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Interactive comment on "Light availability modulates the effects of warming in a marine N_2 fixer" *by* Xiangqi Yi et al.

Anonymous Referee #2

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General comments: This manuscript by Yi et al. examines how light availability (tested at two levels of light intensity) interacts with the effects of warming (along a gradient of three temperatures) in a marine N2 fixer (Trichodesmium erythraeum IMS101) across a time scale of about ten generations. The experiment is in its essence a two-driver question, where either driver might intrinsically decrease or increase metabolic performance, but the cumulative effect is unknown. The findings and the results are straightforward, with a clearly identifiable general trend. While theoretically relevant (e.g. changes in temperature may coincide with changes in light intensity), it is not quite clear why the authors chose these two drivers over other sets of drivers until much later on in the manuscript. It would also have been nice to see a more explicit evaluation over whether the changes in temperature/light level constitutes an environ-

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mental deterioration or amelioration and how that impacts on how they interact. Still, the results are quite interesting, especially since they cover a range of phenotypic traits (growth rates, N2 fixation rates, photosynthetic machinery). However, I have major concerns about how the results are presented: the methods do not indicate how the data were analysed, and the results appear largely as post-hoc output. The latter would indicate that the authors used an ANOVA or similar test, which is indeed indicated more clearly once in line 185, but details are nowhere to be found. For example, a statement about the data is followed up simply by ($p<0.05$,tukey HSD method). It is impossible to glean from this what kind of data were compared and what the original model looked like. As the main question is about interactive effects, and the data are hierarchical in nature (e.g. differently acclimated samples used in a short-term assay), the authors would have needed some kind of mixed model approach. The closest the text ever gets to describing how the data were handled is in line 129 'parameters can be obtained through non-linear least squares regression in R language'. Which packages did the authors use to do so? How did they fit their data to the Eiler curve? Similarly, the authors mention the Sharpe-Schoolfield model, but that would be no easy feat with only 5 temperatures (it is a 4 parameter equation). More information would have been crucial here! It clearly worked well, as the fits in Figure 3 don't look too bad. However, we then need to also know how different these curves are from each other. For this, one needs to either extract the parameters and compare them (and describe how!) or run a non-linear mixed effects model (and describe how). As it stands, the handling and analysis of data is not at all traceable. I will provide suggestions on how to deal with this issue in the detailed comments below.

Technical comments and corrections, further suggestions:

Throughout: please double-check use of singular/plural and use of present tense and past tense. Please be careful with the vocabulary used. What is 'acclimation', what is 'short term'? How are either of these different from 'acute'? Be consistent throughout in how you use these words. You could, for example, define them in the introduction

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and then stick to that definition.

Abstract Line 13: Consider telling the reader which phenotypes from the get go. Line 16: 'range of 23-31' could be misleading, just state the three temperatures Line 16/17: 'when the acclimation . . . [. . .]. . . to growth temperature was evaluated by short-term

Line 22: "cells growing under low light levels while distributed deep in the euphotic zone or under cloudy weather conditions might be more susceptible to ocean warming": I would be careful about that, the study refers to response of acclimated cells at different conditions, not to acute or immediate responses (at least for the growth response), especially when we consider that these cells can actively migrate along the water column.

Line 23: Point out explicitly that this is true for ocean warming occurring on the timescales of a few generations, or, as in your assays, short term responses within the same generation in mere hours. Mention scenarios when this is applicable upfront (mixing, heat waves..)

Introduction Line 29: might not be all that 'obvious' to all readers. Consider elaborating. Line 39: The 1960s are not a century ago yet, plus the literature cited after this statement is pretty recent? Specifically: Is there a reference for the 1960 discovery of diazotrophy in Trichodesmium?

Line 41/42: 'In the IPCC. . .[. . .]' consider rephrasing to 'The IPCC scenario [. . .] predicts..[..]' Line 43: I am not sure Collins et al 2013 is the correct reference here, as it is focused on the long term implications of global climate change, not so much the ocean physics Line 44: 'consequences' on what? Consider elaborating.

Line 50-52: different responses to warming more due to relation between traits and environment, than only "because of the spatial heterogeneity of present temperatures and projected warming". Clarify it is also a matter of local adaptation.

Line 68: clearly state that Trichodesmium is ACTIVELY able to migrate vertically.

Methods:

Line 75: Are three replicate populations enough to assess within species variability? Was this decision based on pilot studies? Were the cultures clonal? Mixed?

Line 77: Would be crucial to know where these three temperatures lie on the thermal tolerance/performance curve. The 2007 and 2014 studies just state that these are temperatures that this specific Trichodesmium can live in?

Line 77: Might have been better to have used a third light intensity toward the Iopt, just for the sake of comparison and to underpin the basic response to temperature of Trichodesmium.

Line 77: 160 μmol quanta m-2 s-1 seem like quite a low light intensity to be saturating, although they report in the supplementary a pilot study that seems confirm the statement. Nevertheless, the cultures for the pilot study were not aerated while it seems to be a constant for Trichodesmium culturing in all other papers (formation of cells' aggregates and consequently maybe self-shading effects?).

Line 84: 'cyanobacteria were floating singly' consider rephrasing to 'cyanobacteria floated as single filaments' Line 85: Was there a round of pre-acclimation prior to the acclimation phase? Pre-acclimation is a crucial step to avoid carry-over from the previous culture conditions. See for example Trimborn et al 2019, Front. Mar. Sci https://doi.org/10.3389/fmars.2019.00167, Schaum and Collins 2014, Proc Biol Sci.281(1793): 20141486, Scheinin et al 2015 https://doi.org/10.1098/rsif.2015.0056, Lenski 2017 The ISME Journal volume 11, pages2181–2194(2017)

Line 86: How were the growth rate curves fitted? Missing info

Line 94: should be 'before applying the natural logarithm' instead of ' before natural logarithm'. Generally, how does using Chla as a proxy for growth deal with cells having more Chla per cell? Line 99: 'acute' as stated above, be mindful of vocabulary used. Define once, then stick to it. Line 102: is 0.5 to read 50 minutes or 30 minutes? This

seems really short for a 25mL vial to equilibrate to the correct temperatures! Line 107: The Padfield paper is pivotal, but it is not about the Schoolfield equation per se (it is about adaptation to warming and uses the Schoolfield as a tool).The second correct reference is Sharpe, P. J. & DeMichele, D. W. Reaction kinetics of poikilotherm development. J. Theor. Biol. 64, 649–670 (1977).

Line 113: Which package was used for the "optimize" function? Which version?

Line 114: If used correctly, the Sharpe-Schoolfield output should not require the 'optimize' function, but simply, rates at Topt can be obtained by re-arranging the equation. It is really not clear at all here how the data were fitted to the Sharpe-Schoolfield (it clearly went well as the figure looks correct). To me, it would make sense to either extract the parameters (Ea, Eh, Topt. Tc) and then compare them via a mixed model (e.g. parameter $\sim$ growthtemp*light with replicate within treatment as the random effect) or fit a non-linear mixed effects model where lnNrate $\sim$ schoolfield.high(ln.c,Ea,Eh,Th,temp=K,Tc=your Tc value) and, to begin with fixed = list(ln.c + Ea + Eh + Th $\sim$ growthtemp*light). You can then compare AICcs of your models (e.g. test also additive effect, each on their own, and just the intercept) and chose the best one. If you compare extracted parameter values, then the MuMin dredge function will come in handy!

Line 116: Why was it not possible to measure N2for samples at 31° C? At what time were the samples taken? I know N2 fixation-related genes show a strong circadian cycle, maybe a similar mechanism is involved?

As the authors stated into the nice small "meta-analysis", there is a huge within strains variation, why don't you used more strains? Alternatively, more isolates instead of three if you wanted to assess for within strains variations?

Line 129: See comments above – how were the data dealt with? Again, you can either extract parameters and compare via a mixed model, or run a non-linear mixed model starting with the most complex model and then working your way down to the

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most simple model. For all other phenotypic traits (the ones where you are not fitting a slope) , a mixed model seams the way to go!

Results: Throughout: When giving a value, also give the standard deviation or standard error. When referring to the result of statistical test, just giving the post-hoc value is not enough, as that only refers to ONE specific pair-wise comparison. If reporting one specific pair-wise comparison, we need to know which one!

Line 140: Might be worth starting out with whether the combined effect of light limitation was indeed interactive, or additive, or if one out of the two described the data best. Without the appropriate reporting of the stats involved, this is impossible to tell. Line 141: see above. Strictly speaking, this is not a temperature range, but three temperatures, 23,27,and 31oC. Line 145: How much is 'slightly'? Line 152: How much higher is higher? Line 159: Is acute the same as short-term here? Pick a word, then stick to it. Line 168: What was the variation around this 1.4 oC increase? Line 178: be mindful of the tense. Should be 'were able to sustain' Line 183: Add SD or standard error to these values Line 185: again, not clear what the p value refers to, or what was actually tested in the two way ANOVA

Discussion Line 191: "negative growth effects" seems a strong statement, maybe better use "reduced"

Line 196: level should be levels

Line 202: "temperature is lower" than surface?

Line 206: maybe I didn't get it, but "respectively" to what?

Line 210: This is a very nice and clear summary (the additive vs interactive bit), however, without the correct statistical approach it is impossible to tell whether the data actually support this conclusion! Line 232: May need a reference here Line 235: Should be equivalents, not equivalent

Line 250: what is the difference here between 'acclimated" and "short-term"? You

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mentioned both “short-term temperature norms” and “acclimation” throughout the paper (e.g. Table S1). Please clarify.

Line 257: ‘a bit different’ is too vague Line 258: not sure if ‘and/or’ is the correct choice of words here. Plus, it should be ‘on the time scales of acclimation processes’ . Consider adding that here, this is approximately 10 generations. Line 259: What about within-strain variation? Line 266: ‘to some extent’ is a bit vague, may need a bit more information here.

Tables Spell out HL and LL as high light and low light You clearly have the data from the light curves in the table, so explaining how you actually got them should not cause too much agony (we hope).

Figures Might be worth mentioning the software the figures were produced in.

Figure 1 The lettering of the subpanels as a, b,c, is highly confusing with the significance levels using the same lettering. Might be easier to present the significance levels as a table? What are the slopes in this graph? How were they fitted?

Figure 2 Spell out what a.u. stands for. Consider adding confidence intervals to model fits

Figure 4 Not clear where the interactions are. Again, the significance levels are a bit distracting and probably better displayed in a table.

Figure 5: a) Probably good idea to highlight the symbol for this study in bold b) –d) why are there no SDs or confidence intervals?

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