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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 ÅŽliwińska-Wilczewska et al.

Anonymous Referee #2 Received and published: 6 March 2018

1. This article is about the physiological characterisation of three Synechococcus strains the Baltic sea. Overall the experiments seem to be well conducted, although

it fails to explain the relevance of such study. The general conclusions should be restricted to the results of the study only. The text can be generally understood, however there are some confusing sentences and paragraphs that perhaps could be improved by proofreading.

REPLY: The authors would like to thank Reviewer 2 for the comments and suggestions, and to inform that appropriate corrections have been made in the revised MS. The authors inform that the revised MS is ready. In the new version, a series of Reviewer's comments were addressed and the text was revised again. Due to that, we hope the present MS is satisfactory.

2. The three strains characterised in this paper presented different pigmentation -it would be useful to know whether those strains are clade representatives (is that information available?), how phylogenetically similar they are or any other reason why they were chosen for the study (are these bloom-forming strains?). REPLY: We modified the text accordingly by adding information about Synechococcus sp. clades. We also explained in more detail why we chose these strains in our study. All the aspects are addressed in the revised MS (L42-58, L79-83).

Here we enclose the part of the revised MS Introduction section, which was extended in order to introduce above mentioned information.

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 pigment composition (Stomp et al., 2007; Haverkamp et al., 2008). Baltic strains of Synechococcus sp. are classified as red strains with phycoerythrin (PE), green strains rich in phycocyanin (PC) and the brown strains containing two different bilin pigments known as phycoerythrobilin (PEB)

and phycourobilin (PUB), which both bind to the apoprotein PE (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. The existence of these different colors picocyanobacteria is commonly found in the Baltic Sea (Andersson et al., 1996; Hajdu et al., 2007; Stomp et al., 2007; Haverkamp et al., 2009; Mazur-Marzec et al., 2013; Larsson et al., 2014; Paczkowska et al., 2017). Picocyanobacterial species are phylogenetically divided into several major clusters. These clusters have been identified, based on photosynthetic pigmentation, nitrogen requirements, motility and salinity preferences (Herdman et al., 2001). Picocyanobacteria that are often found and isolated from marine, brackish and freshwater environments are related to Synechococcus cluster 5 (Herdman et al., 2001). Synechococcus cluster 5 is divided 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 picocyanobacteria has been investigated mainly by analysis of the 16S rRNA gene. However, the phylogenetic tree of Synechococcus sp. is not always consistent with their 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 SzelÄĚg-Wasilewska, 2015; Jasser and Callieri, 2017; ÅŽliwińska-Wilczewska et al., 2017; Barreiro Felpeto et al., 2018). Many different factors, including physical parameters, availability and competition for resources, selective grazing and allelopathic interactions can affect the occurrence of harmful blooms in aquatic ecosystems.

The development of massive algal blooming is a consequence of the interaction between many favorable factors. Synechococcus sp. greatly contributes to these massive blooms, but so far the characteristics of the life cycle of Baltic PCY has not been sufficiently studied. This knowledge needs to be expanded and improved, especially because of bloom toxicity and negative impacts on ecosystems (Jasser and Callieri, 2017; ÅŽliwińska-Wilczewska et al., 2018a). According to the above all, 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 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 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 ÅŽliwińska, 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 ÅŽliwińska, 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.

3. The authors should be consistent when referring to parameters and strains, for example strains are sometimes mentioned by their name and other by their pigment. REPLY: We corrected this aspect. From the Results section onwards, the strains are referred by their names and parameters by their symbols.

4. a) is salinity measured in PSU (practical salinity units)? REPLY: Yes, we measured salinity in PSU (L109). We added this unit in whole MS. L109: Salinity of the media was measured in PSU (practical salinity units).

b) how is that range (3 to 18) compared to Baltic sea water salinity? 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, around, salinity 30), while the lowest mean salinity is observed in the Baltic northernmost regions (around 3 in Bothnian Basin). The concise information about that was introduced to the MS (L117-119) and more detail information was added in Discussion (L625-632).

L117-119: 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).

L625-632: 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.

5. are the temperature and PAR ranges representative of the Baltic sea environment? REPLY:

The temperature conditions applied in the laboratory are representative for the Baltic Sea area (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 (Leppärranta 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. We added the necessary information in L117-123. Additionally, please note that the annotation regarding the laboratory and natural Baltic ecological conditions was also introduced to the Discussion (L616-632).

L616-632: 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 (Sjöqvist et al., 2015). 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 20°C may be timed in the Baltic between June and September with some inter-annual changes (Siegel and Gerth, 2017). SSTs reaching and exceeding 20 °C are also observed in the Baltic basin. For instance, according to Siegel and Gerth (2017), SSTs higher than 20 °C 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 – 25°C) can be defined as representative for the Baltic Sea.

6. relevant bibliography is absent from the introduction (e.g. Flombaum et al. 2013, PNAS, and Six et al. 2007, Genome biol.) REPLY: These studies are cited in the current version, where appropriate.

7. line 43: please include a reference that puts Synechococcus as a major bloom contributor REPLY: We added the necessary information in L60 by including the reference to Beardall, 2008.

8. a) line 56: Sorokin and Zakuskina (2010) studied the bloom in Comacchio lagoon, it is an overclaim to say it is a phenomenon in Europe REPLY: We agree that this fragment did not give detail information and did not justified our motivation to conduct the present research. We modified the text accordingly, by removing this statement.

b) paragraph from line 59 is repetitive and does not give much information, please consider re-phrasing Thank you for this comment and drawing our attention to the occurrence of the repetition. We re-phrased the paragraph and deleted the repetition in the text (L79-83 in the revised MS).

L78-83: 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 few research papers on the brown strain of Baltic Synechococcus sp. (Stal et al., 2003; Haverkamp et al., 2008; 2009; Jodłowska and ÅŽliwińska, 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.

9. The methods section should be more specific. For example, how was the media prepared in order to change the salinity? where any of the components in f/2 media replaced by Tropic marine synthetic sea salt or was it added on top of it? What pore-size filters were used? REPLY: We corrected this aspect and added more specific information in Methods section (L107-112, 153-154, 157-159, 171-172).

Here, we cited the chosen parts of the revised manuscript Material and methods section. These parts include the modifications, which were introduced. The modifications are marked in colors: regarding the comments of Reviewer 1 – in blue; regarding the comments of Reviewer 2 – in green.

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-μm 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 μmol 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 (Feistelet 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 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, 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 (μg mL–1) = 11.236(A665-A750)Va/Vb, Car (μg 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 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 Fo' parameter. Samples were filtered through 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 (Fv/Fm) 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).

10. please state xg rather than rpm (or else specify rotor/centrifuge used) REPLY: The rotor unit has been changed in revised MS (L157-159).

L157–159: 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).

11. growth rate has to be measured during exponential growth. The parameters here calculated only report yield and not growth rate. REPLY: That is right. We calculated the growth rate basing on the abundance difference between the seventh and first days 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 are grateful the Reviewer for this comment.

12. line 131: please put reference or protocol for Chl a and Car extraction REPLY: The reference has been added in the revised MS (L153). L152-154: 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.

13. please change "absorption" for "absorbance" REPLY: We introduced this change (L159, L163).

L159-163: 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(A665-A750)Va/Vb, Car (μg 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.

14. line 147: it is not clear whether the filter or filtrate was used REPLY: The information has been specified in the revised MS (L171-172).

L171-172: Samples were filtered through 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.

15. The results section describes individual strains, but the figures are difficult to interpret. Please consider reviewing labels and legends. REPLY: The modifications were introduced in the revised MS.

16. number of cells and growth should not be used interchangeable REPLY: Thank you for drawing our attention to this. We corrected this aspect in the revised MS.

17. it is not clear what a "positive" or "negative" impact means REPLY: These sentences were rewritten to be more precise. Moreover, the appropriate brief explanation was introduced to the revised MS (L247-249). The explanation is as follows:

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.

18. The "pigment content" section is not clear, please specify in the methods section REPLY: The "pigment content" section was re-phrased. More specific information was also introduced to the Method section (L152-154).

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 (μg mL–1) = 11.236(A665-A750)Va/Vb, Car (μg 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.

19.Table 1 is very difficult to interpret. How were those parameters measured? REPLY: We corrected this aspect and added more specific information in the Result (L401-524 and L527-531) and Discussion (L596-602 and L609-610) sections. The description

of photosynthesis parameters measurement is provided in section 2.5 of the MS, i.e.: Measurements of photosynthesis rate.

Here we enclose only a part of a description in the revised Results section, where the modifications were introduced:

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).

and in the revised Discussion section:

The results showed that T, PAR and salinity influenced the photosynthesis parameters only to a certain degree. There were many not statistically significant multiple comparisons pointed by post hoc tests. However, it was found that generally, in cell-specific estimations, elevated PAR had a negative effect on α and PAR increase influenced the respiration negatively. For each of the studied strains of Synechococcus sp., the highest α and the lowest Rd 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 Pm 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. According 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 ÅŽliwińska, 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 ÅŽliwińska, 2014). Moreover, in our study, photoacclimation mechanisms occurred less frequently in the scenarios with salinity 3 PSU. The changes of photosynthesis parameters (Pm, α, Rd) under different environmental conditions explains the occurrence of different photoacclimation mechanisms. According to our results, Synechococcus strains present different ecophysiological characteristics, however, they all demonstrate their tolerance to elevated PAR (for BA-120 to a certain degree) and T levels and could have effectively acclimated to varied water conditions. These strains were able to change the composition of photosynthetic pigments in order to use light quanta better. The ability of Synechococcus to sustain their growth in low light conditions and their low photo-inhibition in exposure to high light intensities could give picocyanobacteria an advantage in optically changeable waters (Jasser, 2006).

Thereby, we ensure that the Results section has been extended by introducing the detail description of photosynthesis parameters.

20. line 401: what does it mean growth intensity? REPLY: These sentences were re-phrased.

21. line 458: how could these variables be related to the natural conditions in different regions of the Baltic sea? REPLY: We understand that the Reviewer 2 is asking about how the environmental variables applied in laboratory are relatd to the natural conditions in different regions of the Baltic Sea. We re-phrased the paragraph loacated originally between L458 and L466 (see L616-632).

L616-639: Due to occurrence of extremes in salinity and other environmental con-

ditions in the Baltic Sea area, the Baltic inhabitants are highly adapted to different regions and often reach their physiological limits (Sjöqvist et al., 2015). 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 20°C may be timed in the Baltic between June and September with some inter-annual changes (Siegel and Gerth, 2017). SSTs reaching and exceeding 20 °C are also observed in the Baltic basin. For instance, according to Siegel and Gerth (2017), SSTs higher than 20 °C 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 – 25°C) 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 ÅŽliwińska, 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).

22. lines 471-473: unclear, please rephrase or delete REPLY: We re-phrased the fragment in the revised MS (L645-647).

L645-647: 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.

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