THE ROYAL SOCIETY PUBLISHING

PROCEEDINGS B

Resilience of cold-water coral holobionts to thermal stress

Leila Chapron, Pierre E. Galand, Audrey M. Pruski, Erwan Peru, Gilles Vétion, Sarah Robin and Franck Lartaud

Article citation details
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Review timeline

Original submission:	2 March 2021
1st revised submission:	24 September 2021
2nd revised submission:	17 November 2021
Final acceptance:	23 November 2021

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

Review History

RSPB-2021-0501.R0 (Original submission)

Review form: Reviewer 1

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?
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?
No

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
Review Chapron et al.
Resilience of cold water coral holobionts to sea water temperature changes

In this study, Chapron et al. simulated the impact of global warming on deep-water corals. Using samples of Lophelia pertusa and Madrepora oculata from the Mediterranean Sea, the coral physiology (feeding, growth, carbohydrate, lipid, protein, organic matter) and the associated bacterial community diversity (microbiome), was monitored for six months in aquaria.

It was revealed that a temperature increase of two degrees (end of century estimate) shifted the physiology and microbiome of L. pertusa but not of M. oculata. Initially, the microbiome of L. pertusa shifted towards an abundance of Rhodobacterales. It was not clear, but it seems the microbiome of M. oculata also became dominated by Rhodobacterales by, increased temperature. Notably, at higher temperature microbiomes appeared to converge and loose their 'host imprint' i.e. temperature became a stronger driver than host. Subsequently, feeding increased but energy reserves and skeletal growth became reduced. Additional temperature scenarios killed the corals (four degree increase) or resulted in no observable change? (three degrees reduction). Controls at in situ temperature (13 ℃) resulted in little to no change.

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Limitations may also be emphasized more. May this reference on aquaria experiments be helpful to include? Orejas et al. 2019, Cold-Water Coral in Aquaria: Advances and Challenges. A Focus on the Mediterranean. In: Mediterranean Cold-Water Corals: Past, Present and Future. May the corals have been somehow stressed by cutting into nubbins? What about adaptation, in a natural setting temperature change over decades whereas in aquaria over months. May a reference be

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Review form: Reviewer 2

Recommendation
Reject – article is not of sufficient interest (we will consider a transfer to another journal)

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

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

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Marginal

Is the length of the paper justified?
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Decision letter (RSPB-2021-0501.R0)

05-May-2021

Dear Miss Chapron:

I am writing to inform you that your manuscript RSPB-2021-0501 entitled "Resilience of cold water coral holobionts to sea water temperature changes" 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 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 Daniel Costa
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
Thank you for submitting your manuscript "Resilience of cold water coral holobionts to sea water temperature changes" to Proceedings B. Your manuscript has now been evaluated by two expert reviewers and myself. As you will see, both reviewers found the paper to be novel and interesting, on a topic of general interest. However, the reviewers raised concerns about the clarity of the manuscript and, more seriously, about the experimental design. Regarding the experimental design, Reviewer 2 points out that only four tanks were used, one at each temperature, which raises the concern that there could be tank effects that are unrelated to temperature effects. While there were, in fact, replicates of coral nubbins (the number of which in each tank should be reported), all of the nubbins exposed to a given temperature were in the same tank, which is problematic. Another effect of this design is that the microbes from one coral species are free to interact with the other coral species (ie, the two species that were studied were in the same tanks); therefore the observation that the microbiomes become more similar in the non-control temperatures could be because they shared microbes. Regarding the manuscript clarity, both Reviewer 1 and 2 provided several suggestions for improving the manuscript. In my estimation, it is clear that a lot of work went into this study and it represents a significant advance, but the concerns about experimental design are legitimate. The lack of tank replicates should be addressed somehow. If it were possible to include more replicates, that would be ideal, but I realize the amount of time and effort that this experiment required, and repeating it at double or triple the scale is likely impossible. Barring that, a careful revisiting of the statistics used and a defense of the experimental design might be persuasive. In particular, rather that treating temperature as a categorical variables with four values, consider treating it as a continuous variable. Consulting with a statistician may be helpful.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s)
Review Chapron et al.

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Author's Response to Decision Letter for (RSPB-2021-0501.R0)

See Appendices A & B.

RSPB-2021-2117.R0

Review form: Reviewer 1

Recommendation
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Scientific importance: Is the manuscript an original and important contribution to its field?
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Do you have any ethical concerns with this paper?
No

Comments to the Author
Chapron et al. (R1, considered as new submission)

Although improved, it seems further improvements would benefit this manuscript. Generally, try emphasising take home messages more. Attention is suggested given to readability. Clarity of text will also help guide the reader more easily trough all the figures. Regarding the figures, for example, Figure 1 has no error bars. What are the number of replicates? 4 to 25 for Lophelia and 6 to 30 for Madreopora (Ln 87)? Or, more than hundred each (Ln 106 to 110)? Sentence: "The 17°C data could not be used due to the high mortality observed after 2 months" (Ln 220) but there were measurements ahead of 2 months? Figure 3, here error bars shown but what are the number of replicates? Try improving flow of thought (readability). No tables were found in the main document. Consider whether converting any of the figures into a table would improve. The reason for reporting a ten degrees incubation is still a bit confusing. If tied more closely with the North Atlantic corals, i.e. other such corals thrive at this temperature, this may become clearer.

In the Results, the "Coral bacterial communities" paragraph is not so easy to understand. It is felt that especially Result sections readability can be improved (this will help improve understanding of Tables and Figures). Wherever possible, consider try shortening the manuscript because this may help focus.

Discussion, look over, try to improve.

Language can be improved (grammar and style).

Minor comments (line numbers refer to revision)

Ln 1: consider revert back to original title ("Resilience of cold water coral holobionts to sea water temperature changes") or minimum include "temperature". Sorry, I may have confused suggesting changes in the title. Temperature is in focus and "global change" may be too vague.

See also title going with the deposited sequences which also highlight temperature ("Cold water coral exposed to thermal stress").

Ln 19: the plus and minus signs may need support for clarity. Consider if using the term initially introduced in the Abstract "rising temperatures" (or "rising") will clarify its deviation from 13℃ (and not absolute temperature) and may be used instead of such as "higher".

Ln 164: PCR condition not found included: "Amplicon fragments are PCR-amplified using the high-fidelity Phusion polymerase under conditions of 30s at 98°C, 16 cycles of 98°C for 10s, 60°C for 30s, 72°C for 80s and final extension for 5m at 72°C."

Ln 179: I'm sorry, yes samples, I misunderstood "Biological" seems not needed. To simplify "Samples containing less than…"

Ln 190: how many sequences? I.e. what are the sample sizes counts were divided by to obtain normalized data? Show at least range, minimum to maximum.

Ln 196: why were not ANOVA but ANOSIM used for comparison of the bacterial communities? Has this to do with normality? Can this somehow be briefly explained?

Ln 335: should carbohydrates be discussed as well?

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Figure 4: legend, number of replicates? What are those stars above bars representing?

Supplementary: "Chapron et al. [28]…" should this be "Chapron et al. [29]"?

Table S2: in legend, include what differences that are compared. From the text it seems to be bacterial community diversity.

Decision letter (RSPB-2021-2117.R0)

28-Oct-2021

Dear Miss Chapron:

Your manuscript has now been again peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns. We normally do not allow multiple rounds of revision, but your manuscript still needs work before it can be considered further. We are willing to give you one final opportunity to adequately revise your manuscript to address them.

This will be your final opportunity to revise your manuscript so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please 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.

When submitting your revision please upload a file under "Response to Referees" in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the 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.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

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If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article (https://royalsociety.org/journals/authors/author-guidelines/#data). Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

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=(Document not available), 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.

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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. Please try to submit all supplementary material as a single file.

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].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Dr Daniel Costa
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
Thank you for revising your mansucript. The revised version is much improved, but there are still issues remaining that would preclude publication in its current form. In general, the text of the manuscript should be revised throughout to improve clarity and the narrative structure, and sections of the Results and Discussion could be shortened. The main points (take-home messages) could be made more explicit. Related to this, the reviewer suggests that the title be changed again; I would encourage the authors to take this advice but to settle on a title that clearly captures the main message of the paper. The figures and figure legends could be improved; in some cases error bars and number of replicates (n) need to be provided; in other cases consider if tables may be more effective. The methods are missing important details or are still lacking in clarity in places. Please respond to each of the reviewer comments in your revisions.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s).
Chapron et al. (R1, considered as new submission)

Although improved, it seems further improvements would benefit this manuscript. Generally, try emphasising take home messages more. Attention is suggested given to readability. Clarity of text will also help guide the reader more easily trough all the figures. Regarding the figures, for example, Figure 1 has no error bars. What are the number of replicates? 4 to 25 for Lophelia and 6 to 30 for Madreopora (Ln 87)? Or, more than hundred each (Ln 106 to 110)? Sentence: “The 17°C data could not be used due to the high mortality observed after 2 months” (Ln 220) but there were measurements ahead of 2 months? Figure 3, here error bars shown but what are the number of replicates? Try improving flow of thought (readability). No tables were found in the main document. Consider whether converting any of the figures into a table would improve. The reason for reporting a ten degrees incubation is still a bit confusing. If tied more closely with the North Atlantic corals, i.e. other such corals thrive at this temperature, this may become clearer.

In the Results, the “Coral bacterial communities” paragraph is not so easy to understand. It is felt that especially Result sections readability can be improved (this will help improve understanding of Tables and Figures). Wherever possible, consider try shortening the manuscript because this may help focus.

Discussion, look over, try to improve.

Language can be improved (grammar and style).

Minor comments (line numbers refer to revision)

Ln 1: consider revert back to original title ("Resilience of cold water coral holobionts to sea water temperature changes") or minimum include "temperature". Sorry, I may have confused suggesting changes in the title. Temperature is in focus and "global change" may be too vague. See also title going with the deposited sequences which also highlight temperature ("Cold water coral exposed to thermal stress").

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Supplementary: "Chapron et al. [28]…" should this be "Chapron et al. [29]"?

Table S2: in legend, include what differences that are compared. From the text it seems to be bacterial community diversity.

Author's Response to Decision Letter for (RSPB-2021-2117.R0)

See Appendices C & D.

Decision letter (RSPB-2021-2117.R1)

23-Nov-2021

Dear Miss Chapron

I am pleased to inform you that your manuscript entitled "Resilience of cold water coral holobionts to thermal stress" 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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Please remember to make any data sets live prior to publication, and update any links as needed when you receive a proof to check. It is good practice to also add data sets to your reference list.

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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,
Dr Daniel Costa
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Sorbonne Université, Observatoire Océanologique de Banyuls/Mer
Laboratoire d'Ecogéochimie des Environnements Benthiques (UMR 8222)
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www.obs-banyuls.fr

Appendix A

Leila Chapron,
Corresponding author,
chapron@obs-banyuls.fr
chapron.3@osu.edu

13th September 2021

Resubmission: Previous reference number RSPB-2021-0501

Dear Editor,

Please find enclosed our revised manuscript entitled 'Resilience of cold water coral holobionts to global change' by L. Chapron, P.E. Galand, A.M. Pruski, E. Peru, G. Vétion, S. Robin, and F. Lartaud for consideration for publication in *Proceedings of the Royal Society B: Biological Sciences*. This paper has not been published elsewhere and is the original work of the authors.

We would like to thank the reviewers for their positive feedback and their constructive comments. We have answered all their queries and we feel that it improved the quality of the paper. We hope that the editor and reviewers will be satisfied with this new version of our manuscript. A detailed point by point answer to the reviewers' comments is attached and all referred line changes can be found in the document chapronetal_trackedchanges.doc.

Because of the originality of our work, we think that our results will be of considerable interest for marine ecologists, oceanographers, biologists and toxicologists, and more widely to a broad audience interested in knowing more about the impact of global change on marine life.

Thank you for your time and consideration. We hope that you will consider our manuscript for publication in *Proceedings of the Royal Society B: Biological Sciences* and look forward to hearing from you.

Yours sincerely,

Dr. Leila Chapron

Appendix B

Dear Miss Chapron:

I am writing to inform you that your manuscript RSPB-2021-0501 entitled "Resilience of cold water coral holobionts to sea water temperature changes" 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 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 Daniel Costa
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
Thank you for submitting your manuscript "Resilience of cold water coral holobionts to sea water temperature changes" to Proceedings B. Your manuscript has now been evaluated by two expert reviewers and myself. As you will see, both reviewers found the paper to be novel and interesting, on a topic of general interest. However, the reviewers raised concerns about the clarity of the manuscript and, more seriously, about the experimental design. Regarding the experimental design, Reviewer 2 points out that only four tanks were used, one at each temperature, which raises the concern that there could be tank effects that are unrelated to temperature effects. While there were, in fact, replicates of coral nubbins (the number of which in each tank should be reported), all of the nubbins exposed to a given temperature were in the same tank, which is problematic.

We are pleased to read that the reviewers and the associate editor appreciated our work. We acknowledge that our manuscript was maybe not clear enough. We have carefully considered all the comments and improved the text by changing the writing and making statement about the experimental design and its limitations.

Another effect of this design is that the microbes from one coral species are free to interact with the other coral species (ie, the two species that were studied were in the same tanks); therefore the observation that the microbiomes become more similar in the non-control temperatures could be because they shared microbes.

We understand this concern. It is, however, unlikely that the microbes from L. pertusa interacted with the ones from M. oculata as both coral species select a very species-specific microbiome. It has been showed for corals reared in a same tank (Galand et al.Env. Microbiol. 2020) and in situ, where corals grow very close to each other in the same water (Meistertzheim DSR 2016). In the present study, the microbiomes of both species differed significantly from each other in the control (13°C), cooler (10°C) and warmer (15°C) conditions during the whole experiment suggesting no interactions between species. We would also like to emphasis that since the few ASVs shared between species at warmer temperatures were opportunists that did not originate from corals. Finally, by analyzing the microbiome of the surrounding water in the tanks we showed that coral and seawater always had different microbial communities. The seawater in the tanks was renewed continually (more than 1 time a day) reducing the probability for interactions.

Regarding the manuscript clarity, both Reviewer 1 and 2 provided several suggestions for improving the manuscript. In my estimation, it is clear that a lot of work went into this study and it represents a significant advance, but the concerns about experimental design are legitimate. The lack of tank replicates should be addressed somehow. If it were possible to include more replicates, that would be ideal, but I realize the amount of time and effort that this experiment required, and repeating it at double or triple the scale is likely impossible. Barring that, a careful revisiting of the statistics used and a defense of the experimental design might be persuasive. In particular, rather that treating temperature as a categorical variables with four values, consider treating it as a continuous variable. Consulting with a statistician may be helpful.

We are glad to read that the associated editor valued our work and appreciated the extend of the effort needed to obtain these results. We have followed the reviewers' suggestions to improve the quality of the manuscript and address the lack of tank replicates. We now specifically write: 'Our findings nevertheless to be considered with caution as they originate from laboratory experiments in which the replicates for each colony and condition were maintained in the same tank. In addition, such study does not allow to infer the dynamics of the microbiome and energy reserves between sampling times.' Lines 1374-1377. Due to the difficulty and high cost of getting deep sea protected animals, we were limited in the number of replicates that we could use.

Regarding statistics, we have interacted with other biologists and statisticians that worked on thermal experiments and we agreed that the use of temperature as categorical variables can be legitimated since the sampling for each time point induced differences in our coral samples. Using temperature as categorical variables on corals is common in the field (e.g., Mayfield et al., 2012 – JEMBE ; Clause and Roth, 1975 – Marine Biology ; Jokiel et al., Coral Health and Disease, 2003 – SPRINGER). Combining factors 'temperature' and 'time' induce the use of categorical variables as it has been done in several works (Brook et al., 2013 DSRII ; Bonesso et al, 2017 – PeerJ).

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
Review Chapron et al.
Resilience of cold water coral holobionts to sea water temperature changes

In this study, Chapron et al. simulated the impact of global warming on deep-water corals. Using samples of Lophelia pertusa and Madrepora oculata from the Mediterranean Sea, the coral physiology (feeding, growth, carbohydrate, lipid, protein, organic matter) and the associated bacterial community diversity (microbiome), was monitored for six months in aquaria.

It was revealed that a temperature increase of two degrees (end of century estimate) shifted the physiology and microbiome of L. pertusa but not of M. oculata. Initially, the microbiome of L. pertusa shifted towards an abundance of Rhodobacterales. It was not clear, but it seems the microbiome of M. oculata also became dominated by Rhodobacterales by, increased temperature. Notably, at higher temperature microbiomes appeared to converge and loose their 'host imprint' i.e. temperature became a stronger driver than host. Subsequently, feeding increased but energy reserves and skeletal growth became reduced. Additional temperature scenarios killed the corals (four degree increase) or resulted in no observable change? (three degrees reduction). Controls at in situ temperature (13 ℃) resulted in little to no change.

We have clarified our text to highlight that contrary to the stable microbial communities observed within M. oculata, L. pertusa's microbiome changed in globality with temperature. However, when exposed to higher temperature, both L. pertusa and M. oculata exhibited the presence of the ASV24 (order Rhodobacterales) after 1 week with a higher relative abundance in the highest temperature. In the text we now write 'At 15°C and 17°C, both L. pertusa and M. oculata bacterial communities were dominated by the ASV1 and ASV24 (order Rhodobacterales) at 1 week, then by the ASV4 (order Rhodobacterales) and ASV3 (order Acidimicrobiales) at 2 months, with significantly higher abundances at 17°C (Figure S7, Table S3). The ASV23 (Epsilonproteobacteria) also increased in relative abundance at 17°C for both species at 2 months.' Lines 802-806.

This is an interesting study but the manuscript *may become clearer* and the *main findings better conveyed*. It is felt that especially *Result sections* readability can be much improved (more work is needed here). *Shortening* the manuscript may also help focus. The abstract initially mention global warming. Seeing 10 ℃ mentioned later in the abstract may distract the reader. Consider changing the title to "…to global warming". This is just a suggestion. But, why not emphasize the two degree raise in temperature? Impact from a two degree increase (to 15 ℃) seems the most important to report because it seems to be the expected temperature raise by the end of this century and two degrees is also forecasted by the IPPC as a limit beyond which irreversible changes may be difficult to avoid. It is suggested that the other temperatures can be used to indicate response to extreme temperatures (17 ℃, 10

°C). The 13 °C becomes the baseline all temperatures deviate from. *Consider whether this +2 degree focus may be better*.

We have clarified and shortened the result section as suggested to highlight the main findings. We understand the consideration to focus on the +2 degree in the manuscript, but we wanted to keep the response to extreme temperatures (i.e., 17°C and 10°C), for a better overview of coral responses to temperature changes. Particularly, the cold temperature allows us to compare our data with previous studies on these species, and test a potential trend of coral response with temperature, as previously seen for oxygen consumption (Dodds et al., 2007) and calcification (Naumann et al., 2014). This is now clarified in the text: 'The tank at 15°C represented the temperature forecast by the IPCC [2] for the end of the century in the deep Mediterranean Sea, and 17°C simulated extreme warmest conditions. Finally, 10°C was used to investigate if corals maintained their health conditions at colder than present temperatures. This temperature is similar to the one used in earlier studies on Atlantic L. pertusa and M. oculata specimens in relation to oxygen consumption and calcification [11,25].' Lines 144-158.
As advised, we changed the title to 'Resilience of cold water coral holobionts to global change'.

Limitations may also be emphasized more.

We understand the reviewer's concern about the limitation and now write 'Our findings nevertheless to be considered with caution as they originate from laboratory experiments in which the replicates for each colony and condition were maintained in the same tank. In addition, such study does not allow to infer the dynamics of the microbiome and energy reserves between sampling times.' Lines 1374-1377.

May this reference on aquaria experiments be helpful to include? *Orejas et al. 2019*, Cold-Water Coral in Aquaria: Advances and Challenges. A Focus on the Mediterranean. In: Mediterranean Cold-Water Corals: Past, Present and Future. May the corals have been somehow stressed by cutting into nubbins?

We have added additional descriptions of the experimental design, which respects the recommendations made in Orejas et al. 2019. As highlighted in the referred paper, the use of nubbins for coral experiments both in situ and in aquaria is largely widespread. No evidence of stress has been observed earlier, including with various sizes of nubbins (e.g., small and large nubbins exhibit the same growth rates as reported by Lartaud et al., 2017). However, to avoid a potential stress, an acclimation period of 3 months was used after collection and cutting the nubbins prior to the experiment. We have now clarified it in the text 'The nubbins were then maintained in tanks for 3 months prior to the start of the experiment. This ensured a full recovery of the corals from the stress caused by the collection and cutting. It also allowed acclimatization to laboratory conditions.' Lines 134-136.

What about adaptation, in a natural setting temperature change over decades whereas in aquaria over months.

We do not expect to directly reproduce global warming in aquaria experiments, but as other studies in the field, we track the coral responses to changes of temperatures based on a gradual temperature increase (or decrease). As earlier experienced with these species (e.g., Naumann et al., 2014), and to avoid short term acute heat stress on corals, we used a long-term experiment (i.e., several months). It was previously shown that short-term exposures reflect coral stress response rather than response to environmental changes (Form and Riebesell, 2012; Chapron et al., 2018).

May a reference be included for that fluorescent calcein staining (I guess this; Lartaud et al. Aquat Living Resour 2013) to inform on potential inference on growth or microbiome and what does this stain bind, proteins?

The sentence was modified to include the reference cited, and more details on the staining were provided in Supplementary Information. We now write 'The skeletal growth, behavior and energy reserves were measured for each temperature condition. The skeletal growth was assessed through the polyp linear growth rate, based on the use of calcein [27], easily recognized under fluorescent light microscopy (Figure S2), and following the protocol described in Chapron et al. [28]. Calcification rates of corals were also monitored from the buoyant techniques [29]. The detailed protocols of both polyp growth rate and buoyant weight were provided as Supplementary Information.' Lines 195-200.

May a sentence be included to indicate that little is known about what happened between sampling points?

We agreed with the reviewer's that little in known between sampling points for the microbiome and the energy reserves, and now write 'In addition, such study does not allow to infer the dynamics of the microbiome and energy reserves between sampling times.' Lines 1372-1375.

<u>Supplementary text</u>

Skeletal growth, third sentence: calyx (can it be briefly explained what this is?)

Done: "the protective cup within which the polyps sit".

Feeding behavior, first sentence: Coral capture rates and polyp activity were measured for each thermal condition at… (complex sentence why not just polyp activity? Are not all measurements performed at all temperatures and all time points?).
Feeding behavior, second sentence: yes prey capture rates but what about polyp activity? Why polyp prey capture and polyp activity is used may need some clarification.

The title and sentence were clarified. We agree that polyp activity does not necessarily reflects feeding processes. We previously showed that these two parameters are not always correlated (Chapron et al. 2018), likely because polyp activity can be associated to additional physiological processes (e.g. respiration, egestion, production of mucus). For simplicity, the term 'behavior' was used, based on both response of coral prey capture rates and polyp activity measured at each time. We now write 'Behavior was assessed by measuring prey

capture rates and polyp activity at the start of the experiment (T0), at 1 week, and every month until the end of the 6 months experiment.' Lines 201-202.

Energy reserves, first sentence: assessed for what? (help reader understand by saying lipid, carbohydrate and proteins if this is what is meant).

We now write 'The energy reserves were measured as the total organic matter (TOM) and the three main classes of compounds (lipids, proteins and carbohydrates) at the start of the experiment (T0) and at 2 and 6 months.' Lines 207-209.

Energy reserves, third sentence; Bioblock Alpha-1-4-LD (what is this and why product any chemical reaction here? Not just freeze drying? Any chemistry involved should be mentioned because may influence sample.

The coral nubbins were freeze dried using the freeze dryer Bioblock Alpha-1-4-LD. We clarified with 'The coral fragments were freeze-dried with a Bioblock Alpha-1-4-LD at -50°C for a week.' in supplementary information.

Figure 1: legend say M. oculata symbols being stars but circles are shown in figure.

Thank you for noticing a typo error. We now write 'Figure 1: Polyp survival rate (%) for (A) L. pertusa and (B) M. oculata at 10°C, 13°C, 15°C and 17°C during 6 months of experiment. T0: start of the experiment, w: week, m: month.' Lines 361-362.

Figure 2 and 3: use same y-axis scale to ease L. pertusa and M. oculata comparisons?

We understand the reviewer's suggestion but the use of the same scale for both species will make the M. oculata's graph much harder to read (polyp growth rate changes by an order of magnitude).

Figure 3: is both polyp activity and polyp capture rate needed? (using just the most important one may help focus)

As detailed above, polyp activity and prey capture rates are not always linked (Chapron et al., 2018). For a precise characterization of behavior we chose to measure both activity and capture.

Figure 4B: M. oculata y-axis spelling "protides"

Thank you for noticing we now write proteins.

"Table S4. Mean values and standard deviations are presented.", is this what Table S4 shows?

We now clarify with 'Figure S4: Calcification rates (G % day-1) of (A) L. pertusa and (B) M. oculata under different temperature conditions after 6 months of incubation. The values are the means and positive standard deviations.'

Table S2 and S3, numbers not significant seems not needed (help focus). It is suggested to use text to convey that most comparisons were insignificant. Legend may be improved. "One-way analyses of similarities (ANOSIM) between…" (similarities of what?)
Table S2 looks like a control and indicate that the bacterial communities do not change. It seems better to remove Table S2 and just use text to convey this.
Table S3, similar here, for clarity it is suggested to only show significant changes and use text to convey what was not significant and what was measured.

We took into account the reviewer's suggestions and removed the table S2 and changed the name of the rest of the supplementary tables.
We also changed the legend for table S3 (now S2) with 'Table S2: One-way analyses of similarities (ANOSIM) among temperatures within each time point for L. pertusa and M. oculata (global R=0.33, p=0.001) with pairwise tests of differences between Time and Temperature. Significant effects (p-value<0.01) are in bold. All models were run with 9999 permutations.'
Regarding the non-significant changes, we prefer to keep the information. We feel that the results are easy to read through the figure 5 in the main text.

Table S4, (xl sheet, in sheet labelled as Table S1 should probably be S4), unclear how to interpret this table. How can, for example, ASV6 be the most abundant ASV at all times and temperature 13 ℃ for M. oculata when three other ASVs (1, 2, 7) are listed as the most abundant ASVs of M. oculata at 13 ℃? To provide some relative information on abundances percentages of ASV abundance may be included (I presume these are dominant ASVs). In relation to this it would be helpful to know what are the number of sequences per sample? Are all samples normalized to the same sequence number? It seems that Table S4 more or less duplicates Figure S7. Is Figure S7 needed? Figure S7 is not so easy to read and less figures may help focus.

We understand the confusion. We changed the title of the table which is now table S3. We now include relative abundances 'Overall, the ASV1 (order Cellvibrionales) was the most abundant in L. pertusa (14%) and the ASV6 (Spirochaetales) (15%) was the most abundant in M. oculata. Lines 763-764.
The samples were not normalized to the same sequence numbers. As written in the material section 'To enable comparison of the bacterial community compositions and diversities, the sequence data were normalized by dividing counts by sample size.' Lines 331-332.
We understand the reviewer's concern about the Table S4 that is similar to the Figure S7, but we still think that Figure S7 is helpful to highlight relative abundance in some ASV known to be opportunistic and/or pathogens.

Figure S2, how was 100% determined?

Tests were performed to determine the number of pictures extracted from the video needed to represent 100% of the polyp activity. For that, pictures were extracted from the video from every hour to every minute. Within the 2-hour video, this extraction led to the selection of 2 to 120 pictures. Then, we compare the polyp activity extracted from one test video to the different selections made. We found that the selection of 50 pictures (extraction every 2.4

minutes) was sufficient to represent more than 99% of the polyp activity register in the whole video. If needed, more information is available on Chapron et al., 2018 and in the supplementary information within the behavior section.

Figure S4, what is calcification rate? would it be better to use growth rate?

Coral calcification rate is only one of several parameters used to determine growth rate. It's determined with buoyant weight and represents the rate at which the reef-building corals lay down their calcium carbonate skeleton (Jokiel et al., 1978). This is a classical technique used with coral. Other growth parameters include surface and linear extension, and budding rate (details in Lartaud et al., 2019 – Growth Patterns of Mediterranean calcifying cold-water corals, In: Mediterranean Cold-Water Corals: Past, Present and Future). In the present study, the polyp linear growth rate was measured using the calcein staining and is represented in Figure 2.

Figure S5, organic matter, for clarity consider use total organic matter (if that is correct)

We clarify now with Total organic matter as suggested and also corrected it in the main text.

Figure S7, it may be better to remove this figure (looks redundant because of Table S4)

The figure S7 is complementary to the Table S3 (former Table S4) highlighting the relative abundance of each ASV with time (as this information cannot be deduced from the new Table S3). We thus chose to keep this Figure in the Supplementary Material.

Minor comments

Ln 16: fragile? If this is meant, consider something like this …, especially in the relatively warm Mediterranean Sea,…
Rephrased: 'Cold water corals are threatened by global warming, especially in the Mediterranean Sea where they live close to their upper known thermal limit (i. e., 13°C),' Lines 20-21.
Ln 17: complex sentence, can it be made clearer? (is all this information needed?)
We simplified: 'Here, for the first time, temperature effects on Lophelia pertusa and Madrepora oculata holobionts (i.e., the host and its associated microbiome) were investigated.' Lines 22-24.
Ln 22: instead of "impacted" consider use the word "resilient" from the title, i.e. was more resilient
We now write. ', whereas M. oculata was more resilient.' Line 26.
Ln 24: the abstract may become clearer without mentioning the 10℃ experiment (may confuse because warming appear in focus.
The use of a colder temperature (10°C) is now clarified in the text and we think that those results should appear in the Abstract. We have however rephrased it: 'In addition, our results, showing the holobiont's negative response to colder temperatures (-3°C), suggest that Mediterranean corals live close to their thermal optimum. The species-specific response to temperature change highlights that global warming may affect dramatically the main

deep-sea reef-builders, which would alter the associated biodiversity and related ecosystem services.' Lines 28-32.

Ln 34: why Mediterranean Sea more sensitive to global warming? (if because temperatures are relatively high in deep waters try rephrase to include this for clarity)

We now write for clarity 'The Mediterranean Sea, an almost land-locked sea, is particularly exposed to the effects of global warming [5], and deep water temperatures may increase by 1.5°C by the end of the century [2,6].' Lines 57-60.

Ln 41: seems better to turn this sentence around and start with warming since apparently focus of this work (trawling haven not been seen mentioned until now)

We now write 'CWC reefs are threatened by direct anthropogenic activities, and the increase of CO2 concentrations that acidify and warm up seawater.' Lines 65-66.

Ln 42: specific factors of threat mentioned here are these associated with Ln 34 (sensitive) and Ln 16 (fragile)? If so, consider clarifying

We now write for clarity: The impacts of ocean acidification and warming on coral physiology, and particularly for calcification, have been studied recently [9–11]. The integrative effects of temperature at different biological scales in the coral organism are, however, poorly known. Additionally, tolerance to temperature changes could differ between species, as suggested by the opposite responses in calcification rates observed for Dendrophyllia cornigera and the solitary cup coral Desmophyllum dianthus [10,12].' Lines 66-72.

Ln 47: Desmophyllium is Lophelia (might confuse, might be fine). See Ln 64, explanation might be moved to Ln 48.

We moved the explanation Line 73 and now write 'Lophelia pertusa (recently synonymized to Desmophyllum pertusum [13]) and Madrepora oculata,'

Ln 65: upper thermal limit, would an improvement be to mention this in Ln 34 and Ln 16? It seems better to mention the higher temperatures first.

We now write 'Cold water corals are threatened by global warming, especially in the Mediterranean Sea where they live close to their upper known thermal limit (i. e., 13°C),' Lines 20-21.

Ln 88: how often fed? Every second day Artemia and marine snow?

Modified to: 'and they were fed alternatively 3 times per week with Artemia and marine snow plankton diet (ratio 2:1 respectively).' Lines 139-140.

Ln 94: it seems appropriate to explain why 10℃ is used (shift expected towards microbiomes of coral hosts in colder waters? Using 10 ℃ may confuse in a context of global warming).

We understand the reviewer's concern about the 10°C experiment in a context of global warming and now write 'Finally, 10°C was used to investigate if corals maintained their health conditions at colder than present temperatures. This temperature is similar to the one used in earlier studies on Atlantic L. pertusa and M. oculata specimens in relation to oxygen consumption and calcification [11,25].' Lines 155-158.

Ln 98: tank volume 36 L not 80 L as mentioned Ln 88? During 80 L tank conditioning all Lophelia in one tank and all Madrepora in another tank? Is it known whether cutting into fragments may influence physiology and associated bacteria?

No evidence of stressful conditions was observed earlier, including with various sizes of nubbins (e.g., small and large nubbins exhibit the same growth rates as illustrated by Lartaud et al., 2017). However, to avoid this potential risk, an acclimation period of 3 months in 80L tanks was used after collection and cutting the nubbins prior the experiment. We clarify in the text: 'The nubbins were then maintained in tanks for 3 months prior to the start of the

experiment. This ensured a full recovery of the corals from the stress caused by the collection and cutting. It also allowed acclimatization to laboratory conditions.' Lines 134-136.
Then, the nubbins were transferred together into 36L experimental tanks for another 1-month period of acclimatization before the change of seawater temperature. We clarify in the text: '. Coral nubbins from 3 different colonies for each species were transferred and randomly distributed in 36L experimental tanks.' Lines 160-161.

Ln 114: complete nutrient supply? Is the nutrient supply in situ known? Consider remove "complete" or both "complete" and "diverse" and just use "rich" instead

We took into account the reviewer's suggestion and now write 'to provide a rich nutrient supply' Line 177.

Ln 117 to 130: seems unclear. Briefly explain method, experimental temperature («experimental» can be removed for clarity). Is growth rate and calcification rate the same rate? Consider rephrase something like this (if this is meant) «Coral growth was measured as skeletal calcification rate using the protocol… and as polyp growth by length extension using video… (Supplementary Information).» Consider all experimental design to be explained in the foregoing paragraph. In the following, again two related aspects are considered, polyp activity and polyp prey capture. Are these the same activities? Try improve on clarity.

We removed "experimental" as suggested and we created several paragraphs for each measurement and made some changes to enhance the method section.
As explained above, polyp linear growth rate and calcification rates are not the same. The polyp linear growth rates, highlighted by the staining, allows to extrapolate the linear extension of the colony. The calcification rates rather reflect the rate of calcium carbonate accretion. We clarify by adding 'polyp linear growth rates' through the text.
We also clarify the 'coral capture rates' and change it to 'prey capture rates' within the behavior measurements with 'Behavior was assessed by measuring prey capture rates and polyp activity at the start of the experiment (T0), at 1 week, and every month until the end of the 6 months experiment.' Lines 201-202.

Ln 123: Cold-water coral polyp. Seems clearer if «Cold-water» is removed, all are cold-water.

We now write directly 'poly activity' Line 201.

Ln 130: for clarity consider «The energy reserves, carbohydrates, lipids and proteins of…»

We now write 'Lipids were quantified following the colorimetric assay developed by Barnes and Blackstock [31], proteins were determined by the Bradford method [32] and the carbohydrates were determined according to Dubois et al. [33] (Supplementary Information).' Lines 211-214.

Ln 131: freeze drying process. If not just frozen but some chemicals added this may need to be explained but if just freeze-dried «process» seems better removed

As suggested 'process' was removed Line 210.

Ln 146: dedicated? If just an ordinary hammer remove "dedicated" and instead say "sterile" (if that was meant)

We now write 'sterile hammer' Line 287.

Ln 155: PCR chemistry and cycling conditions?

Amplicon fragments are PCR-amplified using the high-fidelity Phusion polymerase under conditions of 30s at 98°C, 16 cycles of 98°C for 10s, 60°C for 30s, 72°C for 80s and final extension for 5m at 72°C.

Ln 164: searching NCBI with this accession returned no sequences (not made public yet?)

It was private but we changed it to public.

Ln 165: model errors? Is this needed? ("model" may confuse)

We changed 'model' with 'analyze' Line 314.

Ln 170: is SIMPER part of the DADA2 software?

The SIMPER analysis is from the Vegan package. We added 'was performed on the ASVs selected by SIMPER analysis from the vegan package.' Line 320.

Ln 171: Samples? Not ASVs?

It was samples. We changed with 'biological samples' for clarity Line 320.

Ln 182: To compare the bacterial community composition and the community diversity (text not clear, here not only to compare it is to enable comparisons of similar sizes…

We took into account the reviewer's suggestion and now write: 'To enable comparison of the bacterial community compositions and diversities, the sequence data were normalized by dividing counts by sample size.' Lines 331-332.

Ln 183: normalized how? Not removed reads by random sampling?

There are different data normalization process and we decided to normalize by dividing the reads with the total number of reads per biological samples.

Ln 278: may be better to report the seawater bacterial community last in this paragraph since paragraph title is "Coral bacterial communities". Readability of the paragraph can be improved.

We rewrote the paragraph for readability from Line 539 to 557.

Ln 288: may be better to start this sentence with "After 2 months…" because may clarify time perspective in focus here (0 weeks, 1 week, 2 months, 6 months).

Change made.

Ln 310: what are the number of sequences or percentage dominance of these ASVs?

We added the percentage dominance of these ASVs: 'Overall, the ASV1 (order Cellvibrionales) was the most abundant in L. pertusa (14%) and the ASV6 (Spirochaetales) (15%) was the most abundant in M. oculata. Lines 763-764.

Ln 327: unusual bacteria appeared at temperatures away from control. This seems important. Consider mentioning the dominant taxa as they could be indicators of dysbiosis.

The dominant taxa for both species are shown lines 763-764: Overall, the ASV1 (order Cellvibrionales) was the most abundant in L. pertusa (14%) and the ASV6 (Spirochaetales) was the most abundant in M. oculata (15%).'

For each species, sampling time and temperature, the dominant ASVs changed and as suggested we now write: 'At 10°C, both species exhibited the same pattern of ASVs abundance. The ASV1 and ASV42 (Rhodobacterales) were the most abundant at 1 week in both species, then the ASV12 (Rhiziobiales) at 2 months, and the ASV49 (Rhodobacterales) at 6 months (Figure S7).

At 15°C and 17°C, both L. pertusa and M. oculata bacterial communities were dominated by the ASV1 and ASV24 (order Rhodobacterales) at 1 week, then by the ASV4 (order Rhodobacterales) and ASV3 (order Acidimicrobiales) at 2 months, with significantly higher abundances at 17°C (Figure S7, Table S3). The ASV23 (Epsilonproteobacteria) also increased in relative abundance at 17°C for both species at 2 months. ASV24 is 100% similar to sequences found in bacteria growing on living [41] or inert surfaces [42].' Lines 790-798.

Ln 353: is two months a rapid change in the six month experimental period?

A change within 2 months of thermal experiment prior to physiological changes, may be considered as rapid. But to avoid confusion we rephrased in the text and wrote: 'Bacteria known to be associated to stressed or diseased corals (Rhodobacterales, Acidimicrobiales, Epsilonproteobacteria) [50,51] appeared rapidly (after 1 week), which might conduct to coral dysbiosis (change in bacterial community composition, including opportunist species) and

death. Dysbiosis appeared prior to changes in the host physiology and the microbial signature could therefore be used as a promising sentinel of coral health.' Lines 1056-1061.

Ln 388: probably mucus and gut and not tissue associated (ephemeral)

To avoid confusion we added: 'Coral bacterial communities for each temperature condition were assessed from three polyps (including tissues, gut and mucus) per colony at the start of the experiment (T0) and at 1 week, 2 months and 6 months.' Lines 281-283.

Ln 424: …which are thought to live close to their upper thermal tolerance limit… consider mentioning this earlier and perhaps already early in the Abstract

We now mention this in the abstract and the introduction.

Referee: 2

Comments to the Author(s)
This manuscript looks at responses of reef-building corals L. pertusa and M. oculata to temperature increases. In addition to the novel exploration of holobiont-level responses, this manuscript also explores skeletal growth, feeding behaviour, energy reserves, and polyp mortality. However, despite the holobiont-approach (which is novel in cold-water coral studies), there were issues with the paper which mean it is not currently suitable for publication. These issues were:
1. The lack of replicates. There were 4 treatments and 4 tanks. Any differences could thus be seen as a tank effect.
We understand the reviewer's concern as this is a critical aspect in aquaria studies on deep sea species. Unfortunately, due to the difficulty and high cost of getting deep sea animals, we were limited in our number of replicates. The originality of the study was to focus on a holobiont approach and the use of several physiological, biochemical and molecular descriptors is consuming a large number of polyps. We are well aware of the potential weakness of our approach compared to more classical design; however, we believe that the experimental design is robust enough and that the results strongly support the role of thermal effect rather than tank effect on the corals.
Several studies have used large tanks with several fragments per colony (i.e., pseudo-replicates) because of the difficulty in getting large enough sample numbers (Naumann et al., 2013; Burdett et al., 2014; Hennige et al., 2014; Gori et al., 2014; Lartaud et al., 2014; Naumann et al., 2014; Chapron et al., 2018; Galand et al., 2018). We have a strong experience in aquaria experiments with cold-water coral species, and have taken a number of measures to reduce the possible bias. To limit the tank effect, all pumped seawater pass through a buffer tank prior to being distributed into experimental tanks. In addition, every tank received the same amount of filtered seawater that was renew more than 1 time a day. Then, a number of abiotic (temperature, seawater currents, pH, salinity, oxygenation) and biotic parameters (amount of nutrients) were monitored daily or weekly in all tanks to verify that the experiment was running under strictly comparable conditions.
In our control tank, microbial communities from the seawater differed from the coral microbiome, which kept their own specific microbiome through the entire study. For warmer temperatures, both corals lost their species specific signature and gained a warming signature, even though they still differ from the seawater microbial community.
We have now specified the limitations of the study: 'Our findings nevertheless to be considered with caution as they originate from laboratory experiments in which the replicates for each colony and condition were maintained in the same tank. In addition, such study does not allow to infer the dynamics of the microbiome and energy reserves between sampling times.' Lines 1374-1377.

2. As the number of fragments/individuals in each tank was not stated, it is not possible to judge the statistical power of the experiment.
We now clearly specify in the method section the number of coral fragments and the number of polyps in each tank for both species with 'A total of 62 nubbins were placed in each tank, with enough distance between nubbins to avoid any contact among the polyps [26]. In total, each experimental tank had 280 polyps of L. pertusa and 350 polyps of M. oculata.' Line 161-164.

3. This experiment placed two species of corals (M. oculata and L. pertusa) together in the same tank and then looked at bacterial compositions of the two, which appear to have interacted. The line: 'The bacterial community composition became similar in both coral species, the original species-specific signature disappeared' was specifically of concern seeing as it appears the corals shared their microbiomes when under stress. Therefore, bacterial changes found could be based not on the temperature increase but on the influence of an interaction with another coral species.

We understand reviewer's concern, however, it is unlikely that the microbiome change was due to the interaction between the two coral species:

1. *the coral fragments (nubbins) were separated inside the tanks to avoid any contact between polyps*
2. *the seawater in the tanks was renew every day and its microbial communities differed from both corals during the entire study and for all temperatures.*
3. *previous studies (Galand et al., 2019; Galand et al., 2020) highlighted that these two coral species placed in the same tank keep their specific microbiome over several months (DNA analysis). This was confirmed here with our control tank (13°C) where both species kept their specific microbiome during the 6 months of the experiment. Corals collected in situ also exhibit systematically species-specificities while they originate from the same site and the same time of collection (Meistertzheim et al., 2016).*
4. *finally, the dominant ASVs at 17°C were opportunistic bacteria. It indicates that the two coral species did not share their microbiome, but that they acquired opportunists from the environment.*

Specific Comments

This paper could be improved with English-language editing.

We edited the manuscript and hope that the reviewer now finds it suitable for publication.

Line 16: 'Could be threatened' and 'Fragile' odd wording

We now write 'Cold water corals are threatened by global warming, especially in the Mediterranean Sea where they live close to their upper known thermal limit (i. e., 13°C),' Lines 20-21.

Line 18: 'reef building species' instead of 'reef builders'

Line 19: state duration instead of just saying 'short and long-time scales'

As suggested by the other reviewer, this sentence was rewritten and the term reef-building species' was removed. We now write 'Here, for the first time, temperature effects on Lophelia pertusa and Madrepora oculata holobionts (i.e., the host and its associated microbiome) were investigated.' Lines 22-24.

Line 20: under predicted temperatures for when? End of century?

We now clarify: 'The Mediterranean Sea, an almost land-locked sea, is particularly exposed to the effects of global warming [5], and deep water temperatures may increase by 1.5°C by the end of the century [2,6]..' Lines 57-60.

Lines 41-43: Citations needed for trawling threats and increased CO2 threat to CWC

Following reviewer1's suggestion we rephrased to better highlight the present topic: "CWC reefs are threatened by direct anthropogenic activities, and the increase of CO2 concentrations that acidify and warm up seawater.' Lines 65-66.

Line 67: Why were 15 and more specifically, 17 degrees chosen? Earlier in the introduction, a temperature increase of 1.5 degrees was mentioned for the region. What was the motivation to select 2 and 4 degrees for this experiment?

The purpose of the study was not to strictly test the response of corals to future deep-sea Mediterranean temperature conditions (+1.5°C), but rather analyze the effects of temperature changes on coral health status, at different level of biological organization. That's why we tested lower and higher temperature conditions, including values close to future conditions, and extrema. We rephrased in the text to avoid misinterpretations.

Line 72: How many months?

We now write 'to provide a more complete view of the physiological pathways that could be affected by global warming, at short (1 week) and longer (2 and 6 months) time scales.' Lines 110-111.

Line 97: 'warmest conditions' still unclear where this forecast is coming from

This temperature was chosen to study the response under extreme warm conditions, to better highlight disruptions in the metabolic pathways inferred by temperature increase. Such type of extreme temperatures was also used by others (see Brooke et al., 2013).

Line 200: Figure 1. Could just be a plot for 17 degrees. No benefit for having other temperatures shown

We think that keeping all temperatures completes the text perfectly without overloading the figure and gives a better representation of the dynamics of mortality over the whole experiment.

Lines 296-298: Where did this increased diversity in L. pertusa's bacterial community come from? Was it influenced by the experimental sea water? Perhaps by M. oculata?

The increase in bacterial diversity with time in aquaria is classically found for L. pertusa (Galand et al., 2018; Galand et al., 2020) and is suggested to reflect the colonization of the host by additional opportunist bacteria induced by the aquaria conditions. This increase in diversity indicated that these corals lost their ability to strongly select specific bacteria, which in turn may reflect a poorer health status.

Lines 444-447: We are uncertain that the conclusion that temperature increase 'could lead to a dominance of M. oculata in future deep-sea reefs' as mentioned in the abstract is valid. Yes, L. pertusa growth rates decreased while M. oculata's stayed the same, but L. pertusa grows faster than M. oculata even with the decrease.

We agree with this comment and modified this section accordingly. We now write: 'In addition, our results showing the holobiont's negative response to colder temperatures (-3°C), suggests that Mediterranean corals live close to their thermal optimum. The species-specific response to temperature change highlights that global warming may affect dramatically the main deep-sea reef-builders, which would alter the associated biodiversity and related ecosystem services.' Lines 28-32.

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Appendix C

Leila Chapron,
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16th November 2021

Resubmission: Previous reference number RSPB-2021-2117

Dear Editor,

Please find enclosed our revised manuscript entitled 'Resilience of cold water coral holobionts to thermal stress' by L. Chapron, P.E. Galand, A.M. Pruski, E. Peru, G. Vétion, S. Robin, and F. Lartaud for consideration for publication in *Proceedings of the Royal Society B: Biological Sciences*. This paper has not been published elsewhere and is the original work of the authors.

We would like to thank again the reviewer for his/her positive feedback and his/her constructive comments. We have answered all his/her queries and we feel that it improved the quality and clarity of the paper. A suggested, we revised and shortened the manuscript, which has been read by a native English speaker to improve the language. We have better emphasized the take home message and hope that the editor and reviewers will be satisfied with this new version of our manuscript. A detailed point by point answer to the reviewers' comments is attached and all referred line changes can be found in the document chapronetal_trackedchanges.doc.

Because of the originality of our work, we think that our results will be of considerable interest for marine ecologists, oceanographers, biologists and toxicologists, and more widely to a broad audience interested in knowing more about the impact of global change on marine life.

Thank you for your time and consideration. We hope that you will consider our manuscript for publication in *Proceedings of the Royal Society B: Biological Sciences* and look forward to hearing from you.

Yours sincerely,

Dr. Leila Chapron

Appendix D

28-Oct-2021

Dear Miss Chapron:

Your manuscript has now been again peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns. We normally do not allow multiple rounds of revision, but your manuscript still needs work before it can be considered further. We are willing to give you one final opportunity to adequately revise your manuscript to address them.

This will be your final opportunity to revise your manuscript so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please 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.

When submitting your revision please upload a file under "Response to Referees" in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the 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.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

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If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials

supporting the results in the article (https://royalsociety.org/journals/authors/author-guidelines/#data). Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

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=(Document not available), 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.

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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. Please try to submit all supplementary material as a single file.

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].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,

Dr Daniel Costa
mailto: proceedingsb@royalsociety.org
Associate Editor

Comments to Author:

Thank you for revising your mansucript. The revised version is much improved, but there are still issues remaining that would preclude publication in its current form. In general, the text of the manuscript should be revised throughout to improve clarity and the narrative structure, and sections of the Results and Discussion could be shortened. The main points (take-home messages) could be made more explicit. Related to this, the reviewer suggests that the title be changed again; I would encourage the authors to take this advice but to settle on a title that clearly captures the main message of the paper. The figures and figure legends could be improved; in some cases error bars and number of replicates (n) need to be provided; in other cases consider if tables may be more effective. The methods are missing important details or are still lacking in clarity in places. Please respond to each of the reviewer comments in your revisions.

We appreciated the last comments of the reviewer and have carefully answered all his/her queries. We have revised the entire manuscript to improve clarity and especially shortened the microbiome part of the result section and the discussion.

We have better emphasized the take home message in the discussion by stating the main findings in the start of the paragraphs:
- The absence of polyp mortality at 15°C suggests that both L. pertusa and M. oculata should tolerate the temperature expected at the end of this century.
- The early change in the microbial communities indicates that dysbiosis can occur rapidly with increasing temperature.
- Our dataset at the holobiont scale could indicate that 15°C is a threshold temperature for the maintenance of L. pertusa fitness.
- On the opposite, M. oculata's skeletal growth was not affected at 15°C.
- An increase of 4°C (i.e., 17°C), rapidly led to high polyp mortality for both L. pertusa and M. oculata. Our results thus suggest that the main reef-builders in the deep-sea are not resilient to larger thermal changes.
- Surprisingly, our holobiont approach suggests that colder temperatures (10°c) are not associated with better health.

In addition, we have changed the title, improved the figures following the comments, replaced one figure with a table and added missing information to the method section.

We created a Data accessibility section in our manuscript with 'Physiological data are available from the Dryad Digital Repository (doi:10.5061/dryad.djh9w0w1h). All sequences were deposited in GenBank under SRA accession number PRJNA648865.' Lines 1036-1062. The temporary private Reviewer URL has been created: https://datadryad.org/stash/share/ZQ7C8vuZfHXpLspUSyEzgf5TeHKBE1Xqd9NJ-tJMEbs.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).
Chapron et al. (R1, considered as new submission)

Although improved, it seems further improvements would benefit this manuscript. Generally, try emphasising take home messages more. Attention is suggested given to readability. Clarity of text will also help guide the reader more easily trough all the figures.
We appreciated the last comments of the reviewer and have carefully answered all his/her queries. We have revised the entire manuscript to improve clarity and especially shortened the microbiome part of the result section and the discussion.
In addition, we have changed the title, improved the figures following the comments, replaced one figure with a table and added missing information to the method section.
We created a Data accessibility section in our manuscript with 'Physiological data are available from the Dryad Digital Repository (doi:10.5061/dryad.djh9w0w1h). All sequences were deposited in GenBank under SRA accession number PRJNA648865.' Lines 1036-1062.
The temporary private Reviewer URL has been created:
https://datadryad.org/stash/share/ZQ7C8vuZfHXpLspUSyEzgf5TeHKBE1Xqd9NJ-tJMEbs.

Regarding the figures, for example, Figure 1 has no error bars. What are the number of replicates? 4 to 25 for Lophelia and 6 to 30 for Madreopora (Ln 87)? Or, more than hundred each (Ln 106 to 110)?
We have changed Figure 1 to introduce the error bars among coral colonies for each species and time point (the first draft of the figure contained the total number of the dead polyp in each tank normalized to the number of total polyps).
We are sorry for the misunderstanding on the number of replicates. We now specify that each nubbin had different numbers of polyps ranging from 4 to 25 per nubbin for L. pertusa and from 6 to 30 per nubbin for M. oculata. We write: 'coral colonies were cut into nubbins that contained 4 to 25 living polyps for L. pertusa and 6 to 30 living polyps for M. oculata' Lines 105-106.
Overall for L. pertusa, for each coral colony in each tank we had 11±1 nubbins which represented 100±10 polyps. For M. oculata, for each coral colony in each tank we had 10 nubbins which represented 110±20 polyps. Thus, with 3 colonies per species, we had in total in each tank 62 nubbins (32 L. pertusa and 30 M. oculata), which represented 630 polyps (280 L. pertusa and 350 M. oculata). We now write: 'A total of 62 nubbins (32 for L. pertusa and 30 for M. oculata) were placed in each tank, with enough distance between nubbins to avoid any contact among the polyps [27]. These 62 nubbins contained overall 280 polyps of L. pertusa and 350 polyps of M. oculata.' Lines 145-148.

Sentence: "The 17°C data could not be used due to the high mortality observed after 2 months" (Ln 220) but there were measurements ahead of 2 months?
All polyps of M. oculata were dead at 17°C after 6 months and the few remaining polyps of L. pertusa were dedicated to assess the coral bacterial communities. Thus, we could not measure growth in both species at 6 months. We, however, analyzed linear polyp growth for fragments that died at 2, 4 and 5 months of exposition at 17°C for L. pertusa and at 2, 3 and 4 months for M. oculata, but the results face severe limitations. First, the exact date of death is difficult to determine as the polyp survival rate was assessed once a month. Then, it is not possible to compare linear polyp growth rates from polyps exposed at 17°C for 2-5 months

with polyps exposed for 6 months at 10°C, 13°C and 15°C due to difference in energy allocation with time in stressed cold water corals (Chapron et al., 2018; Mouchi et al., 2019). Indeed, in the present study, growth rate appears faster during the first months of the experiment (Review Table 1). Due to the low number of replicates, conclusion on the topic are, however, limited and we therefore decided not to show these results in the manuscript to avoid confusion.

Review Table 1: Average polyp linear growth rate ± SD (mm y^{-1}) of L. pertusa and M. oculata for all, apical and subapical polyps, exposed at 17°C for different periods of incubation time. The number in brackets represent the number of replicates.

	17°C	All polyps (mm y^{-1})	Apical polyps (mm y^{-1})	Subapical polyps (mm y^{-1})
Lophelia pertusa	2 months	2.0±1.8 (30)	2.4±2.0 (15)	1.7±1.5 (15)
	4 months	1.1±1.2 (4)	1.2±0 (1)	1.1±1.5 (3)
	5 months	0.8±0.7 (4)	1.2±0.6 (3)	0.3±0 (1)
Madepora oculata	2 months	1.2±0.8 (35)	1.0±0.9 (10)	1.3±0.4 (25)
	3 months	0.6±0.4 (19)	0.5±0.2 (4)	0.8±0.4 (15)
	4 months	0.7±0.5 (10)	0.3±0.2 (2)	0.7±0.6 (8)

Figure 3, here error bars shown but what are the number of replicates?
We now write 'Mean values (from three replicates per time point) and standard deviations are presented' Lines 263-264.

Try improving flow of thought (readability).
We revised the manuscript which has been also read by a native English speaker Dr Rowan McLachlan.

No tables were found in the main document. Consider whether converting any of the figures into a table would improve.
We took into account the reviewer's suggestion and replaced Figure 2 'Linear polyp growth' with a table' Table 1: Average polyp linear growth rates (mm y^{-1}) of L. pertusa and M. oculata for all, apical and subapical polyps, under different temperature conditions after 6 months of incubation. The number in brackets represent the number of replicates.' Lines 310-313. The original Figure 2 is now in the supplemental material (Figure S3).

The reason for reporting a ten degrees incubation is still a bit confusing. If tied more closely with the North Atlantic corals, i.e. other such corals thrive at this temperature, this may become clearer.
For a better explanation on the use of ten degrees in our study we now write: 'Finally, 10°C was used to investigate if corals maintained their health conditions at colder than present Mediterranean temperatures. This temperature corresponds to the thermal conditions of North Atlantic corals that thrive at 10°C. It also allows a direct comparison with earlier studies on the response of L. pertusa and M. oculata's oxygen consumption and calcification at 10°C [11,26].' Lines 119-142.

In the Results, the "Coral bacterial communities" paragraph is not so easy to understand. It is felt that especially Result sections readability can be improved (this will help improve understanding of Tables and Figures).
We think that we improved the readability of the 'Coral bacterial communities' paragraph in the results sections by shortening and rewriting the text. We now write:

'The seawater bacterial communities were similar between all tanks at the start of the experiment (T0), and differed from the coral bacterial composition (Figure 4, SI Appendix, Table S1). At the start of the experiment (T0), L. pertusa and M. oculata had different bacterial communities (Figure 4, SI Appendix, Table S1, ANOSIM, p<0.01).

Under control conditions (13°C), the coral bacterial community compositions for both L. pertusa and M. oculata did not change significantly through time (Figure 4, SI Appendix, ANOSIM, p>0.01).

Under experimental conditions, at 1 week, no change in bacterial community composition was observed in both L. pertusa and M. oculata (SI Appendix, Table S2, ANOSIM p>0.05). At 2 months, the microbiome of L. pertusa at 15°C and 17°C became similar to each other, separated from the 10°C and 13°C microbiomes, and converged in their composition with the microbiome of M. oculata at 17°C. (Figure 4, SI Appendix, Table S2). At 6 months, L. pertusa, microbiome at 10 °C in turn became similar to the microbiomes at 15°C and 17°C. In contrast in M. oculata, the microbiomes at 10°C, 13°C and 15°C were similar to each other and did not change with time (Figure 4, SI Appendix, Table S2). No data was available for M. oculata after 6 months at 17°C as all polyps were dead.
The bacterial communities had always higher diversity in L. pertusa than M. oculata (Shannon diversity index, ANOVA p<0.01). This diversity increased through time at each temperature for L. pertusa, while it remains stable for M. oculata, except at 2 months for 17°C, where an increase was observed (SI Appendix, Figure S7).
At the start of the experiment and in control condition, the ASV1 (order Cellvibrionales) was the most abundant in L. pertusa (14%) and the ASV6 (Spirochaetales) was the most abundant in M. oculata (15%). Most of these ASVs were most closely related to sequences previously retrieved from shallow-water coral species (SI Appendix, Table S3).
At 10°C, the ASV1 and ASV42 (Rhodobacterales) were the most abundant at 1 week in both coral species, then the ASV12 (Rhiziobiales) at 2 months, and the ASV49 (Rhodobacterales) at 6 months (Figure S8).
At 15°C and 17°C, both L. pertusa and M. oculata bacterial communities were dominated by the ASV1 and ASV24 (order Rhodobacterales) at 1 week. Then, they were dominated by the ASV4 (order Rhodobacterales) and ASV3 (order Acidimicrobiales) at 2 months, with significantly higher abundances at 17°C (Figure S8, Table S3). The ASV23 (Epsilonproteobacteria) also increased in relative abundance at 17°C for both species at 2 months. The ASV24 is 100% similar to sequences found in bacteria growing on living [42] or inert surfaces [43].
Most of these dominant ASVs characterizing lower or higher temperatures were not related to sequences previously found on corals (SI Appendix, Table S3).' Lines 398-553.

Wherever possible, consider try shortening the manuscript because this may help focus. Discussion, look over, try to improve. Language can be improved (grammar and style).
We shortened the manuscript and the manuscript has been read by a native English speaker Dr Rowan McLachlan. Line 1081.

Minor comments (line numbers refer to revision)
Ln 1: consider revert back to original title ("Resilience of cold water coral holobionts to sea water temperature changes") or minimum include "temperature". Sorry, I may have confused suggesting changes in the title. Temperature is in focus and "global change" may be too vague. See also title going with the deposited sequences which also highlight temperature ("Cold water coral exposed to thermal stress").
We understand reviewer's concern and now write as title 'Resilience of cold water coral holobionts to thermal stress' Line 1.

Ln 19: the plus and minus signs may need support for clarity. Consider if using the term initially introduced in the Abstract "rising temperatures" (or "rising") will clarify its deviation from 13℃ (and not absolute temperature) and may be used instead of such as "higher".
We followed reviewer' suggestion and now write 'We found that at warmer temperature (+2°C)' Line 21 and 'warmer seawater temperatures (15 and 17°C)' Line 83.
Ln 164: PCR condition not found included: "Amplicon fragments are PCR-amplified using the high-fidelity Phusion polymerase under conditions of 30s at 98°C, 16 cycles of 98°C for 10s, 60°C for 30s, 72°C for 80s and final extension for 5m at 72°C."
We added this sentence in lines 228-230.

Ln 179: I'm sorry, yes samples, I misunderstood "Biological" seems not needed. To simplify "Samples containing less than…"
We understand the confusion and went back to 'Samples containing less than' Lines 245-246.

Ln 190: how many sequences? I.e. what are the sample sizes counts were divided by to obtain normalized data? Show at least range, minimum to maximum.
The normalization of the data depends of the total number of reads per samples which changed among samples. We now write '…data were normalized by dividing counts for each sample by the sample's size to obtain a relative abundance (average of 2500 reads per sample)' Lines 258-259.

Ln 196: why were not ANOVA but ANOSIM used for comparison of the bacterial communities? Has this to do with normality? Can this somehow be briefly explained?
The ANOSIM test is similar to an ANOVA, but it uses a dissimilarity matrix as input instead of raw data. It is also non-parametric, so it's a good test for skewed microbial abundance data. As a non-parametric test, ANOSIM is a very nice complement to an NMDS plot. ANOSIM tests if the differences between two or more groups are significant. We now explain it briefly in the text: 'ANOSIM is a non-parametric test that uses a dissimilarity matrix as input to determine if there are significant differences in microbial community composition between groups of samples.' Lines 264-266.

Ln 335: should carbohydrates be discussed as well?
We did not discuss carbohydrates in detail because it's not a major component of the total energy storage for corals. We now justify it clearly in the discussion: '…with lower skeletal growth, energy reserves (except for carbohydrates, which are not a major component of the total energy storage in corals), altered behavior…' Lines 560-563.

Ln 402: dysbiosis prior to host physiology changes has been shown for tropical corals (see for example Glasl et al. Microbiome 2019).
We added the reference: 'Dysbiosis appeared prior to changes in the host physiology and the microbial signature could therefore be used as a promising sentinel of coral health [XX].

Figure 4: legend, number of replicates? What are those stars above bars representing?
This figure is now Figure 3. We increased the size of the legend in the upper right corner, added the replicate number of nubbins and explaining the significance of the stars. We now write: 'Mean values (from three replicates per time points) and standard deviations are presented. The stars represent the significant differences between values from temperature exposures and the values from the start of the experiment (T0, ANOVA, $p<0.05$).' Lines 391-395.

Supplementary: “Chapron et al. [28]…” should this be “Chapron et al. [29]”?
Thank you for noticing. We changed to ‘Chapron et al., [29]’ in the Supplementary material.

Table S2: in legend, include what differences that are compared. From the text it seems to be bacterial community diversity.
We now write in the supplementary document ‘Table S2: One-way analyses of similarities (ANOSIM) among temperature within each time point for bacterial community composition of L. pertusa and M. oculata (global R=0.33, p=0.001) with pairwise tests of differences between Time and Temperature. Significant effects (p-value<0.01) are in bold. All models were run with 9999 permutations.’

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Abstract: Cold water corals are threatened by global warming, especially in the Mediterranean Sea where they live close to their upper known thermal limit (i. e., 13°C), yet their response to rising temperatures is not well known. Here, temperature effects on Lophelia pertusa and Madrepora oculata holobionts (i.e., the host and its associated microbiome) were investigated. We found that at higher seawater temperatures (+2°C), L. pertusa showed a modification of its microbiome prior to a change in behavior, leading to lower energy reserves and skeletal growth, whereas M. oculata was more resilient. At extreme temperature (+4°C), both species quickly lost their specific bacterial signature followed by lower physiological activity prior to death. In addition, our results showing the holobionts’ negative response to colder temperatures (-3°C), suggest that Mediterranean corals live close to their thermal optimum. The species-specific response to temperature change highlights that global warming may affect dramatically the main deep-sea reef-builders, which would alter the associated biodiversity and related ecosystem services.
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