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

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Abstract. The bloom of picocyanobacteria (PCY), accompanied by a drastic ecological crisis is a new phenomenon in Europe, which requires careful investigation. Therefore, this work examined the response of *Synechococcus* sp. physiology to different environmental conditions. Three strains of *Synechococcus* sp. (red BA-120, green BA-124 and brown BA-132) were cultivated in a laboratory under previously determined environmental conditions. These conditions were as follows: temperature (T) from 10 by 5 to 25°C, salinity from 3 by 5 to 18 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 growth rates 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 found to be true for BA-120, but only to a certain degree as the growth rates 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 the recording and recognition of such small organisms. Before the discovery of picocyanobacteria (PCY) in the oceans by Johnson and Sieburth (1979) and Waterbury et al. (1979) there only existed incidental reports of this fraction of cyanobacteria in aquatic ecosystems. Since then, the number of reported PCY occurrences 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; 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). Later work by 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. Depending on pigment content, *Synechococcus* sp. is classified as red strains with phycoerythrin (PE), green strains rich in phycocyanin (PC) and the brown strains containing phycourobilin (PUB) and PE (Mazur-Marzec et al., 2013). Despite its association with open ocean systems, has become increasingly evident in recent years that *Synechococcus* sp. is a significant contributor to cyanobacterial blooms. 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). Most field or laboratory investigations concerning Baltic *Synechoccocus* sp. are focused on two of the three aforementioned strains: green (BA-124) and red (BA-120) usually neglecting the brown strain (BA-132). These give 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; Jodłowska and Śliwińska, 2014).

Recently, it has been confirmed that PCY are able to excrete harmful and allelopathic substances (e.g., Jakubowska and Szeląg-Wasilewska, 2015; Śliwińska-Wilczewska et al., 2016; 2017). Many different factors, including physical parameters, availability and competition for resources, selective grazing and allelopathic interactions can affect the occurrence of harmful blooms in aquatic ecosystems. The development of massive algal blooming is a consequence of the interaction between many favourable factors. *Synechococcus* sp. greatly contributes to these massive blooms, but so far the characteristics of the life cycle of Baltic PCY has not been sufficiently studied. This knowledge needs to be expanded and improved, especially because of bloom toxicity and negative impacts on ecosystems. Moreover, the blooms of PCY, accompanied by a drastic ecological crisis are a new phenomenon in Europe (Sorokin and Zakuskina, 2010), which also requires careful investigation.

According to the above, phytoplankton is of great interest to scientists in 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 the above, 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 examination. 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 motivation 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 implies that the present study is one of the first in-sight descriptions of BA-132 ecophysiology and its comparison to other strains of the same genus.

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, the study is supposed to provide in-depth information on how different environmental conditions influence the life cycle of picocyanobacteria quantitatively. The initial step of these works was to carry out laboratory experiments with *Synechococcus* sp. cultures. In order to create different environmental conditions in the Baltic Sea range, combinations of physical quantities were determined. 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), and temperature (T) (10, 15, 20, 25°C). 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 growth rate, 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 will help to develop the knowledge of the picocyanobacteria life cycle. Moreover, the PCY

experiments underlie the improved numerical approach to phytoplankton modeling development. On the basis of derived results, the algorithm for picocyanobacterium growth will be created.

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 cultures preparation was carried out as follows. 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). In order to develop the media, first the appropriate amount of Tropic Marine Synthetic Sea Salt was dissolved in distilled water. Salinity of the media was measured by salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany). Then, the biogenic compounds were added and the media were autoclaved. Into 25 mL Erlenmeyer glass flasks, the cells of specific strains were inoculated. The picocyanobacteria cultures were acclimated 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 (10, 15, 20 and 25°C), and salinity (3, 8, 13 and 18). The combination of aforementioned quantities is called a scenario in the present paper. The intensity of PAR was measured using a LI-COR spherical quantum-meter. 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. After acclimation time (2 d), the picocyanobacteria 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. Values of quantities representing the environmental conditions occurred at the same intervals. 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 number of cells (N) in cultures was counted with flow cytometer BD Accuri™ C6 Plus (BD Biosciences, San Jose, CA, USA). Events were recorded in list form. Samples were run at a flow rate of approximately 14 µl min^{-1}. Flow was daily calibrated with Spherotech 6- and 8- Peak Validation Beads (BD, San Jose, USA). This ensures that the cytometer is working properly before running experimental samples. FITC, PE, and PE-Cy5 detectors were daily calibrated with SPHERO™ Rainbow Calibration Particles (BD, San Jose, USA), and the APC channel was calibrated with SPHERO 6-peaks Allophycocyanin Calibration Particles (APC). 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) (Marie et al., 2005). The flow cytometry was used to establish the initial number of picocyanobacteria cells and to estimate the final cells concentration after the incubation period. Based on the final cells concentrations, the growth rates were calculated. The growth rate and cells concentration are different parameters but both lead the researcher to the same conclusions on the growth characteristics. In this paper, the growth rates were analyzed abandoning the separate study on the cell concentrations themselves.

2.3 Determination of the pigments content

The concentration of photosynthetic pigments of analyzed picocyanobacteria was measured by the spectrophotometric method. 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 picocyanobacteria 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. After this time the pigment extract was centrifuged at 10000 rpm for five minutes in order to remove filter particles. The absorption 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 - absorption 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. Before measurements, each sample taken from the culture was filtered through 13 mm glass fibre filters (Whatman GF/C, pore size = 1.2 μm). At the beginning of the experiment, the filter sample was adapted in the dark for about 10 minutes. 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 different cultures, the same as the PAR level was for each incubation. The above is similar to the method used by Campbell et al. (1998).

2.5 Measurements of photosynthesis rate

The measurements of oxygen evolution were carried out on the day seventh of the experiment using a Clark-type oxygen electrode (Chlorolab 2, Hansatech). 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 was fitted to the photosynthetesis irradiance response (*P-E*) curves using equation (Jassby and Platt, 1976) and Statistica® 13.1 software and photosynthetic parameters, i.e., the photosynthetic capacity (P_m), the initial slope of *P-E* curve (α) and the dark respiration (R_d) (Sakshaug et al., 1997).

2.6 Statistical analyses

The effect of light and temperature separately and then their combinations 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 marine *Synechococcus* community (Glover, 1985; Glover et al., 1985; 1986, Joint and Pomroy, 1986; Jasser and Arvola, 2003; Jasser, 2006; Jodłowska and Śliwińska, 2014), as a result it was decided that light and temperature would be the independent variables in ANOVA and post-hoc test analysis. The dependent variable was always the parameter, which had been measured, e.g.: growth rate or pigments concentration.

3 Results

3.1 Growth rates

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

In general, due to post-hoc tests, 2008 multicomparisons (70%) out of all 2880 completed for three strains, indicated the highest statistical significance (Tukey HSD, *** $p < 0.001$), 160 multicomparisons (6%) pointed to the statistical significance of $0.001 <$ ** $p < 0.01$, and 114 (4%) stated for the significance of $0.01 <$ * $p < 0.05$. The rest of the multicomparisons (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, respectively). For BA-120, the growth rate (μ) increased with T in each medium (salinities 3, 8, 13, 18) (Figs. 1a – 1d). The rates varied between 0.07 and 0.34 d^{-1} within the whole scenarios domain. The minimum μ was estimated in salinity 3, T 10°C and PAR 10 μmol photons m^{-2} s^{-1}, whilst the maximum in salinity 18, T 25°C, PAR 190 μmol photons m^{-2} s^{-1}. The decrease in growth rate was observed from PAR 190 μmol photons m^{-2} s^{-1} onwards (Figs. 1a – 1d). This can likely be related to the photo-inhibition of photosystem II (PSII). The above was true for each salinity. It seemed that the most important environmental factor for BA-120 growth was T, with PAR playing an additional role, for instance in the context of photo-inhibition. Multicomparison tests pointed to the strong significance of PAR and T combined in influencing the pigmentation. According to the statistics, 82% of multicomparisons 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, respectively). For BA-124, growth rate increased with T and PAR in all salinities (Figs. 1e – 1h). The growth rates varied between 0.11 and 0.53 d^{-1}. The lowest μ was calculated in salinity 3, T 10°C and PAR 10 μmol photons m^{-2} s^{-1} and the highest growth rate was reached in salinity 18, T 25°C, PAR 280 μmol photons m^{-2} s^{-1}. Intensive cell division was also estimated under the highest T and PAR conditions in salinity 13 with a growth rate of 0.52 d^{-1}. The lowest μ of BA-124 was estimated for the lowest T and PAR condition in each salinity. Generally, cell abundance (Fig. S1 in Supplement) and the growth rates were the highest when compared to BA-120 and BA-132 in relative scenarios. One of the observations was the difference in BA-124 growth between lower and higher PAR and T scenarios. BA-124 seemed to be more sensitive to changes in PAR and T in their lower rather than higher ranges (Figs. 1e – 1h). The highest cell concentrations of BA-124 were noted in moderate- and high-salinity mediums. However, optimum salinities for green strain to grow were 8 and 13. Due to post-hoc analysis, salinity 13 differentiated the conditions for growth under different PAR and T at a lower degree when compared to other salinities. Another feature of BA-124 growth was that the

growth rates in low T and high PAR scenarios were nearly equal to growth rates in high T and low PAR scenarios. This was not observed for red and brown strains. The observation was supported by Tukey's tests, where only few statistically significant differences in growth rates 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 (Tukey HSD, ** p < 0.05 in both cases). Multicomparison tests showed high significance of a combination of PAR and T in affecting growth. According to Tukey HSD tests, 72% of multicomparisons were statistically significant (* p < 0.05) with 82% of them with the highest significance level (*** p < 0.001).

Similar 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, respectively). For BA-132, the positive impact of T and PAR on cells concentration and growth rates was observed in each medium (Figs. 1i – 1l). Salinity played a more significant role here than when compared to BA-124. Note that the higher the salinity, the higher the growth rates of brown strain. What is more, according to the statistical analysis, salinity 18 differentiated the growth rates the most and it was the easiest medium to determine a fixed response of growth rate to the variable environmental conditions in it. In salinity 18, the growth rate 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 (Figs. 1i – 1j). Moreover, in high salinity, the sensitivity of growth rate to T changes was much lower than in low salinities (Fig. 1l). PAR did not determine the growth as strongly as T, which was quite consistent to the observation noted for BA-120. The growth rates vary between 0.05 and 0.38 d^{-1} within all gathered results. The minimum growth rate was observed in salinity 3, 10°C and 10 µmol photons m^{-2} s^{-1}, whilst the maximum in salinity 18, 25°C, 280 µmol photons m^{-2} s^{-1}. The lowest values of BA-132 growth rates 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 growth. According those tests, 84% of multicomparisons were statistically significant (* p < 0.05) with 90% of them with the highest significance (*** p < 0.001).

Concerning all three strains, salinity generally had a positive impact on growth. What is more, the relations between salinity and growth rates for all strains were almost increasing linearly with the highest increase for BA-132, followed by BA-120. The smallest increase in growth along with salinity was noted for BA-124.

3.2 Pigment content

The results showed that for all strains, cell-specific pigment composition (pigment content per cell) was environmentally driven. 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 difficult to determine qualitative and quantitative relations between pigment content and ecological state unequivocally in analyzed scenarios. Nonetheless, there were a few facts in pigmentation changes under environmentally different conditions, which were as follows.

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, respectively). Both PAR and T also affected the Car content in the red 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, respectively). For BA-120 in every scenario the pigmentation changes presented different characteristics when comparing two estimations – mL-specific (Figs. S2A, S3A), and cell-specific (Figs. 2, 3). However, both cell-specific and mL-specific Chl *a* and Car concentrations

decreased with the increase of salinity, which was noticeable especially in cell-specific analysis (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 domain at low PAR. Maximum Chl *a* content was measured under T 25°C and PAR 10 µmol photons m^{-2} s^{-1}. This concerned each salinity. The highest Chl *a* concentration within all scenarios was reached in BA-120 cells in salinity 3 and was equal to 0.339 pg $cell^{-1}$. For other salinities these maximums were as follows: 0.233 pg $cell^{-1}$ (8), 0.164 pg $cell^{-1}$ (13), 0.100 pg $cell^{-1}$ (18). The highest Car content was measured in salinity 3 under T of 20°C and PAR 10 µmol photons $m^{-2}s^{-1}$ and reached 0.160 pg $cell^{-1}$. The lowest concentrations of Chl *a* (0.038 pg $cell^{-1}$) and Car (0.031 pg $cell^{-1}$) were measured in salinity 18, T 25°C, PAR 190 µmol photons $m^{-2}s^{-1}$ and salinity 18, T 15°C, PAR 280 µmol photons $m^{-2}s^{-1}$, respectively. Multicomparison tests indicated the significance of PAR and T combined in shaping the pigmentation. Due to those tests, 52% and 55% of multicomparisons of comparisons in Chl *a* and Car content analysis, 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, 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, respectively) of *Synechococcus* sp. BA-124 significantly. Regarding BA-124, in each scenario the composition of pigments differed between the estimations (mL- or cell-specific). However, generally, PAR and T 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, starting from the lowest and ending at the highest salinity, were as follows: 0.095, 0.102. 0.1760, 0.1480 pg $cell^{-1}$ for Chl *a* and 0.051, 0.067, 0.087, 0.079 pg $cell^{-1}$ for Car. Nonetheless, there were also some exceptions. In salinity 3, 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. On average, salinity 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, T 10°C, PAR 280 µmol photons m^{-2} s^{-1} and were equal to 0.013 pg $cell^{-1}$ and 0.009 pg $cell^{-1}$, for Chl *a* and Car, respectively. Multicomparison tests pointed to the significance of PAR and T combined in influencing the pigmentation. According to the statistics, 47% and 54% of multicomparisons 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, 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, respectively) of *Synechococcus* sp. BA-132 significantly. As for the two other analyzed strains, the cell-specific and mL-specific pigmentation changes differed from each other in every scenario. Nonetheless, salinity had a negative impact on Chl *a* and Car concentrations in both estimations, which was noticeable especially in the cell-specific pigmentation. Note that salinity affected pigment concentrations negatively. This was a consistent feature for all examined strains. 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 and 25°C in salinity 3, 8, 18) under the lowest PAR (10 µmol photons m^{-2} s^{-1}). These maximums were 0.299 pg $cell^{-1}$ in salinity 3, 0.248 pg $cell^{-1}$ in salinity 8, 0.151 pg $cell^{-1}$ in salinity 13, 0.073 pg $cell^{-1}$ in salinity 18. 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, 0.131 pg $cell^{-1}$ in salinity 8, 0.097 pg $cell^{-1}$ in salinity 13, 0.062 pg $cell^{-1}$ in salinity 18.

Minimums of Chl *a* and Car cell-specific contents within all scenarios were estimated in salinity 18, T 15°C and PAR 280 µmol photons m^{-2} s^{-1} being equal to 0.020 pg $cell^{-1}$ and 0.19 pg $cell^{-1}$, 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 for the same conditions of T and PAR. Tukey HSD tests pointed to the significance of PAR and T combined in impacting the pigmentation. According to those tests, 66% and 61% of multicomparisons 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 (Fig. 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 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, 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}. Generally, strong fluctuations were noticeable in F_v/F_m values, which disabled the fixed environmentally driven pattern determination (Fig. 4A). However, there was a constant relation noted between T and PAR and ΦPSII. PAR and T had a negative impact on ΦPSII. The impact was the strongest in low salinity (Fig. 5A). 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 0.8 and was estimated for scenario: salinity 18, T 10°C, PAR 280 µmol photons m^{-2} s^{-1}. 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, T 25°C and PAR 190 µmol photons m^{-2} s^{-1}. Concerning ΦPSII, the greatest of all was ΦPSII = 0.77 estimated in salinity 18. Minimum ΦPSII was measured in salinity 3, T 25°C and PAR 280 µmol photons m^{-2} s^{-1}. Multicomparison 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, 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 relative incubations of other strains (Figs. 4A, 4B, 4C). 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 (Figs. 4B, 5B). The F_v/F_m minimums were measured under the lowest T and highest PAR in each salinity. The lowest value within all scenarios was 0.12 and was observed in salinity 3. 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 0.56 for salinity 3. 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 0.11 (followed by the minimum in salinity 3 being equal to 0.12) and was measured in salinity

13. Maximums of ΦPSII were observed in the highest T and lowest PAR in each medium, similar to F_v/F_m. The greatest ΦPSII of all was 0.54 and was measured in salinity 3. 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, 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, 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 (Fig. 4C). 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 stated for salinity 13 (F_v/F_m = 0.15). In salinity 3, under aforementioned conditions of T and PAR, the F_v/F_m value was also low compared to the others and equaled 0.16. The maximums of F_v/F_m were measured in T 25°C and PAR 10 µmol photons m^{-2} s^{-1}. This was true for all mediums. The highest F_v/F_m were noted in salinities 13 and 18 and equaled 0.74 and 0.73, respectively. This was consistent with the tendency observed in growth rates of BA-132 in low T scenarios, where µ increased strongly with salinity elevation. The lowest ΦPSII were noted under the highest PAR and T conditions in every salinity. The minimum ΦPSII, within all gathered results, was obtained in salinity 3 and equaled 0.28. T and PAR affected ΦPSII negatively (Fig. 5C). 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, 0.79, was noted in salinity 8. The ΦPSII reached generally higher values than F_v/F_m in BA-132 experiments. ΦPSII reached lower values than respective ΦPSII for two other strains (Figs. 5A, 5B, 5C). Multicomparison 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 red strain, F_v/F_m was affected negatively by T, while green and brown strains were affected positively. T 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 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 for 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.

The analysis of photosynthesis characteristics enabled the authors to examine and define the photoacclimation process of all three strains. This was done on the basis of Photosynthesis-Irradiance (*P-E*) curves (exemplification shown in Fig. 6). According to a photoacclimation modeled description, the results of the present study indicated changes in Photosynthetic Units (PSU) sizes as the photoacclimation mechanism, which occurred the most frequently (Table 1). There were also *P-E* curves pointing to some changes in enzymatic reactions and the altering of accessory pigments activity. What is more, changes in PSU numbers were noted as well, but these observations were episodic. In this paper the term ‘OTHER’ stated

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 our results, the process was observed in only four cases in low salinity: BA-120 25°C salinity 3, BA-124 25°C salinity 3, BA-132 10°C salinity 3, and BA-132 25°C salinity 3. 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 stated for OTHER, not for PSUsize. 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. 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., 2007; 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 and the growth intensity 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 cell abundance for two out of the three studied strains of *Synechococcus* sp. The highest cell concentrations were noted at 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 two in high PAR conditions. The decrease in growth rates appeared then, i.e. growth rates for cultures grown under the most elevated PAR were lower than growth rates calculated for cells 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 growth rates under the elevated PAR could have likely been associated with Photosystem II photo-inhibition. This was a result of a few observations, which are described 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 the above value. The results showed that in all synthetically created 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).

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 acclimated 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}.

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 picocyanobacteria (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 picocyanobacteria, such as the BA-124 strain, usually dominate in surface euphotic waters (Stal et al., 2003; Haverkamp et al., 2008; 2009). On the other hand, the strains rich in PE (BA-120 and BA-132), usually occurred deeper (Fahnenstiel et al., 1991; Hauschild et al., 1991; Vörös et al., 1991). Nonetheless, generally PCY, thanks to their high concentration of photosynthetic pigments, may occur in waters under low light intensity (Stal et al., 2003). 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 for BA-120 cell-specific pigments content, there were very high concentrations of Chl *a* observed in the whole T domain under low PAR. This could have implied the photoacclimation type, which was the change in PSU number. This mechanism was also observed in *P-E* curves for scenario with salinity 8 and temperature 20°C.

PAR and T were also the main factors influencing the changes in Chl *a* fluorescence and photosynthesis rates in three strains of *Synechococcus* sp. and may be of a great importance in the context of PCY domination in many aquatic ecosystems during the summer period. Due to Chl a fluorescence parameters results, it should be noted that PAR 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.

Due to our results, on the basis of *P-E* curves, 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 results predominantly derive the two first aforementioned types of recognition (Stal et al., 2003; Jodłowska and Śliwińska, 2014). The present study showed that changes in PSU size occur most frequently (Table 1). The second, ranked by frequency of occurrence, was the altering of accessory pigment activity. PSU number changes in *Synechoccocus* sp. rarely occurred, which is consistent with literature (Jodłowska and Śliwińska, 2014). Moreover, in our study, photoacclimation mechanisms occurred less frequently in the scenarios with salinity 3. Additionally, according to our results, *Synechococcus* strains presented different ecophysiological characteristics, however, they all demonstrated their tolerance to elevated PAR (for BA-120 to a certain degree) and T levels and could have effectively acclimated to varied water conditions. These strains were able to change the composition of photosynthetic pigments in order to use light quanta better. The ability of *Synechococcus* to sustain their growth rate in low light conditions and their low photoinhibition in exposure to high light intensities could give picocyanobacteria an advantage in optically changeable waters (Jasser, 2006).

The presented analysis may derive accurate assumptions regarding the regional distribution of *Synechococcus* sp. 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 brown strain will be higher near the Baltic Sea entrance (Danish Straits) than in Bothnian Bay. Additionally, it was observed that despite high 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 one

least recognized in marine science. Stal et al. (2003) and Haverkamp et al. (2008) pointed to its inhabitation in the Baltic Sea but did not give its characteristics in detail. In more recent research new information has appeared, which has provided a more detailed examination of BA-132 (Jodłowska and Śliwińska, 2014). Nonetheless, this strain still requires careful studies. The present paper derived an analysis of the brown strain ecophysiology itself and its comparison to other strains of the same species.

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 observed feature that T had a positive impact on all strains' growth is also consistent with field studies, which indicate the seasonal cycle of PCY blooms. 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 in 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. 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, picocyanobacteria 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 above winter-spring field research.

The studies of autecology of the PCY community and an understanding of its response to main environmental factors could be 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 will enable long-term numerical studies on the response of PCY to changes in environmental conditions.

5 Conclusions

This study showed both differences and similarities in three strains of Baltic *Synechococcus* sp. ecophysiology. Discrepancies in growth rates, 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 similar for all analyzed strains, offering a reason to associate these features with *Synechococcus* sp. 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. What is more, laboratory results and statistics indicated that for all strains (not just PAR and T), other factors influenced photosynthesis and Chl *a* fluorescence parameters. Additionally, the positive impact of salinity on the growth was observed in each culture. Another similarity was the prevalence of the one mechanism of photoacclimation, which was the change in size of PSU. This

type was followed by the altering of accessory pigments and by a change in PSU number, ranked by frequency of occurrence.

Contrary to that, the main differences were: various patterns of growth rates under different PAR and T conditions in different cultures; various photoacclimation mechanisms observed; inclinations for salinity levels; and changes in pigmentation and different preferences to PAR intensities (BA-124 and BA-132 hold higher PAR than BA-120, for which a photoinhibition point above 190 µmol photons m^{-2} s^{-1} was observed). According to the latest research, PCY are a great contribution to total primary production in the Baltic Sea and may contribute to summer cyanobacteria bloom to a high degree. This explains the authors' motivation to lead an in-depth investigation on Baltic PCY response to a changeable environment. The present research is a first step on the way to gaining new knowledge on *Synechococcus* sp. ecophysiology and is a foundation for further studies.

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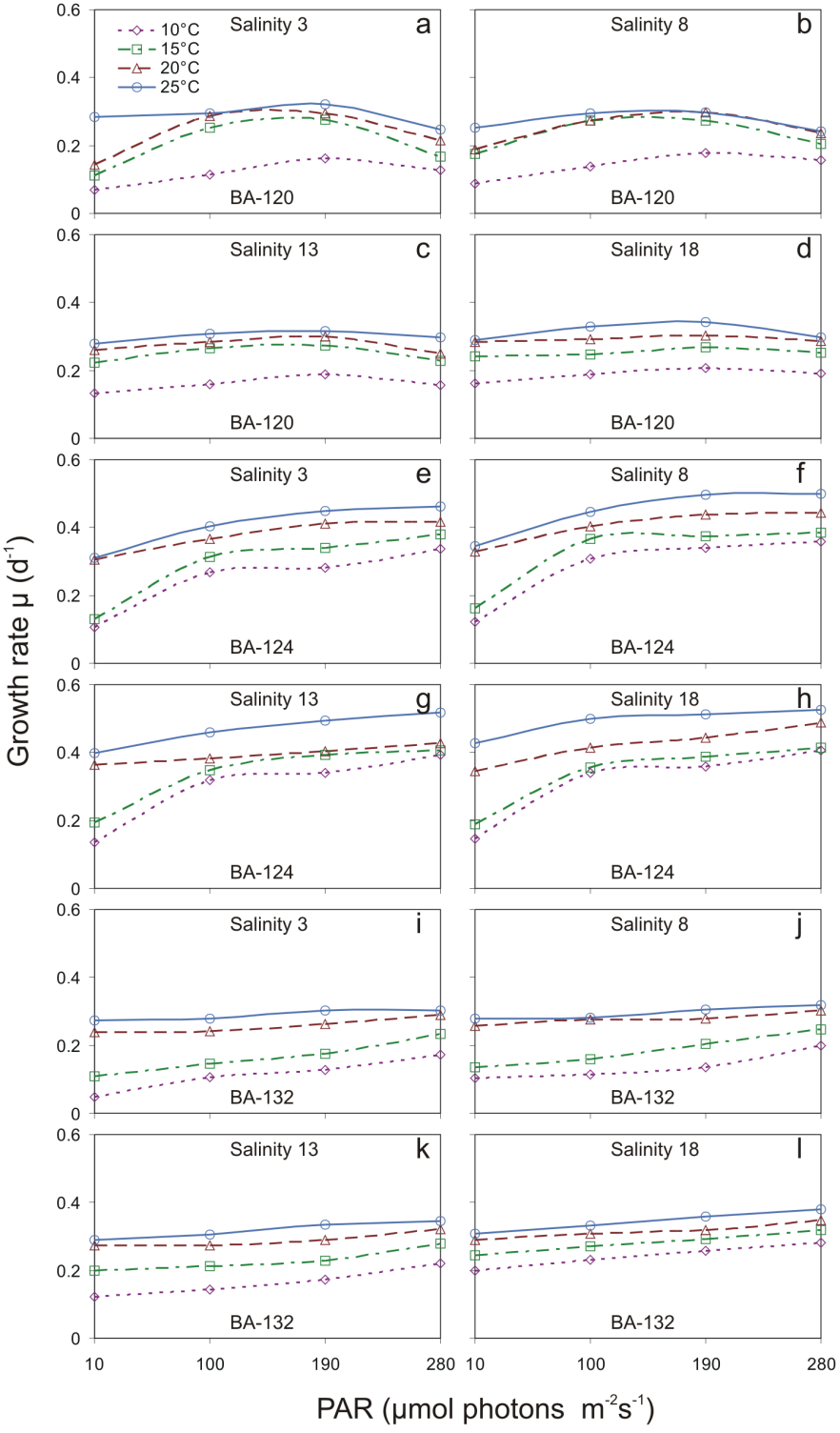

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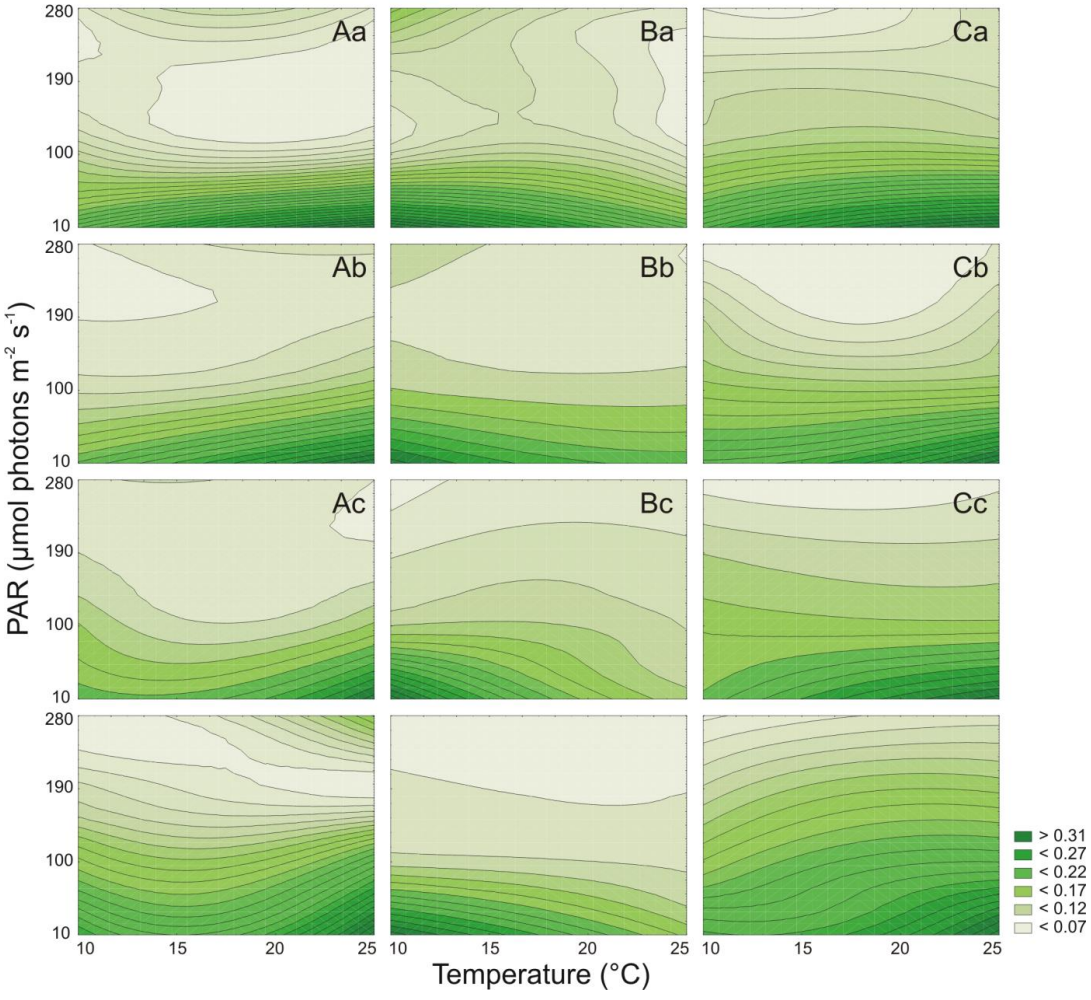
672 **Fig. 1.** Growth rates of strains BA-120 (a-d), BA-124 (e-h), BA-132 (i-l) under different environmental scenarios in 4 mediums: a) b) e) f)
673 i) j) low salinity – 3 and 8; c) g) k) moderate salinity – 13; d) h) l) high salinity – 18.



674
675 **Fig. 2.** Cell-specific Chl *a* (pg cell^{-1}) changes for BA-120 (A), BA-124 (B) and BA-132 (C) under different environmental scenarios in 4
676 mediums: a) salinity – 3; b) salinity – 8; c) salinity – 13; d) salinity – 18.
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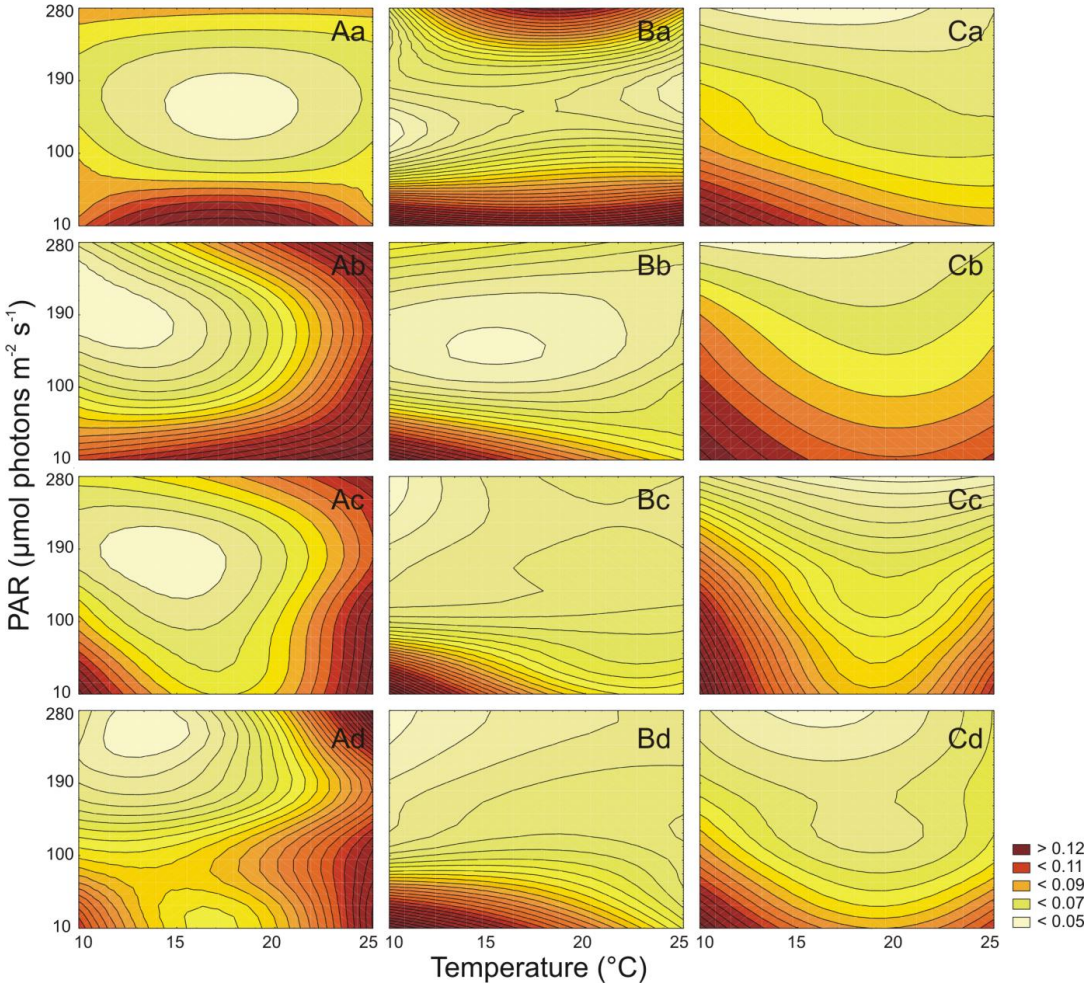

Fig. 3. Cell-specific Car (pg cell^{-1}) changes for BA-120 (A), BA-124 (B) and BA-132 (C) under different environmental scenarios in 4 mediums: a) salinity – 3; b) salinity – 8; c) salinity – 13; d) salinity – 18.

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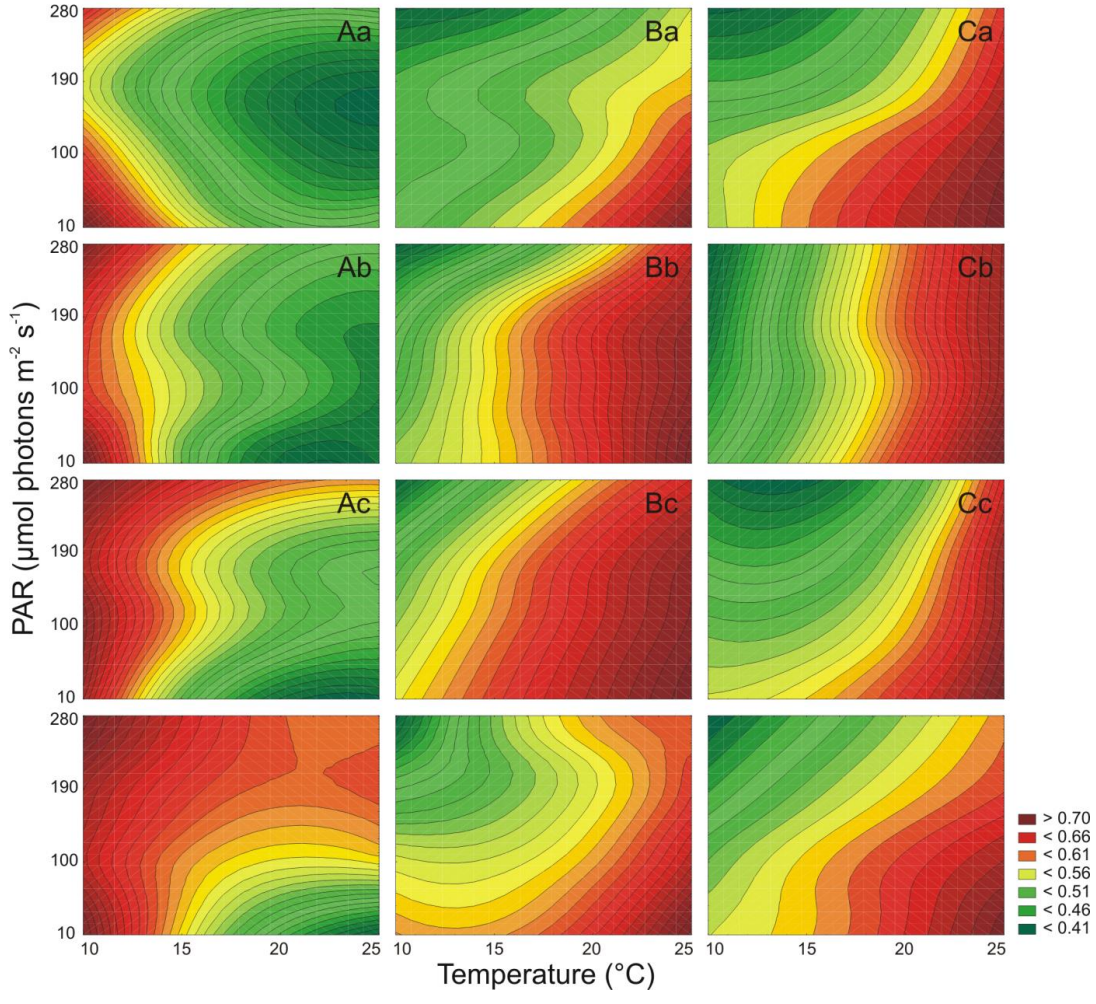

683
684 **Fig. 4.** The maximum photochemical efficiency of PSII in the dark-adapted state (F_v/F_m) of BA-120 (A), BA-124 (B) and BA-132 (C)
685 under different PAR and temperature conditions in 4 types of medium: a) salinity – 3; b) salinity – 8; c) salinity – 13; d) salinity – 18.

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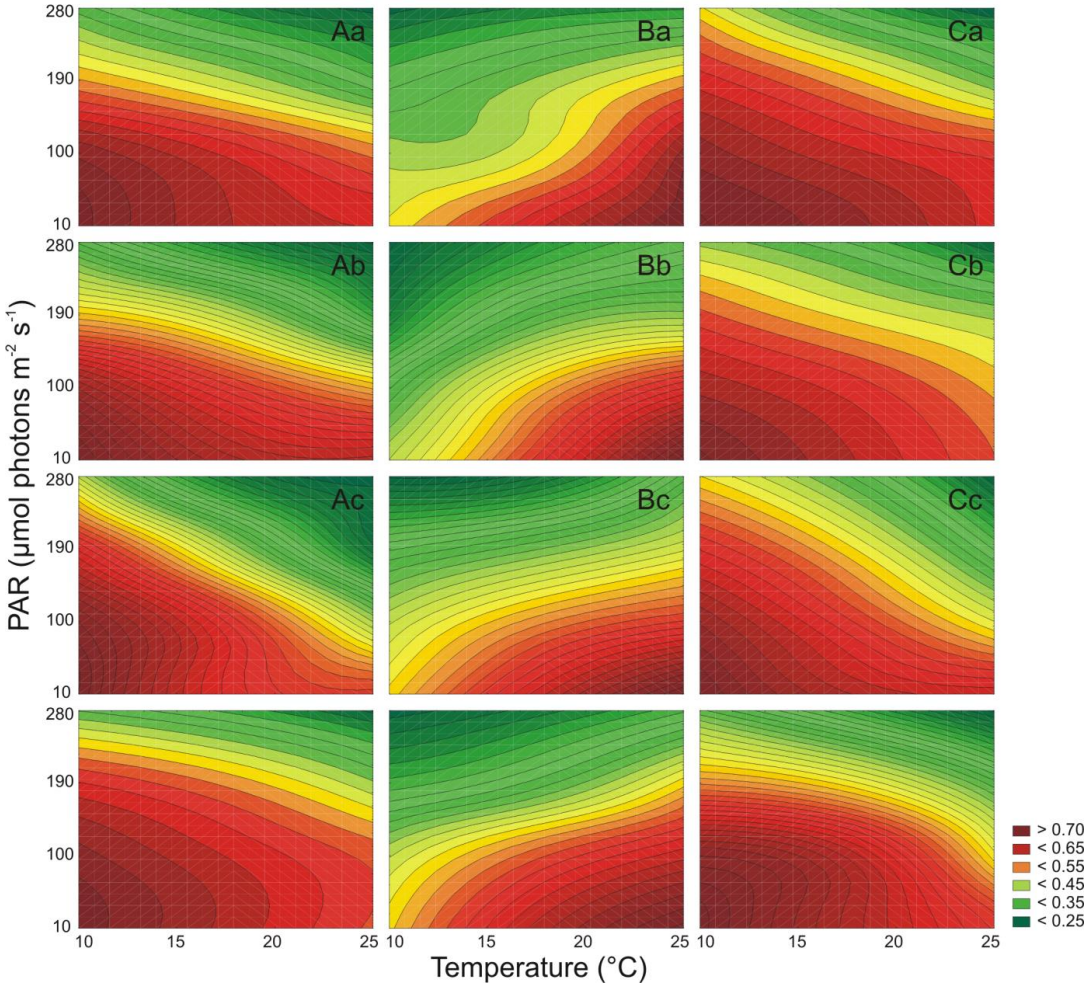

Fig. 5. The photochemical efficiency of PSII under actinic light intensity (ΦPSII) of BA-120 (A), BA-124 (B) and BA-132 (C) under different PAR and temperature conditions in 4 types of medium: a) salinity – 3; b) salinity – 8; c) salinity – 13; d) salinity – 18.

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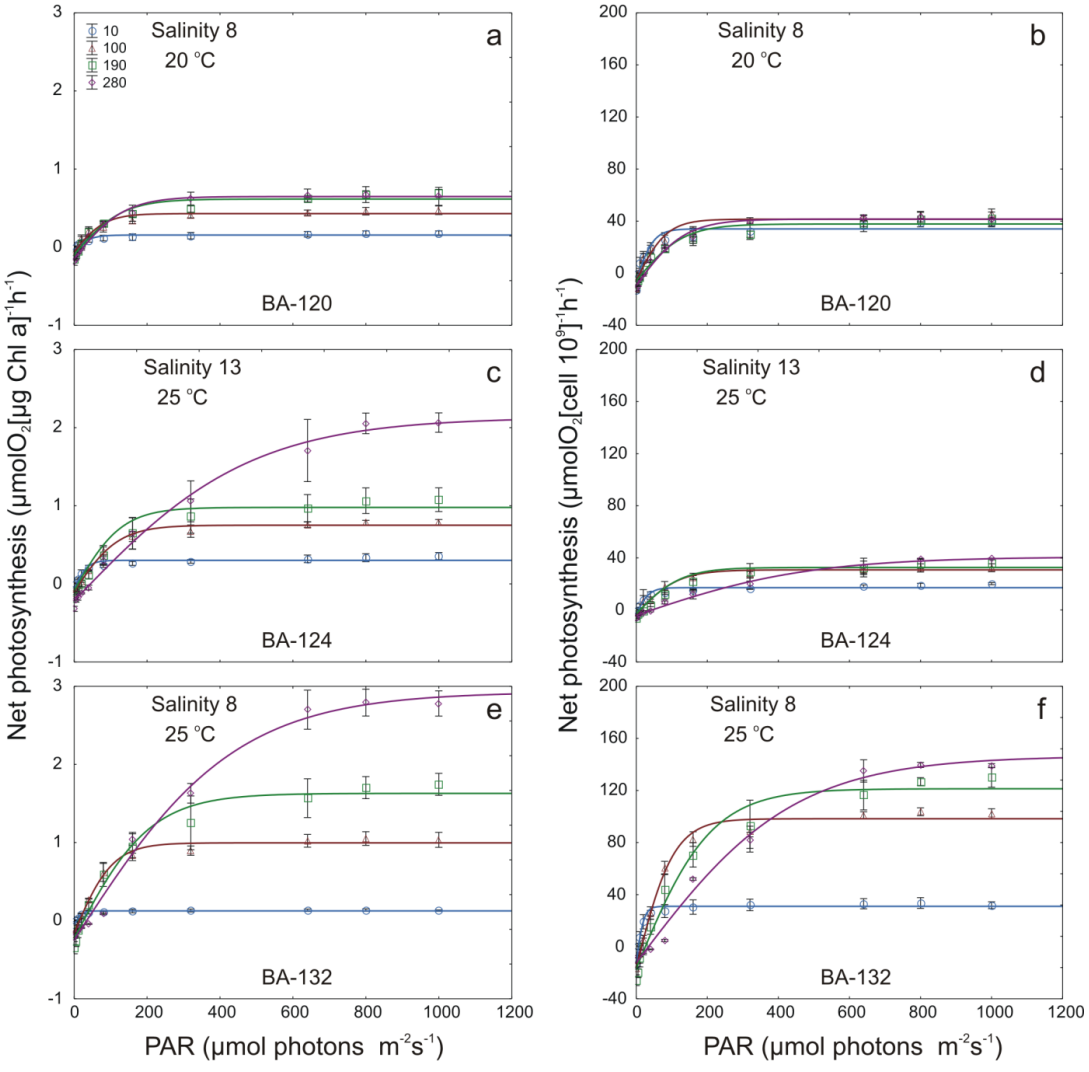

Fig. 6. Selected Chl *a* - specific and cell-specific (right side and left side panel, respectively) net photosynthetic–light response curves for BA-120 (a, b), BA-124 (c, d) and BA-132 (e, f) strains. Curves present examples of three types of photoacclimation observed for *Synechococcus* sp. and these are as follows: change in number of photosynthesis units (PSU) (a, b), change in size of PSU (c, d) and change in accessory pigments activity (e, f).

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710 **Table 1.** Photoacclimation types (mechanisms) for each strain at different ecological conditions. OTHER states for altering of accessory
711 pigments activity or changes in enzymatic reactions; PSUsize states for the change in PSU sizes; PSUno. states for the change in PSU
712 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
713 and ^{brown} for BA-132.

714

CONDITIONS	Salinity 3	Salinity 8	Salinity 13	Salinity 18
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}

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