER, not for PSUsize or PSUno. For BA-124 and BA-132 photoacclimation was noted in the whole T range. All photoacclimation mechanisms observed for different strains are listed in Table 1.

4 Discussion

Picoplanktonic organisms show a lot of adaptations, which enable them to spread in aquatic environments (e.g., Stomp et al., 2007; Jodłowska and Śliwińska, 2014; Larsson et al., 2014; Jasser and Callieri, 2017). What is more, picocyanobacteria often dominate and occupy the niches, which are inaccessible for other photoautotrophs. Owing to the fact that PCY are small-sized cells and consequently possess an advantageous surface area to volume ratio, they can assimilate trace amount of nutrients and effectively absorb light. Therefore, in oligotrophic regions of seas and oceans PCY compete with other cyanobacteria and microalgae and it can determine primary production of the whole marine ecosystem (Six et al., 2007a; Richardson and Jackson, 2007; Worden and Wilken, 2016). This is also true for eutrophic basins (Stal et al., 2003; Haverkamp et al., 2008; 2009; Callieri, 2010; Mazur-Marzec et al., 2013).

The distribution of PCY are determined by their optimal ecological requirements for light and temperature. Due to the presented results, PAR and T had positive effects on the number of cells for two out of the three studied strains of *Synechococcus* sp. The highest cell concentrations were noted in scenarios with the highest T (25°C) and the highest PAR level (280 µmol photons m^{-2} s^{-1}) for BA-124 and BA-132. The BA-120 strain behaved differently when compared to the other strains. For BA-120, the decrease in number of cells was observed in high PAR conditions, i.e. cell abundances for red

strain cultures grown under the most elevated PAR were lower than the number of BA-120 cells measured in cultures grown under 190 µmol photons m^{-2} s^{-1}. According to the results derived from pigmentation, Chl *a* fluorescence and photosynthesis sections of the present study, the decrease in number of cells under the elevated PAR could have likely been associated with Photosystem II photo-inhibition. This is a conclusion of a few observations, which are as follows. Firstly, there was a higher cell-specific Car content observed for 280 µmol photons m^{-2} s^{-1} when compared to 190 µmol photons m^{-2} s^{-1}. Secondly, higher F_v/F_m values were observed for 280 µmol photons m^{-2} s^{-1} when compared to 190 µmol photons m^{-2} s^{-1}, especially for low T scenarios and for all scenarios in the lowest salinity medium. Thirdly, for Chl *a*-specific photosynthesis, P_m increased along with PAR until 190 µmol photons m^{-2} s^{-1}, above which the values started to decrease slightly in all salinity mediums. According to the above, a PAR level of 190 µmol photons m^{-2} s^{-1} could be defined as the PSII photo-inhibition point for the red strain. This implies BA-120 did not lead as effective photosynthesis being grown in PAR of more than 190 µmol photons m^{-2} s^{-1} as the cells grown in PAR levels equal or are beneath 190 µmol photons m^{-2} s^{-1}.

Cyanobacteria are generally recognized to prefer low light intensity for growth (Fogg and Thake, 1987; Ibelings, 1996). Some picoplanktonic organisms demonstrated the ability to survive and resume growth after periods of total darkness. Such a pronounced capacity for survival in the dark would enable these organisms to outlive the seasonal rhythm of winter darkness and sinking into the aphotic zone (Antia, 1976). The investigated strains of *Synechococcus* sp. were found to be well adapted to relatively low and high PAR levels. The latter was especially evident at the high treatment T. This conclusion is consistent with the observations of picocyanobacteria maximum abundance at the euphotic zone in coastal and offshore marine waters (Stal et al., 2003; Callieri, 2010). Moreover, Kana and Glibert (1987a,b) showed that *Synechococcus* sp. could grow at irradiance as high as 2000 µmol photon m^{-2} s^{-1}. Regarding the comparison of abundance values of the analyzed strains, the results showed that in all synthetically developed environmental scenarios, BA-124 was the strain of the highest cell abundance. This is consistent with the Baltic Sea field studies (Mazur-Marzec et al., 2013).

Surface and near-surface populations experience extremely variable light and temperature conditions (Millie et al., 1990), and these factors are the ones that affect the composition of photosynthetic pigments and photosynthesis performance of PCY (Jodłowska and Śliwińska, 2014). Picocyanobacteria with a high concentration of PC are chromatically better adapted to harvest longer wavelengths of PAR than those with PE as a dominating pigment. Therefore, such PCY, 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). Carotenoids have a dual role in the cell: to maintain a high capacity for photosynthetic light absorption and to provide protection against photooxidation (Siefermann-Harms, 1987). 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 regarding BA-120 cell-specific pigments content, there were very high concentrations of Chl *a* observed in the whole T range under low PAR. This could have implied the photoacclimation type, which was the change in PSU number. This mechanism was observed in *P-E* curves for scenario with salinity 8 PSU and temperature 20°C.

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 to 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 and influenced the respiration negatively. For each of the studied strains of *Synechococcus* sp., the highest α and the lowest R_d were 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 inability for the cells incubated in low PAR conditions to be as effective in photosynthesis as the cells grown under high irradiances. On the basis of P-E curves derived in this study, three types of photoacclimation mechanisms of *Synechoccocus* 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 the two first of photoacclimation mechanisms listed above are predominant (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 *Synechoccocus* sp. occurred rarely, which is consistent with literature (Jodłowska and Śliwińska, 2014). Moreover, in this study, salinity 3 PSU was the medium, where the photoacclimation mechanisms in the *Synechococcus* sp. cells were recognized the least frequently. The changes of photosynthesis parameters (P_m, α, R_d) under different environmental conditions explains the occurrence of different photoacclimation mechanisms. According to the results, *Synechococcus* strains present different ecophysiological characteristics, however, they all demonstrate the 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* sp. to sustain its growth in low light conditions and its low photoinhibition in exposure to high light intensities could give PCY an advantage over the other phytoplankton in optically changing waters (Jasser, 2006).

Due to 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 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 the recent research more detailed investigation on BA-132 was provided (Jodłowska and Śliwińska, 2014). Nonetheless, the autecology issue of this strain still requires 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.

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

The studies of autecology of the PCY community and an understanding of its response to main environmental factors is an important step in recognizing the phenomenon of PCY blooms in marine environments. Additionally, the laboratory experiments became a foundation in developing a new approach to Baltic Sea phytoplankton modeling - development of pico-bioalgorithm describing PCY growth, which may enable long-term numerical studies on the response of PCY to changing environment.

5 Conclusions

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

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

Contrary to that, the main differences were: different responses of number of cells to respective environmental conditions in different cultures; various photoacclimation mechanisms observed; and different changes in pigmentation.

According to the latest research, PCY are a great contributor to total primary production in the Baltic Sea and may contribute to summer cyanobacteria bloom at a high degree. This explains the authors' motivation to lead an in-depth investigation on Baltic PCY response to a changing environment. The present research is a first step on the way to deriving new knowledge on *Synechococcus* sp. ecophysiology and is a foundation for further studies.

Acknowledgments

The authors would like to thank the Reviewers and Editor for their valuable comments and suggestions to improve the quality of the paper. The authors would like to thank Simon Bretherton for English language support and Proof Reading Service company for professionally proofread. The authors gratefully thank Jakub Maculewicz (IO UG), for his excellent and professional technical assistance. The author SSW was financially supported by BMN grants, Poland, no. 538-G245-B568-17. This work has been funded by the Polish National Science Centre project (contract number: 2012/07/N/ST10/03485) entitled: "Improved understanding of phytoplankton blooms in the Baltic Sea based on numerical models and existing data sets". The author (AC) received funding from Polish National Science Centre in a doctoral scholarship program (contract number: 2016/20/T/ST10/00214). AC contribution was also supported by the statutory funding of IO PAS.

References

Agawin, N. S., Duarte, C. M., and Agustí, S.: Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production, Limn. Oceanogr., 45(3), 591–600, https://doi.org/10.4319/lo.2000.45.3.0591, 2000.

Antia, N. J.: Effects of temperature on the darkness survival of marine microplanktonic algae, Microb. Ecol., 3, 41–54, 1976.

Barreiro Felpeto, A., Śliwińska-Wilczewska, S., Złoch, I., and Vasconcelos, V.: Light-dependent cytolysis in the allelopathic interaction between picoplanktic and filamentous cyanobacteria, J. Plankton Res., https://doi.org/10.1093/plankt/fby004, 2018.

Beardall, J.: Blooms of *Synechococcus*: An analysis of the problem worldwide and possible causative factors in relation to nuisance blooms in the Gippsland Lakes; Monash University: Clayton, VIC, Australia, 2008; pp. 1–8, 2008.

Becker, S., Singh, A. K., Postius, C., Böger, P., and Ernst, A.: Genetic diversity and distribution of periphytic *Synechococcus* spp. in biofilms and picoplankton of Lake Constance, FEMS Microbiol. Ecol., 49, 181–190, 2004.

Belkin, I. M.: Rapid warming of large marine ecosystems, Prog Oceanogr., 81 (1-4), 207–213, https://doi.org/10.1016/j.pocean.2009.04.011, 2009.

Cai, Y., and Kong, F.: Diversity and dynamics of picocyanobacteria and bloom-forming cyanobacteria in a large shallow eutrophic lake (lake Chaohu, China), J. Limnol., 72(3), 473–484, doi:10.4081/jlimnol.2013.e38, 2013.

Callieri, C.: Picophytoplankton in freshwater ecosystems: The importance of small-sized phototrophs, Freshw. Rev., 1, 1–28, https://doi.org/10.1608/FRJ-1.1.1, 2007.

Callieri, C.: Single cells and microcolonies of freshwater picocyanobacteria: A common ecology, J. Limnol., 69, 257–277, https://doi.org/10.4081/jlimnol.2010.257, 2010.

Callieri, C., and Stockner, J. G.: Freshwater autotrophic picoplankton: A review, J. Limnol., 61, 1–14, https://doi.org/10.4081/jlimnol.2002.1, 2002.

Campbell, D., Hurry, V., Clarke, A. K., Gustafsson, P., and Öquist, G.: Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation, Microbiol. Mol. Biol. Rev., 62(3), 667–683, 1998.

Dutkiewicz, S., Morris, J. J., Follows, M. J., Scott, J., Levitan, O., Dyhrman, S. T., and Berman-Frank, I.: Impact of ocean acidification on the structure of future phytoplankton communities. Nat. Clim. Change., 5(11), 1002–1006,

https://doi.org/10.1038/nclimate2722, 2015.

Everroad, R.C., and Wood, A.M.: Comparative molecular evolution of newly discovered picocyanobacterial strains reveals a phylogenetically informative variable region of beta-phycoerythrin, J. Phycol., 42, 1300–1311, 2006.

Fahnenstiel, G. L., Carrick, H. J., Rogers, C. E., and Sicko-Goad, L.: Red fluorescing phototrophic picoplankton in the Laurentian Great Lakes: What are they and what are they doing?, Int. Rev. Ges. Hydrobiol., 76(4), 603–616, https://doi.org/10.1002/iroh.19910760411, 1991.

Feistel, R., Feistel, S., Nausch, G., Szaron, J., Lysiak-Pastuszak, E., and Ærtebjerg, G.: BALTIC: Monthly time series 1900–2005, edited by: Feistel, R., Nausch, G., and Wasmund, N., State and Evolution of the Baltic Sea, 1952–2005, A Detailed 50-Year Survey of Meteorology and Climate, Physics, Chemistry, Biology, and Marine Environment, John Wiley & Sons, Inc., Hoboken, 311–336, 2008.

Feistel, R., Weinreben, S., Wolf, H., Seitz, S., Spitzer, P., Adel., B., Nausch, G., Schneider, B., and Wright, D. G.: Density and absolute salinity of the Baltic Sea 2006-2009, Ocean Sci., 6, 3–24, www.ocean-sci.bet/6/3/2010/, 2010.

Flombaum, P., Gallegos, J. L., Gordillo, R. A., Rincón, J., Zabala, L. L., Jiao, N., Karl, D. M., Li, W. K. W., Lomas, M. W., Veneziano, D., Vera, C. S., Vrugt J. A., and Martiny A. C.: Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*, Proc. Natl. Acad. Sci., 110(24), 9824–9829, https://doi.org/10.1073/pnas.1307701110, 2013.

Fogg, G. E., and Thake, B. (Eds.): Algal Cultures and Phytoplankton Ecology, University of Wisconsin Press, Madison and Milwaukee, 1987.

Glover, H. E.: The physiology and ecology of marine Cyanobacteria, *Synechococcus* spp., in: Advances in Aquatic Microbiology, Vol. 3, Jannasch, H. W., and Williams Leb, P. J., (Eds.), New York, Academic Press, 49–107, 1985.

Glover, H. E., Phinney, D. A., and Yentsch, C. S.: Photosynthetic characteristics of picoplankton compared with those of larger phytoplankton populations, in various water masses in the Gulf of Maine, Biol. Oceanogr., 3, 223–248, 1985.

Glover, H. E., Campbell, L., and Prézelin, B. B.: Contribution of *Synechococcus* spp. to size-fraction primary productivity in three waters masses in the Northwest Atlantic Ocean, Mar. Biol., 91, 193–203, 1986.

Guillard, R. R. L.: Culture of phytoplankton for feeding marine invertebrates, in: Culture of Marine Invertebrate Animals, Smith, W. L., and Chanley, M. H. (Eds.), Plenum Press, New York, USA, 26–60, 1975.

Hajdu, S., Höglander, H., and Larsson, U.: Phytoplankton vertical distributions and composition in Baltic Sea cyanobacterial blooms, Harmful Algae, 6(2), 189–205, https://doi.org/10.1016/j.hal.2006.07.006, 2007.

Hauschild, C. A., McMurter, H. J. G., and Pick, F. R.: Effect of spectral quality on growth and pigmentation of picocyanobacteria, J. Phycol. 27, 698–702, https://doi.org/10.1111/j.0022-3646.1991.00698.x, 1991.

Haverkamp, T., Acinas, S. G., Doeleman, M., Stomp, M., Huisman, J., and Stal, L. J.: Diversity and phylogeny of Baltic Sea picocyanobacteria inferred from their ITS and phycobiliprotein operons, Environ. Microbiol. 10(1), 174–188, https://doi.org/10.1111/j.1462-2920.2007.01442.x, 2008.

Haverkamp, T. H., Schouten, D., Doeleman, M., Wollenzien, U., Huisman, J., and Stal, L. J.: Colorful microdiversity of *Synechococcus* strains (picocyanobacteria) isolated from the Baltic Sea, The ISME Journal, 3(4), 397–408, 2009.

Herdman, M., Castenholz, R. W., Iteman, I., Waterbury, J. B., and Rippka, R.: The Archaea and the deeply branching and phototrophic bacteria, in: Boone, D. R., Castenholz, R. W. (Eds.), Bergey's Manual of Systematic Bacteriology, 2nd edn. Springer Verlag: Heidelberg, 493–514, 2001.

Ibelings, B. W.: Changes in photosynthesis in response to combined irradiance and temperature stress in cyanobacterial surface waterblooms, J. Phycol., 32, 549–557, https://doi.org/10.1111/j.0022-3646.1996.00549.x, 1996.

Jakubowska, N., and Szeląg-Wasilewska, E.: Toxic Picoplanktonic Cyanobacteria – Review, Mar. Drugs., 13, 1497–1518, https://doi.org/10.3390/md13031497, 2015.

Jassby, A. D., and Platt, T.: Mathematical formulation of the relationship between photosynthesis and light for phytoplankton, Limnol. Oceanogr., 21, 540–547, https://doi.org/10.4319/lo.1976.21.4.0540, 1976.

Jasser, I.: The relationship between autotrophic picoplankton (APP) – The smallest autotrophic component of food web and the trophic status and depth of lakes, Ecohydrol. and Hydrobiol., 6(1-4), 69–77, https://doi.org/10.1016/S1642-3593(06)70128-8, 2006.

Jasser, I., and Arvola, L.: Potential effects of abiotic factors on the abundance of autotrophic picoplankton in four boreal lakes, J. Plankton Res., 25(8), 873–883, https://doi.org/10.1093/plankt/25.8.873, 2003.

Jasser, I., and Callieri, C.: Picocyanobacteria: The smallest cell-size cyanobacteria, in: Handbook on Cyanobacterial Monitoring and Cyanotoxin Analysis, Meriluoto, J., Spoof, L., and Codd G. A. (Eds.),. John Wiley & Sons, Ltd, Chichester, UK,19–27, https://doi.org/10.1002/9781119068761.ch3, 2017.

Jodłowska, S., and Latała, A.: Photoacclimation strategies in the toxic cyanobacterium *Nodularia spumigena* (Nostocales, Cyanobacteria), Phycologia, 49(3), 203–211, https://doi.org/10.2216/PH08-14.1, 2010.

Jodłowska, S., and Śliwińska, S.: Effects of light intensity and temperature on the photosynthetic irradiance response curves and chlorophyll fluorescence in three picocyanobacterial strains of *Synechococcus*, Photosynthetica, 52(2), 223–232, https://doi.org/10.1007/s11099-014-0024-y, 2014.

Johnson, P. W., and Sieburth, J. M.: Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass, Limnol. Oceanogr., 24(5), 928–935, https://doi.org/10.4319/lo.1979.24.5.0928, 1979.

Joint, I. R., and Pomroy, A. J.: Photosynthetic characteristics of nanoplankton and picoplankton from the surface mixed layer, Mar. Biol., 92, 465–474, 1986.

Kana, T. M., and Glibert, P. M.: Effect of irradiances up to 2000 μmol E m^{-2} s^{-1} on marine *Synechococcus* WH7803-I. Growth, pigmentation, and cell composition, Deep-Sea Res., 34(4), 479–495, https://doi.org/10.1016/0198-0149(87)90001-X, 1987a.

Kana, T. M., and Glibert, P. M.: Effect of irradiances up to 2000 μmol E m^{-2} s^{-1} on marine *Synechococcus* WH7803-II. Photosynthetic responses and mechanisms, Deep-Sea Res., 34(4), 497–516, https://doi.org/10.1016/0198-0149(87)90002-1, 1987b.

Kuosa, H.: Occurrence of autotrophic picoplankton along an open sea-inner archipelago gradient in the Gulf of Finland, Baltic Sea, Ophelia, 28, 85–93, 1988.

Latała, A., Jodłowska, S., and Pniewski, F.: Culture collection of Baltic Algae (CCBA) and characteristic of some strains by factorial experiment approach, Algol. Stud., 122, 137–154, https://doi.org/10.1127/1864-1318/2006/0122-0137, 2006.

Larsson, J., Celepli, N., Ininbergs, K., Dupont, C.L., Yooseph, S., Bergman, B., and Ekman, M.: Picocyanobacteria containing a novel pigment gene cluster dominate the brackish water Baltic Sea, The ISME Journal, 8, 1892–1903, https://doi.org/10.1038/ismej.2014.35, 2014.

Leppäranta M., and Myrberg K., Physical Oceanography of the Baltic Sea, Springer, Berlin, pp. 378, 2009.

Marie, D., Simon, N., and Vaulot, D.: Phytoplankton cell counting by flow cytometry, Algal Culturing Techniques, 1., 253–267, 2005.

Mazur-Marzec, H., Sutryk, K., Kobos, J., Hebel, A., Hohlfeld, N., Błaszczyk, A., Toruńska, A., Kaczkowska, M.J., Łysiak-Pastuszak, E., Kraśniewski, W., and Jasser, I.: Occurrence of cyanobacteria and cyanotoxins in the Southern Baltic Proper. Filamentous cyanobacteria *vs.* single-celled picocyanobacteria, Hydrobiologia, 701, 235–252, https://doi.org/10.1007/s10750-012-1278-7, 2013.

Meier, H. E.: Regional ocean climate simulations with a 3D ice-ocean model for the Baltic Sea. Part 2: Results for sea ice, Clim Dyn., 19, 255–266, 2002.

Millie, D. F., Ingram, D. A., and Dionigi, C. P.: Pigment and photosynthetic responses of *Oscillatoria agardhii* (Cyanophyta) to photon flux density and spectral quality, J. Phycol., 26, 660–666, https://doi.org/10.1111/j.0022-3646.1990.00660.x, 1990.

Motwani, N. H., and Gorokhova, E.: Mesozooplankton grazing on picocyanobacteria in the Baltic Sea as inferred from molecular diet analysis, PLoS One, 8(11), e79230, https://doi.org/10.1371/journal.pone.0079230, 2013.

Neumann, T.: Climate-change effects on the Baltic Sea ecosystem: A model study, J. Marine Syst., 81(3), 213–224. https://doi.org/10.1016/j.jmarsys.2009.12.001, 2010.

Paczkowska J., Rowe O., Schlüter L., Legrand C., Karlson B., and Andersson A.: Allochthonous matter: An important factor shaping the phytoplankton community in the Baltic Sea, J. Plankton Res., 39(1), 23–34, https://doi.org/10.1093/plankt/fbw081, 2017.

Pniewski, F. F., Biskup, P., Bubak, I., Richard, P., Latała, A., and Blanchard, G.: Photo-regulation in microphytobenthos from intertidal mudflats and non-tidal coastal shallows, Estuar. Coast. Shelf S. 152, 153–161, https://doi.org/10.1016/j.ecss.2014.11.022, 2015.

Prezelin, B. B.: Light reactions in photosynthesis, in: Physiological Bases of phytoplankton Ecology, Platt, T., (Ed.), Ottawa, Canadian Bulletin of Fisheries and Aquatic Sciences, no. 210, 1–46, 1981.

Prezelin, B. B., and Sweeney, B. M.: Photoadaptation of photosynthesis in two bloom-forming dinoflagellates, in: Toxic Dinoflagellate Blooms, Taylor, D., Seliger, H., (Eds.), Elsevier North Holland, Inc., 101–106, 1979.

Ramus, J.: The capture and transduction of light energy, in: The Biology of Seaweeds, Lobban, C. S., Wynne, M. J., (Eds.). Botanical Monographs, vo. 17, Oxford, Blackwell Scientific Publications, 458–492, 1981.

Richardson, K., Beardall, J., and Raven, J. A.: Adaptation of unicellular algae to irradaince: An analysis of strategies, New Phytol., 93, 157–191, https://doi.org/10.1111/j.1469-8137.1983.tb03422.x, 1983.

Richardson, T. L., and Jackson, G. A.: Small phytoplankton and carbon export from the surface ocean, Science, 315, 838–840, https://doi.org/10.1126/science.1133471, 2007.

Sakshaug, E., Bricaud, A., Dandonneau, Y., Falkowski, P. G., Kiefer, D. A., Legendre, L. L., Morel, A., Parslow, J., and Takahashi, M.: Parameters of photosynthesis: Definitions, theory and interpretation of results, J. Plankton Res., 19, 1637–1670, https://doi.org/10.1093/plankt/19.11.1637, 1997.

Sánchez-Baracaldo, P., Handley, B. A., and Hayes, P. K.: Picocyanobacterial community structure of freshwater lakes and the Baltic Sea revealed by phylogenetic analyses and clade-specific quantitative PCR, Microbiol., 154(11), 3347–3357, https://doi.org/10.1099/mic.0.2008/019836-0, 2008.

Sheskin D. J.: Handbook of Parametric and Nonparametric Statistical Procedures: Third Edition, CRC Press Company, London and New York, 867–980, 2000.

Sieburth J. M. N., Smatacek V., and Lenz J.: Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions, Limnol Oceanogr., 23, 1256–126, https://doi.org/10.4319/lo.1978.23.6.1256, 1978.

Siefermann-Harms, D.: The light-harvesting and protective functions of carotenoids in photosynthetic membranes, Physiol. Plant., 69, 561–568, https://doi.org/10.1111/j.1399-3054.1987.tb09240.x, 1987.

Siegel, H., and Gerth M.: Sea surface temperature in the Baltic Sea in 2016, HELCOM Baltic Sea Environment Fact Sheets 2017, Online [Date Viewed: March 15, 2018], http://www.helcom.fi/baltic-sea-trends/environment-fact-sheets/.

Six, C., Finkel, Z. V., Irwin, A. J., and Campbell, D. A.: Light variability illuminates niche-partitioning among marine picocyanobacteria, PLoS One 2(12), e1341, https://doi.org/10.1371/journal.pone.0001341, 2007a.

Six, C., Thomas, J. C., Garczarek, L., Ostrowski, M., Dufresne, A., Blot, N., Scanlan, D. J., and Partensky, F.: Diversity and evolution of phycobilisomes in marine *Synechococcus* spp.: a comparative genomics study, Genome Biol., 8(12), R259, https://doi.org/10.1186/gb-2007-8-12-r259, 2007b.

Śliwińska-Wilczewska, S., Maculewicz, J., Barreiro Felpeto, A., Vasconcelos, V., and Latała, A.: Allelopathic activity of the picocyanobacterium *Synechococcus* sp. on filamentous cyanobacteria, J. Exp. Mar. Biol. Ecol., 496, 16–21, https://doi.org/10.1016/j.jembe.2017.07.008, 2017.

Śliwińska-Wilczewska, S., Maculewicz, J., Barreiro Felpeto, A., and Latała, A.: Allelopathic and bloom-forming picocyanobacteria in a changing world, Toxins, 10, 48; https://doi.org/10.3390/toxins10010048, 2018a.

Śliwińska-Wilczewska, S., Barreiro Felpeto, A., Maculewicz, J., Sobczyk, A., Vasconcelos, V., and Latała A.: Allelopathic activity of the picocyanobacterium *Synechococcus* sp. on unicellular eukaryote planktonic microalgae, Mar. Freshwater Res., 69, 1–8. https://doi.org/10.1071/MF18024, 2018b.

Snoeijs-Leijonmalm, P., and Andrén, E.: Why is the Baltic Sea so special to live in?, in: Biological Oceanography of the Baltic Sea, Snoeijs-Leijonmalm, P., Schubert, H., and Radziejewska, T. (Eds.), Springer, Dordrecht, 23–84, 2017.

Sorokin, P. Y., Sorokin, Y. I., Boscolo, R., and Giovanardi, O.: Bloom of picocyanobacteria in the Venice lagoon during summer–autumn 2001: ecological sequences, Hydrobiologia, 523(1-3), 71–85, 2004.

Sorokin, Y. I., and Zakuskina, O. Y.: Features of the Comacchio ecosystem transformed during persistent bloom of picocyanobacteria, J. Oceanogr., 66, 373–387, 2010.

Stal, L. J., Albertano, P., Bergman, B., Bröckel, K., Gallon, J. R., Hayes, P. K., Sivonen, K., and Walsby, A. E.: BASIC: Baltic Sea cyanobacteria. An investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea - Responses to a changing environment, Cont. Shelf Res., 23, 1695–1714, https://doi.org/10.1016/j.csr.2003.06.001, 2003.

Stal, L. J., Staal, M., and Villbrandt, M.: Nutrient control of cyanobacterial blooms in the Baltic Sea, Aquat. Microb. Ecol., 18, 165–173, 1999.

Stal, L. J., and Walsby, A. E.: Photosynthesis and nitrogen fixation in a cyanobacterial bloom in the Baltic Sea, Eur. J. Phycol., 35, 97–108, https://doi.org/10.1080/09670260010001735681, 2000.

Stawiarski, B., Buitenhuis, E. T., and Le Quèrè, C.: The physiological response of picophytoplankton to temperature and its model representation, Front. Mar. Sci., 3, 164, https://doi.org/10.3389/fmars.2016.00164, 2016.

Stockner, J. G.: Phototrophic picoplankton: An overview from marine and freshwater ecosystems, Limnol. Oceanogr., 33, 765–775, https://doi.org/10.4319/lo.1988.33.4part2.0765, 1988.

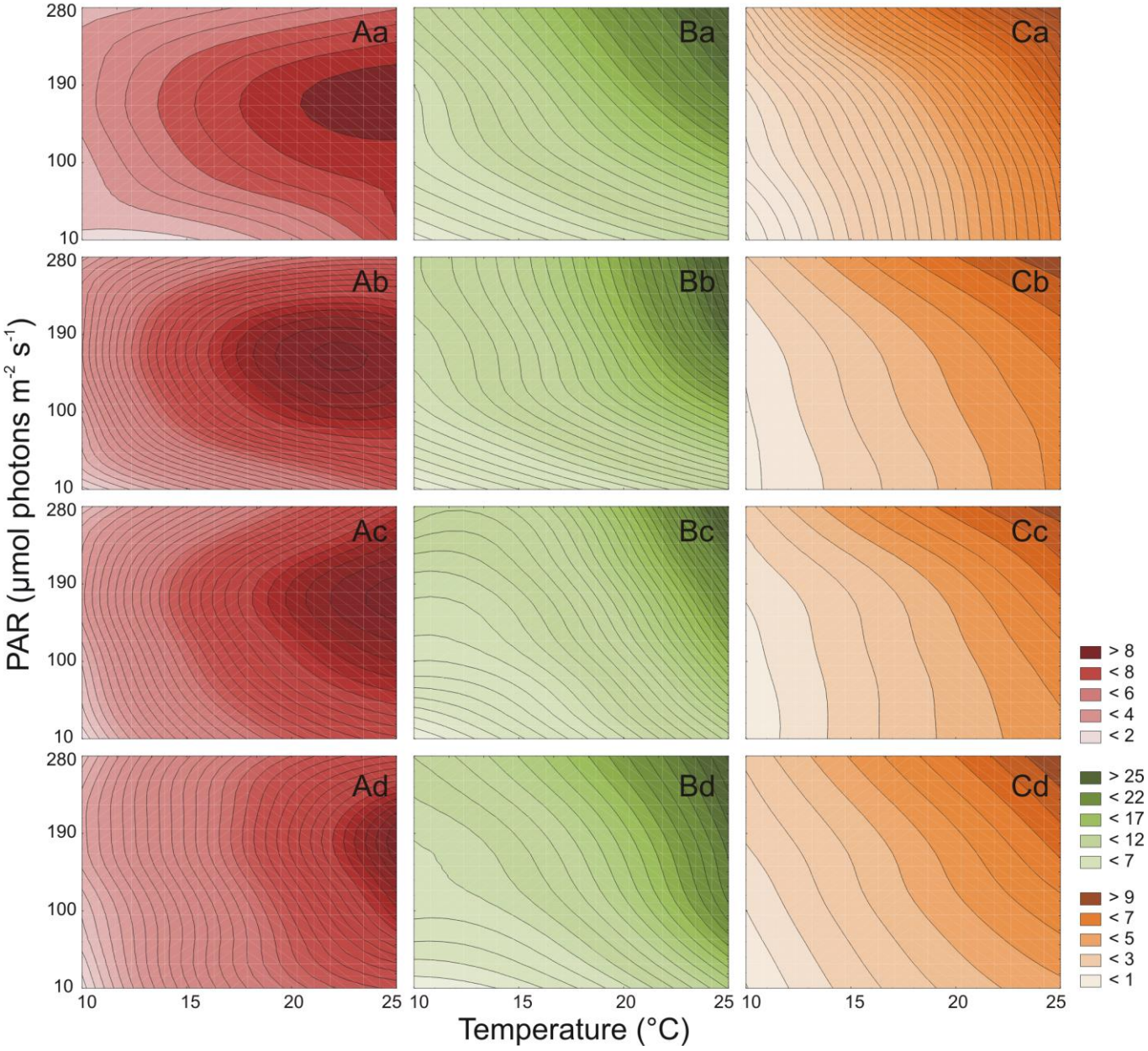
Stomp, M., Huisman, J., Vörös, L., Pick, F. R., Laamanen, M., Haverkamp, T., and Stal, L. J.: Colourful coexistence of red and green picocyanobacteria in lakes and seas, Ecol. Lett., 10, 290–298, https://doi.org/10.1111/j.1461-0248.2007.01026.x, 2007.

Strickland, I. D. H., and Parsons T. R.: A practical handbook of seawater analysis, J. Fish Res. Board Can., 167, 1–310, 1972.

Vörös, L., Gulyas, P., and Nemeth, J.: Occurrence, dynamics and production of picoplankton in Hungarian shallow lakes, Int. Rev. Ges. Hydrobiol., 76, 617–629, https://doi.org/10.1002/iroh.19910760412, 1991.

Waterbury, J. B., Watson, S. W., Guillard, R. R., and Brand, L. E.: Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium, Nature, 277(5694), 293–294, https://doi.org/10.1038/277293a0, 1979.

Worden, A. Z., and Wilken, S.: A plankton bloom shifts as the ocean warms, Science, 354(6310), 287–288, https://doi.org/10.1126/science.aaj1751, 2016.



912

913 **Figure 1.** Cell number (10^6 cell mL^{-1}) for three *Synechococcus* sp. strains: BA-120 (A), BA-124 (B) and BA-132 (C) under

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

915

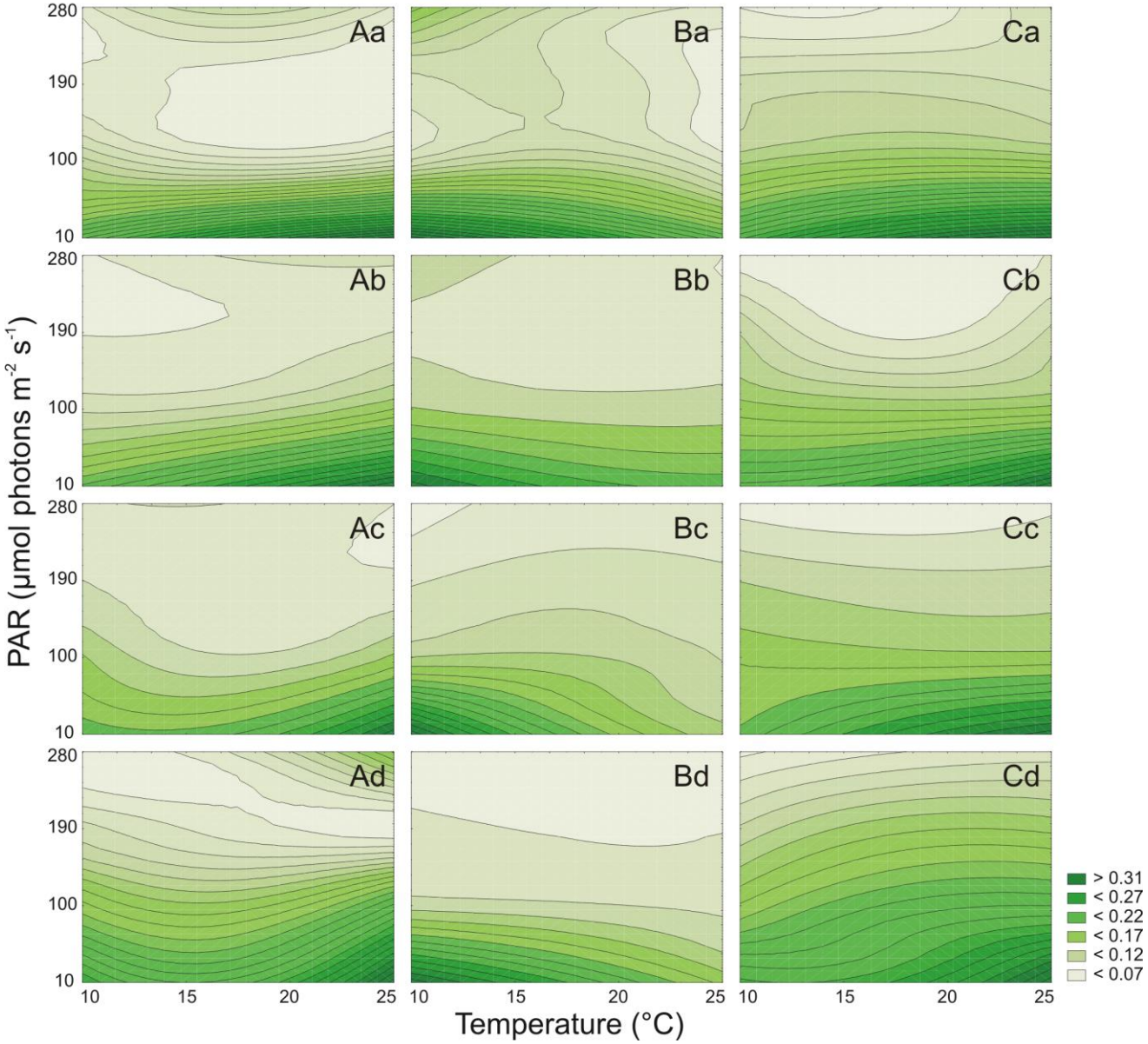


Figure 2. Cell-specific Chl *a* (pg cell^{-1}) changes for three *Synechococcus* sp. strains: BA-120 (A), BA-124 (B) and BA-132 (C) under different PAR and temperature conditions in 4 salinity mediums : 3 PSU (a), 8 PSU (b), 13 PSU (c) and 18 PSU (d).

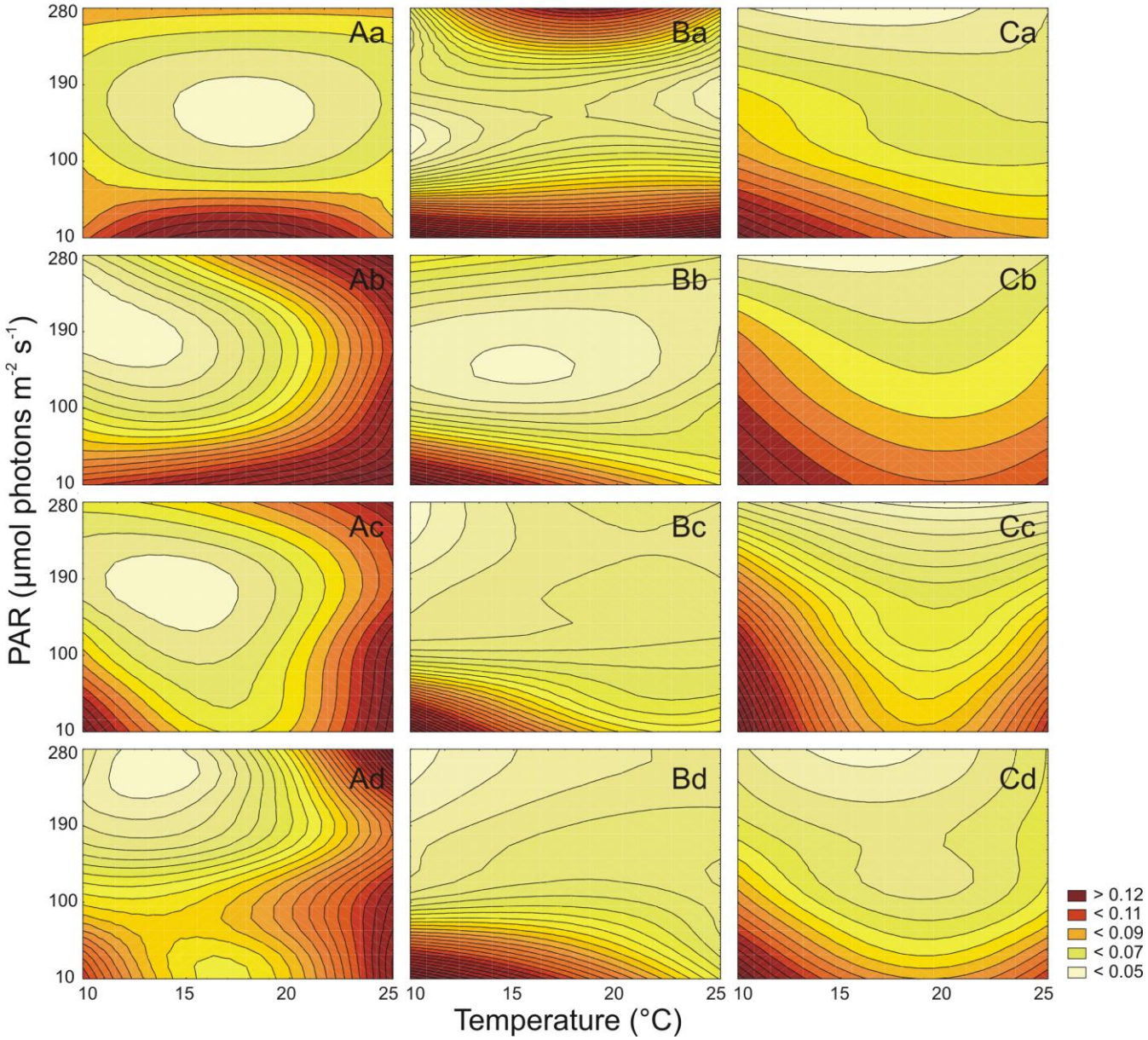


Figure 3. Cell-specific Car (pg cell^{-1}) changes for three *Synechococcus* sp. strains: BA-120 (A), BA-124 (B) and BA-132 (C) under different PAR and temperature conditions in 4 salinity mediums: 3 PSU (a), 8 PSU (b), 13 PSU (c) and 18 PSU (d).

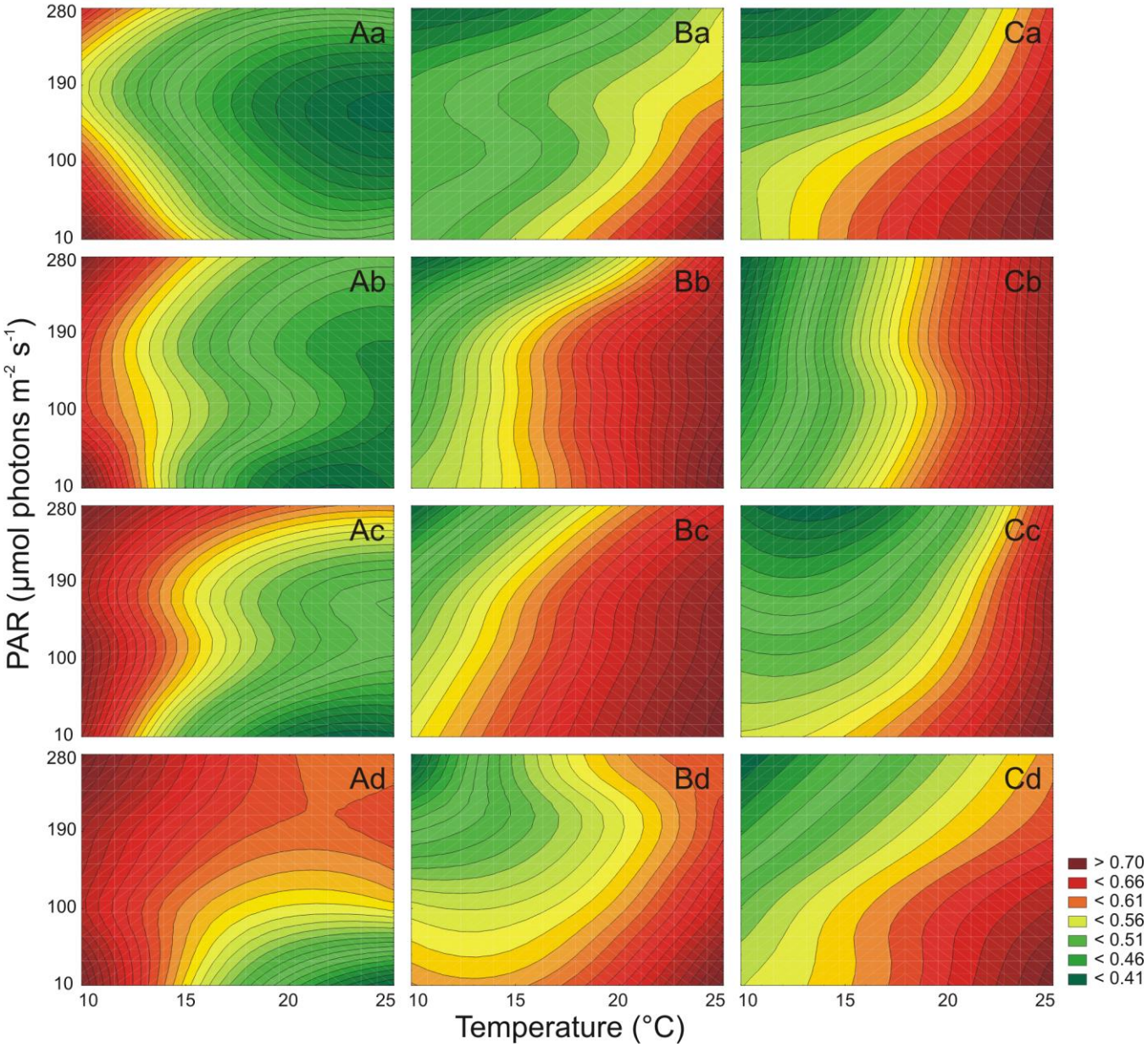


Figure 4. The maximum photochemical efficiency of PSII in the dark-adapted state (F_v/F_m) for three *Synechococcus* sp. strains: BA-120 (A), BA-124 (B) and BA-132 (C) under different PAR and temperature conditions in 4 salinity mediums: 3 PSU (a), 8 PSU (b), 13 PSU (c) and 18 PSU (d).

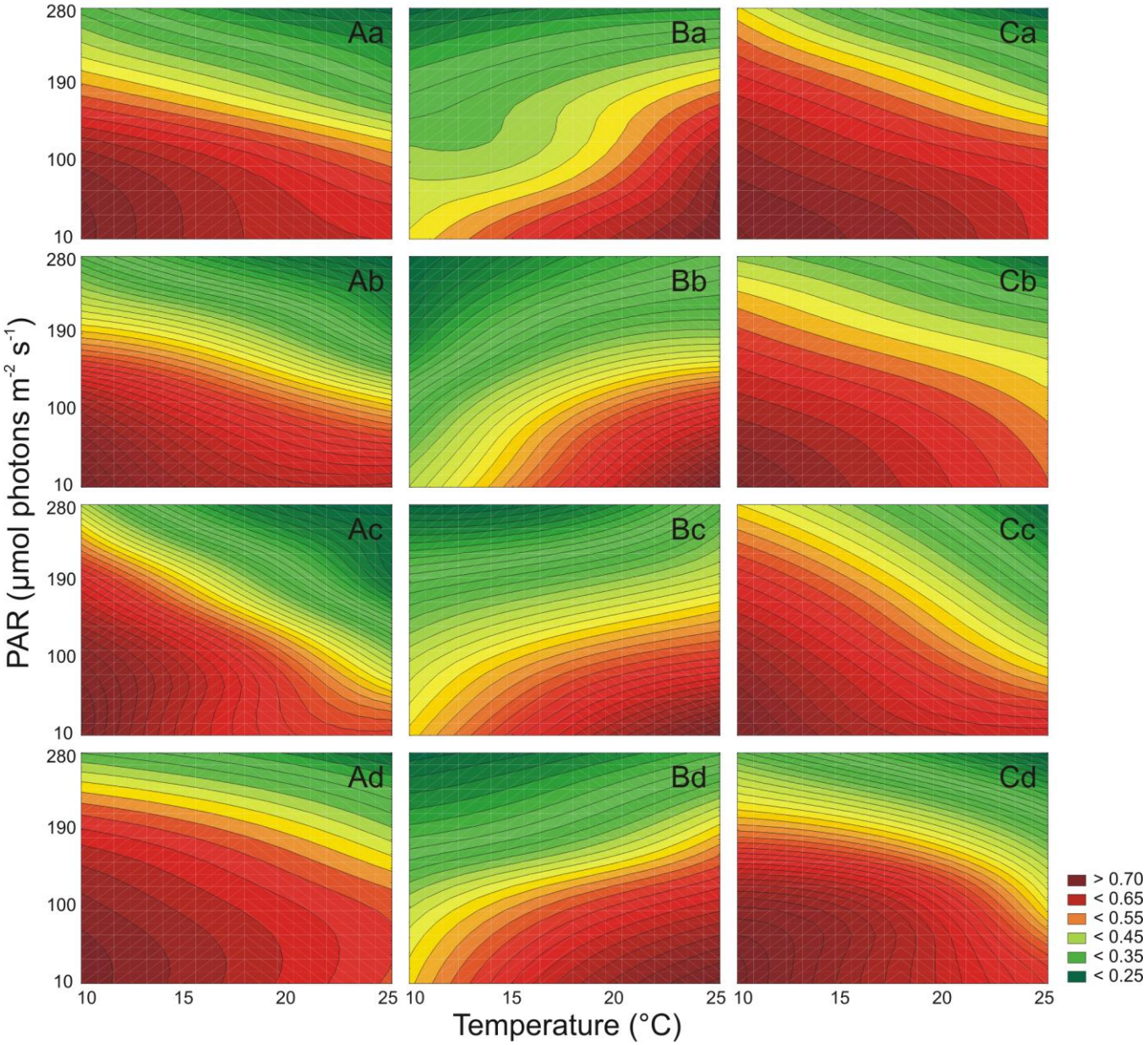
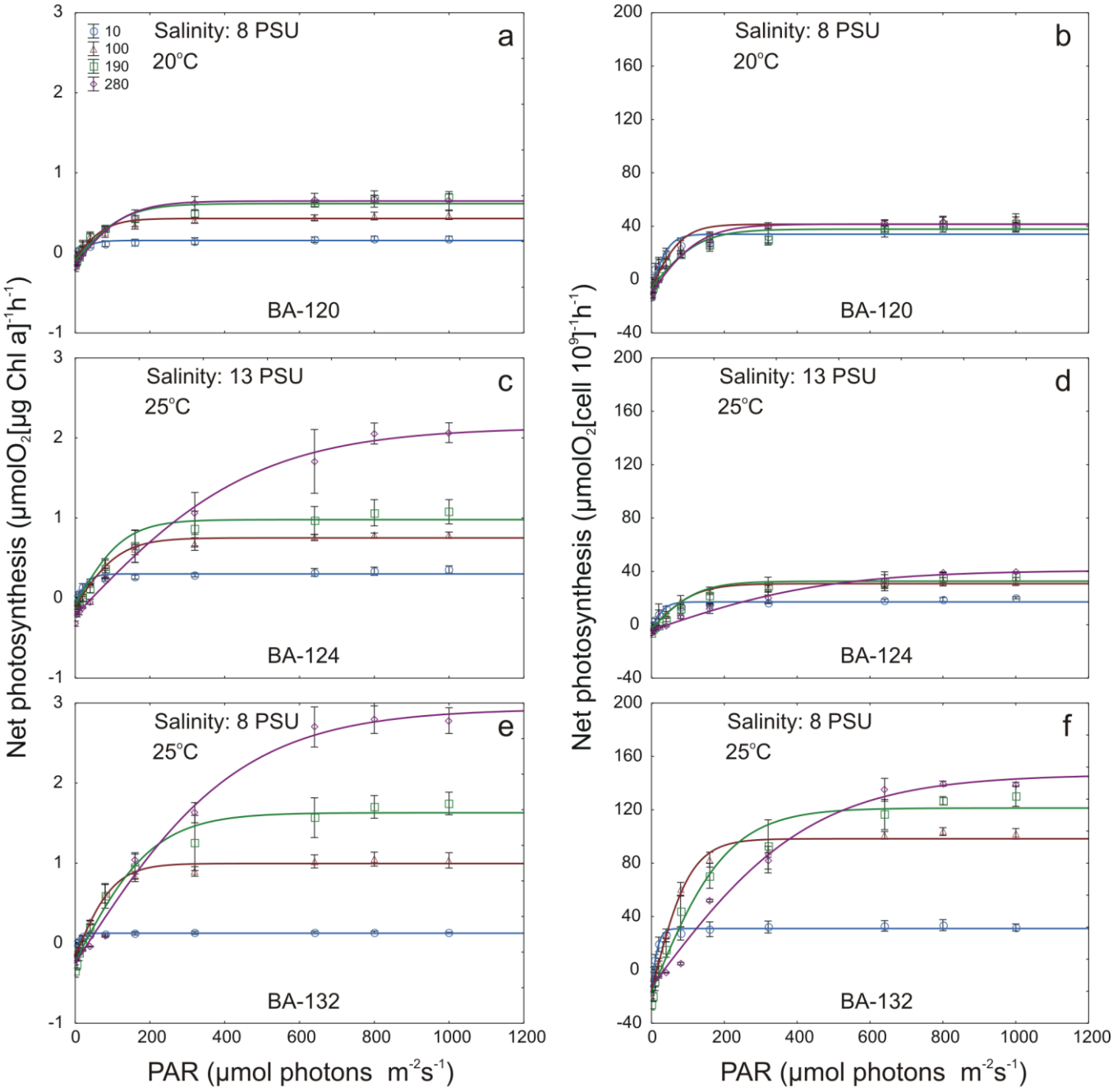


Figure 5. The photochemical efficiency of PSII under actinic light intensity (ΦPSII) for three *Synechococcus* sp. strains: BA-120 (A), BA-124 (B) and BA-132 (C) under different PAR and temperature conditions in 4 salinity mediums: 3 PSU (a), 8 PSU (b), 13 PSU (c) and 18 PSU (d).



939
940 **Figure 6.** Selected Chl *a* - specific and cell-specific (right side and left side panel, respectively) net photosynthetic–light
941 response curves for three *Synechococcus* sp. strains: BA-120 (a, b), BA-124 (c, d) and BA-132 (e, f) strains. Curves present
942 examples of three types of photoacclimation observed for *Synechococcus* sp. and these are as follows: change in number of
943 photosynthesis units (PSU) (a, b), change in size of PSU (c, d) and change in accessory pigments activity (e, f).
944
945
946
947
948
949
950
951
952
953
954
955

Table 1. Photoacclimation types (mechanisms) for three *Synechococcus* sp. strains: BA-120, BA-124 and BA-132 at different ecological conditions. OTHER stands for altering of accessory pigments activity or changes in enzymatic reactions; PSUsize stands for the change in PSU sizes; PSUno. stands 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}

Supplement of

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

Sylwia Śliwińska-Wilczewska et al.

Correspondence to: Agata Cieszyńska (acieszynska@iopan.gda.pl, cieszynska.agata@gmail.com)

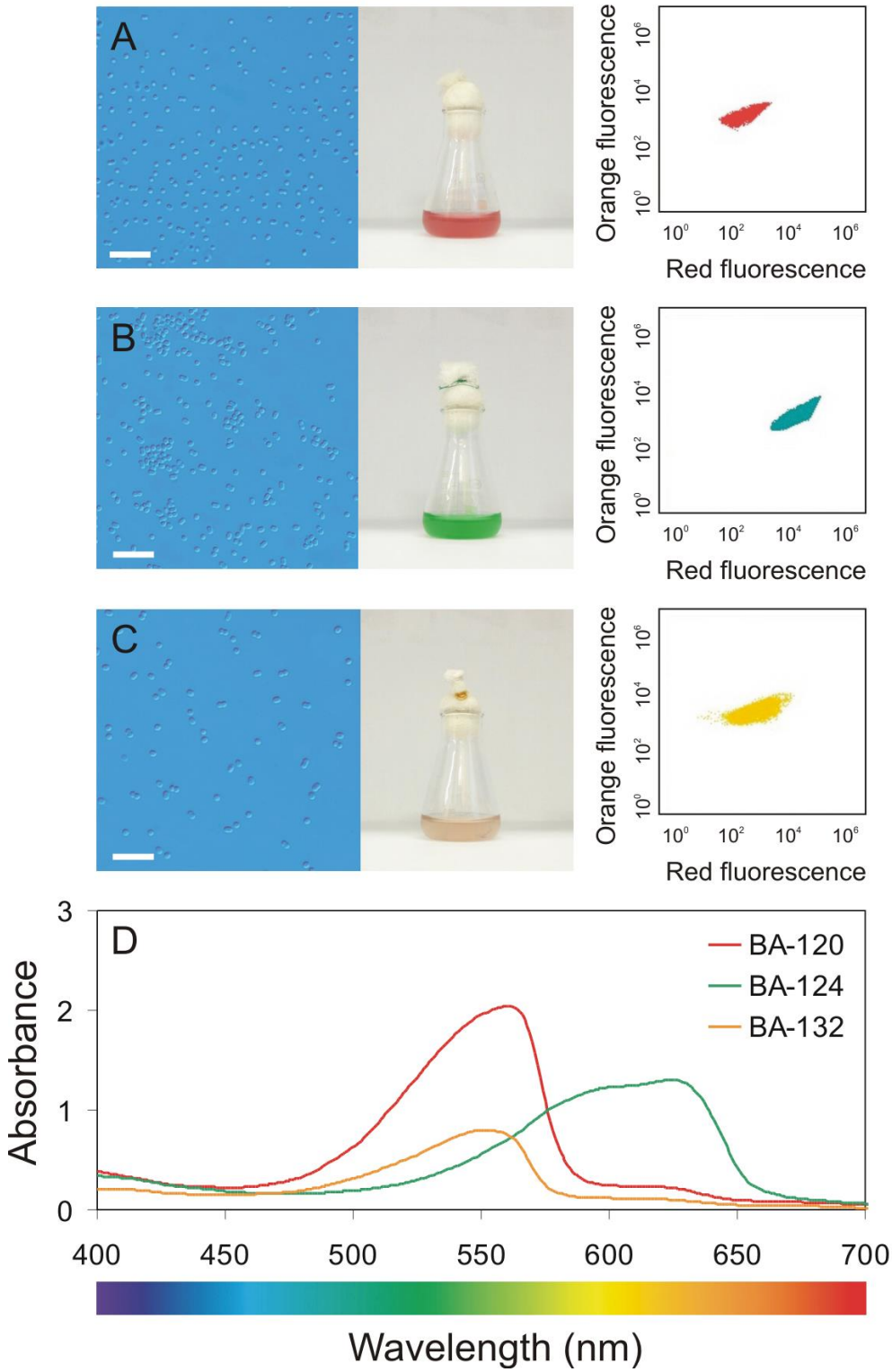


Figure S1: Left-side top panel (A, B, C) – light microscope photographs of three *Synechococcus* sp. strains (scale bar = 10 µm) along with the photographs of the cultures in 25-mL glass Erlenmeyer flasks; right-side top panel – scatter plots of orange fluorescence vs. red fluorescence analyzed using a BD Accuri™ C6 Plus flow cytometer and bottom panel (D) – PAR absorption spectra determined for the mixture of phycobilin pigments for each *Synechococcus* sp. strain.

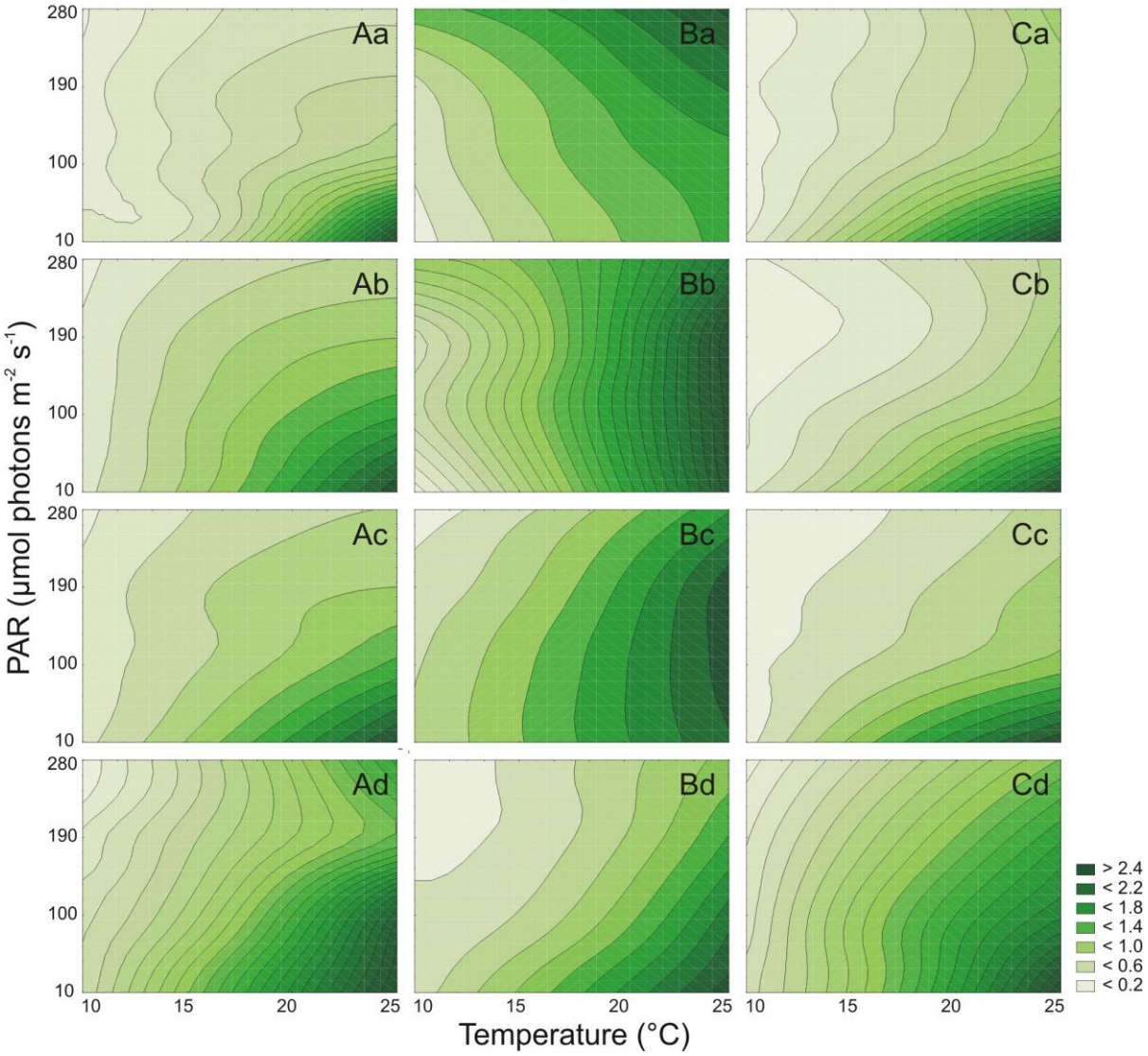


Figure S2: Chl *a* (µg mL^{-1}) changes for three *Synechococcus* sp. strains: BA-120 (A), BA-124 (B) and BA-132 (C) under different PAR and temperature conditions in 4 salinity mediums: 3 PSU (a), 8 PSU (b), 13 PSU (c) and 18 PSU (d).

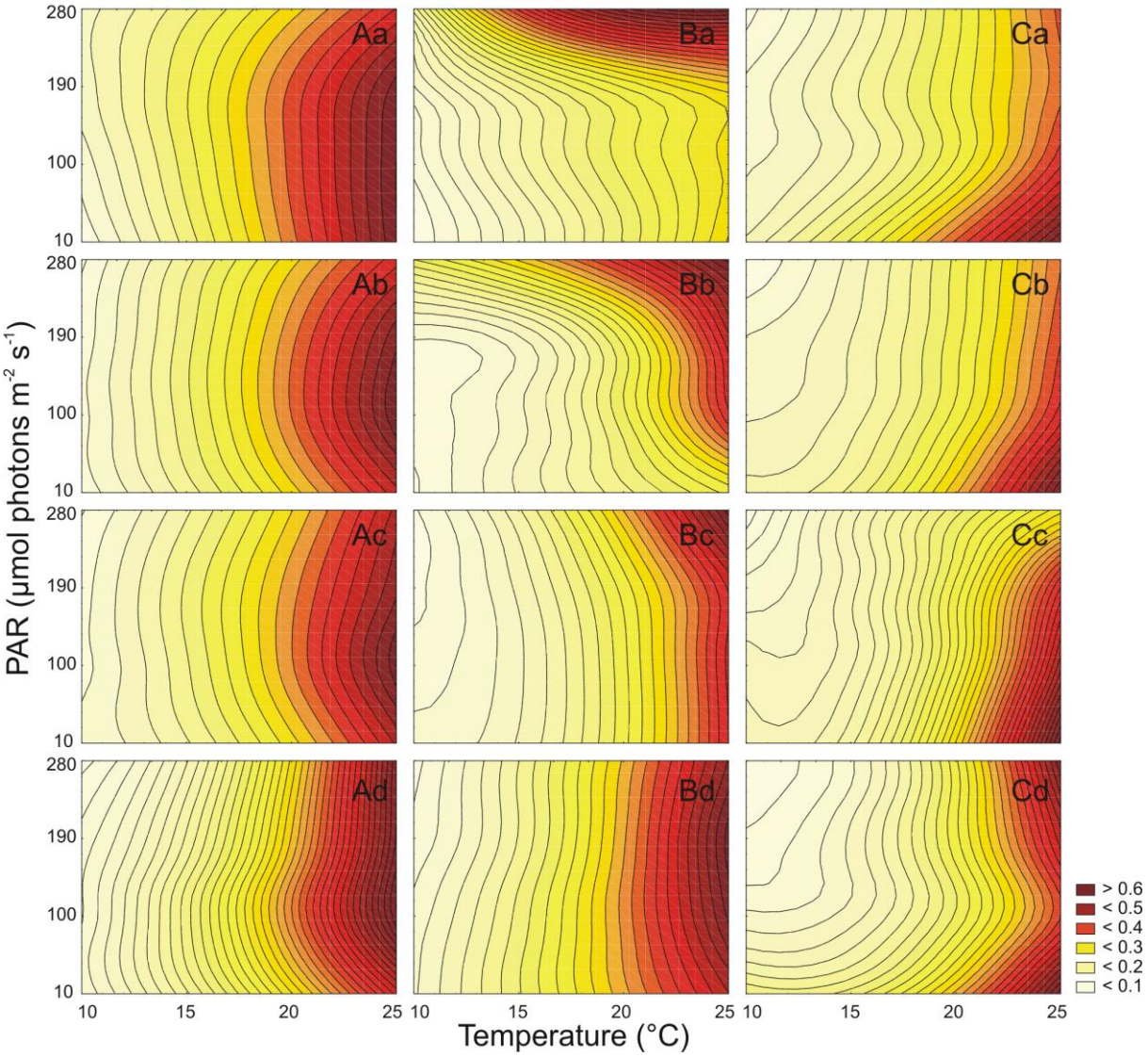


Figure S3: Car (µg mL^{-1}) changes for three *Synechococcus* sp. strains: BA-120 (A), BA-124 (B) and BA-132 (C) under different PAR and temperature conditions in 4 salinity mediums: 3 PSU (a), 8 PSU (b), 13 PSU (c) and 18 PSU (d).

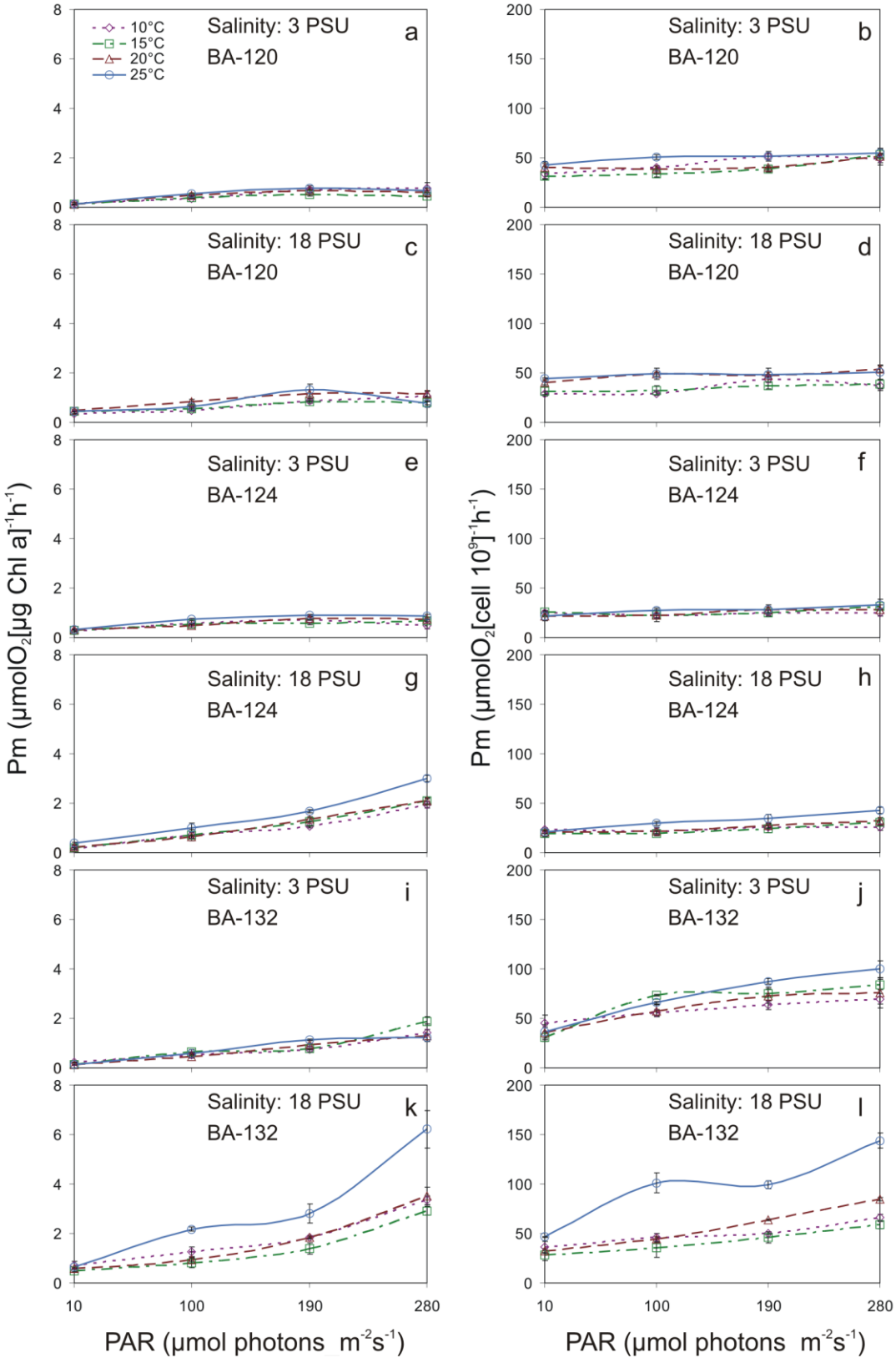


Figure S4: The Chl *a*-specific (left side panel) and cell-specific (right side panel) photosynthesis capacity (P_m) at two extreme salinities (3 and 18 PSU) under different PAR and temperature conditions for three *Synechococcus* sp. strains: BA-120 (a-d), BA-124 (e-h) and BA-132 (i-l).

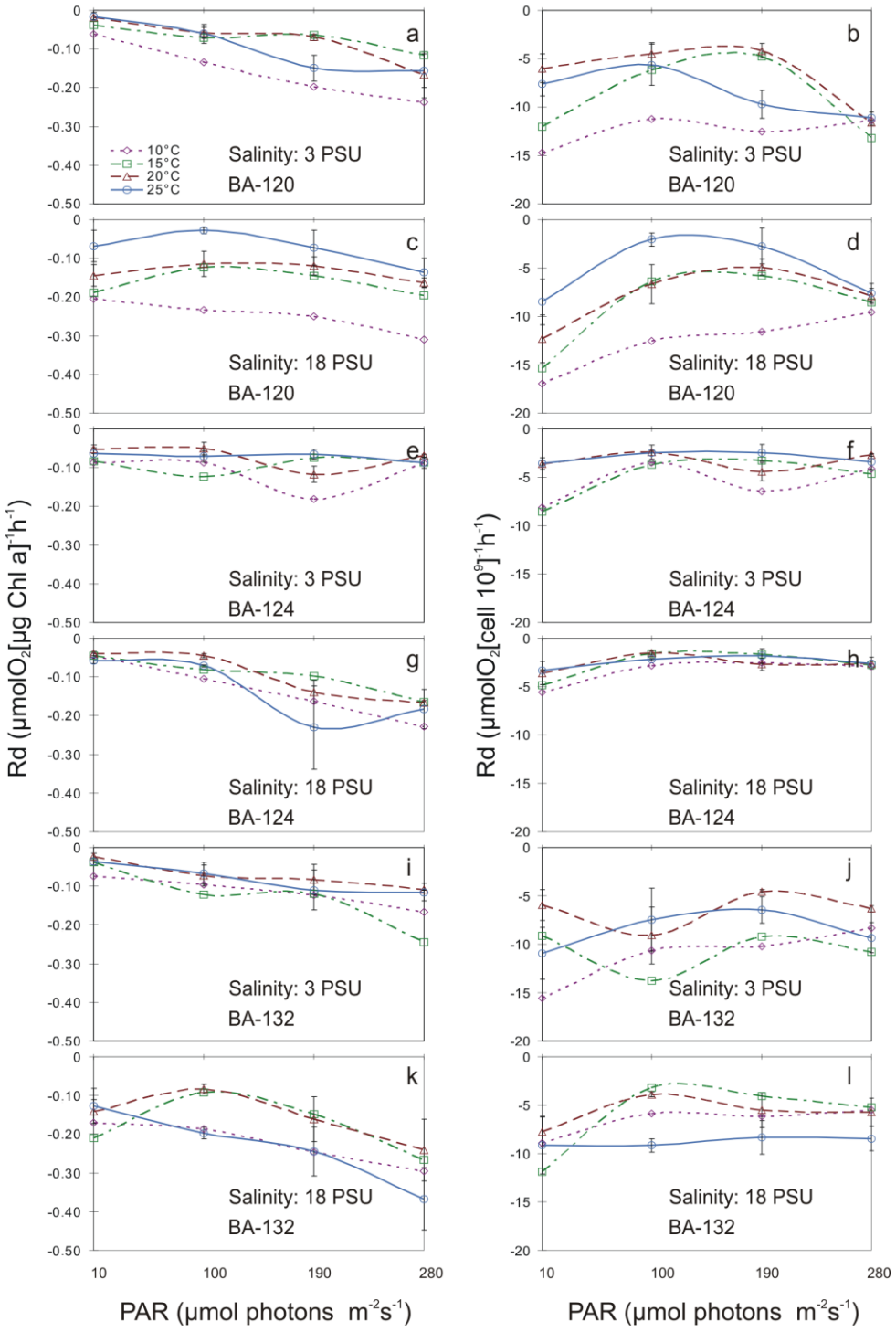


Figure S5: The Chl *a*-specific (left side panel) and cell-specific (right side panel) dark respiration (R_d) at two extreme salinities (3 and 18 PSU) under different PAR and temperature conditions for three *Synechococcus* sp. strains: BA-120 (a-d), BA-124 (e-h) and BA-132 (i-l).

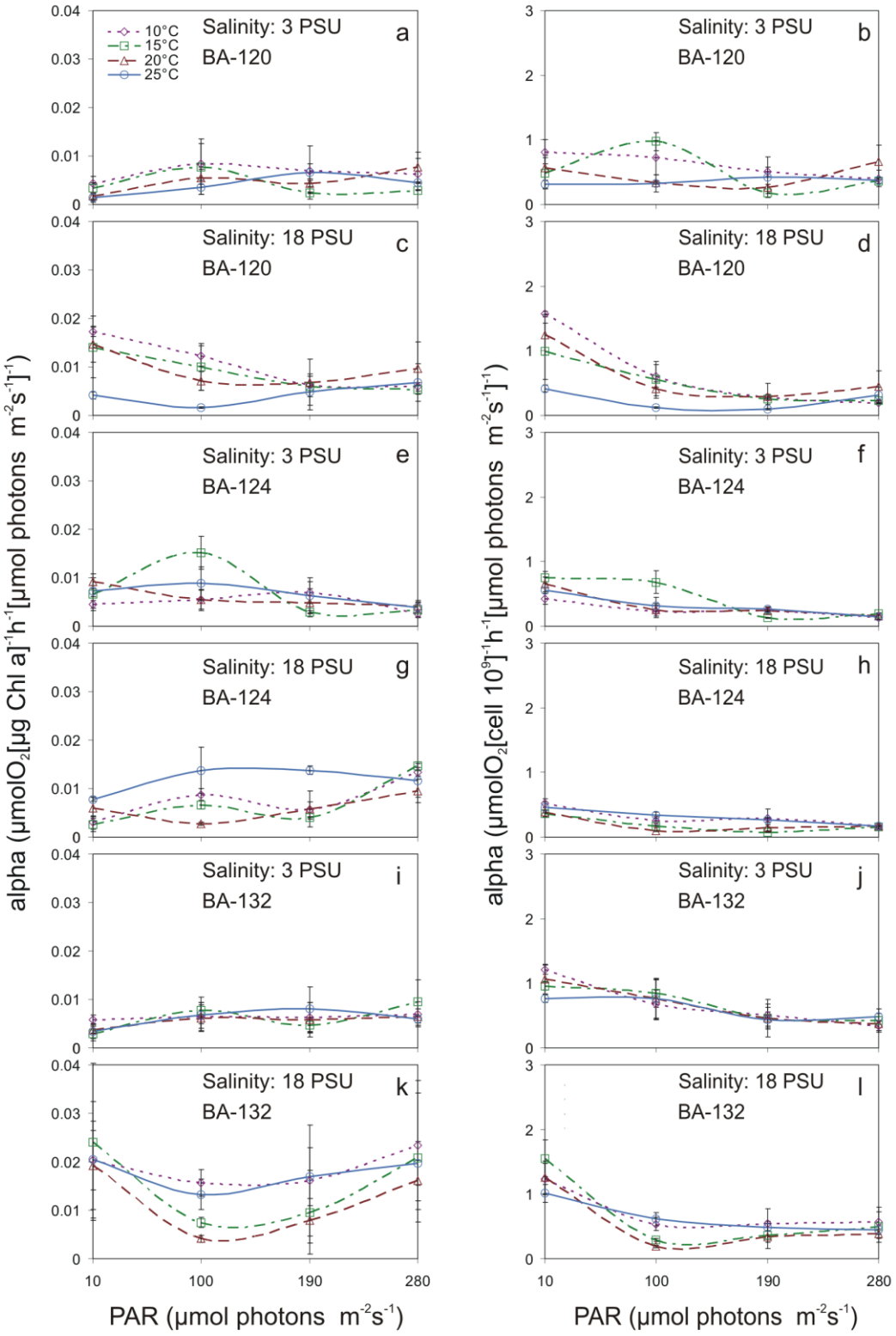


Figure S6: The Chl *a*-specific (left side panel) and cell-specific (right side panel) photosynthetic efficiency at limiting irradiance (*αlpha*) at two extreme salinities (3 and 18 PSU) under different PAR and temperature conditions for three *Synechococcus* sp. strains: BA-120 (a-d), BA-124 (e-h) and BA-132 (i-l).