

Influence of past climatic change on phylogeography and demographic history of narwhals, *Monodon monoceros*

Marie Louis, Mikkel Skovrind, Jose Alfredo Samaniego Castruita, Cristina Garilao, Kristin Kaschner, Shyam Gopalakrishnan, James S. Haile, Christian Lydersen, Kit M. Kovacs, Eva Garde, Mads Peter Heide-Jørgensen, Lianne Postma, Steven H. Ferguson, Eske Willerslev and Eline D. Lorenzen

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Original submission: 20 December 2019
1st revised submission: 1 March 2020
2nd revised submission: 28 March 2020
Final acceptance: 30 March 2020

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

Review History

RSPB-2019-2964.R0 (Original submission)

Review form: Reviewer 1

Recommendation

Accept with minor revision (please list in comments)

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

Good

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Excellent

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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.

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Decision letter (RSPB-2019-2964.R0)

10-Feb-2020

Dear Miss Louis:

Your manuscript has now been 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 with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision 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:

Research ethics:

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. 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=\(Document not available\)](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.

For more information please see our open data policy <http://royalsocietypublishing.org/data-sharing>.

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,
Professor Gary Carvalho
mailto:proceedingsb@royalsociety.org

Associate Editor
Comments to Author:

The two reviews are both positive and provide thorough reviews. Both then actually provide pretty detailed comments, which I think will help clarify and balance the ms, and these comments need addressing carefully and thoroughly.

Reviewer(s)' Comments to Author:

Referee: 1

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Author's Response to Decision Letter for (RSPB-2019-2964.R0)

See Appendix A.

RSPB-2019-2964.R1 (Revision)

Review form: Reviewer 1

Recommendation

Accept with minor revision (please list in comments)

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

Excellent

General interest: Is the paper of sufficient general interest?

Excellent

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

Excellent

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

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

Is it accessible?

No

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

Excellent work addressing the points of both of us reviewers, particularly I appreciated the efforts the authors are making to disseminate their findings to Indigenous folks. Also my apologies for sending you down the rabbit hole for trying to calculate SD for haplotype and nucleotide diversity for Figure 2. I remember running into difficulties trying to calculate this myself using Nei's analytical formulas, and had thought the `genetic_diversity_diffs` R script you used for the narwhal populations could calculate this (but I see it only assesses whether populations are significantly different from each other). Your decision on which plot to include is a fair one!

Also good justification for not mentioning matrilocality in this manuscript. I think this had been solidified in my brain by mention of it in Whitehead's (1998) paper on 'cultural hitchhiking', and I was unaware that it wasn't based on explicit observations, so thanks for informing me!

Again, great work on this manuscript, and I'm looking forward to seeing it out in the literature. I have a few very, very minor comments below:

Abstract

Line 35: I wonder if it would be more accurate to say "limited geographic structuring" rather than "a lack of geographic structuring"?

Methods:

Line 185: I wonder if saying "as well as for the physeterid sperm whale" instead of "as well as for sperm whales" will make it clear to folks less familiar with cetaceans that the sperm whale is not a delphinid.

Results:

Line 265-266: I feel the authors should summarise the patterns found that are shown in Supp Table S4, because if the results aren't going to be mentioned here, then perhaps the tests themselves are superfluous.

Line 329-330: I suggest commenting not just on decline in habitat, but potential isolation of subpopulations e.g. looks like populations will potentially be isolated on each side of Baffin Island.

Discussion:

Line 435: Suggest changing "significant" to "strong" or another word that cannot be conflated with statistical significance (as some of the differentiation between stocks was indeed significant).

Figures and supplementary materials:

Figure 2 legend: Need to indicate what the different colours for the data points associated with each species mean (e.g. grey vs dark). What determines the order that the delphinids have been plotted in?

Genbank accession numbers need to be added to Table S1 and S2 before publication, other than that, no comments on the supplementary materials.

Review form: Reviewer 2 (Phillip Morin)

Recommendation

Accept as is

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

Good

General interest: Is the paper of sufficient general interest?

Excellent

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

Excellent

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

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

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

The authors have adequately responded to previous reviews and the manuscript is now acceptable for publication. Congratulations on a nicely written and valuable study.

Decision letter (RSPB-2019-2964.R1)

23-Mar-2020

Dear Miss Louis

I am pleased to inform you that your Review manuscript RSPB-2019-2964.R1 entitled "Influence of past climatic change on phylogeography and demographic history of narwhals, *Monodon monoceros*" has been accepted for publication in Proceedings B.

The referee(s) do not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let me know immediately.

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You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, upload a new version through your Author Centre.

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

3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g. `authorname_procb_ESM_figures.pdf`

All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please see: <https://royalsociety.org/journals/authors/author-guidelines/>

4) Data-Sharing and data citation

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

If you wish to submit your data to Dryad (<http://datadryad.org/>) and have not already done so you can submit your data via this link

<http://datadryad.org/submit?journalID=RSPB&manu=RSPB-2019-2964.R1> which will take you to your unique entry in the Dryad repository.

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

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

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

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

Associate Editor Board Member: 1

Comments to Author:

Both reviewers are happy with the revisions made, thank you for the care taken in these responses. One reviewer suggests a small number of further and minor alterations be considered, and I think this should be done.

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s)

The authors have adequately responded to previous reviews and the manuscript is now acceptable for publication. Congratulations on a nicely written and valuable study.

Referee: 1

Comments to the Author(s)

Excellent work addressing the points of both of us reviewers, particularly I appreciated the efforts the authors are making to disseminate their findings to Indigenous folks. Also my apologies for sending you down the rabbit hole for trying to calculate SD for haplotype and nucleotide diversity for Figure 2. I remember running into difficulties trying to calculate this myself using Nei's analytical formulas, and had thought the `genetic_diversity_diffs` R script you used for the narwhal populations could calculate this (but I see it only assesses whether populations are significantly different from each other). Your decision on which plot to include is a fair one!

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Genbank accession numbers need to be added to Table S1 and S2 before publication, other than that, no comments on the supplementary materials.

Author's Response to Decision Letter for (RSPB-2019-2964.R1)

See Appendix B.

Decision letter (RSPB-2019-2964.R2)

30-Mar-2020

Dear Miss Louis

I am pleased to inform you that your manuscript entitled "Influence of past climatic change on phylogeography and demographic history of narwhals, *Monodon monoceros*" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

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

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

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Electronic supplementary material:

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

You are allowed to post any version of your manuscript on a personal website, repository or preprint server. However, the work remains under media embargo and you should not discuss it with the press until the date of publication. Please visit <https://royalsociety.org/journals/ethics-policies/media-embargo> for more information.

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

Sincerely,

Editor, Proceedings B

mailto:proceedingsb@royalsociety.org

Appendix A

Dear Miss Louis:

Your manuscript has now been 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 with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision 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:

Research ethics:

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. 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=\(Document not available\)](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.

For more information please see our open data policy <http://royalsocietypublishing.org/data-sharing>.

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,

Professor Gary Carvalho
mailto: proceedingsb@royalsociety.org

Associate Editor

Comments to Author:

The two reviews are both positive and provide thorough reviews. Both then actually provide pretty

detailed comments, which I think will help clarify and balance the ms, and these comments need addressing carefully and thoroughly.

>> Dear Professor Carvalho,

Thank you for your decision letter and your comments. Please find attached a revised version of our manuscript. The reviewer comments were very thorough and helpful, and we have taken all input into account.

Please find below our detailed responses to the reviewers' comments.

Sincerely,

Marie Louis and Eline Lorenzen, on behalf of all co-authors

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

The authors use a very comprehensively sampled (in terms of range and number of samples – cetaceans being very tricky to sample, polar cetaceans even more so) dataset for narwhals, to demonstrate the direct impact of climate change on mitogenomic phylogeny and diversity. The figures were both informative, and beautiful to look at. The language throughout the manuscript was excellent. In some places I would like a little more context/interpretation of patterns and methodology choices (e.g. the use of mitogenomes rather than other markers), but I believe the analyses conducted are solid and support the general interpretation of the authors that shifts in distribution due to climate change have directly impacted the phylogeny and diversity of narwhal mtDNA. I think with the general interest in the effects of climate change on Arctic ecosystems, this paper would be of high interest to a broad range of biologists. I include some more detailed comments below (and hopefully you can figure out what I'm talking about based on the approximate page positions).

>> Dear reviewer 1,

Thank you for your very helpful comments. We agree that our manuscript needed more context and discussion of results and methodological choices, and we have taken all your input into account. Specifically, we now include a sentence in the Introduction on why we used mitogenomes (L118-120), and on the impact this may have on our results (L378-380) in the Discussion. We also account for alternative explanations of our Bayesian skyline analysis results (L391-393). We have detailed our response to each point raised below.

And many apologies that page numbers were not included in our first submission – this was a formatting error when moving the document from google docs to word; we understand this made the review process much more time consuming.

Abstract: Excellent. Normally I find something more to pick on!

Introduction:

-- Bottom of page 3: A recent paper (which probably came out after this paper was submitted) also described a similar phenomenon (population expansion of ice-obligates) in penguins in the Southern Ocean, just in case you would like to further bolster evidence that ice-landscapes can be very influential on Ne of species.

Cole, T.L., Dutoit, L., Dussex, N., Hart, T., Alexander, A., Younger, J.L., Clucas, G.V., Frugone, M.J., Chereil, Y., Cuthbert, R. and Ellenberg, U., 2019. Receding ice drove parallel expansions in Southern Ocean penguins. *Proceedings of the National Academy of Sciences*, 116(52), pp.26690-26696.

>> Thank you for the reference, we included it in the revision L81.

-- Top of page 5: Given the mitogenome is maternally inherited, and narwhals are thought to be somewhat matrifocal, I believe you need to address (a) why you chose to use the mitogenome for this study, rather than say RADseq or some kind of reduced representation nuclear marker and (b) address the impact that matrifocality may have on some of your population inferences. The latter point might be more suited for the discussion instead of the introduction, but I think it is important to address.

>> comment a): we added the following sentence L118-120: “Here, we present complete mitochondrial genomes (mitogenomes) from 121 narwhals sampled across their range (figure 1a); our study focuses on mitogenomes, as they are a useful marker for inferring phylogeographic and evolutionary processes in species with low genetic diversity (e.g. [31,32]).”

We have not included explicitly that narwhals are matrifocal as this has never been explicitly tested, as far as we know.

comment b): We also clarified in the discussion that the significant genetic differences we see could be linked to maternally transmitted site fidelity 378-380: “Mitogenomes are maternally inherited, and the genetic differentiation of Svalbard and East Greenland from animals in other areas could reflect maternally transmitted site fidelity “.

-- Top of page 5, last sentence of the introduction: You also use these SDMs to explain the patterns of diversity (e.g. the presence of the two distinct lineages likely corresponds to isolation on either side of Greenland following the last interglacial period), so suggest you expand this sentence to reflect that you use the SDMs not only to interpret patterns looking backwards, but also to look at what ongoing climate change is likely to mean for this species. One last comment about the SDMs: it should be possible to generate an SDM for a future time point based on current warming projections. This might be of general interest and add value to your paper (e.g. where are narwhals likely to be found in 2100 etc).

>> We added the work “ongoing” to the sentence L122-124 “To address the resilience of narwhal populations to near-future projections, we use species distribution models to reconstruct their demographic history and assess the impact of past and ongoing climate shifts.”

We have added the SDM estimate for year 2100 to Figure 4, and included a few sentences on the findings in the main text, in the Results (L329-330): “The estimate for year 2100 shows a 1.6° northwards shift and decline in suitable habitat size of 25% relative to the present.” And in the Discussion (L443-446): “Although associated with a large degree of uncertainty, our habitat suitability estimates for year 2100 indicate a 25% decline and 1.6° northwards shift in habitat availability, suggesting narwhal habitat is likely to decrease in size as sea temperatures rise and sea ice continues to decline.”

Results support a decrease in narwhal suitable habitat in the future.

Methods:

-- I did not pick up on any issues in the methods, but have some comments on methods/results located in the Supplementary Text (below).

Results:

-- Check tense throughout results – some seems to be in present tense rather than past tense.

>> Everything has been changed to past tense.

-- Page 9, ‘Haplotype network’: The final sentence of this section is not entirely accurate, as there are other locations that have closely related haplotypes. The key difference is that ‘Svalbard (n=5) was the only sampling location where all individuals shared haplotypes that were closely related, separated by ...’

>> We changed the sentence to L259-262 “Svalbard (n=5) was the only location where all individuals shared haplotypes that were closely related, separated by eight mutations at most.”

-- Page 9, ‘Diversity statistics’: Suggest giving some context to your comparison to sperm whales by mentioning that they are a cetacean species previously found to have exceedingly low levels of mitogenomic diversity (e.g. Alexander et al. (2016); Morin et al. (2018)).

>> We modified the sentence to L273-276 “The value estimated in narwhals was similar to the range-wide estimate of another toothed whale, the sperm whale, which has previously been reported to have extremely low levels of mitogenomic diversity [32].”

-- Page 9, ‘Fixation statistics’: “Pairwise fixation index estimations” or “Pairwise fixation index estimates”?

>> We modified it to “Pairwise fixation index estimates”.

-- Page 10, ‘Phylogenetic analysis’: ‘genetic clades’ seems a bit redundant – would think just ‘clades’ could suffice. Also in this paragraph, suggest mentioning that the clade with the Svalbard samples is the clade towards the top of the tree in Fig 3a and Fig S4.

>> We modified “genetic clades” to “clades” in “Phylogenetic analysis”. We added the following L302-303 “This clade, situated towards the top of the tree, diverged 22 kya (95% HPD: 43-8 kya)”.

Discussion:

Page 12, last paragraph: This is where I feel like the authors might need to mention some caveats about their Bayesian skyline model rather than interpreting it solely in terms of ‘female effective population size’. Bayesian skyline models, just like PSMC, are based on coalescent rates. This means the detected increase in ‘Nef’ could be reflecting increased population size, but could also be reflecting increased population structure as more habitat opened up following the LGM followed by site fidelity to new locations. Both processes are consistent with a reduction in sea-ice extent and support the authors' link to climate, but I think this paragraph in particular needs a little more nuance to talk about the alternative reasons for an apparent increase in Nef.

>> We agree with the reviewers and we added the following sentence in the discussion L391-394: “Although Bayesian skyline models are based on coalescence rates, and an increase in *Nef* could therefore reflect changes in population structure, a population expansion is supported by the star-like topology of most groups in our haplotype network (figure 1b).”

Acknowledgements/Ethics: Although it is excellent that hunters who assisted with sampling were acknowledged, were any additional efforts made to consult with and inform traditional land owners about your intended research and findings? (i.e. in line with growing expectations about equitable benefit sharing, including knowledge e.g. Nagoya Protocol).

>> A summary of our findings will be included in non-scientific language (both in Danish and in Greenlandic) in a book edited by the Greenlandic Institute of Natural Resources (GINR). The book is intended for hunters and policy makers in Greenland, and will present all the data and results collected by GINR on narwhals these past decades.

Figures and Tables:

-- Figure 2: Suggest adding standard error bars or similar to the plot, as sample size differs markedly between each of these species. Also, the colour of the dots seems to indicate whether the species is matrilineal/matrifocal. This description needs to be added to the figure legend.

>> SD cannot be calculated as far as we know when using the option: “excluding sites with gaps and missing data only in each pairwise comparison” in DnaSP. We would like to use this option as it is well suited to our compiled dataset due to relatively high amount of missing data in some of the published datasets.

We also checked R packages, including the package ape and pegas and it is not possible to compute variance either due to the way variance is calculated. When using option “excluding sites with gaps and missing data only in each pairwise comparison”, that is `pairwise.deletion = TRUE`, the sequences analysed will have different missing sites and thus different lengths among pairwise comparisons. The variance of the estimated diversity uses formula (10.9) from Nei (1987). And this formula applies only if all sequences are of the same lengths.

However, we also computed nucleotide diversity, excluding all sites with gaps and missing data, for which we can compute variance. This option masked a site in all individuals when there is a N. Some dataset from other species, such as *Stenella attenuata* have a relatively high amount of missing data, in particular in the control region, and thus their nucleotide diversity is drastically reduced. We have copied the plot below but we prefer to include the plot with the option “excluding sites with gaps and missing data only in each pairwise comparison” in the manuscript as the way missing data are treated is most suitable to dataset with different levels of completeness, even if variance is not calculated.

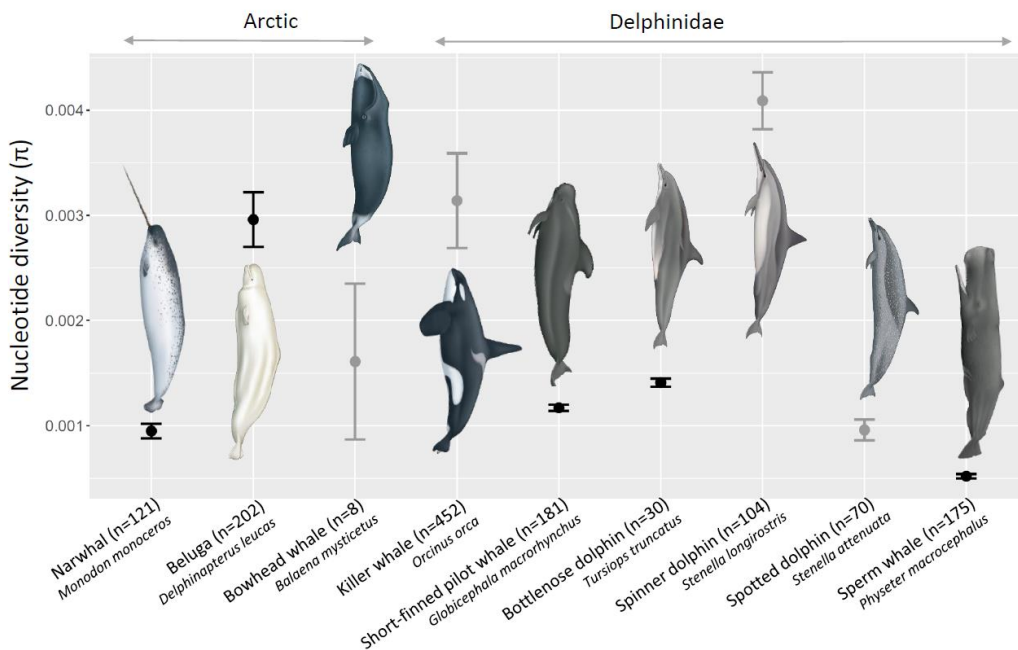


Figure. Mean nucleotide diversity (π) from published, population-level cetacean mitogenome studies from the Arctic and elsewhere. A site was masked if it had a missing data in one individual. The number of samples comprising each data set is shown. Cetacean illustrations by Uko Gorter.

Matrifocality in narwhals has never been explicitly tested and it has recently been shown not to be the case in the closely related beluga (Corry Crowe et al. submitted ms). We therefore would prefer not to indicate the matrifocal information in the legend.

-- Figure 3: Suggest slightly modifying legend for (b) to state “121 mitogenomes” rather than just “mitogenomes” (to make it clear that your skyline was correctly based over the population sample rather than just haplotypes). Good correspondence of PSMC and skyline plot data (especially give the lower effective population size of mtDNA relative to nuclear DNA).

>> We have included “121 mitogenomes” in the legend.

Supplementary Text:

-- Page 1, 'DNA extraction, amplification and sequencing': missing closing parentheses for "i.e. Blunt-End Single-Tube library building for modern and ancient DNA [1]."

>> Corrected

-- Page 5, 'Odontocete phylogeny' and 'Narwhal phylogeny': I'd suggest that comparing the parameter estimates (and topologies) from your two different runs would assess convergence, but looking at whether your ESS values are above 200 is actually assessing stationarity of the chains. (e.g. the wording you have in the 'Bayesian skyline analysis' section on Page 6 is better).

>> We agree and have modified the text to "We assessed stationarity by examining ESS values in Tracer v1.7.1 [31] and convergence by comparing posterior distributions between the two chains."

-- Page 6, 'Bayesian skyline analysis': "We also ran" rather than "We also run"

>> Corrected

-- Page 7, 'Results': I would appreciate a quick discussion/table on the mitogenome bioinformatics QC. Were all sequences fully resolved with no Ns? Were any apparent indels present in protein-coding regions? In addition, given your skimming approach, it is unlikely that "> 10 reads where a single nucleotide did not represent > 80% of the reads" result from PCR artefacts, and may instead reflect heteroplasmy (would be interesting to know the rates in narwhals)

>> We agree that the quality-checks statistics were missing and we have now included them in the supplementary text. We have a low percentage of missing data that is 0.83%, and we are therefore confident this is not affecting our results.

Indels seemed to occur mainly in areas where they were surrounded by Ns or poly-nucleotides. Most of them likely resulted from differences in sequencing platforms between the reference mitogenome and our data, in particular in poly-nucleotide regions. As they may be spurious, we prefer not to include those details in the manuscript.

We have added the following text on the QC in the supplementary material:

"The mitogenome sequence had 16,383 sites, including 16,030 sites with no missing data or gaps across all individuals. 353 sites had missing data; 15,879 sites were invariables and 151 sites were variable. Over all 121 individuals and the full 13,838 bp sequence, representing 1,982,343 nucleotides, 16,438 nucleotides were called Ns, representing 0.83% of the nucleotides. Missing data mainly occurred at the ends of the mitogenome sequences and in the control region for the samples with the lowest coverage. We called 489 nucleotides as Ns as there was < 10 reads with one or more nucleotide variations, or > 10 reads with a single nucleotide not representing > 80% of the reads. This mainly occurred in the control region and might represent heteroplasmy."

-- Page 9, 'References': Species name needs to be italicized for Ref [22]

>> Done

Supplementary Figures and Tables:

-- I have no comments on the Supplementary Figures, well done!

-- Table S1: Suggest using the following format for date, given the confusion Americans cause with their opposite day/month format: 00-MON-YEAR. Also need to explain what "sample_CGG_ID" stands for (all other column headers seem self-explanatory).

>> We have modified the date format as you suggest. We have included the following text in the legend "Sample_CGG_ID in the sample ID refer to the Centre for GeoGenetics database sample ID at the University of Copenhagen while sample_ID refers to the sample ID provided by the source institution".

-- Table S2: 'Baird's beaked whale' has inconsistent capitalization in comparison to the rest of the table.

>> Corrected

-- Table S4: Need to explicitly state that the blue shading corresponds to significant differences.

>> Corrected. We added "Differences were considered significant if they were <0.05 and are indicated by blue shading and bold font."

-- Table S5: What does the colour gradient represent?

>> We added the following in the legend: "Colour gradient from light yellow to red indicates the strength of the F_{ST} values in bins of 0.1 and significant values ($p < 0.05$) are indicated in bold font."

-- Table S6: Appears to be missing from the manuscript.

>> We have now added the table to the manuscript.

-- Table S7 and Table S8: Spelling of 'partitioned' in legend

>> Corrected

-- Table S8: The Supplementary Text suggests two chains were run for this analysis. Why are the combined chains not presented as for Table S6 and Table S7?

>> This was an error and the combined chain is now presented in Table S8.

As an aside: a plea from the reviewing community. Even if the journal doesn't request it, please include line numbers for your manuscript. This greatly facilitates referring to specific parts of the manuscript, and reduces some of the time needed to give a comprehensive (and hopefully helpful!) review. Again, nice work on this manuscript!

>> We do apologize, this was an unfortunate oversight on our part when we formatted the google doc document into a Microsoft Word document.

Referee: 2

Comments to the Author(s)

The authors present analysis of large set of full mitochondrial genomes from across the range of the Narwhal. The analytical methods are appropriate to address the questions, including time-calibrated

phylogenetic analysis and historical demography based on Bayesian skyline plot, and the results are interpreted with the aid of habitat models that indicate concordance of population change with habitat change since the last glacial maximum.

The Manuscript is very well written, with clear descriptions of most methods and results, and sufficient supplemental materials showing the strengths and limits of the analyses. The methods section is oddly short, with some methods relegated entirely to supplemental materials, while others are presented in some detail in the text (with additional detail in the supplemental files). The results are supported by the data, and provide strong context for the effect of climate change on Narwals and other Arctic (and Antarctic) adapted marine mammals. The one issue I have with the results is that the population divergence metrics results (Phi-ST and FST) are not presented in the paper, and are mostly dismissed in the discussion even though there are some interesting patterns and strong divergence among some populations (see detailed comments).

>> Dear reviewer 2,

Thank you for your very useful comments. We agree that some methods were too short in the main text, and we have added more details, in particular for the laboratory and sequencing procedures (L140-147) and bio-informatics (L151-155) as detailed below. We also included more text in the results/discussion about the population divergence metrics results (L374-383).

Please find below our detailed comments below to each of the issues you raised.

Detailed comments:

Page 4, paragraph 2: The paragraph starts with “of the eleven Arctic marine mammal species...”, but only 3 were previously mentioned, so this is a little confusing. I think it would be useful for the authors to first indicate that there are 11 Arctic marine mammals, and what they are (or at least what groups, e.g., pinnipeds, polar bear).

>> We changed the sentence to L93-96 “Eleven marine mammal species are found in the Arctic; in addition to the three cetacean species, these include six pinniped species and polar bears. Narwhals are considered one of the most sensitive of these Arctic marine mammals to sea ice loss and associated trophic cascades, and also to increases in human activities as sea ice disappears [5,19].”

Page 5: Why are the methods so brief? Is the journal pushing all methods to supplemental materials? If not, the methods section for DNA extraction, amplification and sequencing is too abbreviated. Even if the details are provided in supplemental materials, the main text should include a brief outline of all methods, including the library preparation, whether (and how) mitochondrial DNA was enriched prior to sequencing, how many samples were pooled per library, and sequencing method. The “Bioinformatic” section is also very vague, and needs more explanation. Simply saying “reads were processed using PALEOMIX” is not sufficient. What does processing do, and what quality checks or parameters were used? The reader can go to the supplemental materials for full details, but should not have to do that for a simple overview of the methods. Without them, it’s really difficult to read the paper itself and evaluate the appropriateness of the methods and strength of the results.

>> We agree that the methods section was too short, this was done to ensure that we complied with the page length limit of the journal. We have now included an overview of both the laboratory and bioinformatics methods within the main text.

We have added the following text for the laboratory methods in the main text (L140-147):

“In short, we extracted DNA from tissue samples using the Qiagen Blood and Tissue Kit, with minor modifications, and fragmented the DNA to ~350-550 base pair (bp). Libraries were prepared and sequenced according to two different protocols. For 84 samples, libraries were built using the Illumina NeoPrep and sequenced on an Illumina HiSeq 2500 with the 80bp SE technology. For 37 samples, libraries were built using the BEST protocol (i.e. Blunt-End Single-Tube library building for modern and ancient DNA) [34] and sequenced on an Illumina HiSeq Xten with the 150bp PE technology. Full details on DNA extraction, library preparation and sequencing are included in the supplementary text.”

We do not have details about how many samples were pooled per lane as they were sequenced together with other projects.

We have added the following text for the bioinformatics in the main text L150-157:

“After demultiplexing, sequencing reads were processed using PALEOMIX v1.2.13.1 [35] (see supplementary text for details). Briefly, the PALEOMIX pipeline (i) trimmed read ends for residual adapters and low-quality stretches, (ii) mapped reads to the published narwhal mitogenome reference (Genbank accession number: NC_005279) [36] using bwa v0.7.15 [37], requiring a minimum mapping quality of 30, (iii) removed duplicates and (iv) re-aligned indels. We built consensus sequences using the FastaAlternateReferenceMaker function in GATK [38]. We aligned sequences using the ClustalW algorithm in MEGA X [39]. Our QC procedures are described in supplementary text.”

Page 6 and Figure 2: Is there a reason short-finned pilot whales were not included in the analysis of mitogenome diversity? Nucleotide diversity across the global sample and within clades was reported by Van Cise et al. 2019 (Table 1; DOI: 10.1111/mec.15107). Since it's a closely related odontocete, it seems like a good species to include.

>> This manuscript was not yet published when we first compiled our figure. We agree it is a good species to include and we have included it in Figure 2. We added the corresponding information in the text in the methods L181-185.

Page 7: Based on the methods and results, it appeared that only phi-ST was used (not FST), but when I looked at the supplemental materials, results from both metrics were provided in Table S5. Given that there is low differentiation among haplotypes, it is unlikely that phi-ST will have any more power to detect differentiation than the frequency-based metric FST, and it would be interesting to discuss both, even if only one is presented in the main text. It's also worth noting that both metrics will suffer from low power due to the high haplotypic diversity. The majority of samples have unique haplotypes, so frequency-based measures essentially ignore those haplotypes. Despite this lack of power, there are quite a few significant results and large values, so I think these results should be

shown in a table in the paper, so that both the statistical difference from zero, and the magnitude of the divergence can be evaluated. The color coding of the table should also be explained, and the use of bold font to indicate statistical significance ($p < 0.05$). Contrary to the conclusions of the authors, I think the divergence metrics show that there is substantial genetic isolation of populations outside of the eastern Canadian Arctic, where all four of the Baffin Bay/West Greenland (Melville Bay), eastern Greenland, and Svalbard populations are significantly divergent from most others.

>> We have added in the methods that both Φ_{ST} and F_{ST} were estimated and changed the previous sentence “To address levels of genetic differentiation among summer localities, we estimated Φ_{ST} values, which take distance between haplotypes into account, using Arlequin [43]” to L188-189 “To address levels of genetic differentiation among summer localities, we estimated Φ_{ST} and F_{ST} using Arlequin [47]”.

We have added explanations about the colour coding and the fact that bold font was used to indicate statistical significance ($p < 0.05$) to the legend of Table S5: “The colour gradient from light yellow to red indicates the strength of the F_{ST} values in bins of 0.1 and significant values ($p < 0.05$) are indicated in bold font.”

We would prefer to keep Table S5 in the supplementary material, as we think it is good to be cautious about those results given that our sample size for each stock is relatively low for frequency-based statistics.

We have added the following text in the results regarding F_{ST} values, which we did not present in the text previously L280-282: “Based on Φ_{ST} , we found that Svalbard and Melville Bay stocks were significantly differentiated from all other stocks; F_{ST} values mainly differentiated Melville Bay from the rest (supplementary table S5).”

We mentioned previously in our results that Svalbard, Melville Bay and East Greenland are significantly differentiated from most other stocks “Pairwise fixation index estimates Φ_{ST} indicate significant differentiation among Svalbard and Melville Bay stocks and all the other localities. Narwhals from East Greenland are significantly differentiated from six other stocks (supplementary table S5).” However, we exercised caution regarding Melville Bay: “For the Melville Bay stock, closely related individuals could have been sampled, as six out of ten samples were collected on the same day. The six samples include closely related sequences, separated by seven mutations at most across the mitogenome. This could skew the results by increasing differentiation among stocks, if individuals within a group are more related than by chance.”

We have amended the text in the discussion to emphasize the significant differences of the Svalbard and East Greenland stocks from the others as well as the differentiation of the Melville Bay stock – although we exercise caution with the latter as mentioned earlier.

We added “across their range” to the sentence L372-374: “However, for narwhals, these processes are either not strong enough, or have not been in place for a long enough duration, to significantly alter the genetic pattern that has been shaped by longer-term climatic processes across their range”.

We modify the sentence “The exception is the distinctness of individuals from Svalbard, and to a lesser extent from East Greenland (supplementary table S5).” to L374-376 “The exception is the distinctness of individuals from Svalbard and from East Greenland (supplementary table S5).”

We also added a sentence regarding the differentiation of Melville Bay in the discussion L383-385 “Narwhals from Melville Bay are also significantly differentiated from all the other stocks, but we cannot exclude this could be due to the sampling of related individuals”.

Given that genetic differentiation may be due to our sampling scheme for the Melville Bay stock and the fourth stock outside of the Canadian Arctic (Inglefield Bredning) is not significantly differentiated from the other stocks apart from the Svalbard, East Greenland and Melville Bay stocks, we prefer not to state that “there is substantial genetic isolation of populations outside of the eastern Canadian Arctic”.

Results section:

The only description of the resulting mitogenome assemblies was the average and range of depth of coverage. Were all mitogenome sequences complete? If not, what was the number with missing or ambiguous data, and range of the number of N’s? Were there any repeat regions that were not clearly resolved in all samples? Was there any evidence of heteroplasmy? These are all issues that have been seen in other studies and can affect the strength of results, so it’s important to address them.

>> >> We agree that the quality-checks statistics were missing and we have now included them in the supplementary text. We have a low percentage of missing data (0.83%) and are therefore confident this is not affecting our results. We used the backtrack algorithm in bwa, which only keeps reads uniquely mapping to a position in the genome, therefore limiting the occurrence of repeats.

We have added the following text in the supplementary material:

“The mitogenome sequence had 16,383 sites, including 16,030 sites with no missing data or gaps across all individuals. 353 sites had missing data; 15,879 sites were invariables and 151 sites were variable. Over all 121 individuals and the full 13,838 bp sequence, representing 1,982,343 nucleotides, 16,438 nucleotides were called Ns, representing 0.83% of the nucleotides. Missing data mainly occurred at the ends of the mitogenome sequences and in the control region for the samples with the lowest coverage. We called 489 nucleotides as Ns as there was < 10 reads with one or more nucleotide variations, or > 10 reads with a single nucleotide not representing > 80% of the reads. This mainly occurred in the control regions and might represent heteroplasmy.”

Page 10 (Bayesian skyline analysis): The ~3kyr discrepancy between results based on the different data sets (full vs. 3rd position coding regions) is much less than the confidence intervals for each. This should be pointed out to make it clear that the hypothesis that the difference could be due to purifying selection is just a hypothesis, and there is currently no real support for a difference between the two data sets.

>> We agree and we amended and moved the text to the supplementary material “Our analysis using third codon positions only of the protein coding regions indicate a more recent increase in *Nef*, starting ~6 kya (supplementary figure S6), although confidence intervals in the two analyses largely overlap. The discrepancy between results based on the two different data sets might reflect purifying selection on the first and second codon positions of the protein coding regions but this hypothesis is not statistically supported”.

Figure 3b and c: The two colors used for the PSMC (blue) and Skyline (blue-green) plots are too similar. The $\delta^{18}\text{O}$ levels in 3c are not discussed in the paper so their meaning is not obvious to the reader. Why did the authors choose to show this proxy for temperature rather than a plot of global temperature inferred from this and other metrics?

>> We have changed the colors in Figure 3b – the PSMC plot is now in brown. We have amended the legend to “and a nuclear genome (brown line; lighter brown indicates bootstrap values)”

$\delta^{18}\text{O}$ levels are a good proxy for temperature, that is why we included this data on our plot. We have added the following text in the figure legend: “(c) $\delta^{18}\text{O}$ levels from Zachos et al. 2001 as a proxy for global temperatures [66].” We also now refer to Figure 3c in the discussion and clarify the meaning of $\delta^{18}\text{O}$ levels, at L394-396: “The timing of the expansion is coincident with temperature shifts associated with the onset of the Holocene (as shown by $\delta^{18}\text{O}$ levels, figure 3c), and an increase and northwards shift in the amount of available suitable habitat between the LGM and the present (figure 4)”.

Appendix B

Associate Editor Board Member: 1

Comments to Author:

Both reviewers are happy with the revisions made, thank you for the care taken in these responses. One reviewer suggests a small number of further and minor alterations be considered, and I think this should be done.

>> Dear Professor Carvalho,

Thank you for your decision letter. Please find attached the final version of our manuscript. We have taken the comments from Reviewer 1 into account.

Sincerely,

Marie Louis and Eline Lorenzen, on behalf of all co-authors

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s)

The authors have adequately responded to previous reviews and the manuscript is now acceptable for publication. Congratulations on a nicely written and valuable study.

>> Dear reviewer 2,

Thank you for your reviewing our manuscript again and for your comment.

Referee: 1

Comments to the Author(s)

Excellent work addressing the points of both of us reviewers, particularly I appreciated the efforts the authors are making to disseminate their findings to Indigenous folks. Also my apologies for sending you down the rabbit hole for trying to calculate SD for haplotype and nucleotide diversity for Figure 2. I remember running into difficulties trying to calculate this myself using Nei's analytical formulas, and had thought the `genetic_diversity_diffs` R script you used for the narwhal populations could calculate this (but I see it only assesses whether populations are significantly different from each other). Your decision on which plot to include is a fair one!

Also good justification for not mentioning matrilocality in this manuscript. I think this had been solidified in my brain by mention of it in Whitehead's (1998) paper on 'cultural hitchhiking', and I was unaware that it wasn't based on explicit observations, so thanks for informing me!

Again, great work on this manuscript, and I'm looking forward to seeing it out in the literature. I have a few very, very minor comments below:

>> Dear reviewer 1,

Thank you for your helpful comments. We have detailed our response to your comments below.

Abstract

Line 35: I wonder if it would be more accurate to say "limited geographic structuring" rather than "a lack of geographic structuring"?

>> We made the change.

Methods:

Line 185: I wonder if saying "as well as for the physeterid sperm whale" instead of "as well as for sperm whales" will make it clear to folks less familiar with cetaceans that the sperm whale is not a delphinid.

>> We made the change.

Results:

Line 265-266: I feel the authors should summarise the patterns found that are shown in Supp Table S4, because if the results aren't going to be mentioned here, then perhaps the tests themselves are superfluous.

>> We added the following text in the main manuscript: "Nucleotide diversity was the highest in East Greenland and the lowest in Svalbard (figure S2a)." Before the sentence. "We present the values per summer locality in figures S2a-b and the test for significant differences in diversity between stocks in supplementary table S4 and text."

And the following text in the supplementary material:

"Diversity statistics

Haplotype diversity did not significantly differ among localities (table S4). Nucleotide diversity was significantly different in eight comparisons (table S4). The value in East Greenland was significantly higher than in four other localities, and the value in Svalbard was significantly lower than in three other localities. Nucleotide diversity in Northern Hudson Bay was significantly higher than in Eclipse Sound."

Line 329-330: I suggest commenting not just on decline in habitat, but potential isolation of subpopulations e.g. looks like populations will potentially be isolated on each side of Baffin Island.

>> We prefer not to include text on the locations of suitable habitat given the uncertainty associated with the 2100 projection.

Discussion:

Line 435: Suggest changing “significant” to “strong” or another word that cannot be conflated with statistical significance (as some of the differentiation between stocks was indeed significant).

>> Done

Figures and supplementary materials:

Figure 2 legend: Need to indicate what the different colours for the data points associated with each species mean (e.g. grey vs dark). What determines the order that the delphinids have been plotted in?

>> We have added the following text: “Dark dots indicate range-wide data and grey dots local data.” We have changed the order slightly so that the delphinids are order by increasing nucleotide diversity value and grouped by range-scale and local-scale data.

Genbank accession numbers need to be added to Table S1 and S2 before publication, other than that, no comments on the supplementary materials.

>> The Genbank accession numbers have been added to Tables S1 and S2.