

## Archaeological mitogenomes illuminate the historical ecology of sea otters (*Enhydra lutris*) and the viability of reintroduction

Hannah P. Wellman, Rita M. Austin, Nihan D. Dagtas, Madonna L. Moss, Torben C. Rick and Courtney A. Hofman

### Article citation details

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### Review timeline

Original submission: 24 July 2020  
1st revised submission: 20 September 2020  
2nd revised submission: 9 November 2020  
Final acceptance: 9 November 2020

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

## Review History

### RSPB-2020-1787.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?**

Acceptable

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

Marginal

**Is the length of the paper justified?**

Yes

**Should the paper be seen by a specialist statistical reviewer?**

No

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

No

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

**Is it accessible?**

N/A

**Is it clear?**

N/A

**Is it adequate?**

N/A

**Do you have any ethical concerns with this paper?**

No

#### **Comments to the Author**

Wellman et al. present an interesting study on pre-extirpation genetic diversity in sea otters, based on full mitogenomes reconstructed from 18 archaeological dentine samples, and 16 historical dental calculus samples. They conclude that future reintroduction efforts in Oregon should also include individuals from extant northern populations. The study is of wide interest given the ecological role of sea otters in kelp ecosystems, as well as their socio-economical significance for Indigenous populations and the various implications of reintroduction for different stakeholders. The authors do an overall good job of addressing these aspects while putting their results also in an archaeological and population genetics/history context.

This study also presents the first full mitogenomes obtained from non-human dental calculus samples, providing new possibilities for non-destructive sampling of precious material from natural history collections. However, the missing description of their sampling protocol (see below) is problematic and prevents me from better assessing this aspect.

The manuscript is well written but some clarifications are needed to make it more understandable and easier to follow (see below).

- Page 4, lines 29-31: it's not entirely clear to me if you consider the historical specimens pre- or post-fur trade. I assume it's post, given the dates provided at page 3, line 30 and page 5, line 10, but this could be clarified a bit further. You should also explain, here and/or in the discussion, why you did not include modern data/samples of northern populations. I'm also missing more precise information on the age of the historical samples throughout the manuscript.

- Page 4, line 42: you mention gene flow here, have you looked at this in your own data?

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- Page 6, lines 14-16: is this 10 samples per archaeological site?

- I find the color coding in the figures a bit difficult to follow. The explanations in the figure legends are also on the minimal side, especially for figure 1. I have to go back to the text a lot to understand the details and I think more extensive figure legends are necessary.
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- Supplementary table 1: please add age (or age range) information for the samples.
- Supplementary File ModAlign.fa: is it correct that these sequences are 47bp long? From the text I was under the impression that they should be 222bp long.

## Review form: Reviewer 2

### **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?**

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?**

Yes

**Is it clear?**

Yes

**Is it adequate?**

Yes

**Do you have any ethical concerns with this paper?**

No

**Comments to the Author**

Review of "Archaeological mitogenomes illuminate the historical ecology of sea otters (*Enhydra lutris*) and the viability of reintroduction"

Overall this research was well conceived and designed and the ms is concise and well written. I think more explanation is needed as to the differences between the archaeological samples and the historical when compared to the modern samples. More discussion needs to be included as to why the historical samples were chosen and how they inform the hypothesis. In addition more discussion about differences/similarities between historical and archaeological samples in OR would be interesting as this represents a time series of genetics in sea otters separated by almost 2000 years. Overall great paper.

## Specific comments:

Page 4 Lines 30-31: "historical 19-20th century Oregon sea otter specimens to 31 determine how these pre-fur trade sea otters relate to post-fur trade populations". A reference here is needed as to when the sea otters were extinct from Oregon. How many 20th century otters could be in your dataset when most otters were extinct from the PNW coast before protection in 1911. See Scheffer, V. B. (1940). The sea otter on the Washington coast. The Pacific Northwest Quarterly, 31(4), 370-388.

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Page 6 lines 14-21-this paragraph reads like methods to me. I suggest it be moved to that section. Also I don't understand why the historical samples are treated like the archaeological samples in comparison to the modern CA samples. They are post fur trade extirpations and only a few generations removed from the modern samples thus I would not place them in the same category as the archaeological samples. More discussion is needed on why these samples were chosen to compare to the modern samples.

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Page 7 lines 7-9-“ Archaeological Oregon haplotype ten (OR 10), for example, is more closely associated with the historical Russian sea 9 otter”. Authors should note that this same finding of Archaeological Oregon samples clustering with Russia was also reported in Larson et al., 2012. Page 7 lines 18-19-“ Such teeth could have been traded widely and represent wealth and hunting prowess when incorporated into artwork” this statement needs a reference or some other first nations context, pers. comm., etc.

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## Decision letter (RSPB-2020-1787.R0)

07-Sep-2020

Dear Ms Wellman:

I am writing to inform you that your manuscript RSPB-2020-1787 entitled "Archaeological mitogenomes illuminate the historical ecology of sea otters (*Enhydra lutris*) and the viability of reintroduction" has, in its current form, been rejected for publication in Proceedings B.

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

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

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

- 1) A 'response to referees' document including details of how you have responded to the comments, and the adjustments you have made.
- 2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
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Sincerely,  
Dr Daniel Costa

mailto: proceedingsb@royalsociety.org

Associate Editor

Comments to Author:

This is an innovative piece of research that I have enjoyed reading. The use of DNA coming from museum and extant samples is original, and its application to inform conservation policies important. I believe the analyses and their interpretation to be robust.

I'd like to encourage the authors to follow the suggestions of the reviewers. Mainly, I think they should add the description of the new protocol (may I suggest as Supp material), improve the figures legends, and provide more information on the age of the historical samples. Also, this paper offers a unique chance to give a comparison between the different types of samples.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

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## Author's Response to Decision Letter for (RSPB-2020-1787.R0)

See Appendix A.

## RSPB-2020-2343.R0

### Review form: Reviewer 2

#### **Recommendation**

Accept with minor revision (please list in comments)

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

Excellent

#### **General interest: Is the paper of sufficient general interest?**

Good

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

Good

#### **Is the length of the paper justified?**

Yes

#### **Should the paper be seen by a specialist statistical reviewer?**

No

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

No



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

**Is it accessible?**

Yes

**Is it clear?**

Yes

**Is it adequate?**

Yes

**Do you have any ethical concerns with this paper?**

No

### **Comments to the Author**

Great job on the revision of this important paper. I have just some minor suggested edits:

Page 3

line 21: Change to "reintroductions".

Line 23: delete "much" and replace with "a large portion". Much of the previously occupied sea otter habitat has become reoccupied but there remains a large portion of previously occupied habitat between southern WA and central CA where sea otters remain absent.

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Page 9

Line 1-indent paragraph.

## **Decision letter (RSPB-2020-2343.R0)**

03-Nov-2020

Dear Ms Wellman

I am pleased to inform you that your manuscript RSPB-2020-2343 entitled "Archaeological mitogenomes illuminate the historical ecology of sea otters (*Enhydra lutris*) and the viability of reintroduction" has been accepted for publication in Proceedings B.

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. Because the schedule for publication is very tight, it is a condition of publication that

you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

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

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

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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. PowerPoint files are not accepted.
- 3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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

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

#### 5) Data accessibility section and data citation

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

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:

- DNA sequences: Genbank accessions F234391-F234402
- Phylogenetic data: TreeBASE accession number S9123
- Final DNA sequence assembly uploaded as online supplemental material
- Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311

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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. Please see <https://royalsociety.org/journals/ethics-policies/data-sharing-mining/> for more details.

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

Sincerely,

Dr Daniel Costa

mailto:proceedingsb@royalsociety.org

Associate Editor

Board Member

Comments to Author:

I want to congratulate the authors for their efforts in improving this manuscript.

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s).

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## Author's Response to Decision Letter for (RSPB-2020-2343.R0)

See Appendix B.

### Decision letter (RSPB-2020-2343.R1)

09-Nov-2020

Dear Ms Wellman

I am pleased to inform you that your manuscript entitled "Archaeological mitogenomes illuminate the historical ecology of sea otters (*Enhydra lutris*) and the viability of reintroduction" 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.

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

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

# Appendix A

## Reviewer 1

**R1\_1** Page 4, lines 29-31: it's not entirely clear to me if you consider the historical specimens pre- or post-fur trade. I assume it's post, given the dates provided at page 3, line 30 and page 5, line 10, but this could be clarified a bit further. You should also explain, here and/or in the discussion, why you did not include modern data/samples of northern populations. I'm also missing more precise information on the age of the historical samples throughout the manuscript.

**Thank you for raising this point. We have clarified on page 4, lines 28-32 that the 19<sup>th</sup> c. sea otters represent fur trade era otters and that the 20<sup>th</sup> c. and modern specimens represent post fur-trade otters:**

“We present a long temporal perspective and dataset by recovering complete mitogenomes from archaeological (Late Holocene) and 19<sup>th</sup> century fur trade Oregon sea otters and compare them to post-fur trade (20<sup>th</sup> century and modern) sea otters to determine the relationships between populations.”

**Modern SE Alaska, B.C., and Washington state sea otters were reintroduced from the Amchitka/Aleutian populations in the 1960s. Because we had dental calculus samples from the 1960s Amchitka otters, we did not sample additional modern specimens to reduce impact on collections. We have expanded upon this reasoning in the “Current Study” by including the following text on page 5, lines 16-20:**

“These historical mitogenomes include sea otters from the end of the fur trade (just prior to extirpation) and the post-fur trade era, including several 1960s Amchitka Island sea otters. Amchitka sea otters were reintroduced to Southeast Alaska, British Columbia, and Washington, and therefore likely reflect present genetic diversity in those areas (16).”

**We have also included more specific age ranges throughout the manuscript when referring to the historical/dental calculus sea otters, particularly in the results and discussion.**

**R1\_2** Page 4, line 42: you mention gene flow here, have you looked at this in your own data?

**Formal analysis of gene flow (e.g. calculating effective population size,  $F_{st}$ ,  $nei$ 's distance, etc.) may not be useful for two reasons. First, the mitochondrial genome is inherited maternally as a single locus without recombination, unlike the microsatellites used by Larson et al., the study that we mention. Second, these analyses would require establishing populations of multiple individuals within the dataset that would likely violate assumptions given the temporal and geographic breadth of the study. This second point is especially challenging as the location of deposition of the archaeological samples may not reflect the location of the source population, especially if the animals were traded from another locality. We double-checked the literature and could not find any studies similar to ours that also measured gene flow using the methods listed above.**

**R1\_3** Page 5, line 25: please provide references for aDNA anti-contamination procedures. **We have provided additional references on page 5 line 35, and added these to the Supplementary Information, page 2 line 15.**

**R1\_4** Page 5, line 28: the description of the calculus sampling protocol appears to be missing from the supplementary information. This is quite important given that you present this as a new technique (page 5, line 2). **Thank you for pointing out this oversight. Sampling procedures are now described in Supplementary Information in the “Genetic Analysis” section, page 2 lines 19-26. We also provided additional information about the tooth dentine sampling methodology.**

**R1\_5** Page 6, lines 14-16: is this 10 samples per archaeological site? **That is correct -- we have revised to state this more clearly, page 5 lines 9-11.**

**R1\_6** I find the color coding in the figures a bit difficult to follow. The explanations in the figure legends are also on the minimal side, especially for figure 1. I have to go back to the text a lot to understand the details and I think more extensive figure legends are necessary. **Thank you for your feedback; we have revised the figure legend and simplified the map itself.**

**R1\_7** Figure 1 legend: probable typo in "used in study". I think age (ranges) for samples would be appropriate here and it may also be beneficial to show that the dentine samples originated from two different sites. **Thank you for the suggestion. We added age ranges to the figure legend and caption, and added two circles to mark the two archaeological sites in Oregon.**

**R1\_8** Figure 2: Are all "OR" haplotypes archaeological? **We have revised the figure labels to denote haplotypes based on haplotype number, so the geographic designations are now based on the color legend.**

**R1\_9** Figure 3: adding calendar years for the three periods would make this figure more readable, and if it makes sense, consider mentioning why the modern period is omitted. **Thank you for these suggestions. We have revised both the figure and caption accordingly.**

**R1\_10** I would recommend adding a paragraph on the historical samples, like you did for the archaeological samples, in the supplementary information. **This is an excellent suggestion; we added a section on “Historical Dental Calculus Samples” in the supplementary information.**

**R1\_11** Supplementary information, mitogenome capture: "PippenPrep" should probably be replaced with "PippinPrep". **We have revised accordingly.**

**R1\_12** Supplementary table 1: please add age (or age range) information for the samples. **We have included a column containing “Estimated Age” in Supplementary Table 1.**

**R1\_13** Supplementary File ModAlign.fa: is it correct that these sequences are 47bp long? From the text I was under the impression that they should be 222bp long. **Thank you for pointing this out, we uploaded the incorrect file. The sequences are 14 kbp long, because they are the complete mitogenome with some trimming as described in our methods. The correct file is now included.**

Referee: 2

**R2\_1** Page 4 Lines 30-31: “historical 19-20th century Oregon sea otter specimens to determine how these pre-fur trade sea otters relate to post-fur trade populations”. A reference here is needed as to when the sea otters were extinct from Oregon. How many 20th century otters could be in your dataset when most otters were extinct from the PNW coast before protection in 1911. See Scheffer, V. B. (1940). The sea otter on the Washington coast. The Pacific Northwest Quarterly, 31(4), 370-388. **There are few reliable sources with details on the extirpation in Oregon, but we have included an estimate from Karl Kenyon’s book on page 4, lines 20-21. We also corrected a typo: 19<sup>th</sup>-20<sup>th</sup> century otters referred to all the dental calculus specimens, but as you correctly note, 20<sup>th</sup> century sea otters are not pre-fur trade. We corrected this on page 4 lines 28-32. We also added the Scheffer citation to page 5, line 18 as well as in Supplementary Information on page 1, line 26.**

**R2\_2** Page 4 Line 37-38: “mtDNA from 16 archaeological Oregon sea otters yielding four haplotypes: 11 matched the California genotype, two matched Alaska, and two were unique”: I read this as 1 haplotype was CA, 2 were Alaska, and 2 were unique=5 haplotypes yet it is stated here that Valentine found 4 haplotypes in pre fur trade Oregon otters. Also it states that 16 sea otters were typed and 11+2+2=15, so the math is wrong there as well. Please correct or explain further. **Thank you for bringing this to our attention, we have revised to correct and clarify on page 4, lines 37-41.**

**R2\_3** Page 6 lines 14-21-this paragraph reads like methods to me. I suggest it be moved to that section. **We have moved this paragraph to the methods.** Also I don’t understand why the historical samples are treated like the archaeological samples in comparison to the modern CA samples. They are post fur trade extirpations and only a few generations removed from the modern samples thus I would not place them in the same category as the archaeological samples. More discussion is needed on why these samples were chosen to compare to the modern samples. **The dental calculus is treated like the archaeological samples because the calculus was obtained from museum collections and is not fresh tissue. Calculus from skeletonized specimens is expected to have fragmented and damaged DNA and is subject to contamination and degradation through handling and post-mortem processing (our fragment length plot in Supplementary Information, Fig. S4, shows similar fragment lengths in both dentine and calculus). Despite the recent age of some of these specimens, ancient DNA protocols are the best approach for recovering the degraded**



**DNA in dental calculus (Austin et al. 2019). We have included the following text to explain this on page 5 Lines 43-45:**

“Due to differential preservation of endogenous DNA in dental calculus (62) and documented degradation of DNA in calculus museum specimens (63) the historical DNA was extracted and sequenced following ancient DNA protocols and workflows.”

**As to why we chose to use these museum specimens, we have added some sentences explaining our logic in the section “Current study” page 5 lines 16-20:**

“These historical mitogenomes include sea otters from the end of the fur trade (just prior to extirpation) and the post-fur trade era, including several 1960s Amchitka Island sea otters. Amchitka sea otters were reintroduced to Southeast Alaska, British Columbia, and Washington, and therefore likely reflect present genetic diversity in those areas (16).”

**R2\_4** Page 6 lines 25-28- “The network analysis yielded 27 mitogenome haplotypes. Ten haplotypes were identified among the archaeological samples from Oregon, five represent the historical/modern California mitogenomes, and six represent historical Alaska mitogenomes (Supplementary Information 28 Table S3)”. Again the math doesn’t add up here (10+5+6=21 not 27). In figure 2 there is 27 haplotypes, why in eth text just call out the ones above? More explanation needed here. **Thank you for pointing this out, we have revised to include all the haplotypes with the following text on page 6 lines 29-33:**

“The network analysis yielded 27 haplotypes: ten (haplotypes 7-16) represent archaeological Oregon individuals, five (haplotypes 23-27) represent historical/modern California, six (haplotypes 1-6) represent historical Alaska, two (haplotypes 19 and 20) represent historical Oregon, and haplotypes 18, 17, 22, and 21 represent historical Washington, Russia, Japan, and British Columbia, respectively (Table S3).”

**R2\_5** Page 7 lines 5-7-“ As hypothesized, the archaeological and historical Oregon sea otter haplotypes are positioned between the California and northern haplotypes within the network analysis, but Oregon haplotypes appear more closely related to northern sea otters.” The authors should state when sea otters were thought to be extinct in Oregon. There is evidence of the last Washington sea otter shot in Washington was in 1911 by Scheffer. Also explain the differences between the archaeological and historical Oregon sea otters that are separated in some cases by 1000s of years. **We have added more text addressing the clustering/relationships within the network under a subheading specifically addressing “Mitogenome haplotype distributions” and discussing these relationships before moving on to a discussion about how sea otter acquisition may have factored into the results on page 7, lines 5-24:**

*“Mitogenome haplotype distributions* The mitogenome results provide new insights into archaeological/pre-extirpation Oregon sea otters. As hypothesized, the Oregon sea otter haplotypes are distinct from California haplotypes, and form several clusters with northern haplotypes in the network analysis.

Archaeological Oregon haplotypes 7, 8, and 9 (representing a total of seven individuals from both sites) are more closely related to the Alaska haplotypes (specifically haplotype 5, a 1949 Amchitka Island sea otter). Archaeological haplotypes 10-15 cluster with the historical Washington and B.C. haplotypes (18 and 21) and historical Oregon haplotype 19, all of which date close to extirpation (c. 1874-1898); this clustering is unsurprising given documented gene flow between northern populations prior to fur trade bottlenecks (16). Historical Oregon haplotype 20 (c. 1859) is comparatively distant from this historical/archaeological cluster, but was collected from Port Orford in southern Oregon, possibly reflecting variation on a latitudinal cline. Archaeological Oregon haplotype 16 is also distinct: it is closely associated with Russia haplotype 17 (collected 1911), and prior studies have indicated gene flow between archaeological Oregon and Russia populations occurred (16). (Interestingly, Japan (haplotype 22, no date) is separated from all other haplotypes including Russia, while the reference genome from a sea otter from the Toba Aquarium in Japan shares haplotype 6 with a 1977 historical Alaska sea otter). Overall, the distribution of haplotypes within the network analysis indicate close associations between the archaeological Oregon sea otters and pre-extirpation sea otters from northern populations, especially those immediately north of Oregon (Washington and B.C.), as well as the post-fur trade historical Alaska sea otters used for reintroductions.”

**R2\_6** Page 7 lines 7-9-“ Archaeological Oregon haplotype ten (OR 10), for example, is more closely associated with the historical Russian sea 9 otter”. Authors should note that this same finding of Archaeological Oregon samples clustering with Russia was also reported in Larson et al., 2012. **We have carefully re-read Larson et al. 2012 but we do not see where Larson et al. state this. They state that gene flow may have occurred primarily between OR and northern populations in addition to southern populations, but the Fst distances do not suggest an especially close clustering relative to the other northern populations (OR/RU Fst=.113 versus OR/WA .188, OR/AK .134, etc., all are < .20 Fst). We did include a sentence to point out that Oregon/Russia gene flow has been documented on page 7, lines 17-18.**

**R2\_7** Page 7 lines 18-19-“ Such teeth could have been traded widely and represent wealth and hunting prowess when incorporated into artwork” this statement needs a reference or some other first nations context, pers. comm., etc.

**We have rephrased and included additional citations, on page 7, lines 35-36:**

“Such teeth could have been traded widely, perhaps as a symbol of the wealth/status associated with sea otter pelts (29,32,33).”

**R2\_8** Page 7 lines 39-42-“ the Oregon sea otters used by Valentine et al. (39) came from archaeological sites along the central and southern Oregon coast. Valentine et al. did find two northern haplotypes in their archaeological Oregon sea otters, while the Oregon sea otters analyzed in this study did not match California haplotypes.” In this discussion it would increase the clarity if the authors add that Larson et al., 2012 used samples from archaeological sites that included the north, central and south central coast of Oregon and found both CA and WA/Alaska signatures. **We have added a sentence to address this important comment on page 8, lines 15-16.**

# Appendix B

## Response to Reviewers

We have noted our changes in bold, and have included the manuscript with changes tracked below.

Page 3

line 21: Change to “reintroductions”. **Changed to “reintroductions.”**

Line 23: delete “much” and replace with “a large portion”. Much of the previously occupied sea otter habitat has become reoccupied but there remains a large portion of previously occupied habitat between southern WA and central CA where sea otters remain absent. **This is a good point, we have re-worded accordingly.**

Line 30-Most references cite the fur trade as starting in 1741 with the Bering expedition returning to the east (Russia and Asia) and ending in 1911 with the signing of the North Pacific Fur Seal Convention that also protected sea otters. Although most of the hunting occurred during the time you mentioned it does not accurately reflect the entire time the fur trade was active. **This is a good point. We have revised to include these standard/better recognized dates.**

Line 34-Change to “structuring”. **We have changed to “structuring.”**

Page 4

Line 18-Start sentence with “However”. **We have edited accordingly.**

Page 8 (**This is page 7 in our draft, in case there is confusion when reviewing our changes**).

Line 17-Remove “(“ at beginning of sentence. **We have revised accordingly.**

Lines 27-29-the otters themselves may not actually have had to physically move between Alaska and Oregon to have similar genetic signatures between the groups rather the similarity between Alaska and Oregon could have been attributed to connectivity and thus geneflow between populations up and down the coast. A line suggesting that alternative hypothesis here is recommended. **We have expanded more upon gene flow contributing to the genetic connectivity.**

Page 9 (**This is page 8 in our draft**).

Line 1-indent paragraph. **The page break is such that it looks like this should be indented, but the paragraph is immediately following a sub-heading and is therefore not indented and consistent with the formatting throughout the rest of the manuscript.**