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PROCEEDINGS B

Differences in mitochondrial efficiency explain individual variation in growth performance

Karine Salin, Eugenia M. Villasevil, Graeme J. Anderson, Simon G. Lamarre, Chloé A. Melanson, Ian McCarthy, Colin Selman and Neil B. Metcalfe

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Review timeline
Original submission: 4 April 2019
1st revised submission: 21 June 2019
2nd revised submission: 1 August 2019
Final acceptance: 1 August 2019

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

Review History

RSPB-2019-0771.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?
Excellent

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

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
See attached (Appendix A)

Review form: Reviewer 2 (Tony Hickey)

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

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

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?
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?
No

Is it clear?
No

Is it adequate?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author
I have read the manuscript RSPB-2019-0771, titled "Differences in mitochondrial efficiency explain individual variation in growth rates" by Salin et al. It is an interesting data set and shows some very important relationships between mitochondrial efficiency and growth/protein synthesis in brown trout. The relationship revealed in this study is interesting as it links mitochondrial efficiency to greater protein synthesis at least in liver. This is perhaps a first. For the most part it is well written and well structured.
I do however have some major issues in that there is information missing from the methods, and this could impact the interpretations.
My main issue is that the concentrations and descriptions of substrates are missing from the manuscript. The ATP/O ratios are on the low side as these should be around 1.5 (with succinate)-2-3 (glutamate or pyruvate), and the maximal ATP/O ratio in this study is around 1.4. This suggests there are some issues with the mitochondria, i.e. they are partially uncoupled or they are using succinate, or there is some issue with the calculation of the ATP flux. It is also hard to tell what the cause of "efficiency" differences result from in this study because there is a lack of information.

If multiple substrates have been used there may not be a difference in proton leak, there may be a difference in respiratory complex flux, i.e. complex I may be functional at a lesser level than complex II.
This will alter the ATP/O ratio. CI chains will translocate 10 protons while CII will move 6. This will change the ATP/O ratio and there may be no difference in proton conductance. In addition super-complex composition differs in rat liver mitochondria if fasted or fed, and exercised and this can impact flux (useful ref https://www.cell.com/cell-metabolism/pdfExtended/S1550-4131(16)30582-4). Therefore, it is necessary to see the substrates, and also concentrations. These appear to be missing.
It would also be useful to have additional data and traces, which could be supplemental if space is limiting. Some representative traces of oxygen flux and ATP synthesis could be useful and comparisons of fluxes between the two groups could be useful as well. i.e. what are the RCRs (reasonable predictor of quality) and oxygen fluxes?

Some minor issues
Line 63: GTP is used for DNA synthesis technically. But maybe state ATP is ultimately the energy source...
Line 69: … and energy rich substrate oxidised by mitochondria (i.e. pyruvate, glutamate, acetyl-CoA...)

Line 209: concentrations and range of substrates are missing
Line 215: to molecular oxygen?
Line 326: Are there any other studies that support this?
Line 353: decreased ROS

Decision letter (RSPB-2019-0771.R0)

13-May-2019

Dear Dr Salin:

I am writing to inform you that your manuscript RSPB-2019-0771 entitled "Differences in mitochondrial efficiency explain individual variation in growth rates" 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.

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,
Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
In this paper, Salin and coauthors examine the proximate factors that explain variation in individual growth rates, in brown trout fed two different diets (high and low food rations); and

then measured metabolic efficiency in terms of the amount of ATP generated per molecule of oxygen (ATP/O ratio) consumed by the liver and muscle mitochondria, and the rate of protein synthesis in these same tissues. The striking result, is that even within a food ration treatment, there is tremendous variation (10-fold difference) in growth rates among individuals, and these differences can be traced to mitochondrial efficiency in the liver.

Two referees found this paper to present interesting and novel results, and noted that the paper was well written. I agree. Referee 1 highlighted some ambiguities in the discussion of the paper, which require clarification (the referee's comments are provided in an attached document). Referee 2 noted some major issues that need to be attended to:

Explanation of some key pieces of methods is missing from the manuscript (concentrations and identity of substrates used).

ATP/O ratios are lower than expected suggesting issues with the mitochondria assayed. It is also unclear what is driving the efficiency differences -- these details require attention.

Also, representative traces of oxygen flux and ATP synthesis for the tissues and ration states should also be presented in the supplementary material, to enable the reader to directly interpret the data.

Please prepare a careful revision that attends to each the referees' comments.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
See attached

Referee: 2

Comments to the Author(s)
I have read the manuscript RSPB-2019-0771, titled “Differences in mitochondrial efficiency explain individual variation in growth rates” by Salin et al. It is an interesting data set and shows some very important relationships between mitochondrial efficiency and growth/protein synthesis in brown trout. The relationship revealed in this study is interesting as it links mitochondrial efficiency to greater protein synthesis at least in liver. This is perhaps a first. For the most part it is well written and well structured.
I do however have some major issues in that there is information missing from the methods, and this could impact the interpretations.
My main issue is that the concentrations and descriptions of substrates are missing from the manuscript. The ATP/O ratios are on the low side as these should be around 1.5 (with succinate)-2-3 (glutamate or pyruvate), and the maximal ATP/O ratio in this study is around 1.4. This suggests there are some issues with the mitochondria, i.e. they are partially uncoupled or they are using succinate, or there is some issue with the calculation of the ATP flux. It is also hard to tell what the cause of “efficiency” differences result from in this study because there is a lack of information.

If multiple substrates have been used there may not be a difference in proton leak, there may be a difference in respiratory complex flux, i.e. complex I may be functional at a lesser level than complex II.

This will alter the ATP/O ratio. CI chains will translocate 10 protons while CII will move 6. This will change the ATP/O ratio and there may be no difference in proton conductance. In addition super-complex composition differs in rat liver mitochondria if fasted or fed, and exercised and this can impact flux (useful ref https://www.cell.com/cell-metabolism/pdfExtended/S1550-4131(16)30582-4). Therefore, it is necessary to see the substrates, and also concentrations. These appear to be missing.
It would also be useful to have additional data and traces, which could be supplemental if space is limiting. Some representative traces of oxygen flux and ATP synthesis could be useful and comparisons of fluxes between the two groups could be useful as well. i.e. what are the RCRs (reasonable predictor of quality) and oxygen fluxes?

Some minor issues
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Line 209: concentrations and range of substrates are missing
Line 215: to molecular oxygen?
Line 326: Are there any other studies that support this?
Line 353: decreased ROS

Author's Response to Decision Letter for (RSPB-2019-0771.R0)

See Appendix B.

RSPB-2019-1466.R0 (Revision)

Review form: Reviewer 1

Recommendation
Accept as is

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

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

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

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?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
I'm happy with the revisions.

Decision letter (RSPB-2019-1466.R0)

26-Jul-2019

Dear Dr Salin

I am pleased to inform you that your Review manuscript RSPB-2019-1466 entitled "Differences in mitochondrial efficiency explain individual variation in growth performance" has been accepted for publication in Proceedings B.

The referee does not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. 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 me know immediately.

To upload your 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 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, upload a new version through your Author Centre.

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. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g authorname_procb_ESM_figures.pdf
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. Please see: https://royalsociety.org/journals/authors/author-guidelines/

4) Data-Sharing and data citation
It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=RSPB-2019-1466 which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

5) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

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

Sincerely,

Professor Hans Heesterbeek
mailto:proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
Dear Dr Salin,

Many thanks for providing such a thorough response to the referees' comments, and for incorporating these into the revised manuscript. I believe the revised paper makes an important contribution to the field. Best wishes

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).
I'm happy with the revisions.

Decision letter (RSPB-2019-1466.R1)

01-Aug-2019

Dear Dr Salin

I am pleased to inform you that your manuscript entitled "Differences in mitochondrial efficiency explain individual variation in growth performance" 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.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

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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.

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

I have reviewed Salin et al. 's manuscript titled 'Differences in mitochondrial efficiency explain individual variation in growth rates.' This study evaluates the relationship between food intake, mitochondrial performance, and growth rate in brown trout. The authors found that the high variation in the growth rate of fishes within each of 2 dietary treatment groups was driven by variation in mitochondrial performance. This is an exciting result and the first study to directly show that individual differences in the rate of growth, on standardize intake, is driven by the capacity of the mitochondria. This is an exciting, carefully designed, and well-written paper that will be of interest to the Proceedings B readership. The results needed additional clarification, but honestly, I found the set and conclusions in the rest of the manuscript to be spot on. Barring a few paragraphs in the results, the authors did an excellent job submitted a highly polished paper.

In the final three paragraphs of the results section, several of the statements were hard to tie to a specific line in table 2. For a few lines – it wasn't clear to me if the statement was erroneous or if I wasn't clear on what like of data I should be referring to.

- Example of unclear wording:
    - *'Differences between individuals in growth performance were positively linked to variation in mitochondrial properties rather than to fractional rates of protein synthesis (table 2). Not surprisingly, food intake had a positive effect on specific growth rates, with fish on average growing threefold faster at the high compared to the low ration.'*
        - I see liver ATP/O as associated with specific growth rate, and muscle Ks is also be associated with specific growth rate, but that isn't consistent with your description.
    - *'Growth efficiency varied among individuals from -0.13 to 2.23 gain in body mass per mass of food eaten (table S1) but did not differ between low and high food fish (table 2; LMM, effect of food intake, P > 0.05)'*.
        - I don't see food intake as a line of data in the table, is this not table 2?

- For clarification, for each paragraph, clearly state what 'dependent variable you are referring to, i.e., 'specific growth rate'- not growth performance, 'growth efficiency' (this was fine), and 'specific protein gain.'
- Make sure what 'source of variation' you are talking about is clear; I suggest either using the exact name for the source of variation you listed in the table or put it in parentheses next to the description if the exact same wording is not used.

One additional stylistic suggestion. The resolution in the statistics should match the resolution in the data. In many cases, the authors appeared to include many more significant figures in their stats than they had in their data. - i.e., in table S1 the low food intake growth efficiency was x=1.39. Presumably, these came from measures in grams to the hundredths decimal place – resulting in 3 significant figures. The f statistic for this line of data had five significant figures, suggesting that the stats have greater resolution than the data that it is based on – which could not be accurate. I strongly recommend consistency.

Congrats on a nice job on an important study.

Appendix B

AUTHORS' RESPONSES TO ASSOCIATE EDITOR AND REVIEWERS:

Associate editor' comments to the authors:

Comment**:** In this paper, Salin and coauthors examine the proximate factors that explain variation in individual growth rates, in brown trout fed two different diets (high and low food rations); and then measured metabolic efficiency in terms of the amount of ATP generated per molecule of oxygen (ATP/O ratio) consumed by the liver and muscle mitochondria, and the rate of protein synthesis in these same tissues. The striking result, is that even within a food ration treatment, there is tremendous variation (10-fold difference) in growth rates among individuals, and these differences can be traced to mitochondrial efficiency in the liver. Two referees found this paper to present interesting and novel results, and noted that the paper was well written. I agree.

Response: We thank the associate editor for their handling of this manuscript and their insightful and thoughtful critiques. We have attempted to address all associate editor and reviewer suggestions and concerns. These critiques are much appreciated and we think that the resulting changes in the text have significantly improved the message delivered in the revised manuscript. Specific responses to individual editor and reviewer comments are detailed below.

Comment**:** Referee 1 highlighted some ambiguities in the discussion of the paper, which require clarification (the referee's comments are provided in an attached document).

Response: We have amended the results section such that the dependent variables and the source of variation are now clearly stated (Lines 262 to 283). We have also edited the results section so that the names of variables in the text and tables are now consistent.

Comment**:** Referee 2 noted some major issues that need to be attended to: Explanation of some key pieces of methods is missing from the manuscript (concentrations and identity of substrates used).

Response: We did provide information on the full protocol – including concentrations and identity of substrates and inhibitors used – in the supplementary material (ESM: Lines 121 – 132); we felt that this was the best place for it, but can move it to the main text if the editor would prefer this. In addition, we did provide in the main text a reference that publishes a detailed outline of the methodology (Main Document: line 203).

Comment**:** ATP/O ratios are lower than expected suggesting issues with the mitochondria assayed. It is also unclear what is driving the efficiency differences -- these details require attention.

Response: In our study, the ATP/O ratios were calculated as the amount of ATP generated per unit of oxygen consumed and these values are usually lower that the theoretical maximum ATP/O value (Brand 2005). This is mainly explained by the fact that there is a significant rate of oxygen consumption that is used to counter the proton leak rather than to synthesize ATP (Brand, Harper and Taylor 1993). The theoretical maximum ATP/O value is calculated based on mitochondrial

stochiometry that excludes any proton leak. However, this proton leak is physiological and significant, so making the theoretical maximum ATP/O value unrealistic. The extent of the rate of oxygen consumption that is required to offset the proton leak differs between individual animals (Salin et al. 2016a), and this variation in proton leak respiration is likely to drive the differences in ATP/O ratio that we have observed between individuals in this study.

Comment**:** Also, representative traces of oxygen flux and ATP synthesis for the tissues and ration states should also be presented in the supplementary material, to enable the reader to directly interpret the data.

Response: Done – we have included representative traces as requested (electronic supplementary material: Figure S3, pages 10).

Reviewers' comments to the authors:

Referee: 1

Comment**:** I have reviewed Salin et al. 's manuscript titled 'Differences in mitochondrial efficiency explain individual variation in growth rates.' This study evaluates the relationship between food intake, mitochondrial performance, and growth rate in brown trout. The authors found that the high variation in the growth rate of fishes within each of 2 dietary treatment groups was driven by variation in mitochondrial performance. This is an exciting result and the first study to directly show that individual differences in the rate of growth, on standardize intake, is driven by the capacity of the mitochondria. This is an exciting, carefully designed, and well-written paper that will be of interest to the Proceedings B readership. The results needed additional clarification, but honestly, I found the set and conclusions in the rest of the manuscript to be spot on. Barring a few paragraphs in the results, the authors did an excellent job submitted a highly polished paper.

Response: We thank the referee for their time and effort invested in the review of our work. We have addressed each of their concerns in full as outlined below.

Comment**:** In the final three paragraphs of the results section, several of the statements were hard to tie to a specific line in table 2. For a few lines – it wasn't clear to me if the statement was erroneous or if I wasn't clear on what like of data I should be referring to.

- Example of unclear wording:
    - 'Differences between individuals in growth performance were positively linked to variation in mitochondrial properties rather than to fractional rates of protein synthesis (table 2). Not surprisingly, food intake had a positive effect on specific growth rates, with fish on average growing threefold faster at the high compared to the low ration.'

- I see liver ATP/O as associated with specific growth rate, and muscle Ks is also be associated with specific growth rate, but that isn't consistent with your description.

Response: Done - thank you for this comment, we have now corrected the wording and we hope that it is now clear (Lines 271-273, lines 293-294, lines 324-326, lines 333-337).

Comment**:**

- 'Growth efficiency varied among individuals from -0.13 to 2.23 gain in body mass per mass of food eaten (table S1) but did not differ between low and high food fish (table 2; LMM, effect of food intake, $P > 0.05$)'.
  - I don't see food intake as a line of data in the table, is this not table 2?

Response: Done - we apologise for this error. This is now corrected (Line 276).

Comment: For clarification, for each paragraph, clearly state what 'dependent variable you are referring to, i.e., 'specific growth rate'- not growth performance, 'growth efficiency' (this was fine), and 'specific protein gain.' Make sure what 'source of variation' you are talking about is clear; I suggest either using the exact name for the source of variation you listed in the table or put it in parentheses next to the description if the exact same wording is not used.

Response: Done - we have now replaced "growth performance" with the dependent variable we are referring to (Lines 262-264). We have also edited the results section so that the variables in the text and tables are now named with consistency (Lines 290 and 291).

Comment**:** One additional stylistic suggestion. The resolution in the statistics should match the resolution in the data. In many cases, the authors appeared to include many more significant figures in their stats than they had in their data. - i.e., in table S1 the low food intake growth efficiency was x=1.39. Presumably, these came from measures in grams to the hundredths decimal place – resulting in 3 significant figures. The f statistic for this line of data had five significant figures, suggesting that the stats have greater resolution than the data that it is based on – which could not be accurate. I strongly recommend consistency.

Response: Done - Thanks for pointing out this error, this has now been corrected as you have suggested.

Comment**:** Congrats on a nice job on an important study.

Response: Thank you for your careful reading of our manuscript and for your comments/suggestions that have improved manuscript quality.

Referee: 2

Comments to the Author(s)

Comments: I have read the manuscript RSPB-2019-0771, titled “Differences in mitochondrial efficiency explain individual variation in growth rates” by Salin et al. It is an interesting data set and shows some very important relationships between mitochondrial efficiency and growth/protein synthesis in brown trout. The relationship revealed in this study is interesting as it links mitochondrial efficiency to greater protein synthesis at least in liver. This is perhaps a first. For the most part it is well written and well structured. I do however have some major issues in that there is information missing from the methods, and this could impact the interpretations.

Comments: My main issue is that the concentrations and descriptions of substrates are missing from the manuscript.

Response: We take the referee’s point that it would be good to have these details in the main text of the manuscript, however, since the article is already at the upper word limit we think it would be more judicious to give priority to other key information in the main document and to provide details on the concentrations and descriptions of the substrates in the electronic supplementary material – ESM (Lines 121 to 132) as the titration protocol has already been published in full detail elsewhere (Adjusted to homogate of fish tissue: Salin et al. 2016b, Original protocol: Chinopoulos et al. 2014).

Comments: The ATP/O ratios are on the low side as these should be around 1.5 (with succinate)-2-3 (glutamate or pyruvate), and the maximum ATP/O ratio in this study is around 1.4. This suggests there are some issues with the mitochondria, i.e. they are partially uncoupled or they are using succinate, or there is some issue with the calculation of the ATP flux.

Response: In our study, the calculated ATP/O ratios were 1.07 ± 0.02 and 1.47 ± 0.12 in the mitochondria of liver and muscle, respectively. We agree that these effective ATP/O ratios are somewhat lower than the theoretical maximum ATP/O ratio for succinate as a fuel (1.636 (Brand 2005), after correction for ATP stochiometry based on (Watt et al. 2010)), and lower than the theoretical maximum value with pyruvate malate as the fuel (2.727; (Brand 2005), after correction for ATP stochiometry (Watt et al. 2010)).

Although our values were lower than the theoretical maximum ones, there are a number of reasons to suggest that the theoretical values might not be approriate and our mitochondria and calculation of the ATP flux are correct.

- **Empirical evaluation of the ATP/O ratio is expected to be lower than the theoretical maximum ATP/O ratio**. In our study, the ATP/O ratios were calculated as the amount of ATP generated per unit of oxygen consumed and they are usually lower that the expected maximum ATP/O value (Brand 2005). This is mainly explained by the fact that there is a significant rate of oxygen consumption that is used to counter the proton leak and not to synthesize ATP (Brand et al. 1993). The theoretical maximum ATP/O values are calculated based on mitochondrial stochiometry that excludes any proton leak. However, this proton leak is physiologic and significant, so making the theoretical maximum ATP/O value excessively high. The extent of this proton leak differs between

individual animals (Salin et al. 2016a), and this variation in proton leak is likely to drive the differences in ATP/O ratio that we have observed between individuals in this study.

- **Limitations in the comparison of ATP/O ratio between animal models, tissues, mitochondrial preparation and experimental conditions**: In the study by Brand (2005), theoretical maximum values were determined in isolated mammalian mitochondria, and unfortunately there are few comparable data on effective ATP/O ratios in fish. Those that exist make it clear that direct comparisons with mammalian data are not straightforward: effective ATP/O ratios calculated from complex I or complex II substrates have been found to be lower (Strobel et al. 2013, Power et al. 2014, Bryant, Chung and Schulte 2018) or even higher (Power et al. 2014, Strobel et al. 2013) than the theoretical maximum ATP/O ratio calculated in Brand (2005). These differences may arise due to a number of confounding factors including differences in the substrates, assay temperature, method for measuring ATP, mitochondrial preparation, tissue and species specificity (Hinkle 2005).

- **Validation of our ATP assay and ATP/O calculation**: We have shown that our assay method (mitochondrial preparation, mitochondrial experimental conditions and calculation of fluxes and ATP/O ratio) is highly repeatable (Salin et al. 2016b) and sensitive enough to detect differences between treatment groups. We provide more details on the quality of the mitochondria below.

Comments: It is also hard to tell what the cause of "efficiency" differences result from in this study because there is a lack of information. If multiple substrates have been used there may not be a difference in proton leak, there may be a difference in respiratory complex flux, i.e. complex I may be functional at a lesser level than complex II. This will alter the ATP/O ratio. CI chains will translocate 10 protons while CII will move 6. This will change the ATP/O ratio and there may be no difference in proton conductance. In addition super-complex composition differs in rat liver mitochondria if fasted or fed, and exercised and this can impact flux (useful ref https://www.cell.com/cell-metabolism/pdfExtended/S1550-4131(16)30582-4).

Response: We agree with the referee that substrate preferences and super-complex dynamics can greatly influence mitochondrial efficiency. However, in this study we did not attempt to measure any of the parameters that cause differences in mitochondrial efficiency, so we cannot track the contribution of the different complexes to substrate oxidation, as well as any changes in the super-complex composition. The purpose of our study was to determine whether differences in mitochondrial efficiency between individual are related to variation in growth performance. Further work will be needed to understand the mechanisms that underlie the observed variation in mitochondrial efficiency.

Comments: Therefore, it is necessary to see the substrates, and also concentrations. These appear to be missing.

Response: All the details on the titration protocol (including substrates and concentrations) are provided in the ESM (Lines 121 to 132).

Comment: It would also be useful to have additional data and traces, which could be supplemental if space is limiting. Some representative traces of oxygen flux and ATP synthesis could be useful.

Response: All the data will be made available from a Dryad Digital Repository, and fluxes of ATP and Oxygen will be included in this data table. Traces of oxygen flux and magnesium fluorescence for liver and muscle homogenate of fish have been added to the electronic supplementary material (pages 10 – Figure S3). Since the calibration curve of magnesium in our experimental conditions is quadratic, it was not possible to generate traces for ATP flux since (to our knowledge) the Datlab software can only handle linear calibration curves.

Comment: Comparisons of fluxes between the two groups could be useful as well.

Response: We take this point, but since the article is already at the upper word limit we think it would be more judicious to give priority to other key information as the main point to the article is to understand causes of individual differences. Again, those data will be made available from a Dryad Digital Repository.

Comment: What are the RCRs (reasonable predictor of quality) and oxygen fluxes?

Response: RCR values were very high (means ± standard error: Muscle: 10.86 ± 0.46 (n = 58); Liver = 13.70 ± 0.42 (n = 59)) which indicates a very high quality preparation, although comparison of RCR values between fish homogenate and isolated mammalian mitochondria is not straightforward due to the number of confounding factors (as discussed above for the effective ATP/O ratio). The protocol of homogenization of liver and muscle of juvenile brown trout has previously been validated, with similarly high RCR values under similar condition of temperature and substrates (Salin et al. 2016a). Likewise, RCR values were very good under our experimental conditions for the ATP assay (in presence of Mg Green probe, Salin et al. (2018b)). Increases in membrane potential between state 3 and state 4 (measured in the presence of safranin probe in a separate experiment in trout) is an additional strong indicator of the quality of the mitochondria in our experimental system (Salin et al. 2018a). We can thus assert that the mitochondria in our experimental conditions were of good quality. RCR data will be made available from a Dryad Digital Repository.

Some minor issues

Comments: Line 63: GTP is used for DNA synthesis technically. But maybe state ATP is ultimately the energy source...

Response: As far as we know, ATP molecules remain the main source of energy for DNA synthesis; GTP is a material substrate.

Comments: Line 69: … and energy rich substrate oxidised by mitochondria (i.e. pyruvate, glutamate, acetyl-CoA...)

Response: Done (line 68).

Comments: Line 209: concentrations and range of substrates are missing

Response: All the details on the titration protocol are provided in the ESM (Lines 121 to 132).

Comments: Line 215: to molecular oxygen?

Response: Yes, the ratio of ATP production is divided by twofold the rate of molecular oxygen. This is now clarified (Line 214).

Comments: Line 326: Are there any other studies that support this?

Response: There is at least one study that shows a tissue-specific response of the rate of protein synthesis (Ks) to experimental treatment (Cassidy, Saulnier and Lamarre 2016), but we are not aware of any other work looking at the correlation of protein synthesis rates across different tissues in the same individual.

Comments: Line 353: decreased ROS

Response: Done (line 318).

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