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**All data and annotated code for data import, transformations and analyses are available on the figshare data repository (DOI: 10.6084/m9.figshare.25959061).**

4. We note that you have referenced (Campbell, unpub) on page 17, which has currently not yet been accepted for publication. Please remove this from your References and amend this to state in the body of your manuscript: (ie “Bewick et al. [Unpublished]”) as detailed online in our guide for authors  
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**We removed these from the manuscript file.**

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**Our reference list is complete.**

Reviewers' comments:

Reviewer #1: This manuscript presents a helpful expansion of our knowledge of the ecological tolerance ranges of several Prochlorococcus ecotypes. It is especially interesting in its implications for range expansion of this genus under potential future conditions, although this angle could be expanded on in my opinion, as I explain below.  
  
Specific Comments:  
  
The abstract seems to omit any discussion of the conclusions/bigger picture of the study.

**Thank you for your comment. We added text to discuss the main conclusions of this study.**  
  
Line 55: LLII/III and LLIV are not the “oldest” clades, but the most basal or early branching.

**We edited the text in the revised manuscript: "Clades LLII and LLIII, including cultured strain SS120, are grouped together as early branching phylogenetic lineage in the P. marinus radiation, with a preference for low light."**

**Line: 64**  
  
Line 369: The authors invoke “limitations in the annotation process” to explain the lack of evidence for MED4 ATP synthase. What does this mean? The MED4 genome surely contains ATP synthase genes.

**We agree that the MED4 genome includes genes encoding ATP Synthase. Upon verifying our annotation assignments, we discovered the problem with the absence of ATP Synthase from Clade HLI was not due to the limitations of the process but rather a mislabel of the column in figure 2. 'ATP Synthase' should have been labelled 'RUBISCO' and 'RUBISCO' should have been labelled 'ATP Synthase'. However, in the Ocean Protein Portal database no RUBISCO peptides are annotated as coming from MED4. This likely reflects an annotation assignment issue because the RUBISCO proteins are highly conserved, so assignments to strains or clades of Prochlorococcus may be ambiguous.**

**We edited figure #2 to add 'jitter' offsets up to 15% of full axes scales, to visualize over-laid data points.**

**We edited the text in the revised manuscript to reflect the limitation of the annotation process as it pertains to the highly conserved RUBISCO complex: "Though present and expressed in the genome of MED4 [65], the absence of proteins annotated as RUBISCO complex for clade HLI, compared to annotated detections of RUBISCO across the other three clades, suggests limitations in the annotation process assigning highly conserved protein sequences to clades."**

**Lines: 394 to 398**  
  
Paragraph starting L363: Why is there no discussion of the Rubisco data? Or especially the absence thereof at low O2 for ecotypes HL1 and LL1? Since Rubisco is one of the most abundant proteins in a photosynthesizing cell, does this indicate that these ecotypes are present but not actively fixing carbon under these conditions? Also, is it worth noting that LLIV is the only variety to be detected at all in the deepest low O2 site -- which would appear to be consistent with subsequent findings in this study?

**We revised the text from lines 398 to 401 to incorporate these helpful observations. The following was added: "Notwithstanding limitations on assignments of proteins to clades, detections of the abundant carbon fixation complex RUBISCO derived from clades HLI, LLI and LLII/III were notably absent from stations at low [O2], suggesting limited capacity for carbon fixation by clades HLI, LLI and LLII/LLIII under low [O2] habitats."**  
  
Figures 3 through 8: These figures are obviously the crux of the results in this paper, but as they are currently presented it is relatively difficult to think clearly about them. I feel like they could be combined into 2 or 3 full page figures which might make it easier to make the relevant comparisons; either by combining the two types of figure (growth rates and model fits) for each ecotype, or else all the growth rate curves together in one figure and all the model plots together in another.

**Thank you for this suggestion. We combined the growth rate figure with the GAM model for each strain to facilitate comparisons.**  
  
I'm also not sure I understand why the red light data is relevant -- perhaps by moving it to supplemental, it would be easier to compile these data into compound figures?

**We briefly motivated the inclusion of the red light growth trials, as mechanistically informative, at line 432:**

**"As mentioned, growth rate trials under red light, although not representative of \*P. marinus\* niches, are mechanistically informative [@murphyPhotoinactivationPhotosystemII2017] regarding photoinactivation of PSII."**

**Through the Discussion, notably under the discussion of the GAM models and responses to growth rate to increasing PUR, we return to the responses to red light to show that some 'high light' limitations on growth are interpretable as photoinhibition of PSII, which is more severe under blue, compared to red, light, notably at lines 646-650.**  
  
I also wonder if the data from the model fits could be used to predict which of the three ecotypes would prevail (have the highest growth rate) across the parameter space depicted, resulting in another figure showing something like "zones of exclusion" for each ecotype, which would be interesting in terms of predictive power. One could easily imagine applying those "zones of exclusion" to a map of the actual ocean at various depths, or even comparing current with projected future conditions of spreading OMZs. This is merely a suggestion -- I don't expect the authors to necessarily do this, but I think if they chose to it would make the manuscript of broader interest.

**Thank you for your comment. We added a new figure (Fig 10) of potential clade occupancy in temperate and equatorial seasonal and depth niches, in future warmed oceans under full oxygen or oxygen minimum zones.**  
  
L482, 536, 548: some experiments were not performed “due to time constraints”. I'm not sure I understand this, although I am interpreting it along the lines of "the person doing the experiments graduated"… In any case, it seems like these particularly treatments were unlikely to grow, so it's not important that they were omitted -- but maybe there is a better way to phrase this that doesn’t sound so fishy?

**Thank you for this suggestion. We edited the text at lines: 454, 518, 586**  
  
Figure S4 — It is very hard to see the fit lines other than the pooled fit. Consider using different colors and solid lines since this is online.

**We edited the figure to clarify the fit.**  
  
Table 1: The trees described in the legend should be included in the supplemental material.

**Thank you for bringing this to our attention. We clarified the wording on line 720 of the revised manuscript. "Protein homologies for FtsH isoforms between \*Prochlorococcus marinus\* and \*Synechocystis\* were extracted from bonisteelStrainSpecificDifferences2018."**

Fig 11: Despite the fact that it is explained in the legend, the figure should probably indicate that O2 values are log Km, or else put actual values on the log-scale Y axis. Also, the axis should indicate the units for Km. Finally, why is the red band so narrow compared to the green and blue?

**-We apologize for the omissions. We revised the axis labels to make the log scaling more noticeable. The narrowness of the red bar (spanning 230 to 280 uM) results from the log scaling of the y axis. This is now Figure 8.**

L757: The authors should specify that this is a DNA repair ligase, not the DNA ligase that seals Okazaki fragments during DNA replication (assuming this is the case).

**Thank you for your comment, the revised text now reads "DNA repair ligase".**

**Lines: 800, 803, 815**  
  
L 758: Photolyase should not have been relevant in the lab experiments because there is no UV light emitted (AFAIK) by the bulbs used in the Multicultivator. Also, it is unlikely that the absence of these two genes is sufficient to explain why these strains can’t grow at the ocean’s surface.

**We agree. The following was added in the revised manuscript: "Our growth rate experiments did not include wavebands in the UV range, so we do not know whether the absence of deoxyribodipyrimidine photolyase would inhibit the growth rates of P. marinus SS120 and MIT9313 under low [O2] if exposed to full spectrum, ocean surface light. "**

**Lines: 806-809**

**To address the second part of your comment, the revised sentence now reads: "Conversely, P. marinus SS120 and MIT9313 lack genes encoding deoxyribodipyrimidine photolyase and DNA repair ligase (ATP), which may explain, in part, why these two strains cannot tolerate growth under full 250 µM [O2] and high light, as found at the ocean surface."**

**Lines: 802-805**

Fig 12: Phosphoglycolate phosphatase is a major enzyme in the photorespiration pathway — what does it have to do with DNA repair? There is evidence that 2-phosphoglycolate causes oxidative stress, but I don’t think this enzyme is properly a DNA repair enzyme.

**Thank you for bringing this to our attention, we removed phosphoglycolate phosphatase from the figure as it is indeed not directly involved in DNA repair but in clearing DNA damage repair products. This is now Figure 9.**

**Upon revisiting the interpretation of this figure, we edited the text: "Prochlorococcus marinus NATL2A and MED4 possess the largest, most complete suites of genes encoding DNA repair enzymes."**

**Lines: 796**  
  
Summary and Conclusion: I feel like the authors really need to expand their discussion of how these findings apply to the future ocean. Where are OMZs likely to expand? Are there specific examples of regions where LLIV might expand to the surface? What would it mean for LLIV to become competitive against HLI and HLII? I think this is likely to be similar to scenarios where Synechococcus may outcompete Prochlorococcus, since LLIV is quite similar to Syn in terms of size and genome repertoire. I'm not sure where exactly such a discussion should end up, but I think it is important that the authors try to "seal the deal" a bit better in this concluding section and relate the findings back to the issues laid out in the introduction.

**We added the following to the Summary and Conclusion section:**

**"In warming oceans, P. marinus clades will differentially expand into new regions. Competition among clades will be driven not simply by light levels, but by their differing capacities to tolerate and exploit combinations of photoperiods, light levels, and [O2]. Clade HLI (including MED4) is excluded from short photoperiod regimes, typical of temperate winters at light attenuated depths. In contrast, clade LLIV (including MIT9313) may exploit higher light niches under expanding OMZ conditions, where low O2 relieves the stresses of oxidative stress and PSII photoinhibition."**

**Lines:942-948**

Reviewer #2: Savoie et al describe a beautiful and extensive set of detailed Prochlorococcus growth rate measurements for three strains, representing distinct ecotypes, under varied conditions of light and oxygen concentration. The work adds a lot of interesting and useful information concerning the impact of varied photoperiods, wavelengths/PUR, and oxygen concentrations to the literature. These data are also connected to hypotheses about the potential role of DNA repair and PSII maintenance to explain the results. The methods are appropriately detailed, and the experiments appear to have been done with great care. The data are available in a usable form.  
  
My primary concern with the manuscript comes from the way the results are framed throughout. The authors describe results as informing about the growth of Prochlorococcus across a matrix of different conditions. However, my interpretation of the methods is that the authors took from a maintenance culture kept under standard growth conditions and then immediately transferred them into the Multicultivator to look at responses. This is of course completely valid, but extensive experience by many in the field (e.g. described in Moore et al 2007 Limn. Oceanog. Methods and elsewhere) is that these cells typically require multiple generations to acclimate to new growth conditions. I read these data as more reflecting the immediate response of Prochlorococcus to environmental changes. It is quite conceivable that some of these strains may exhibit a different growth rate than what you report if allowed to gradually acclimate to their new condition rather than directly move them. I would suggest that the framing and interpretation of the paper should reflect this. I also have no idea whether this might have influenced the surprising observations of MIT9313 growth at relatively high PAR. Global change scenarios would presumably give cells many generations to physiologically acclimate to differences (as well as to evolutionarily adapt).

**Thank you for highlighting this issue. We added the following in the materials and methods section of the revised manuscript:**

**"Each tube containing 70 mL of Pro99 media was inoculated around mid day of the 12 h maintenance photoperiod with 10 mL of growing maintenance preculture, to reach a starting OD680 of approximately 0.020. The tubes containing the cultures were then placed in the Multicultivator water bath set at 22°C, sparged with the experimental [O2], and kept at low light until late afternoon. Cultures were then in the dark for 12 to 16 hr until the photoregime of a sinusoidal photoperiod commenced the following morning, reaching peak PAR at noon each day. Cultures thus took approximately 24 h to move gradually from maintenance photoregime to the peak PAR of the experimental photoregime. Cultures were grown for 7 to 14 days, until they reached stationary phase at OD680 of approximately 0.4 to 0.8 after approximately 5 generations of growth."**

**Lines: 237-247**

**We added the following in the results and discussion section of the revised manuscript:**

**"We implemented measures to minimize shock to cultures from exposure to experimental growth conditions by inoculating them the day before the experiment began, employing a sinusoidal photoperiod with a gradual increase in PAR exposure, and extracting exponential growth rates from logistic curves fit over approximately 5 generations of growth, to accommodate multiple generations to acclimate to the imposed growth conditions (Moore et al. 2007)."**

**Line: 434-439**  
  
This reviewer found the manuscript to be quite difficult to read as I kept wanting to understand how different strains were responding to similar types of variables, which made me have to constantly flip through and reread parts of the manuscript to find the information. Establishing a clearer overall summary narrative early on, as opposed to presenting a laundry list of data, might help. For instance, more clearly motivating the questions driving the analyses in each section would help; many paragraphs simply start with “Figure X shows…”. But, the organization of the paper is of course up to the authors.

**In response to reviewer 1 and reviewer 2 we have combined the growth rate figure with the GAM model for each strain to faciliate comparisons. These are now figures 3-5.**

**We edited the abstract, results and discussion texts to better convey the motivations and findings of the study.**  
  
Minor points:  
  
General note: the manuscript text at some points refers broadly to “growth”, and other places is more specific about drawing conclusions about “growth rate”. I would suggest being clearer in some way that you are focused on growth rate and not growth yield.

**Thank you for your suggestion. We edited the text to use 'growth rate' throughout.**  
  
40: Despite \_its\_ small cell size (?)

**We edited the text: "Despite its small cell size, "**

**Line: 48**

56: “Affinity” for low light? Consider rephrasing

**We edited the text in the revised manuscript: "Clades LLII and LLIII, including cultured strain SS120, are grouped together as early branching phylogenetic lineage in the \*P. marinus\* radiation, with a preference for low light."**

**Line: 63**  
  
84: Follett et al 2022 PNAS showed that temp is not the only factor influencing poleward issues - ecological interactions may further impact poleward shift during warming

**Thank you for bringing this to our attention. We added the following text to the revised manuscript: "Follett et al. , however, model interactions of heterotrophic bacteria that may influence latitudinal expansions of \*P. marinus\*"**

**Line: 93**  
  
199: Could you please state more about why you are confident in this assignment? What validation or metric leads you to this?

**Thank you for noting that this needs further clarification.**

**We edited the paragraph for clarity in the revised manuscript adding: "While the accuracy of strain specific protein annotations is limited due to the high conservation of the target protein complexes, the two step approach of peptide-to-spectrum matching using deep paired metagenomics, does assign proteins at the level of clades."**

**Lines: 215-218**  
  
209: It seems overly general to blame the culturing difficulties solely on having reduced genomes – in this context, it is the lack of just one gene (catalase) that matters, and reduced genomes reflect an outcome of the ultimate suite of selective pressures acting on these systems.

**Thank you for your comment. We edited the text in the revised manuscript: "*Prochlorococcus marinus* remain challenging to culture at high densities or under fluctuating environments, partially due to their dependence upon mutualistic heterotrophic bacteria to detoxify reactive oxygen species [48,49]."**

**Lines: 220-222**

363: Could the authors comment somewhere on why you took this proteomic approach to examine the baseline distribution of Pro across O2 and depth, as opposed to (or at least in concert with) the extensive metagenomic, amplicon, qPCR, etc datasets of ecotype abundances available?

**Thank you for your comment. Co-authors, Aurora Mattison and Dr. Amanda Cockshutt, used the metaproteomics database as an extension of their earlier studies on the functional implications of differential protein allocations across different Prochlorococcus strains [Zorz et al. 2015, Bonisteel et al. 2018]. Those studies indeed motivated the design of our growth matrix. Extending the study to include metagonomic analyses is valid but is beyond our scope.**

**We edited the text in the revised manuscript: "Our analyses utilized a proteomics dataset; however, alternate approaches, such as metagenomics or metatranscriptomics, could have been employed to analyze ecotype abundances using the TARA Oceans [@pesantOpenScienceResources2015] dataset."**

**Lines: 408-411**  
  
407: growth -> grew?

**We edited the text: "The growth rate for P. marinus MED4, clade HLI, under 250 µM O2, increased with higher imposed PAR and longer photoperiods (Fig 3A), across all spectral wavebands."**

**Line: 447**  
  
428: How many replicates were carried out for most experiments? Perhaps I just missed it, but please be sure this information is clear in the methods

**Thank you for this comment. In this work we used 1 to 3 replicates depending on the experimental conditions. Specific number of replicates (shown as small dots in Figures (3 - 5)).**

**We edited the text in the revised manuscript: "Consistent absence of growth of some strains under some levels of photoperiod, PAR, or [O2] meant we completed 268 growth rate factor treatment combinations with 1 to 3 replicates."**

**Lines: 266-268**  
  
431, and generally throughout the paper: Would the authors care to comment on any connection between the differences in blue vs red light growth and the underlying amount of energy input from these different wavelengths?

**As outlined in responses to questions from Review 1, the implications of blue vs. red light wavebands indeed relate to the higher energy per photon in the blue light band. In particular blue light is absorbed well by chlorophyll, but is also directly absorbed by the manganese cluster of PSII, provoking one major path of photoinactivation, as discussed in Murphy et al. 2017, and cited at lines 307, 434, 648, 663, 676, and 894 .**

**In contrast, red light is absorbed well by chlorophyll, driving photosynthesis, but does not provoke direct photoinactivation of the PSII manganese cluster, lowering the metabolic cost of maintaining the photosynthetic system, compared to equivalent rates of photosynthesis driven by blue light.**

**These issues are now discussed in relation to figures 6 and S4, with a given amount of absorbed red light PUR provoking more growth than the equivalent absorbed blue light PUR. In particular:**

**"...MED4 achieved more growth per unit diel cumulative PUR (Fig 6A and B), consistent with Murphy \*et al\*. [@murphyPhotoinactivationPhotosystemII2017], who showed a lower cost for growth under red light, for MED4, because red light provokes less photoinactivation of PSII, than equivalent levels of blue light..."**

**Lines: 647-650**  
  
Fig 4 and related figures: It might be useful to plot the specific measured points within the countour plot

**Thank you for your suggestion. We combined the growth rate figure with the GAM model for each strain. We believe this will aid the reader with interpreting both plots. These are now figures 3 to 5.**  
  
685: The lack of detected FtsH could simply be a factor of low overall spectral counts and detection limits in the dataset; please provide more context to help the reader understand whether this could be just a small number problem with respect to MIT9313 in low O2 samples; Fig. 10 seems to just show a binary detected/not detected value.

**Thank you for your suggestion. We agree that detection limits vary for different target proteins, particularly for detection of single proteins such as FtsH isoforms. Such issues are less problematic for summed detections of multiple proteins assigned to multi-protein complexes such as PSII. An analyses of mass spectrophotometric proteomic detection limits is beyond the scope of the paper. We, however, edited figures 2 and 7 to add 'jitter' offsets up to 15% of full axes scales, to visualize multiple previously over-laid data points.**