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PROCEEDINGS B

A life-history trade-off gene with antagonistic pleiotropic effects on reproduction and survival in limiting environments

Rani M. S. Saggere, Christopher W. J. Lee, Irina C. W. Chan, Dion G. Durnford and Aurora M. Nedelcu

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Review timeline

Original submission:	3 July 2021
1st revised submission:	9 December 2021
2nd revised submission:	22 December 2021
Final acceptance:	22 December 2021

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2021-1516.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
The manuscript analyzes the life history trade-off between reproduction and survival in a unicellular algal species. Specifically, it focuses on the hypothesis of antagonistic pleiotropy in this process. By analyzing mutants of the RLS1 gene that are unable to suppress their reproduction in phosphate deprived environment, the authors can shed light on this relationship between life history traits. I think the study is well done but have two main comments that should be addressed:
1. The statistical analysis needs to be better explained and described in the manuscript
2. I believe the authors should discuss a) the choice of nutrient deprivation/starvation using phosphate in the context of other nutrients such as nitrogenous compounds and b) the implications of that choice for their results in the discussion

Major point 1: I did not see any specific details on the statistical analysis in the materials and methods of the paper. Furthermore, in the results section, no statistical analysis was presented. This should be addressed. Also, when comparing the growth curves under different conditions/mutant strains, the authors should provide some estimates of variance in their data. Are these curves they present averages or estimates? What are the confidence intervals and would be for example the confidence interval around the difference in growth response (population growth - comparison of final carrying capacity and exponential growth timing).
Major point 2: microalgae can metabolize and use a variety of different types of nutrients. Specifically, they can produce polyphosphates, which require a large amount of energy to produce and that energy can be drawn from metabolic processes. The authors are investigating antagonistic pleiotropy, which they interpret in the context of downregulation of photosynthesis. It is however also possible that the increased demand for polyphosphate production may draw energy from other sources and therefore create energy mediated trade-offs. I believe it would be valuable to discuss these scenarios at least in general terms. More importantly, it would be valuable for other researchers to gain insight into the decision of depriving algae of phosphate rather than other nutrient sources. As well, the authors should discuss some of the potential limitations of using just phosphate limitation

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Line 125: 4 tech reps on hemocytometer meaning - 4 counts on the same slide within the same aliquot, or 4 different aliquots onto different slides? Which counts as a technical replicate? I'm not sure I could replicate this.
Include t test results in results section, also methods. What statistical program was used? What were the predictor & response variables?

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Line 276: Good iteration of importance of work and strength of evidence

Line 296: Not sure about ending on this question unless it gets answered later in the text. If not, elaborate more to direct future research (ex "future research should investigate how RLS1 regulates these two life history traits")

Lines 324-334: I found this a little confusing; Could use a little more description about why they believe the data supports the third scenario

Line 339: affects -> affect

Lines 354-357: Ending to discussion could be strengthened - Could use a final sentence to tie the last section of the discussion together more clearly.

Line 360-363: Kind of a long and awkward sentence, consider revising for clarity
Line 363: Why tie in yeast here at the end? This was only briefly discussed in the introduction and could be motivated better. Could be a bit broader here to encapsulate the other organisms mentioned in discussion (nematodes, V carteri... What about bacteria?)
Good summary of results and conclusions

Line 369-372: awk and long, consider condensing and simplifying.

Line 427: "et al" - can you find the other authors? Same comment throughout the ref list.

Line 454 ref error (period after initial)
Throughout ref list, capitalization of titles
Spacing in ref list - spaces between initials or not?
Journal abbreviations - period or not

Line 516: incomplete ref

Figure feedback:

Fig 2: Not sure it makes sense to have diff line types when you're already splitting them up by matrix; what new info does this show? I suggest keeping the same line type throughout the

panels to reduce clutter of the figure

Fig 4: I think fig 4a could be restructured to keep time on x axis and keep y axes consistent across to make them easier to compare

Fig 4 caption: is fig 4 really showing "comparisons"? Maybe "Growth curves"? I think "comparison" kind of implies a box plot or similar figure type. This doesn't indicate that the values are means, nor does it present the error. Cell counts and fluorescence readings can vary *a lot* between readings.

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Marginal

Is the length of the paper justified?
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Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
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Comments to the Author
In this study, the authors obtain an RLS1 mutant from a collection and carry out growth assays of this and a wild type strain in growth media with and without phosphate. In addition, they carry

out viability assays of the two strains. The basic idea is to test the hypothesis that RSL1 mediates the regulatory decision about whether to devote resources to short term reproduction or long-term survival. While the authors present data that supports this hypothesis, another strong conclusion of this study is that one of the reasons that photosynthesis is down regulated is in order to avoid oxidative damage. I think that this particular conclusion is not reasonable given the citations and data presented in this study, see my detailed comments.

1. I found the writing to be excellent, the manuscript was very clear especially in the results. The authors have done a good job of motivating and providing context for their results, so that everything is very easy to understand. I did find the concluding sentence in the abstract a bit tricky to understand: "We propose a model where the antagonistic effect involves the downregulation of photosynthesis, which mediates the acclimation response to nutrient deprivation in all photosynthetic organisms in order to avoid oxidative damage and increase survival, though at a cost to immediate reproduction." Reading this sentence, I am not clear what mediates the acclimation response- the antagonistic effect (down regulation) or photosynthesis itself? This sentence could be rewritten to remove the ambiguity and also to break this into two sentences.

2. line 155 I would like to know why this data is not shown. This knockdown strain is referred to again in line 188 - I know that "data not shown" is sometimes mentioned in other studies, but I think that the authors should show the data from the knock down strain if they are going to repeatedly use it to support their claims.

3. Line 174 Here the discussion about the role of ROS is worded as if it has been shown that photosynthesis is downregulated in order to reduce the production of ROS. This is different from the demonstration that photosynthesis results in the production of ROS. The authors are claiming that photosynthesis is down regulated because of the damaging effect of photosynthesis. And later, the conclusion is that viability of the mutant is low because of the uncontrolled production of ROS. However, when I followed the citations used by the authors I don't think this link has been firmly established. For example, the sentence "Thus under nutrient limitation to decrease the potential damaging effect of excess light energy, photosynthesis is downregulated (Wykoff et al 1998)". Wykoff does not mention ROS except in the intro, where they write "A lack of coordination could result in the generation of toxic, reduced O2 species" and then cite Asada 1994. The next sentence " This acclimation process is a general stress response that coordinates nutrient availability with the metabolism of the cell and its growth and division potential, resulting in a temporary inhibition of cell division…(Grossman 2000). Reading Grossman, esp their section on phosphate limitation, the focus is on down regulation as a means to deal with nutrient limitation, not as a stress response. The simplest explanation for the downregulation of photosynthesis in the context of low nutrient availability, is that stopping the production of the photosynthetic machinery conserves nutrient resources. While, I think that it is reasonable to think that Ros play a role in the downregulation of photosynthesis, this is not as well established as it appears in the in the author's writing- in fact I don't that this assertion has been experimentally tested. The authors need to either find citations that demonstrate that the downregulation of photosynthesis is a direct response of high levels of Ros or they need to remove this assertion. Alternatively, they may have their own experimental data that demonstrate this.

Line 248 again here the authors assert that the non-reproductive and lowered metabolic state prevents oxidative damage. I would like the authors to provide the citations or show the experimental data that support these claims.

Line 265 this concluding paragraph again assumes that the reduced viability is due to the accumulation of Ros in the RLS1 mutant. However, the reduced viability could be because these cells don't have the nutrient reserves to start growing again in favourable conditions. I would like the authors to either present this as a more likely explanation for this experimental result, or

explain why it isn't correct.

Line 306. Meager et al is cited in support of this statement: “In C. reinhardtii, when nutrients (e.g., phosphorus, sulphur, nitrogen) are limited, imbalances between excitation energy and cell’s reducing power result in the down-regulation of photosynthesis while maintaining the capacity for light dissipation, as an adaptive response to avoid potential light-induced, oxidative damage (Meager et al 2021)”. This study mentions ROS in the first line of the intro, but does not show any data pertaining ROS and photosynthesis.

Line 366 here the authors state that a nutrient limiting environments RLS1 down regulates photosynthesis to avoid oxidative damage again without any citations or supporting data.

4. Line 219 I think this growth essay is referring to a direct competition essay with the two strains of mixed in coculture this needs to be explained a little more clearly to distinguish it from the previous growth experiments. Or when I look closely at the data is figure 3 simply the re plotting of the same data from figure 2?

5. Line 242 “anaddotional” needs to be corrected (an additional?)

Decision letter (RSPB-2021-1516.R0)

16-Sep-2021

Dear Dr Nedelcu:

I am writing to inform you that your manuscript RSPB-2021-1516 entitled "A life history trade-off gene with antagonistic pleiotropic effects on reproduction and survival in limiting environments" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

1) A ‘response to referees’ document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
3) Line numbers in your main document.
4) Data - please see our policies on data sharing to ensure that you are complying (https://royalsociety.org/journals/authors/author-guidelines/#data).

To upload a resubmitted manuscript, log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

Sincerely,
Dr Locke Rowe
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
This manuscript addresses an interesting theoretical question and has the potential to be of high interest. The first reviewer has raised some minor points that could be addressed in revision. Specifically, the statistical analysis performed needs to be better described in the manuscript and the choice of nutrient deprivation using phosphate instead other nutrients such as nitrogenous compounds and the implications of that choice should be discussed. The 2nd reviewer raises a more substantive point that would require major revision and resubmission. Specifically, there is little evidence that photosynthesis is down regulated because of high levels of ROS in the 3 studies cited as supporting this assertion. So the study tests the hypothesis that RSL1 mediates the regulatory decision about whether to devote resources to short term reproduction or long-term survival and the data presented supports this hypothesis, but the additional strong conclusion of the ms.is that photosynthesis is down regulated in order to avoid oxidative damage. The reviewer raises the important point that this conclusion is not reasonable given the citations and data presented in this study and that the response may simply be due to conservation of nutrient resources. I agree with the reviewer that the authors need to either find citations that demonstrate that the downregulation of photosynthesis is a direct response of high levels of ROS, provide experimental data supporting this assertion, or modify the study conclusions.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s)
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Author's Response to Decision Letter for (RSPB-2021-1516.R0)

See Appendix A.

RSPB-2021-2669.R0

Review form: Reviewer 2

Recommendation
Reject – article is scientifically unsound

Scientific importance: Is the manuscript an original and important contribution to its field?
Marginal

General interest: Is the paper of sufficient general interest?
Acceptable

Quality of the paper: Is the overall quality of the paper suitable?
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Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
Having read through the response, the authors seemed to be concerned that I have misrepresented what they have said,

"We believe that this is a misunderstanding. We did not "claim that photosynthesis is downregulated BECAUSE of the damaging effect of photosynthesis", or the "downregulation of photosynthesis is a direct RESPONSE of high levels of Ros" as indicated in the referee's comment. Rather, we stated that "under nutrient limitation, TO DECREASE the potential damaging effect of excess light energy, photosynthesis is downregulated (Wykoff et al 1998)"

This is a very fine distinction. So avoid confusion I will use the authors exact words:

"Thus, to decrease the potential damaging effect of excess light energy under nutrient limitation – and increase survival, photosynthesis is down-regulated"

What I am saying that it has not been demonstrated that photosynthesis is down-regulated to decrease the potential damaging effect of excess light energy.

In other words, this sentence (the authors sentence from the manuscript) "Thus, to decrease the potential damaging effect of excess light energy under nutrient limitation – and increase survival, photosynthesis is down-regulated"

does not follow from this (what the authors wrote in the response):

"What is generally accepted in the field is that the downregulation of the photosynthesis under nutrient deprivation avoids the potential damaging effects of ROS produced as a result of imbalances between excitation energy and reducing power in the absence of nutrients in the light."

The authors have provided more references and quotes. Having looked at these I cannot agree that this has been shown. The quotes that you provide refer to circumstantial evidence and speculation. Eg

"This SUGGESTS that either the formation of O2 radicals generated by photosynthetic electron transport or the hyperreduction of electron transport components downstream of the QB-binding site, or both, leads to a loss of viability in the sac1 mutant."

"Indeed, when the snrk2.1 mutant is starved for S, it APPEARS to show molecular responses, such as the accumulation of transcripts associated with oxidative damage, ROS production, and apoptosis that are typical of organisms experiencing extreme environmental conditions"

Furthermore, with the decreased demand for reductant, the cell would tend to accumulate high potential electrons and excited chlorophyll molecules that WOULD interact with oxygen, creating reactive oxygen species (e.g. superoxides and singlet oxygen). These species COULD cause extensive cellular damage and also function as regulatory signals that modulate metabolic activity.

The words I highlight (some of which the authors also highlighted) emphasise that these are speculative sentences, often from discussions and introductions of papers.

You cite this paper Takeuchi and Benning 2019, the title is "Nitrogen-dependent coordination of cell cycle, quiescence and TAG accumulation in Chlamydomonas" and the quote is from the background section. Where is the paper providing the strong evidence for this "generally accepted conclusion"?

If the papers that are being cited are being tentative, then the authors of this paper need to be tentative too. If this is so well established, why did the authors not just point me to a figure in a results section of one these papers?

In conclusion, the authors need to remove these sentences or parts of sentences.

Line 167 "Thus, to decrease the potential damaging effect of excess light energy under nutrient 168 limitation – and increase survival, photosynthesis is down-regulated"

Line 310: "In addition to down-regulating photosynthesis to avoid damage and increase survival, the general acclimation response in…"

Decision letter (RSPB-2021-2669.R0)

21-Dec-2021

Dear Dr Nedelcu

I am pleased to inform you that your manuscript RSPB-2021-2669 entitled "A life history trade-off gene with antagonistic pleiotropic effects on reproduction and survival in limiting environments" has been accepted for publication in Proceedings B.

The AE has recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to their comments and revise your manuscript, and the accept decision is contingent on these revisions. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

To revise your manuscript, log into https://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Before uploading your revised files please make sure that you have:

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

5) Data accessibility section and data citation
It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository (https://royalsociety.org/journals/authors/author-guidelines/#data).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:
• DNA sequences: Genbank accessions F234391-F234402
• Phylogenetic data: TreeBASE accession number S9123
• Final DNA sequence assembly uploaded as online supplemental material
• Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311
NB. From April 1 2013, peer reviewed articles based on research funded wholly or partly by RCUK must include, if applicable, a statement on how the underlying research materials – such as data, samples or models – can be accessed. This statement should be included in the data accessibility section.

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Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Dr Locke Rowe
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
This manuscript addresses an interesting theoretical question and has the potential to be of high interest. Both reviewers think the paper has strong merit and is worthy of publication and the authors have done a good job of addressing their criticisms but there is one remaining point of contention with the more critical reviewer. In their initial review, the critical reviewer raised the point that there is little evidence that photosynthesis is down regulated because of high levels of ROS in the 3 studies cited as supporting this assertion that were key in setting up some of the concluding statements. The current study tests the hypothesis that RSL1 mediates the regulatory decision about whether to devote resources to short term reproduction or long-term survival and the data presented supports this hypothesis, but the additional strong conclusion of the ms. is that photosynthesis is down regulated in order to avoid oxidative damage. The reviewer thinks this conclusion is not reasonable given the citations and data presented in this study and that the response may simply be due to conservation of nutrient resources. This author response is based on a pretty fine parsing of the language, that the conclusion was the downregulation of photosynthesis was done to AVOID ROS production but not BECAUSE of ROS production as a proximate mechanism. Fair enough. I have read all of the cited articles and responses and I agree with the reviewer that despite the sound theoretical basis and frequent reference to ROS the only experimental evidence for ROS impacts was an upregulation of transcripts related to oxidative

stress in one paper cited in another part of the manuscript. So I agree with the authors about the very reasonable potential for ROS production but agree with the reviewer that this is apparently a well-considered hypothesis rather than something that has been confirmed experimentally and note the careful language used by the citations (probably, could, suggests, etc.) and the more important fact that the authors did not assess the mechanisms underlying loss of viability in the current study and therefore can't draw any conclusions about those mechanisms from their data. I would like to ask the authors to address the reviewers point by revising the language of the 2 remaining sentences identified by the reviewer as potentially overstating the findings so that it better acknowledges the reviewer's point (photosynthesis downregulation, survival impacts and RSL1 association have been shown but adaptive use to avoid ROS production is presumed) and use language more similar to the citations. Please add the citation showing increase in OS related transcripts under nutrient deprivation [36] to the citations for the contested point (line 308).

Line 167 "Thus, to decrease the potential damaging effect of excess light energy under nutrient limitation – and increase survival, photosynthesis is down-regulated"

Line 310: "In addition to down-regulating photosynthesis to avoid damage and increase survival, the general acclimation response in…"

Reviewer(s)' Comments to Author:
Referee: 2
Comments to the Author(s).
Having read through the response, the authors seemed to be concerned that I have misrepresented what they have said,

"We believe that this is a misunderstanding. We did not "claim that photosynthesis is downregulated BECAUSE of the damaging effect of photosynthesis", or the "downregulation of photosynthesis is a direct RESPONSE of high levels of Ros" as
indicated in the referee's comment. Rather, we stated that "under nutrient limitation, TO DECREASE the potential damaging effect of excess light energy, photosynthesis is downregulated (Wykoff et al 1998)"

This is a very fine distinction. So avoid confusion I will use the authors exact words:

"Thus, to decrease the potential damaging effect of excess light energy under nutrient limitation – and increase survival, photosynthesis is down-regulated"

What I am saying that it has not been demonstrated that photosynthesis is down-regulated to decrease the potential damaging effect of excess light energy.

In other words, this sentence (the authors sentence from the manuscript) "Thus, to decrease the potential damaging effect of excess light energy under nutrient limitation – and increase survival, photosynthesis is down-regulated"

does not follow from this (what the authors wrote in the response):

"What is generally accepted in the field is that the downregulation of the photosynthesis under nutrient deprivation avoids the potential damaging effects of ROS produced as a result of imbalances between excitation energy and reducing power in the absence of nutrients in the light."

The authors have provided more references and quotes. Having looked at these I cannot agree that this has been shown. The quotes that you provide refer to circumstantial evidence and speculation. Eg

“This SUGGESTS that either the formation of O2 radicals generated by photosynthetic electron transport or the hyperreduction of electron transport components downstream of the QB-binding site, or both, leads to a loss of viability in the sac1 mutant.”

“Indeed, when the snrk2.1 mutant is starved for S, it APPEARS to show molecular responses, such as the accumulation of transcripts associated with oxidative damage, ROS production, and apoptosis that are typical of organisms experiencing extreme environmental conditions”

Furthermore, with the decreased demand for reductant, the cell would tend to accumulate high potential electrons and excited chlorophyll molecules that WOULD interact with oxygen, creating reactive oxygen species (e.g. superoxides and singlet oxygen). These species COULD cause extensive cellular damage and also function as regulatory signals that modulate metabolic activity.

The words I highlight (some of which the authors also highlighted) emphasise that these are speculative sentences, often from discussions and introductions of papers.

You cite this paper Takeuchi and Benning 2019, the title is “Nitrogen-dependent coordination of cell cycle, quiescence and TAG accumulation in Chlamydomonas” and the quote is from the background section. Where is the paper providing the strong evidence for this “generally accepted conclusion”?

If the papers that are being cited are being tentative, then the authors of this paper need to be tentative too. If this is so well established, why did the authors not just point me to a figure in a results section of one these papers?

In conclusion, the authors need to remove these sentences or parts of sentences.

Line 167 “Thus, to decrease the potential damaging effect of excess light energy under nutrient 168 limitation – and increase survival, photosynthesis is down-regulated”

Line 310: “In addition to down-regulating photosynthesis to avoid damage and increase survival, the general acclimation response in…”

Author's Response to Decision Letter for (RSPB-2021-2669.R0)

See Appendix B.

Decision letter (RSPB-2021-2669.R1)

22-Dec-2021

Dear Dr Nedelcu

I am pleased to inform you that your manuscript entitled "A life history trade-off gene with antagonistic pleiotropic effects on reproduction and survival in limiting environments" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page

length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

Associate Editor
Board Member: 1
Comments to Author:
This manuscript addresses an interesting theoretical question and has the potential to be of high interest.

The first reviewer has raised some minor points that could be addressed in revision. Specifically, the statistical analysis performed needs to be better described in the manuscript

- Additional analyses and information on the statistical analyses used were included in this revised manuscript.

and the choice of nutrient deprivation using phosphate instead other nutrients such as nitrogenous compounds and the implications of that choice should be discussed.

- The choice of phosphate deprivation (relative to other nutrients) and the implications are now fully explained in the manuscript.

The 2nd reviewer raises a more substantive point that would require major revision and resubmission. Specifically, there is little evidence that photosynthesis is down regulated because of high levels of ROS in the 3 studies cited as supporting this assertion. So the study tests the hypothesis that RSL1 mediates the regulatory decision about whether to devote resources to short term reproduction or long-term survival and the data presented supports this hypothesis, but the additional strong conclusion of the ms.is that photosynthesis is down regulated in order to avoid oxidative damage. The reviewer raises the important point that this conclusion is not reasonable given the citations and data presented in this study and that the response may simply be due to conservation of nutrient resources. I agree with the reviewer that the authors need to either find citations that demonstrate that the downregulation of photosynthesis is a direct response of high levels of ROS, provide experimental data supporting this assertion, or modify the study conclusions.

- We appreciate the reviewer's concern. However, there seems to have been a misunderstanding regarding the interpretation of the link between ROS and the downregulation of photosynthesis. Specifically, we did NOT state or implied that the downregulation IS **IN RESPONSE** to high levels of ROS and/or oxidative damage; but rather **TO AVOID** this possibility. This is a generally accepted idea – as stated in the references we included in the manuscript. However, at low levels, ROS can act as signaling molecules, and in photosynthetic organisms they are known to be involved in regulating many processes related to photosynthesis. We made this clear (both in our response below and the text) and added additional references.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
The manuscript analyzes the life history trade-off between reproduction and survival in a unicellular algal species. Specifically, it focuses on the hypothesis of antagonistic pleiotropy in this process. By analyzing mutants of the RLS1 gene that are unable to suppress their reproduction in phosphate deprived environment, the authors can shed light on this relationship between life history traits. I think the study is well done but have two main comments that should be addressed:
1. The statistical analysis needs to be better explained and described in the manuscript
2. I believe the authors should discuss a) the choice of nutrient deprivation/starvation using phosphate in the context of other nutrients such as nitrogenous compounds and b) the implications of that choice for their results in the discussion

Major point 1: I did not see any specific details on the statistical analysis in the materials and methods of the paper. Furthermore, in the results section, no statistical analysis was presented. This should be addressed.

- Due to word limits, we only mentioned the use of a t-test, p values and the meaning of error bars in Figure legends ("Error bars represent 2xSE (3 biological replicates); *** denotes p <0.005 (t-test; final time point)").
- In this revised version, we included a specific Methods section on statistical analyses and also indicated the significance values in the Results section.

Also, when comparing the growth curves under different conditions/mutant strains, the authors should provide some estimates of variance in their data. Are these curves they present averages or estimates? What are the confidence intervals and would be for example the confidence interval around the difference in growth response (population growth - comparison of final carrying capacity and exponential growth timing).

- We assume the errors bars were not easily visible because of the labels (triangles and circles); we removed the labels and decreased the thickness of the lines for a better visualization of the error bars. As mentioned in the methods, the curves are based on averages of 3 biological replicates. We redid the statistical analyses and included more information in both figures and text.

Major point 2: microalgae can metabolize and use a variety of different types of nutrients. Specifically, they can produce polyphosphates, which require a large amount of energy to produce and that energy can be drawn from metabolic processes. The authors are investigating antagonistic pleiotropy, which they interpret in the context of downregulation of

photosynthesis. It is however also possible that the increased demand for polyphosphate production may draw energy from other sources and therefore create energy mediated trade-offs. I believe it would be valuable to discuss these scenarios at least in general terms.

- We were not able to find any studies suggesting that polyphosphates can accumulate in Chlamydomonas under phosphate deprivation (though it seems they can accumulate under S- and N-deprivation). Rather, it seems that polyphosphates accumulate after cultures are pre-conditioned to P-deprivation and then transferred to phosphate-repleted medium (which we have not done); the so-called luxury-uptake or over-plus response that cells utilize/mobilize during P-deprivation (Sanz-Lukue et al. 2020). Regardless, if the trade-off were based on energy related to the synthesis of polyphosphates, the response to phosphate deprivation in the RLS1 mutant should be independent of light; which we found to not be the case.

More importantly, it would be valuable for other researchers to gain insight into the decision of depriving algae of phosphate rather than other nutrient sources. As well, the authors should discuss some of the potential limitations of using just phosphate limitation

- We have previously shown that RLS1 is also induced under sulphur-deprivation, during stationary phase, and extended periods of dark (Nedelcu 2009); which was mentioned in the manuscript. We have included additional explanation for our decision to only use phosphate deprivation in these experiments. However, we appreciate the point raised by the reviewer and made clear that the antagonistic effect we observed for RLS1 when cultures are deprived by phosphate cannot necessarily be extended to all nutrients.

Below are some additional minor issues we discovered when reviewing the manuscript:
Line 18 & 40: Topic sentence - same in abstract and introduction, consider revising

Line 67 - use of "lastly" doesn't seem to make sense here

- replaced with “Furthermore,”

Line 124 - weird wording "by daily" - suggest rephrasing

- rephrased: “assessed daily… by counting the cells…”

Line 125: 4 tech reps on hemocytometer meaning - 4 counts on the same slide within the same aliquot, or 4 different aliquots onto different slides? Which counts as a technical replicate? I'm not sure I could replicate this.

- rephrased: “4 aliquots per culture”

Include t test results in results section, also methods. What statistical program was used? What were the predictor & response variables?

- T-test results were included in the Results; and additional information about the statistical analyses and the program was included in the Methods.

Line 201- 203: "Specifically, under an LD regime, the mutant population entered the stationary phase earlier (day 4 vs day 6) and achieved only ca. 75% of the wild-type population size (8.3x106 cells/ml vs 1.1x107 ; Figure 2c)." -> The claim that Mu entered stationary phase significantly before wildtype seems dubious; I think this claim could be strengthened w a statistical test

- We performed two-way ANOVA analyses and included the statistical significance in the text and figure.

Line 224 - "significant" -> significantly

- Replaced with “significantly”

Line 242: "anaddotional" -> "an additional"

- corrected

Line 246: "Under long-term nutrient deprivation" - need to define/explain this more - what constitutes 'long term'?

- Statement was rephrased to “under nutrient-deprivation”, as quiescence can be triggered soon after nutrient levels decrease (eg, by day 2 of N deprivation, greater than 70% of the population arrests; Tsai et al 2018)

Line 276: Good iteration of importance of work and strength of evidence

Line 296: Not sure about ending on this question unless it gets answered later in the text. If not, elaborate more to direct future research (ex "future research should investigate how RLS1 regulates these two life history traits")

- The question is answered in the next section – “RLS1’s life history trade-off activity is linked to photosynthesis”. To ensure this is clear, we added the sentence “Below we suggest that RLS1’s role as a life history trade-off gene involves the down-regulation of photosynthesis (and thus growth and reproduction) to increase survival in limiting conditions. “

Lines 324-334: I found this a little confusing; Could use a little more description about why they believe the data supports the third scenario

- We re-wrote that section and included additional information to support our conclusion

Line 339: affects -> affect

- Corrected

Lines 354-357: Ending to discussion could be strengthened - Could use a final sentence to tie the last section of the discussion together more clearly.

- we agree; and we added a sentence to the end of the discussion section.

Line 360-363: Kind of a long and awkward sentence, consider revising for clarity

- We agree; sentence was revised/simplified

Line 363: Why tie in yeast here at the end? This was only briefly discussed in the introduction and could be motivated better. Could be a bit broader here to encapsulate the other organisms mentioned in discussion (nematodes, V carteri... What about bacteria?)

- We have removed the reference to yeast; we summarized our findings in *C. reinhardtii* and compared it to the nematode example

Good summary of results and conclusions

Line 369-372: awk and long, consider condensing and simplifying.

- we agree; sentence was revised/simplified

Line 427: "et al" - can you find the other authors? Same comment throughout the ref list.

- the reference style was revised to include up to 5 authors – as required by the journal.

Line 454 ref error (period after initial)
Throughout ref list, capitalization of titles
Spacing in ref list - spaces between initials or not?
Journal abbreviations - period or not

- the reference style was revised/updated to conform to the journal style

Line 516: incomplete ref

- references completed

Figure feedback:

Fig 2: Not sure it makes sense to have diff line types when you're already splitting them up by matrix; what new info does this show? I suggest keeping the same line type throughout the panels to reduce clutter of the figure

- lines have been kept the same, as suggested

Fig 4: I think fig 4a could be restructured to keep time on x axis and keep y axes consistent across to make them easier to compare

- figure was restructured, as suggested

Fig 4 caption: is fig 4 really showing "comparisons"? Maybe "Growth curves"? I think "comparison" kind of implies a box plot or similar figure type. This doesn't indicate that the values are means, nor does it present the error. Cell counts and fluorescence readings can vary *a lot* between readings.

- we assume the comment refers to Fig 2 caption (as Fig 4 is not showing growth curves). The values are means and the error bars were shown. Unfortunately, because they were rather small they were fully or partially hidden under the symbols; we removed the symbols and made the line thinner to increase the visibility of error bars.

Referee: 2

Comments to the Author(s)

In this study, the authors obtain an RLS1 mutant from a collection and carry out growth assays of this and a wild type strain in growth media with and without phosphate. In addition, they carry out viability assays of the two strains. The basic idea is to test the hypothesis that RSL1 mediates the regulatory decision about whether to devote resources to short term reproduction or long-term survival. While the authors present data that supports this hypothesis, another strong conclusion of this study is that one of the reasons that photosynthesis is down regulated is in order to avoid oxidative damage. I think that this particular conclusion is not reasonable given the citations and data presented in this study, see my detailed comments.

> - We are not arguing/concluding that one of the reasons that photosynthesis is down regulated is in order to avoid oxidative damage. This idea is a rather well-accepted concept based on both theoretical and experimental data (see details below). We are suggesting that RLS1 is involved (in a central role) in this process.

1. I found the writing to be excellent, the manuscript was very clear especially in the results. The authors have done a good job of motivating and providing context for their results, so that everything is very easy to understand.

I did find the concluding sentence in the abstract a bit tricky to understand: “We propose a model where the antagonistic effect involves the downregulation of photosynthesis, which mediates the acclimation response to nutrient deprivation in all photosynthetic organisms in order to avoid oxidative damage and increase survival, though at a cost to immediate reproduction.” Reading this sentence, I am not clear what mediates the acclimation response- the antagonistic effect (down regulation) or photosynthesis itself? This sentence could be rewritten to remove the ambiguity and also to break this into two sentences.

> - The sentence was re-written to remove ambiguity; and also to fit into the word-limit imposed by the journal

2.line 155 I would like to know why this data is not shown. This knockdown strain is referred to again in line 188 - I know that “data not shown” is sometimes mentioned in other studies, but I think that the authors should show the data from the knock down strain if they are going to repeatedly use it to support their claims.

> - The data on the knockdown strain were part of a project that we initiated a few years back, in collaboration with a different lab. Since the genomic mutant became available, we switched to using this mutant because is more stable than RNAi transformants and is publicly available. To show those data we would have to also include a rather large

section on the development of the transformant; also, we have not kept the transformant in culture and so we would not be able to provide it if requested. In this revised version, we have removed the mention of the transformant to avoid similar questions. We believe the genomic mutant provides strong evidence on its own.

3.Line 174 Here the discussion about the role of ROS is worded as if it has been shown that photosynthesis is downregulated in order to reduce the production of ROS.

- Yes, it has been shown. The references included in the statement (Wykoff et al 1998) discusses this based on findings from a mutant deficient in acclimation to sulphur (also see discussion below); we have included the original reference and added another more recent reference discussing another acclimation mutant.

This is different from the demonstration that photosynthesis results in the production of ROS. The authors are claiming that photosynthesis is down regulated because of the damaging effect of photosynthesis.

- We have not claimed that. What is generally accepted in the field is that the downregulation of the photosynthesis under nutrient deprivation avoids the potential damaging effects of ROS produced as a result of imbalances between excitation energy and reducing power in the absence of nutrients in the light (see discussion below)

And later, the conclusion is that viability of the mutant is low because of the uncontrolled production of ROS.

- Because other mutants defective in the acclimation response have low viability due photooxidative damage, we suggest that RLS1 mutant's viability (whose reproduction is not suppressed during light, as it is expected for a cell that is able to properly acclimate to nutrient deprivation) is also decreased because of such damage

However, when I followed the citations used by the authors I don't think this link has been firmly established. For example, the sentence "Thus under nutrient limitation to decrease the potential damaging effect of excess light energy, photosynthesis is downregulated (Wykoff et al 1998)". Wykoff does not mention ROS except in the intro, where they write "A lack of coordination could result in the generation of toxic, reduced O2 species" and then cite Asada 1994. The next sentence " This acclimation process is a general stress response that coordinates nutrient availability with the metabolism of the cell and its growth and division potential, resulting in a temporary inhibition of cell division…(Grossman 2000). Reading Grossman, esp their section on phosphate limitation, the focus is on down regulation as a means to deal with nutrient limitation, not as a stress response. The simplest explanation for the downregulation of photosynthesis in the context of low nutrient availability, is that stopping the production of the photosynthetic machinery conserves nutrient resources. While, I think that it is reasonable to think that Ros play a role in the downregulation of photosynthesis, this is not as well

established as it appears in the in the author's writing- in fact I don't that this assertion has been experimentally tested. The authors need to either find citations that demonstrate that the downregulation of photosynthesis is a direct response of high levels of Ros or they need to remove this assertion. Alternatively, they may have their own experimental data that demonstrate this.

> - We believe that this is a misunderstanding. We did not "claim that photosynthesis is downregulated **BECAUSE** of the damaging effect of photosynthesis", or the "downregulation of photosynthesis is a direct **RESPONSE** of high levels of Ros" as indicated in the referee's comment.

Rather, we stated that "under nutrient limitation, **TO DECREASE** the potential damaging effect of excess light energy, photosynthesis is downregulated (Wykoff et al 1998)". In other words, if under nutrient stress photosynthesis is NOT downregulated, damaging ROS **CAN** be generated; so, the INABILITY to down-regulate photosynthesis under nutrient stress can have damaging effects.

The original manuscript included the following statement and references: "For instance, mutants that are **unable** to down-regulate the photosynthetic electron transport during nutrient deprivation die sooner than the wild-type when grown in the light due to accumulation of photooxidative damage; but when maintained in the dark, they can survive nutrient-deprivation as well as the wild-type strains do (Davies et al. 1996; Moseley et al. 2006)." So, there is evidence that the inability to down-regulate photosynthesis during nutrient deprivation results in photooxidative damage. Hence, the generally accepted conclusion is that the downregulation of photosynthesis is a response to avoid such damage; however, the downregulation itself is not due to ROS.

Wykoff et al 1998 mention the formation of oxygen radicals in such mutants (see below) - based on Davies et al 1996; in this revised version we have added the reference Davies et al 1996 to the Wykoff et al 1998, and includes a more recent study with a different acclimation mutant (see below).

Wykoff et al 1998: "The reduction in photosynthetic electron flow that develops during nutrient limitation of C. reinhardtii cells is an active process and is necessary for survival. A sac1 mutant strain becomes light sensitive during S deprivation because it cannot alter photosynthetic electron transport. S starvation of sac1, as in wild-type cells, results in the induction of both qE and qT. These processes may be triggered by metabolic changes in nutrient-starved cells that are independent of the SacI signal-transduction pathway. However, in contrast to wild- type cells, the level of damaged PSII centers in the sac1 mutant reflects cell death and the mutant strain is unable to form PSII Q_B-nonreducing centers. DCMU, which phenocopies the formation of Q_B-nonreducing centers, rescues the lethal phenotype (Davies et al., 1996). **This suggests that either the formation of O_2 radicals generated by photosynthetic electron transport or**

the hyperreduction of electron transport components downstream of the Q_B-binding site, or both, leads to a loss of viability in the sac1 mutant. "

Gonzalez-Ballester et al (2010): "Indeed, when the snrk2.1 mutant is starved for S, it appears to show molecular responses, such as the accumulation of transcripts associated with oxidative damage, ROS production, and apoptosis that are typical of organisms experiencing extreme environmental conditions"

> -Regarding the referee's statement "Reading Grossman, esp their section on phosphate limitation, **the focus is on down regulation as a means to deal with nutrient limitation, not as a stress response.":** Downregulation of photosynthesis is not a stress response per se; rather is a response to AVOID potential stress resulting from imbalances between … see paragraph below from Grossman:

"A dramatic slowing of anabolic processes leads to a reduced need for ATP and NADPH generated by photosynthetic electron transport. Hence, even when nutrient-deprived cells are grown in moderate or low light, the photosynthetic electron transport chain will be fully reduced. The redox potential of the cell will increase as a consequence of hyper-reduction of the plastoquinone pool (and other photosynthetic electron carriers). This increase in intracellular redox potential will have a global effect on cellular metabolism. Furthermore, **with the decreased demand for reductant, the cell would tend to accumulate high potential electrons and excited chlorophyll molecules that WOULD interact with oxygen, creating reactive oxygen species (e.g. superoxides and singlet oxygen). These species COULD cause extensive cellular damage and also function as regulatory signals that modulate metabolic activity.**

> -Regarding the referee's statement "The simplest explanation for the downregulation of photosynthesis in the context of low nutrient availability, is that stopping the production of the photosynthetic machinery conserves nutrient resources.", see the paragraphs from Grossman, explaining that the **downregulation of photosynthesis allows the cells to more effectively dissipate excess absorbed excitation energy; and that is an active process critical to maintain cell viability in the light and involves the regulation of the photosynthetic electron transport (not the "production of the photosynthetic machinery"):**

"During nutrient limitation anabolic processes are slowed and NADPH is not rapidly recycled. This results in decreased photosynthetic electron flow and reduction of the plastoquinone pool. The reduced plastoquinone pool triggers phosphorylation of the light harvesting complex of photosystem II which results in the redirection of energy absorbed by the light harvesting pigments from photosystem II to photo- system I (the photosynthetic apparatus makes a transition from state 1 to state 2). **This can be beneficial to nutrient-deprived organisms since it decreases the production of NADPH, favors ATP production through cyclic electron transport and allows the cells to more effectively dissipate excess absorbed excitation energy.** P700, the reaction center of photosystem I, can quench excess absorbed light energy,

decreasing the potential toxic effect of light that occurs under conditions when the cells can neither grow or rapidly recycle the pool of NADPH. "

And: "One hallmark of the general responses to nutrient deprivation in Chlamydomonas is a marked decline in photosynthetic activity (Badger et al. 1980; Peltier and Schmidt 1991; Plumley and Schmidt 1989; Spalding et al. 1983c; Wykoff et al. 1998). **This decrease in photosynthetic activity is critical to sustain cell viability when nutrient levels fall.** This is illustrated by the phenotype of the sac1 mutant, which is defective for both the specific and general responses of Chlamydomonas to sulfur deprivation; this strain dies in the light within 2 d of being transferred to sulfur-free medium. **The decline in photosynthesis during exposure of wild-type cells to sulfur-limited growth is an active process controlled by the Sac1 polypeptide. Regulation of photosynthetic electron transport appears to be a critical aspect of tailoring the metabolism of the cell to nutrient availability. "**

Line 248 again here the authors assert that the non-reproductive and lowered metabolic state prevents oxidative damage. I would like the authors to provide the citations or show the experimental data that support these claims.

- The statement was based on the reference Takeuchi and Benning 2019, which was right before the start of the sentence in the original manuscript (see quotes below); we re-wrote that statement for clarity and included an additional reference (Tsai et al. 2018; see excerpt below)

Takeuchi and Benning 2019: "The entry into the quiescence cycle in the early G1 phase before genome replication is likely important for the maintenance of viability during quiescence and the successful reentry into the cell division cycle in response to growth-promoting cues. **Because quiescent cells cannot effectively dilute out molecules such as DNA damaged by reactive oxygen species (ROS) through growth and cell division, replace them through active synthesis, or repair them by energy-costly mechanisms, the condensation of chromosomes facilitates the preservation of genomic integrity and promotes survival** [37, 38, 62]."

"The maintenance of a quiescent state is an active process. The repression of genes associated with cell cycle progression, DNA synthesis and replication must be maintained in order to prevent the premature entry into the cell division cycle in the absence of nutrient(s), such as N. **The effective management of damaging reactive oxygen species (ROS) and the achievement of redox homeostasis are necessary to promote cellular survival during the non-dividing, energy-limited state**. "

Tsai et al 2018: "During quiescence, a plethora of metabolic adjustments has to take place. For example, because quiescent cells do not grow they cannot dilute out reactive oxygen species (ROS) as readily as actively growing and dividing cells. These are toxic to proteins or other macromolecules that cannot be replaced by rapid resynthesis during quiescence. Therefore, quiescent cells require specialized ROS-dissipating mechanisms to maintain redox

homeostasis." And "For photosynthetic organisms**, there is an additional challenge when entering quiescence: to reduce the highly redox-susceptible photosynthetic machinery in a way that it can be restored rapidly as conditions improve.** These include transcriptional modifications, such as down-regulation of photosynthetic genes"

Line 265 this concluding paragraph again assumes that the reduced viability is due to the accumulation of Ros in the RLS1 mutant. However, the reduced viability could be because these cells don't have the nutrient reserves to start growing again in favourable conditions. I would like the authors to either present this as a more likely explanation for this experimental result, or explain why it isn't correct.

- The concluding statement stated that it is "likely due to the accumulation of oxidative damage", and references Takeuci et al, who state that "The effective management of damaging reactive oxygen species (ROS) and the achievement of redox homeostasis are necessary to promote cellular survival during the non-dividing, energy-limited state " . We have no information (are not aware of such data) that suggests a need for "nutrient reserves" to allow cells to start growing again in favourable conditions. If nutrients become available, those nutrients should be enough to restore growth…

Line 306. Meager et al is cited in support of this statement: "In C. reinhardtii, when nutrients (e.g., phosphorus, sulphur, nitrogen) are limited, imbalances between excitation energy and cell's reducing power result in the down-regulation of photosynthesis while maintaining the capacity for light dissipation, as an adaptive response to avoid potential light-induced, oxidative damage (Meager et al 2021)". This study mentions ROS in the first line of the intro, but does not show any data pertaining ROS and photosynthesis.

- Again, our statement does not say that ROS are responsible for the downregulation of photosynthesis; rather, redox changes associated with imbalances between excitation energy and cell's reducing power result in the down-regulation of photosynthesis to AVOID POTENTIAL light-induced, oxidative damage. The study cited shows that during stationary phase (ie, low nutrients) there are **clear changes in the photosynthetic apparatus and function**, including the downregulation of chlorophyll (which can be a potential cellular photo-toxin when the absorbed light energy is diverted inappropriately to oxygen, leading to the production of ROS; Hörtensteiner and Kräutler 2011), and the expression of a stress protein that has been shown to minimize the potential for light-induced ROS production under stress (see discussion in Meager et al; and Damoo and Durnford 2021). The study showed such changes and discusses their implications for avoiding photooxidative damage during stationary phase. We included additional references, including Grossman's review

Line 366 here the authors state that a nutrient limiting environments RLS1 down regulates photosynthesis to avoid oxidative damage again without any citations or supporting data.

- The statement was based on (as discussed in the manuscript and this response) other mutants that cannot down-regulate photosynthesis in nutrient-limiting conditions and die in the light but not dark – suggesting that they succumb to photo-oxidative damage (see above excerpts from Grossman). However, we changed the concluding statemenet to "Our data show that in the unicellular species, *C. reinhardtii*, a single gene – *RLS1*, can adaptively adjust both survival and reproduction as part of the general acclimation response that ensures survival at a cost to immediate reproduction, by regulating photosynthetic activities in response to nutrient and light availability."

4.Line 219 I think this growth essay is referring to a direct competition essay with the two strains of mixed in coculture this needs to be explained a little more clearly to distinguish it from the previous growth experiments. Or when I look closely at the data is figure 3 simply the re plotting of the same data from figure 2?

- Yes; figure 3 is a replotting of data from figure 2 to allow direct comparisons between strains; as opposed to comparisons within strains (in figure 2). The text was edited to avoid this misunderstanding.

5.Line 242 "anaddotional" needs to be corrected (an additional?)

- corrected

Appendix B

Associate Editor
Comments to Author:

I would like to ask the authors to address the reviewers point by revising the language of the 2 remaining sentences identified by the reviewer as potentially overstating the findings so that it better acknowledges the reviewer's point (photosynthesis downregulation, survival impacts and RSL1 association have been shown but adaptive use to avoid ROS production is presumed) and use language more similar to the citations.

- The sentences have been revised – see below; and in the appended manuscript

Please add the citation showing increase in OS related transcripts under nutrient deprivation [36] to the citations for the contested point (line 308).

- The reference was added; see appended manuscript

Line 167 "Thus, to decrease the potential damaging effect of excess light energy under nutrient limitation – and increase survival, photosynthesis is down-regulated"

- changed to "Thus, it has been suggested that in order to decrease the potential damaging effect of excess light energy under nutrient limitation – and ***increase survival***, photosynthesis needs to be down-regulated [30,32,36]"

Line 310: "In addition to down-regulating photosynthesis to avoid damage and increase survival, the general acclimation response in…"

- changed to" In addition to down-regulating photosynthesis ~~to avoid damage and increase survival~~, the general acclimation response in *C. reinhardtii* also involves the temporary cessation of reproduction [31]"