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# *Interactive comment on* "Ecophysiological characteristics of red, green and brown strains of the Baltic picocyanobacterium *Synechococcus* sp. – a laboratory study" *by* Sylwia Śliwińska-Wilczewska et al.

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Interactive comment on "Ecophysiological characteristics of red, green and brown strains of the Baltic picocyanobacterium Synechococcus sp. – a laboratory study" by Sylwia Sliwinska-Wilczewska et al.

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REPLY: The authors would like to thank professor Cristiana Callieri for her comments and suggestions, and to inform that appropriate modifications have been made in the



revised MS. What is more, the authors inform that the revised MS is ready.

In the revised version, we improved the Material and methods section and introduced more details there. We revised the whole MS and added a photograph of the cultures together with the absorption spectra and the scatter plot of the orange vs. red fluorescence. This is a new Figure in supplementary material (Fig. S1, which we enclose as a part of the Supplement in the interactive response). We hope the revised version will be satisfactory.

1. Please read with attention the text and the legends and be more precise in the description of the experiments and of the results. REPLY: We read the whole MS carefully and afterwards introduced some modifications (in blue in the revised MS). The results are described more precisely now.

2. It would be interesting to show in the supplementary material the photograph of the cultures together with the absorption spectra and the fluorescence spectra to be sure of the PUB presence in the brown cultures. REPLY: The new figure (Figure S1) was added in Supplementary material.

Figure S1: Left-side top panel (A, B, C) – light microscope photographs of three Synechococcus sp. strains (scale bar = 10 $\mu$m) 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$^{TM}$ C6 Plus flow cytometer and bottom panel (D) – PAR absorption spectra obtained for the mixture of phycobilin pigments for each Synechococcus sp. strain

3. If I well understood you kept the cultures at the different condition combinations for 2 days for acclimation then you used these cultures as inoculum with an initial number of 106 cells ml-1 and the experiment lasted 1 week and at the end you made the measurement. In this way you calculate the growth not from a curve with different points but with a line from T0 to T7. I wonder why you did not sampled every day to have a better pattern of what happened in the cultures? REPLY: That is right. We

calculated the growth rate basing on the abundance difference between the seventh and first day of the experiment (line from T0 to T7). The rationale for that was our intention to focus on the population yield, as the first idea. However, in the context of the present MS we agree that the modification of our approach is needed to be done. Concerning above, we modified this aspect and in the revised MS not a growth rate but the change in number of picocyanobacteria cells within a course of a week is described. We thank the Reviewer for this comment. We ensure that in further work every time we want to analyze the growth rate itself we will use a curve with everyday measurements.

4. Why did you use so low flux (14 $\mu$L min–1) with Accuri C6? REPLY: Selection of this flow rate was based on previous introductory experiments on determining the most relevant effectiveness. We used the procedure proposed and described by ÅŻliwińska-Wilczewska et al. (2018b).

In the revised MS, we added the sentence (L137-138): Selection of the flow rate was based on previous introductory experiments to determine the most relevant effectiveness.

5. I would appreciate to know the tresholds you used to count Pcy and the fluorescences you finally selected. REPLY: In the revised MS, we added the sentences (L138-142): 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, 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.

6. In general, the description of the methods should be improved and more detailed. I do not understand line 122-125 were you declare not to consider the cell number but the cell growth: please explain better this concept. REPLY: In the revised MS,



we improved and introduced more details in Material and methods section (L116-117, L136-142, L147-148, L152-154, 157-159, L171-172).

Here, we cited the chosen parts of the revised manuscript Material and methods section. These parts include the modifications, which we introduced.

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, the appropriate amount of Tropic Marine Synthetic Sea Salt was dissolved in distilled water. The final salinity was 3, 8, 13 and 18 PSU, measured with salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany). Salinity of the media was measured in PSU (practical salinity units). 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). Culture media was prepared with artificial seawater filtered through a 0.45-$\mu$m filters (Macherey-Nagel MN GF-5) using a vacuum pump (600 mbar) and 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 $\mu$mol photons m–2 s–1), temperature (T) (10, 15, 20 and 25°C), and salinity (3, 8, 13 and 18 PSU). 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 (Feistel et al., 2008; 2009; Siegel and Gerth, 2017). Regarding PAR, its levels has been generated the highest possible to be achieved in the laboratory. These values are generally lower than mean PAR intensities being observed in the summertime in the Baltic (Leppäranta and Myrberg, 2009). 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. 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 106 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 $\pm$ 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) according to the procedure proposed by ÅŽliwińska-Wilczewska et al. (2018b). Events were recorded in list form. Samples were run at a flow rate of approximately 14 $\mu$L 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 dis-



criminator (Fig. S1) and to select a high threshold, 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 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). The flow cytometry was used to establish the initial number of picocyanobacteria cells and to measure the final cells concentration after the incubation period.

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. 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. To remove cell debris and filter out the particles, the extracts were centrifuged at 10,000 rpm (8496 $\times$ 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 ($\mu$g mL–1) = 11.236(A665-A750)Va/Vb, Car ($\mu$g mL–1) = 4(A480-A750)Va/Vb, where: Va - extract volume (in this

study 5 mL), Vb - sample volume (in this study 4 mL), and Ax - 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 $\mu$mol photons m–2 s–1 in 50 steps) and a saturating pulse (0 – 20000 $\mu$mol 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 Fo' parameter. Samples were filtered through 13-mm glass fiber filters (Whatman GF/C, pore size = 1.2 $\mu$m). 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 (Fv/Fm) and the photochemical efficiency of PSII under actinic light intensity ($\Phi$PSII) 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).

We also removed the sentences from L122-125: 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.

7. A revision for the language is necessary REPLY: The MS was checked and corrected by the professional Proof Reading Service company before submitting, however, the text has been revised again, considering Reviewer's suggestion.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2018-19/bg-2018-19-AC3-supplement.pdf

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-19, 2018.