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.

C. Callieri (Referee)
c.callieri@ise.cnr.it
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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.

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). We hope the revised version will be satisfactory. All the modifications in the MS are marked in blue color.

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). 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.

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 10^6 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 µL 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 Śliwińska-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).

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.

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.

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. All the modifications in the MS are marked in green color. 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).

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.

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.

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 (L625-630).

5. are the temperature and PAR ranges representative of the Baltic sea environment?
REPLY:
We added the necessary information in L117-123.

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.
Additionally, please note that the annotation regarding the laboratory and natural Baltic ecological conditions was also introduced to the Discussion (L616-632).

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 (L34; L45).

7. line 43: please include a reference that puts *Synechococcus* as a major bloom contributor
REPLY:
We added the necessary information in L60.

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).

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).

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).

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).

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

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).

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:
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).

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.

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 slightly (see L616-632).

22. lines 471-473: unclear, please rephrase or delete

REPLY:

We re-phrased the fragment in the revised MS (L645-647).