

The authors would like to thank Dr Douglas Campbell for his comments and suggestions, and to inform that appropriate modifications have been made in the revised MS. We hope that the present version is satisfactory. [All the modifications in the manuscript are marked in blue color.](#)

### **1. COMMENT:**

Figure 1: Information rich figure.

The 3 strains show different salinity/temperature/light niches

BA120 peaks at ~190 uE, 25C, with niche volume widest at 3-8 PSU

BA124 peaks at ~250 uE, 25C, limited effect of salinity

BA132 peaks at ~280, 25C, widest niche at 3 PSU

Given the distributions one might have wished that the temperature scale went higher to better delimit upper temperature limits; a suggestion for future studies.

### **1. REPLY:**

The Authors are grateful for the comment. The motivation underlying the arrangement for scenarios conditions ranges was the will to reflect the general Baltic conditions, to make the study comparable with other known studies on picocyanobacteria autecology, and to make the best use of the laboratory equipment accessible for the Authors at that moment. The Authors do not want to discard the picocyanobacteria studies and they plan to extend the research in the future.

### **2. COMMENT**

Figure 2: information rich figure.

Chl peaks at low light. different temperature effects across strains.

### **2. REPLY:**

The Authors added this short information into the text (L: 534-546).

*In this study, the pigment content was generally the highest under the low PAR treatment for all strains. This was a striking observation, however, with some exceptions. For instance, concerning Cars, BA-120 cell-specific Car reached high concentrations in the whole light range for high T. This was pronounced in mediums of moderate and high salinity (8, 13, 18). Moreover, in medium 3, BA-124 demonstrated high cell-specific Car concentration under the highest analyzed light level. The cell-specific Car peaking in the lowest light was pronounced the most in BA-132 cultures. This is consistent with literature (Jodłowska and Latała, 2010). Regarding cell-specific Chl a peaks, they were noticeable in the low PAR range for all strains with no exceptions. The difference between the strains were various effects of temperature. For BA-120 and BA-132 the highest cell-specific Chl a concentrations were estimated in the highest T, while for BA-124 oppositely, i.e. for the lowest T. This has not been observed before (according to Authors' best knowledge, not reported in the literature yet). Moreover, the Car/Chl a ratio increase along the PAR increase was observed. This, together with low pigment contents in under high PAR, is a very interesting observation, which makes the motivation for further studies on Synechococcus sp. stronger. The Authors plan to extend*

*their research on picocyanobacteria in the future (the pigment content composition analysis, proportion of Chl a and carotenoids – Zeaxanthin,  $\beta$ -carotene – and Phycobilins).*

### **3. COMMENT**

Figure 3. Interesting.

One wonders why Car cell-1 peaks at low light for most strains.

### **3. REPLY:**

The observations are described in Resets section (3.2. Pigment content) provided with details. The pigmentation was generally the highest for all strains in the low light range. Please, refer to Reply 2. and a new fragment added in the text (L: 534-546).

### **4. COMMENT**

Fig. 6 nice to include both chl-1 and cell-1 specific O<sub>2</sub> rates; clearly different.

### **4. REPLY:**

The Authors would like to thank for this comment. They were very interested in what they would obtain for the *P-E* curve trajectory (demonstrating O<sub>2</sub> rates) in Chl-specific and cell-specific domains. The results enabled to conclude on photoadaptation mechanisms. In order to analyze the *P-E* curves, the Authors needed to define Chl *a*- and cell-specific photosynthesis parameters.

### **5. COMMENT**

Abstract

Line 18: 'realistic', not 'real'

### **5. REPLY:**

We corrected this aspect (L: 18).

### **6. COMMENT**

introduction;

Line 51: 'morphotypes'?? perhaps 'pigment types'? 'morpho' implies morphology

Same comment on line 63; is 'pigment' part of 'morphology'; maybe 'phenotype'

### **6. REPLY:**

We corrected these aspects (L: 51 and L: 62).

### **7. COMMENT**

Materials & Methods:

"The PCY cultures were adapted to the various synthetic environmental conditions for two days. "

How many rounds of cell division occurred during the 2 days of acclimation?

What was the growth state of the cultures before the inoculation into the treatment condition? Some combinations might have still been in lag phase after 2 days, depending upon the state of the preculture and the severity of the stress.

#### **7. REPLY:**

The Authors would like to thank for this comment. They did not realize, this fragment of Materials and Methods section could have introduced a confusion or misled the Reader. They introduced slight modifications in the text (L: 136-138).

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<sup>-1</sup>. The acclimation cultures used for inoculation were isolated from the logarithmic growth phase. Due to that, being of some combinations still in lag phase after 2 days can be excluded. During the acclimation time, cell division rate for the strains was about 1 day<sup>-1</sup>, averagely. It was enough to enable the cells to acclimate to environmental conditions without the risk of stress severity.

#### **8. COMMENT**

"The salinity was 120 controlled by salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany). "

Controlled by? Or verified by? Controlled by implies a chemostat type titrator. I think the authors mean they used a salinometer to measure the salinity, not ongoing adjustment of salinity?

#### **8. REPLY:**

The Authors thank Dr. Campbell for drawing their attention to that. The salinity was verified by salinometer, of course. This aspect was clarified in the text (L: 123).

#### **9. COMMENT**

What was the photoperiod, and when in the subjective photoperiod were measures taken?

#### **9. REPLY:**

We added the sentences (L: 121-122):

*The strains were incubated under a 16:8 h light:dark cycle. The measurements of all strains were taken when the experiment incubations completed (after full 7 days) at the same time during the light:dark cycle (in the light phase).*

#### **10. COMMENT**

"In order to achieve the most reliable results, test cultures were grown in three replicas and were incubated for one week at 135 each combination of light, temperature and salinity. "

OK, good.

How does this relate to the earlier statement about 2 days?

## **10. REPLY:**

'Test cultures' are right cultures to carry the experiment on them. 2 days referred to acclimation cultures (not test ones). The test cultures grown in three replicas under specific conditions for 7 days and then the measurements were taken.

## **11. COMMENT**

Figure 1, and first section of results:  
Were all replicates initially inoculated at equal cell densities?

## **11. REPLY:**

Yes, the initial cells number, i.e. the culture density just after inoculation, was the same for all replicas.

The Authors added this short information into the text (L: 141).

## **12. COMMENT**

Line 142

The flow cytometry was used to establish the initial number of picocyanobacteria cells and to measure the final cells concentration after the incubation period.

It would be more generally useful to express:

$\mu = (\ln(\text{Cells}_{\text{final}}) - \ln(\text{Cells}_{\text{initial}})) / \text{elapsed time}$

This assumes steady exponential growth over the entire time window.

Then,  $\ln 2 / \mu = \text{apparent generation time}$ .

Then elapsed time/generation time = number of generations achieved under each treatment.  
Or, the ratio of  $\text{Cells}_{\text{final}} / \text{Cells}_{\text{initial}}$ , as a direct measure of the fold change in biomass.

Any of these metrics would give better comparability across studies.

Instead doing statistics on the achieved final number of cells, without clear reference to the starting number of cells, is methodologically odd and makes comparisons with other studies difficult.

## **12. REPLY:**

In the first version of the manuscript the analysis were done on the basis of the growth rate. However, that time, the Reviewer recommended the Authors to change the attitude and to do the analysis on the abundances. This is what the Authors followed. What is more, please note that the study is not without a clear reference to the starting number of cells. The Authors pointed to the starting abundances in the text, precisely.

Nevertheless, thought through the suggestions of Dr. Campbell deeply, the Authors decided to add a fragment in the Material and Method section (L: 143-145), which says:

*Additionally, to broaden the understanding and comparison possibilities, the number of generations (number of generations = elapsed time (t) /doubling time (d)) were demonstrated in supplementary materials (Fig. S1).*

### **13. COMMENT**

The masses of statistical comparisons make the results almost unreadable.

I wonder if the authors should lift out the masses of statistical comparisons into tabular or supplement form, and use a much shorter text to describe the main quantitative and qualitative findings, with reference to a table of statistical tests.

### **13.REPLY:**

The Authors inform they modified the Results section and the tables with statistics were added to the supplementary material (Tables S1 – S11)

### **14. COMMENT**

Discussion:

"Carotenoids have a dual role in the cell: to maintain a high 599 capacity for photosynthetic light absorption and to provide protection against photooxidation "

I do not know of any evidence that carotenoids can serve in photosynthetic light absorption in cyanobacteria.

If that is true it needs to be backed by a citation.

### **14. REPLY:**

We added citations.