On behalf of all the authors of this paper, we would like to express our sincere gratitude to the two Editors, Ilana Berman-Frank and Elisa Schaum, and two Reviewers for their efforts reviewing and improving this manuscript.

**COMMENTS TO THE AUTHORS**

Associate Editor:

Deputy Editor: 1

Comments to the Authors:

Dear authors,

your manuscript has been assessed by two expert reviewers and the editorial panel. All agree that this is an interesting, well written manuscript. Both reviewers have been very thorough and as a result, there are now a fair few points to address during the revision process.

Additionally, I was wondering whether a more process-based title might work better, e.g. a title highlighting the differences between PhycoCyanin and PhycoErythrin-rich picocyanobacteria more explicitly.

Response: Thank you for these positive statements. We have addressed all comments, and propose a new title. We hope that this new version of the manuscript will be satisfactory.

Associate Editor: 2

Comments to the Authors:

The manuscript has been reviewed by two experts in the field who have both agreed that this is an important manuscript with novel information. Prior to acceptance, the reviewers have raised several issues that should be addressed that will help improve and clarify different parts of the manuscript. Please see the detailed reviews and revise accordingly, or provide explanations if no revision is made.

Response: We appreciate these positive comments We have treated all comments with due attention. We hope that this new version of the manuscript will be satisfactory.

We thank Reviewer 1 for their time and valuable comments. The changes in the manuscript addressing comments from Reviewer 1 are marked in blue.

Reviewer\_1

Comment: This manuscript reports on studies of the photobiology of strains of marine *Synechococcus* that are phycocyanin or phycoerythrin rich. The authors have compared responses of these strains to light intensity and photoperiod and the total photon dose. They measured growth rates and a range of photobiological parameters such as effective absorption cross-sectional area of PSII, as well as photosynthetically usable radiation (PUR) and the ratio of PUR to incoming photosynthetically active radiation (PAR). The latter is not often used, but is a highly useful parameter.

The experiments are well designed and are, on the whole, clearly described. The results are presented adequately and discussed thoroughly.

The authors are clear in the conclusions they draw from their data and my only criticisms are about minor points of presentation.

Response: We appreciate these positive comments.

Comment: I assume from the main data and supplementary information that the authors measured PUR under each specific growth condition to get PUR/PAR ratios. However, since absorbance spectra for only one condition of light treatment for one PE and one PC rich strain are shown in Fig 2, I think that, for clarity, this needs to be explicitly stated in the methods. Adding information about which light conditions were used for the data shown in Fig 2 to the legend would be appropriate.

Response: Thank you. We added the relevant text to the Materials & Methods (refer to track changes version: L236-238). The legend description in Fig. 2 also defines the light conditions applicable to the representative spectra presented. All 480 spectra are available on https://github.com/FundyPhytoPhys/BalticPhotoperiod, now cited in the Materials & Methods.

Comment: Line 119: what is a 'Pre-culture'? Mother cultures used to inoculate experimental tubes are still 'cultures'. Pre-experimental cultures would make more sense, or just say “Picocyanobacterial strains were maintained….”

Response: Thank you. We have made appropriate corrections (L147).

Comment: Line 149: 8000 x g?

Response: We apologize for the error. we corrected it to 8000 x g. This fragment has also been moved to the Supplement, as requested by Reviewer\_2.

Comment: Line 628: The first use of phylogeny here is superfluous

Response: Thank you. We corrected this mistake.

Comment: Line 434 and elsewhere: units for effective absorption cross section of PSII (σPSIIʹ) are given as nm2 quanta−1) – is this correct? Should it be nm2 μmol quanta−1? Or quantum −1?

Response: Thank you. The unit nm2 quanta−1 is correct. Some publications and processing softwares use an equivalent non-SI formulation of A2 quanta−1 (5 nm2 quanta−1 = 500 A2 quanta−1). Other publications have used a short-hand of nm2 PSII−1 or A2 PSII−1, which includes an implicit ratio of 1 quanta PSII−1. Author DA Campbell apologizes for past sins of inconsistent units.

Minor points of expression:

Comment: Line 49: ‘pigments of a given cyanobacteria or algae’ should be ‘pigments of a given

cyanobacterium or alga’ or ‘pigments of given cyanobacteria or algae’

Response: Thank you. We corrected this issue.

Comment: Line 81: Italicise *Synechococcus*.

Response: Thank you for your insightful reading. We corrected this mistake.

Comment: Line 231: “…was measured using a Trilogy Laboratory Fluorometer…”

Response: Done.

Comment: Line 232: “…equipped with a Chlorophyll In-Vivo Module..”

Response: Done.

We thank Reviewer 2 for their time and valuable comments. The relevant changes in the manuscript are marked in green.

Reviewer\_2

Comments to the Authors LO-24-0249

General comments

Comment: This paper focuses on physiological differences between strains of PC rich vs. PE rich *Synechococcus* in their response to photic regimes and growth phases. The work is technically sound and well executed. The paper is generally well written. Overall, the results based on these culture experiments point to the potential importance of photoperiod as well as PUR/PAR in explaining the distribution of PC strains. This would seem to be an important aspect of their ecology that has not been fully considered.

Response: Thank you for these positive statements, in the revised version we strive to emphasize the importance of photoperiod as a factor with differential influences on growth across strains, and across light levels.

Comment: I think this could be more clearly brought forward in the conclusion to the paper. The remarkably high growth rates achieved at 22 C is indeed worth highlighting as a major finding along with behaviour in response to longer photoperiods. However, I would recommend that the authors not generalize based on 2 strains of each type that are all coastal, when there is clearly so much diversity across strains. This generalization needs to be downplayed in the abstract as suggested further below. One generalization that is possible for PC vs PE strains is their light quality niche differentiation (Stomp et al. and references within), but whether this extends to photoperiod length remains to be determined. *Synechococcus* assemblages in coastal areas would tend to be dominated by PC rich strains by virtue of the higher turbidity of these areas relative to the open ocean, perhaps regardless of photoperiod. Based on the present study it would seem that PE rich strains are more susceptible to photoinhibition, and it may indeed be possible to generalize here in reference to other studies based on different strains. I would suggest a concerted literature search of culture studies of PC vs PE to provide further support for this generalization.  Using field data as support here is complicated by many other factors that can affect the outcome of competition between species and strains of species.

Response: Thank you. We emphasized in the revised Discussion and Conclusions that we recorded the fastest known growth rate for picocyanobacteria at a temperature of 22°C (refer to track changes version: L606, L610-616, L749). We corrected the Abstract to avoid over-generalizing our results to all PC and PE strains (L27-42). We also highlighted the role of photoperiod in revised Discussion and Conclusion (L581-583, L672-675, L752-753) and added literature focused on culture studies of PC vs PE (L602-605) *Synechococcus*.

Comment: My other general comment centers around the growth conditions of batch culture (see below) – under the conditions of these experiments, what is the main factor that is triggering the onset of the stationary phase? is there N limitation, or light limitation (from self-shading) within the cultures once stationary reached?  Would picocyanobacteria ever be in stationary under natural conditions? Likely not because their growth rates are very much in sync with loss rates from protozoan grazers - they are essentially kept in exponential growth. Natural conditions are thus more like those of a chemostat.

Response: Thank you for this important comment. We removed the passage on cyanobacterial growth phases from the Introduction and moved some parts to Materials & Methods, Results, and Discussion. Due to this comment and the Editor's suggestion, we also decided to change the title (L1-3, L212-214, L367-374, L652-663).

We added text (L 367-374)

"Not all cultures were grown long enough to reach full stationary phase, but onset of stationary phase, when determined, occurred fairly consistently when cultures reached ~ 0.5 OD720 (PC-rich) or ~ 0.65 OD720 (PE-rich), no matter the level of culture PAR. It is therefore unlikely that onset of light limitation imposed stationary phase on the cultures, which remained optically fairly thin, with even illumination to each tube from the PSI MultiCultivator array of LED. Based upon parallel studies re-launching growth after stationary phase by dilution with fresh media, with the same strains, under the same growth conditions (unpub.), we hypothesize that nutrient limitation imposes the transition to stationary phase."

Specific comments follow.

Title, Abstract:

Comment: Line 25. I think that the forecasting is based on temperature responses as opposed to changes in the depth of the mixing zone which would affect the overall photic regimes.  I think here the authors are alluding to the work of Flombaum et al., which is in reference to projected ocean temperatures rather than changes in light regimes.

Response: Thank you for this comment. We changed this sentence.

Comment: Line 31 here I would specify.. whereas the two PC-rich strains showed…

Response: Thank you. We corrected this issue.

Comment: Line 33. change to “found that all four coastal strains….showed…

Response: Thank you. We changed this sentence.

Introduction  
Comment: Line 51-52. This is not unique to cyanobacteria. Ditto the next sentence (line 54-55) - the challenge of prolonged darkness is not faced just by cyanobacteria at high latitudes. Up until this point the introduction refers to phytoplankton more generally. But the whole first introductory paragraph is pertinent to all phytoplankton taxa even the last two lines (63-64). I would make this clear. The next para introduces Synechococcus …So start here by focusing the background context to this group of phytoplankton, specifically. It is easy to justify the focus on picocyanobacteria, given that they typically dominate the productivity of the open oceans.

Response: We thank the Reviewer\_2 for this insightful comment. As suggested by the Reviewer, we changed the Introduction. In the new version of manuscript, the first paragraph focuses on general phytoplankton, the second more clearly on *Synechococcus*.

Comment: Line 37 define μ or put in brackets when referring to growth rate in line 30 (if this is also μ expressed per unit chlorophyll?).

Response: Thank you. We added brackets when referring to growth rate.

Comment: Line 38. Rephrase coastal picocyanobacteria may easily expand into longer photic regimes…

Response: Thank you. We rephrased this sentence.

Comment: Line 38-39. This brings us back to the first sentence of the abstract…which makes it seem as if the present work is simply confirmatory when I believe it is not. This study brings in an additional explanation for a potential expansion of marine picocyanobacteria in the face of climate change, that is in addition to temperature.

Response: Thank you for this comment. We changed the abstract (L27-42). Additionally, we added a statement to the end of the Introduction (L134-136).

Comment: Line 87. This has more to do with the light quality (turbidity) of coastal areas, than the light levels.

Response: Thank you. We changed this sentence.

Comment: Line 93. This paragraph might be better placed after the one staring line 105? It does not seem to flow well here.

Response: Thank you for this comment. We agree. We removed this paragraph from the Introduction and moved the content, with modifications, to the Material and Methods (L212-214) or Discussion (L652-663).

Comment: Line 93. Is a batch culture condition the best analogy for growth in nature? Especially when it comes to *Synechococcus*? I think the point about the additional pre-stationary phase is well taken but perhaps move to methods.

Response: Thank you for this important comment. We agree. We added information to Materials & Methods (L212-214) and moved this fragment with modifications to the Discussion (L652-663). Due to this and the Editor's suggestion, we also decided to change the title (L1-3).

Materials & Methods

Comment: Line 142 “fiercely”?! replace or just delete this clause. Or change to “pH showed little fluctuation and remained between ~ 8 -9”.

Response: Thank you for this comment. We changed this sentence (L169-170).

Comment: Line 147. The intro should provide an inkling as to why DNA was extracted…but I see that this was simply to include the phylogenetics of the strains used. Is this line 155 necessary to meet the goals of the paper? Just include this info when strains are described at the start.

Response: Thank you for this important comment. We agree. We moved the fragment about phylogenetics to where strains were introduced in Materials & Methods (L143-146). We also added a more detailed description to the legend caption in Fig. S1.

Comment: Line 165. Explain the use of the difference between the 2 wavelengths. What information does this provide with respect to growth. It appears further down but explain here and provide a reference for this proxy for chlorophyll a.

Response: Thank you for this comment. We explained this fragment and provided a reference for proxy for chlorophyll *a* (L191-194).

Comment: Line 171-172. Fix grammar.

Response: Done.

Comment: Fig 1. I can’t see why the flat part of the curve of the logistic fit is called pre-stationary. Looks like stationary to me…perhaps show Fig. 1 and include the stationary phase so that it is possible to understand the distinction.

Response: Thank you for this comment. We added an explanation in the Materials & Methods (L212-214).

Comment: Line 203-204. Remove brackets from Morel 1978.

Response: Thank you. We fixed this issue.

Comment: Fig. 2. Add in the name or number of the strains shown. There were 2 strains of each type, show the patterns for both. It is interesting that for the PE rich strain the relative absorbance of PE peak is much lower under pre-stationary, this would not be expected if light was more limiting (as cell density increased) which suggests that perhaps nitrogen was limiting at this stage?

Response: Thank you. We added the strain numbers to the figure caption, and added appropriate statement to the Discussion (L672-675).

Results

Comment: Line 336-227. Fix grammar in this sentence. There were significant effects of all three independent variables on growth rates as well as significant interactions between variables.  
(these effects are all highly significant based on the p values in Table S2 that really should be provided as the p values are listed as zero …this of course is not possible. Please fix Table S2 and elsewhere when p values are listed as <0.000 (e.g. Table S1, Table S3…).

Response: Thank you. We fixed Tables in the Supplementary materials.

Comment: Line 377. I would not refer to 2 of the 4 strains as “exceptions”. There would appear to be a lot of individual strain variation in many of these endpoints and given that only 2 PC rich and 2 PE strains this makes it hard to say what is the “exception” to more “normal”/general results.  
Response: Thank you. We corrected this issue.

Discussion

Comment: Line 550-553 this is shown well in Fig 5. Cite figures within the paper rather than sup. material when possible.

Response: Thank you. We made corrections to the text.

Comment: Line 554-556. Yes, this is true and the notion that PE strains are somehow more typical of low light conditions is not a valid generalization. Observations that they may be better adapted for lower light may simply be a consequence of the light quality rather than the light quantity. I have not done a search but there are other studies comparing PE to PC strains with respect to high light requirements, no?

Response: Thank you very much for your insightful comment, which helped us improve the manuscript. We added appropriate text to the Discussion (L602-605).

Comment: Line 559. Yes, these growth rates are very high, particularly given the temperature of 22 °C. When comparing to the lit. specify the temperature used in those growth experiments because at higher temp higher growth rates are typically seen. The references here to lit. studies should include the temperature at which max. growth rates measured.

Response: Thank you. We added the information about temperature to each example cited (L606-616).

Comment: 9 figures is a bit much, but I like Fig 9. If a figure has to be moved to sup material, given the audience of typical audience of L&O which may not be so interested in specifics of photo-physiology, perhaps Fig. 7 can be moved to sup. material.

Response: Thank you. We understand the Reviewer's concern. However, we believe that both Figure 7 and Figure 9 are both needed in this manuscript, since the combination of high resolution growth measurements, with functional evaluations, is not common. Thus, we prefer to leave the current layout and number of figures, as we believe that they form a logical whole. However, if necessary, we will move Fig. 7 to the Supplement.

Figures/Tables

Several are mentioned above. But in addition

Comment: Fig.1 include in legend light regime used (photoperiod in particular)  
Response: Thank you. We corrected this issue.

Comment: Fix the p values of 0 in the tables S1 etc.,,,

Response: Thank you. We corrected Tables in the Supplementary materials.