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PROCEEDINGS B

Predicting the fate of eDNA in the environment and implications for studying biodiversity

Jori B. Harrison, Jennifer M. Sunday and Sean M. Rogers

Article citation details
Proc. R. Soc. B **286**: 20191409.
http://dx.doi.org/10.1098/rspb.2019.1409

Review timeline

Original submission:	16 June 2019
1st revised submission:	2 August 2019
2nd revised submission:	27 September 2019
3rd revised submission:	25 October 2019
Final acceptance:	25 October 2019

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

Review History

RSPB-2019-1409.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?
Acceptable

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

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

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 review paper by Harrison et al. is a discussion of the major processes that eDNA may undergo between organism and collection; including shedding, persistence and attenuation, and transport within different environments. The paper is useful as a summary of processes that may affect eDNA result. The authors don't recognize that value of a simple positive eDNA finding of a given organism in a given environment. In a number of instances this can be important in its own right and may not be really influenced by the processes outlined. Where they become important is mainly in regard to quantitative estimates based on eDNA. The paper does not really provide much direct suggestions for solutions; rather outline some quit obvious recommendations. From this perspective the paper is disappointing.
The first sentence in abstract "Environmental DNA applications are transforming the standard of characterizing aquatic biodiversity via the presence, location, and abundance of DNA collected from environmental samples" is misleading as the review is not on aquatic systems only and several interesting eDNA results are found in other settings.

The references used are in a number of places surprising as they are not referring to the original papers; e.g. the founding paper of eDNA is completely missing.

It be good if the authors make a better effort of citing the original papers regarding the topic discussed and come up with a clearer approaches for how to address the problems they outline.

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?
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

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Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
Harrison et al. lay out a detailed and well-organized cross-disciplinary review of information relevant to eDNA removal from the sampling environment. While there are many eDNA reviews published in the last few years, this manuscript stands out as a unique contribution by an in-depth synthesis of information from forensics, aDNA, and cell biology as well as a thorough integration of the eDNA literature. I think this will be a valuable and important contribution.
I have just a few comments and suggestions:
The conceptual framework here is from Barnes and Turner 2016. This should be explicitly acknowledged in the first paragraph (right now this paper is just cited as a description of eDNA applications in the second sentence).
Line 85-87. These biphasic models of decay have a quick rate followed by a slow rate – where the decay constant (k2) is much smaller than the original constant. This sentence says the opposite.
Figure 2. This gives the impression that a biphasic model is only appropriate for freshwater, rather than that it has only been modeled this way in freshwater. The lack of biphasic modeling for marine and tap water should be noted in the caption to avoid this.
Line 185. This study (22) is an artificial system. Should be noted here that whether this occurs in natural systems to a detectable extent has not been demonstrated.
Line 186. This assumes free eDNA. But on line 165: “Most eDNA is found in fragment sizes

reflective of intact tissue or free mitochondria." These need to be reconciled.
Line 218. May want to include Fremier et al. 2019. Stream transport and retention of environmental DNA pulse releases in relation to hydrogeomorphic scaling factors. Environmental Science & Technology 53:6640-6649.
Line 313. This has large implications for the application of sampling methods across areas and the need for pilot studies. Would be great to be more explicit about that here.
Line 329. Typo – visuals.
Reference 5 – missing part of the title.

Review form: Reviewer 3

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

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

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

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

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

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Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
This review discusses the "ecology" of eDNA with particular attention on its interactions with the environment. The authors are also providing several important recommendations for future research.

The review presents few new ideas. It is clearly written and well documented but it is not particularly well presented, developed and justified. Many sections do not have sufficient depth to place the new ideas firmly in the context of what is well known. Moreover, the review is not sufficiently justified to properly highlight the novel elements. The title is promising on "predicting the fate of eDNA in the environment…" but the review, in the present form, is unlikely to bring the eDNA community much closer to understanding this topic or achieving this important goal. Despite these shortcomings, the review contains important new ideas, and recommendations.

Abstract. It is not clear how this review distinguishes itself from other reviews on the "ecology" of eDNA.

Introduction. The authors could provide a more incisive overview of what is known about the fate of eDNA in the environment by discussing sharply the most relevant reviews (particularly Barnes and Turner 2016) by clearly pointing what their review brings to the eDNA community. A clear definition of eDNA would help since most authors use the term eDNA in a loose sense to refer to both extra-organismal and organismal DNA captured from the environment.

Objective of the review (lines 47-55). This section left me a little unclear about the scope of this review or its immediate need, relevance and novel aspect. Are the authors considering terrestrial environments or only aquatic environments?

Decay. This section is very relevant but it would be useful to provide a more in depth discussion on how the multiple processes (mechanisms) involved in the eDNA decay could be investigated. The same comment applies to the recommendation section.

Lines 116-117. Good point, however microbial biofilms are also incorporating extracellular DNA in their extracellular matrix, as a structural component. This can enhance the persistence of bacterial extracellular DNA or exogenous eDNA.

Spontaneous degradation.
It is not clear what 'spontaneous' degradation / decomposition realy is and how this process results in ancient DNA (aDNA). This is counterintuitive. Thus, an explanation would help.

Transport of eDNA
This is a relevant section but a little superficial and not adding much to the current knowledge on the problems related to the eDNA. The single paragraph included in this section is not fully developed. Is eDNA transport restricted to aquatic environments?

In general, the manuscript is highly subdivided and, as a result, only superficially touches on many introduced aspects related to the eDNA 'ecology'.

Recommendations.
Recommendation 1. Provide appropriate references for the need of better transport estimates.

Recommendation 2. Clarify the type of experiment: degradation experiment, etc.
What are the important "natural features" that such experiments should consider?

Decision letter (RSPB-2019-1409.R0)

10-Jul-2019

Dear Dr Rogers:

Your manuscript has now been peer reviewed the referees' comments (not including confidential comments to the Editor) are included at the end of this email for your reference. As you will see, the reviewers are somewhat divided in their opinions, particularly with regard to the added value your review brings in terms of novelty and solutions. However, given Proceedings B's broad audience, I think there's still merit in a review that brings all these issues together in one article. So, I think there is a reasonable chance that you can address the other criticisms raised by the reviewers, and I 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 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.

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

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

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly

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

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

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

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

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,
Innes
Prof Innes Cuthill
Reviews Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
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Referee: 3

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Recommendations.
Recommendation 1. Provide appropriate references for the need of better transport estimates.

Recommendation 2. Clarify the type of experiment: degradation experiment, etc.
What are the important "natural features" that such experiments should consider?

Author's Response to Decision Letter for (RSPB-2019-1409.R0)

See Appendix A.

RSPB-2019-1409.R1 (Revision)

Review form: Reviewer 1

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

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

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

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

Is the length of the paper justified?
Yes

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

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

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

Is it accessible?
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
.

Review form: Reviewer 4

Recommendation
Major revision is needed (please make suggestions 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

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Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
Please see review document attached (See Appendix B).

Decision letter (RSPB-2019-1409.R1)

29-Aug-2019

Dear Dr Rogers:

Your revised paper has now been peer reviewed (one previous referee, one new one) and, as you

will see, both arkebasically happy with the manuscript, but there are some further revisions that would really put the icing on the cake. So, I would like to invite you to make those further revisions in the light of the comments at the end of this email.

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), 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,
Innes

Prof. Innes Cuthill
Reviews Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Reviewer(s)' Comments to Author:

Referee: 4

Comments to the Author(s)
Please see review document attached.

Referee: 1

Comments to the Author(s)
I think the authors have done a good job dealing with the concerns of both reviewers. I have two edits they need to do still:

They state:, “While eDNA is sometimes used to refer to any DNA recovered from a wide variety of environmental samples, this review focuses on shed macro- organismal DNA collected from aquatic environments; “

eDNA is a term generally used for all types of environmental samples not specifically for aquatic samples. Thus the authors need to state that while eDNA is commonly used for DNA studies of all types of environmental samples (modern and ancient) their focus is on aquatic samples. This needs clarified.

Further The authors state: “We acknowledge that we should have more clearly referenced Barnes and Turner 2016 earlier in the MS, as this study helped form the conceptual framework for several aspects of our review, and to Ficetola 2008, which was the founding paper of eDNA (and not previously cited). We have Included a reference to Ficetola 2008 in line 3. We now also cite Barnes and Turner 2016 in conjunction with the ecology of eDNA (line 45)”

The authors haven't done a very thorough literature search. The first eDNA study is that of Willerslev et al. Science 2003. It’s actually referred to in Ficetola 2008. This should be corrected.

Author's Response to Decision Letter for (RSPB-2019-1409.R1)

See Appendix C.

RSPB-2019-1409.R2 (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?
Acceptable

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

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

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
I think the paper is ready to be accepted with one edit:

In the sentence:
The application of environmental DNA (eDNA) has experienced immense growth in recent years, and is now used widely in community ecology, palaeo-environmental research, biomonitoring, conservation biology, and invasion ecology [1,2].

The authors need including reference 3 in [1,2]. I stated that already in my first review.

Review form: Reviewer 4

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

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Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
I am grateful to the authors for cogently responding to all of my concerns. I am in full support of accepting this paper, and thank then for their contributions to the field of eDNA.

Decision letter (RSPB-2019-1409.R2)

18-Oct-2019

Dear Dr Rogers

I am pleased to inform you that your manuscript RSPB-2019-1409.R2 entitled "Predicting the fate of eDNA in the environment and implications for studying biodiversity" has been accepted for publication in Proceedings B.

The referees have recommended publication, but referee 1 also suggests a couple of very minor revisions to your manuscript. Therefore, I invite you to respond to the 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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1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

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

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

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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.

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

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=(Document not available) which will take you to your unique entry in the Dryad repository. If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

6) 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 revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Innes

Innes Cuthill
Reviews Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Reviewer(s)' Comments to Author:

Referee: 4

Comments to the Author(s)
I am grateful to the authors for cogently responding to all of my concerns. I am in full support of accepting this paper, and thank then for their contributions to the field of eDNA.

Referee: 1

Comments to the Author(s)
I think the paper is ready to be accepted with one edit:

In the sentence:
The application of environmental DNA (eDNA) has experienced immense growth in recent years, and is now used widely in community ecology, palaeo-environmental research, biomonitoring, conservation biology, and invasion ecology [1,2].

The authors need including reference 3 in [1,2]. I stated that already in my first review.

Decision letter (RSPB-2019-1409.R3)

25-Oct-2019

Dear Dr Rogers

I am pleased to inform you that your manuscript entitled "Predicting the fate of eDNA in the environment and implications for studying biodiversity" 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 during this period, 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

Your article has been estimated as being 10 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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

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

Sincerely,

Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

UNIVERSITY OF CALGARY

FACULTY OF SCIENCE

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Professor Innes Cuthill, University of Bristol
Reviews Editor
Proceedings of the Royal Society B, Biological Sciences

July 31, 2019

Dear Professor Cuthill,

We were pleased to hear that our manuscript was generally well-received by yourself and the three referees. Nonetheless, there were several suggested edits to improve the paper and broaden the scope of the review for PRSB. Indeed, we found all the comments and suggestions constructive and very helpful towards improving the clarity and the value of the paper. We have now revised the paper fully in light of these comments and systematically list our responses/revisions to each point raised below. We hope the revised version meets the high standards of PRSB.

Sincerely,
Sean Rogers on behalf of all authors

Reviews Editor

Q1. Your manuscript has now been peer reviewed the referees' comments (not including confidential comments to the Editor) are included at the end of this email for your reference. As you will see, the reviewers are somewhat divided in their opinions, particularly with regard to the added value your review brings in terms of novelty and solutions. However, given Proceedings B's broad audience, I think there's still merit in a review that brings all these issues together in one article. So, I think there is a reasonable chance that you can address the other criticisms raised by the reviewers, and I would like to invite you to revise your manuscript to address them.
R1. We appreciate the opportunity to revise with the suggested edits to bring these issues together towards further enhancing the value of the review. We have edited the introduction to highlight the novelty and value of bringing together the underlying fundamentals of environmental DNA with the most recent models that predict its fate under environmental change. We have also clarified and expanded on our recommendations to provide clear, practical steps for eDNA practitioners. The MS is significantly improved by fully addressing the concerns of the Reviewers.

Referee 1

Q1. The review paper by Harrison et al. is a discussion of the major processes that eDNA may undergo between organism and collection; including shedding, persistence and attenuation, and transport within different environments. The paper is useful as a summary of processes that may affect eDNA result.
R1: We are pleased that the referee found the paper useful for the broad audience of PRSB.

Q2: The authors don't recognize that value of a simple positive eDNA finding of a given organism in a given environment. In a number of instances this can be important in its own right and may not be really influenced

by the processes outlined. Where they become important is mainly in regard to quantitative estimates based on eDNA.

R2: We agree with the Reviewer about the value of a positive result and that we could have been clearer on how the processes discussed in this review can affect positive/negative eDNA surveys, rather than simply quantitative surveys. Indeed, the processes and mechanisms that we describe strongly affect where and when a sample will provide a simple positive, and are thus applicable to all eDNA surveys. Therefore, we now include the line "*For presence-absence eDNA surveys, these mechanisms influence how long eDNA can be detected for, and how far away from the organisms location eDNA can be detected at*" (Lines 41-42) to draw greater attention to this connection and broaden the scope.

Q3: The paper does not really provide much direct suggestions for solutions; rather outline some quit obvious recommendations.

R3: We agree that the review would benefit from more direct recommendations. We have therefore updated our recommendation along the lines of the suggested edits for environment-specific parameterization to clearly delineate that we suggest considering lentic, lotic, and marine ecosystems as separate for eDNA parameterization. We have also included, as an additional recommendation, specific environmental variables that we believe eDNA practitioners should collect and publish along with their results (Table 1). One of the strengths of our review is that while some of these recommendations seem obvious, our review demonstrates that they are yet to be subscribed within the eDNA field. In fact, very few studies integrate hydrological modeling of any kind (as noted by Referee 2). Our recommendation for the environment-specific study of eDNA, including the need for experimental studies under this framework will have significant impacts on the application of eDNA methods. Our review demonstrates that following these recommendations will help propel the field of eDNA further towards solutions to these longstanding problems and risks with eDNA.

Q4: The first sentence in abstract "Environmental DNA applications are transforming the standard of characterizing aquatic biodiversity via the presence, location, and abundance of DNA collected from environmental samples" is misleading as the review is not on aquatic systems only and several interesting eDNA results are found in other settings.

R4: Our review did focus on aquatic systems, although we included discussion of other substrate types where it is likely to impact eDNA in aquatic systems (e.g. in benthic sediments). We acknowledge that this could have been more explicit in our objectives and have now included the lines, "*While eDNA is sometimes used to refer to any DNA recovered from a wide variety of environmental samples, this review focuses on shed macro-organismal DNA collected from aquatic environments; we discuss other environments only where they are likely to impact collection from aquatic environments (e.g. within the benthic substrate)*." (Line 31-34) to emphasize the reviewer's point and clarify that our observations in multiple settings are interesting and broad in scope.

Q5: The references used are in a number of places surprising as they are not referring to the original papers; e.g. the founding paper of eDNA is completely missing.

R5: We acknowledge that we should have more clearly referenced Barnes and Turner 2016 earlier in the MS, as this study helped form the conceptual framework for several aspects of our review, and to Ficetola 2008, which was the founding paper of eDNA (and not previously cited). We have Included a reference to Ficetola 2008 in line 3. We now also cite Barnes and Turner 2016 in conjunction with the ecology of eDNA (line 45), and

describe the framework from that article that describes the ecology of eDNA as the "*origin*, *state*, *transport*, and *fate* of eDNA", as well as their findings that there is a complex relationship between these processes and environmental factors (lines 44-49).

Q6: It be good if the authors make a better effort of citing the original papers regarding the topic discussed and come up with a clearer approaches for how to address the problems they outline.
R6: We were admittedly challenged to include all relevant references within the scope of a broad review. We have chosen to include Barnes and Turner (2016) and Ficetola (2008). As we described above, we have also clarified and expanded upon our recommendations so as to provide practical actions for eDNA researchers in the future.

Referee 2

Q1: Harrison et al. lay out a detailed and well-organized cross-disciplinary review of information relevant to eDNA removal from the sampling environment. While there are many eDNA reviews published in the last few years, this manuscript stands out as a unique contribution by an in-depth synthesis of information from forensics, aDNA, and cell biology as well as a thorough integration of the eDNA literature. I think this will be a valuable and important contribution. I have just a few comments and suggestions:
R1: We are very pleased that the referee found the manuscript to be a valuable and important contribution. Below, we address the comments and suggestions that the referee provided in detail.

Q2: The conceptual framework here is from Barnes and Turner 2016. This should be explicitly acknowledged in the first paragraph (right now this paper is just cited as a description of eDNA applications in the second sentence).
R2: We agree that we should have been more explicit describing the framework from Barnes & Turner and have updated the introduction consistent with the changes requested by the first Referees.

Q3: Line 85-87. These biphasic models of decay have a quick rate followed by a slow rate – where the decay constant (k2) is much smaller than the original constant. This sentence says the opposite.
R3: We have fixed this sentence.

Q4: Figure 2. This gives the impression that a biphasic model is only appropriate for freshwater, rather than that it has only been modeled this way in freshwater. The lack of biphasic modeling for marine and tap water should be noted in the caption to avoid this.
R4: We appreciate this suggestion and have updated the caption with the sentence "*Biphasic decay predictions are only included for freshwater environments but are likely applicable to marine and laboratory conditions as well*" to clarify this observation.

Q5: Line 185. This study (22) is an artificial system. Should be noted here that whether this occurs in natural systems to a detectable extent has not been demonstrated.

R5: The referee is correct that resuspension has not been proven to cause 'false' positives at later time points, it has merely been suspected of doing so. We have edited the statement to read *"…as it is suspected that adsorbed DNA can be resuspended into the water column after aquatic eDNA has degraded"* (Line 195-196)

Q6: Line 186. This assumes free eDNA. But on line 165: "Most eDNA is found in fragment sizes reflective of intact tissue or free mitochondria." These need to be reconciled.
R6: We agree with this point and have updated the relevant section to discuss the adhesion of both DNA molecules and biomolecules/cell surfaces. (Lines 191-199).

Q7: Line 218. May want to include Fremier et al. 2019. Stream transport and retention of environmental DNA pulse releases in relation to hydrogeomorphic scaling factors. Environmental Science & Technology 53:6640-6649.
R7: We appreciate this suggestion and have added the line "*Additional hydrological factors may improve the accuracy or precision of such models, with one recent study identifying stream slope, average stream-scale form, and longitudinal roughness as significant explanatory variables [71*]", integrating this reference (Lines 249-251)

Q8: Line 313. This has large implications for the application of sampling methods across areas and the need for pilot studies. Would be great to be more explicit about that here.
R8: We agree. As we have described above, we have revised and expanded this recommendation, and we have included the line "*While this may have significant implications for eDNA applications in new systems and the need for increased basic research, we believe the marked difference in eDNA dynamics between environments necessitates this division."* (lines 336-338) to call attention to this point.

Q9: Line 329. Typo – visuals.
R9: We have fixed this typo.

Q10: Reference 5 – missing part of the title.
R10: We have fixed this reference and thank the referee for their attention to detail.

Referee 3

Q1: This review discusses the "ecology" of eDNA with particular attention on its interactions with the environment. The authors are also providing several important recommendations for future research. The review presents few new ideas. It is clearly written and well documented but it is not particularly well presented, developed and justified. Many sections do not have sufficient depth to place the new ideas firmly in the context of what is well known. Moreover, the review is not sufficiently justified to properly highlight the novel elements. The title is promising on "predicting the fate of eDNA in the environment…" but the review, in the present form, is unlikely to bring the eDNA community much closer to understanding this topic or achieving this important goal. Despite these shortcomings, the review contains important new ideas, and recommendations.
R1: We are grateful for this referees' constructive comments. Below, we have discussed the referee's specific comments where the depth and novelty of the manuscript can be improved. In general, we have extensively

edited the introduction to highlight the novelty and justification for a broad review. In particular, and consistent with the other Referees, we feel there is value to addressing the multitude of elements comprising the ecology of eDNA within a single review; particularly since (as this review shows) these factors are often interconnected in their effects. The comments from this Referee also suggest that the very predictions surrounding the fate of eDNA are novel, new ideas that will be of interest, particularly from the recommendations made.

Q2: Abstract. It is not clear how this review distinguishes itself from other reviews on the "ecology" of eDNA.
R2: Thank you. We agree that abstract did not clearly showcase the novelty of the manuscript. We have updated the abstract to highlight how our review focuses on the environments in which eDNA is found, and how that environment will interact with the different processes that eDNA undergoes.

Q3: Introduction. The authors could provide a more incisive overview of what is known about the fate of eDNA in the environment by discussing sharply the most relevant reviews (particularly Barnes and Turner 2016) by clearly pointing what their review brings to the eDNA community. A clear definition of eDNA would help since most authors use the term eDNA in a loose sense to refer to both extra-organismal and organismal DNA captured from the environment.
R3: We have now included a description of the most Barnes and Turner 2016 (described earlier to in our comments to Referee 1). We clearly discuss how our review distinguishes itself by discussing the mechanistic processes present in the spectrum of aquatic environments from which eDNA is collected. We have also included a working definition of eDNA (included above in our comments to Referee 1) for this paper and thank the referee for their suggestion.

Q4: Objective of the review (lines 47-55). This section left me a little unclear about the scope of this review or its immediate need, relevance and novel aspect. Are the authors considering terrestrial environments or only aquatic environments?
R4: We agree that we should have been clearer describing the objective of the paper, and have extensively edited the introduction to highlight the relevance and novelty of the manuscript. We have also clarified the scope and focus of the manuscript on lines 58-60 by including the lines "*We focus on a mechanistic understanding of processes that affect eDNA within the environment and discuss how those mechanisms are or are not captured in different transport and decay models currently used to predict the behaviour of eDNA*." We have also described what environments we are considering within this review on lines 32-34 (described above).

Q5: Decay. This section is very relevant but it would be useful to provide a more in depth discussion on how the multiple processes (mechanisms) involved in the eDNA decay could be investigated. The same comment applies to the recommendation section.
R5: We agree that this section is very relevant. We also agree that a discussion of the methodologies that could be used to study biological mechanisms relevant to any facet of the ecology of eDNA would be useful. However, the scope of such a discussion is better served by a separate review focused on that specific question, as there are a tremendous variety of methods over several distinct disciplines that can be used to study the multiple processes involved in eDNA decay. To emphasize this point, we now include the line "*We encourage eDNA researchers to embrace cross-disciplinary collaborations; especially incorporating*

methodologies from disciplines such as forensic taphonomy and geochemistry to understand the mechanisms responsible for the decay and attenuation of eDNA" (lines 353-356) within our recommendation 5 to direct researchers to where these methodologies could be found.

Q6: Lines 116-117. Good point, however microbial biofilms are also incorporating extracellular DNA in their extracellular matrix, as a structural component. This can enhance the persistence of bacterial extracellular DNA or exogenous eDNA.
R6: We appreciate the suggestion and have now included the lines "*Adhesion is also observed in the presence of bacterial biofilms, which use exogenous DNA as structural elements [89] and may promote the persistence of eDNA*" (lines 204-206) to incorporate this comment.

Q7: Spontaneous degradation. It is not clear what 'spontaneous' degradation / decomposition realy is and how this process results in ancient DNA (aDNA). This is counterintuitive. Thus, an explanation would help.
R7: We use the term spontaneous degradation using the definition of a 'spontaneous process', i.e. one that proceeds without external sources of energy or influence. We added the term '*non-enzymatically catalyzed*' to help define these terms (lines 132-133) and edited the connection to ancient DNA to so that it now reads *"leading to the fragmented and chemically modified DNA strands characteristic of ancient DNA (aDNA)."* (lines 133-134)

Q8: Transport of eDNA. This is a relevant section but a little superficial and not adding much to the current knowledge on the problems related to the eDNA. The single paragraph included in this section is not fully developed. Is eDNA transport restricted to aquatic environments?
R8: We agree with the relevance of this section that comprises 40% of the text in the current manuscript, with six subsections discussing different aspects of eDNA transport. We acknowledge that, given the number of sections within the manuscript, subsection titles should have been explicitly named to convey section hierarchy, and have updated the section headings to improve clarity. We have also clarified the scope of the paper within the introduction that while eDNA processes (including transport) may occur in a variety of environments, this focus of this manuscript was on aquatic environments (lines 31-34).

Q9: In general, the manuscript is highly subdivided and, as a result, only superficially touches on many introduced aspects related to the eDNA 'ecology'.
R9: We agree that predicting the interaction of DNA with the environment (ecology) will be a significant field, hence the importance of the review. While the balance between depth, scope, and length can always be challenging, we feel that the benefit of our manuscript is that by including origin, decay, transport, and adsorption within one review, we can also address the interactions between these processes to give the readership a broader understanding of the factors affecting the fate of eDNA in the environment. We acknowledge that each of these processes is complex, and not always well understood. The transport of eDNA alone (currently 40% of the MS, but as the Referee suggests, highlights areas to explore) is highly associated with eDNA ecology and currently understudied

Q10: Recommendation 1. Provide appropriate references for the need of better transport estimates.
R10: We have added these references (line 321).

Q11: Recommendation 2. Clarify the type of experiment: degradation experiment, etc. What are the important “natural features” that such experiments should consider?
R11: We have updated this recommendation to specify that we are referring to any experiment that studies processes that affect eDNA (line 331), and that natural features should be “*designed with an understanding of the potential mechanisms that impact these processes*.” (line 332-333)

Appendix B

Review for RSPB-2019-1409.R1

Summary

Harrison et al. present a succinct review of the processes and patterns that control environmental DNA (eDNA) transport and persistence in the environment, and also discuss several main recommendations for authors pursuing eDNA methods for biodiversity estimates. While other papers (e.g., Barnes & Turner) have discussed the "ecology of eDNA" to review the influence of various biotic and abiotic parameters on detection, this review focuses more specifically on the fate and transport of eDNA particles within the environment, which in my eyes is distinct. The authors present a literature review and also discuss several recommendations for eDNA practitioners to consider towards eDNA study design and interpretation.

Overall, I found this paper to be a timely review, as several recent studies have highlighted the importance of context-dependency on eDNA transport and movement within aquatic environments that must be considered before implementing the method. While I have some comments for the authors that should be addressed before publication, I would look forward to seeing this paper in print. I have provided some comments that I hope the authors find insightful.

Minor Comments

Line 27 (and throughout the manuscript): This is minor, but I tend to prefer not to begin a sentence with eDNA as it begins with a lower-case letter.

Line 41: You may also want to cite the following papers: Jerde et al. 2016, Jane et al. 2015, and Shogren et al. 2017. These works showed that eDNA concentrations decreased over distance. This is important as it provides evidence that eDNA particles are not "conservative" in nature, but rather interact with the environment. In effect, while downstream transport may indeed integrate the biodiversity signals (as in Deiner et al. 2016), some may be lost during the downstream movement of water.

Line 44: Rewrite sentence to: "For presence-absence surveys, these mechanisms influence how long eDNA can be detected, and how far away the source location may be"

Paragraph beginning Line 68: Because the review covers various aquatic environments (freshwater streams and rivers, marine environments, etc.), you may want to introduce this within this paragraph of your review.

Section beginning at Line 98: In my experience, the difference between decay and degradation, and whether these processes are distinct, is often confusing to readers. It may be helpful for the authors to define both terms.

Paragraph at line 106: It may be worth making the point that the implications of multiphasic degradation are that there are 1. Many processes influencing how eDNA is degrading over time and 2. There are different labilities of the different eDNA compartments. This would help introduce the remainder of this section and provide clarity on why Figure 2 is meaningful.
Lines 113-115: If Figure 2 is to remain in the paper, the implications of monophasic and multiphasic degradation should be further explained. Please also see comment below regarding the figure itself.

Line 184: "well-developed hydrological models" should be clarified. Do you simply mean "mechanistic models" that describe how water moves eDNA into/out of various ecosystem compartments?

Line 202: Please briefly expand on what you mean by "similar dynamics"

Line 264: Hydrological factors should be changed to "geomorphological factors". Hydrological factors are defined as flow conditions (water discharge), hyporheic exchange, etc.

In general, I was excited by the authors efforts to provide recommendations for potential eDNA practitioners. However, I was often left wondering exactly what techniques, models, or experiments would fill the knowledge gap. While I don't expect the authors to frame these as "in order to understand eDNA you must do XYZ", offering up some suggestions to move the field forward seems like a useful endeavor. For example, at Line 331: While I agree that implementing hydrological models may better parameterize eDNA transport, the authors could provide some suggestions for these models. Again, for Recommendation 2 at Line 338, some of these controlled experiments could help constrain degradation rates (as in Figure 2).

Figures and Tables:
To me, the figures did not seem to be publication quality and could use substantial improvements before publication. I have provided some suggestions for improvement below:

Figure 1: I appreciate the introduction of a conceptual diagram to show how various factors may influence eDNA detection. But given the strength of your review in describing the factors in varying aquatic environments, this figure seemed like a missed opportunity. Rather than one diagram alone, could you instead introduce several conceptual diagrams describing eDNA transport in: 1. Freshwater lakes and ponds, 2. Freshwater rivers and streams, and 3. Marine environments? This would make this figure substantially more useful to the community and greatly improve the overall impact of the paper. The color coding for each different "influencer" on the fate of eDNA could be kept so highlight the differences between environments. I also wonder if these could somehow map to the recommendations that the authors discuss?

Figure 2: I understand the general point of this figure, but it may be more impactful if this again maps back to your review of lentic, lotic, and marine ecosystems. This may also add to your overall recommendations section, but perhaps posing that decay rates are still not well-constrained is a powerful open-ended question.

Table 1: several recent studies have shown that hyporheic exchange influences eDNA transport in lotic ecosystems. As an additional suggested data, could you add hydrologic and hydraulic variables to this table?

Appendix C

UNIVERSITY OF CALGARY

FACULTY OF SCIENCE

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Prof. Innes Cuthill
Reviews Editor, Proceedings B

September 20, 2019

Dear Innes,

We were pleased with the recommendations of the Editor and Associate Editor. We have made most of the recommended minor edits and have fully responded to all of the suggestions made. We believe the MS is now much improved and continues to be of interest to a broad readership.

Sincerely,
Sean Rogers and co-authors

Reviewer Edits; Second Round
Referee 1:

Q1: eDNA is a term generally used for all types of environmental samples not specifically for aquatic samples. Thus the authors need to state that while eDNA is commonly used for DNA studies of all types of environmental samples (modern and ancient) their focus is on aquatic samples. This needs clarified.

R1: We thank the reviewer for the suggestion and now clarify In lines 32-33, *"While eDNA refers to any DNA recovered from any environmental sample (e.g. water [2], sediments [4,5], or air [6]), this review focuses on shed macro-organismal DNA collected from aquatic environments."*

Q2. The authors haven't done a very thorough literature search. The first eDNA study is that of Willerslev et al. Science 2003. It's actually referred to in Ficetola 2008. This should be corrected.

R2: We were aware both that the term 'environmental DNA' was first described in the 1980s to describe microbial DNA isolated from environmental samples (e.g. Trevors and Van Elsas 1989) and that Willerslev et al. was the first study to identify macro-organisms through (sediment) eDNA. However, we originally chose to reference Ficetola et al. as they were the first paper to use describe extant macro-organism communities from aquatic samples. Nonetheless, we agree with the Referee that a reference to Willerslev et al. 2003 on line 33 would be relevant.

Referee 4:
Summary
Q1: Overall, I found this paper to be a timely review, as several recent studies have highlighted the importance of context-dependency on eDNA transport and movement within aquatic environments that must be considered before implementing the method. While I have some comments for the authors that should be addressed before publication, I would look forward to seeing this paper in print. I have provided some comments that I hope the authors find insightful.

R1: We thank the Reviewer for this feedback

Minor Comments

Q2: Line 27 (and throughout the manuscript): This is minor, but I tend to prefer not to begin a sentence with eDNA as it begins with a lower-case letter.

R2: We appreciate this suggestion and have made two edits (on lines 24 and 202) to rephrase sentences beginning with eDNA.

Q3: Line 41: You may also want to cite the following papers: Jerde et al. 2016, Jane et al. 2015, and Shogren et al. 2017.

R3: Agreed. Although we refer to these papers later in the manuscript, we appreciate the suggestion and agree that introducing this evidence earlier in the manuscript strengthens the justification for the manuscript itself. We have therefore added the suggested references.

Q4: Line 44: Rewrite sentence to: “For presence-absence surveys, these mechanisms influence how long eDNA can be detected, and how far away the source location may be”

R4: We have made this change and thank the referee for their suggestion.

Q5: Paragraph beginning Line 68: Because the review covers various aquatic environments (freshwater streams and rivers, marine environments, etc.), you may want to introduce this within this paragraph of your review.

R5: We have changed the sentence at line 59 to now read “We focus on a mechanistic understanding of processes that affect eDNA within **lentic, lotic, and marine** environments “.

Q6: Section beginning at Line 98: In my experience, the difference between decay and degradation, and whether these processes are distinct, is often confusing to readers. It may be helpful for the authors to define both terms.

R6: We agree and now briefly define on lines 89-91.

Q7: Paragraph at line 106: It may be worth making the point that the implications of multiphasic degradation are that there are 1. Many processes influencing how eDNA is degrading over time and 2. There are different labilities of the different eDNA compartments. This would help introduce the remainder of this section and provide clarity on why Figure 2 is meaningful.

R7: We thank the reviewer for this useful feedback. We have included the line “as different cellular compartments may have different labilities, and individual degradation mechanisms may separately impact eDNA decay rates” on lines 108-109 and have expanded on the implications of multiphasic decay within the same paragraph (described below).

Q8: Lines 113-115: If Figure 2 is to remain in the paper, the implications of monophasic and multiphasic degradation should be further explained. Please also see comment below regarding the figure itself.

R8: We have updated lines 109-114 to now read:
"If eDNA decay follows a multiphasic decay pattern, a monophasic model can either significantly underestimate the residency time of low concentration eDNA (if parameterized using data from the initial rapid decay phase) or significantly overestimate the initial eDNA concentration [32]. Without consideration, this may lead eDNA practitioners to conclude their organisms of study were present much more recently or in higher abundances than were actually present. "

Q9: Line 184: "well-developed hydrological models" should be clarified. Do you simply mean "mechanistic models" that describe how water moves eDNA into/out of various ecosystem compartments?

R9: We have removed the term 'well-developed' from this sentence.

Q10: Line 202: Please briefly expand on what you mean by "similar dynamics"

R10: We have rephrased this sentence to read "eDNA transport seems to follow similar transport dynamics as fine particulate organic matter" to be clear that we are referring to transport dynamics.

Q11: Line 264: Hydrological factors should be changed to "geomorphological factors". Hydrological factors are defined as flow conditions (water discharge), hyporheic exchange, etc.

R11: We have changed this term.

Q12: In general, I was excited by the authors efforts to provide recommendations for potential eDNA practitioners. However, I was often left wondering exactly what techniques, models, or experiments would fill the knowledge gap. While I don't expect the authors to frame these as "in order to understand eDNA you must do XYZ", offering up some suggestions to move the field forward seems like a useful endeavor. For example, at Line 331: While I agree that implementing hydrological models may better parameterize eDNA transport, the authors could provide some suggestions for these models. Again, for Recommendation 2 at Line 338, some of these controlled experiments could help constrain degradation rates (as in Figure 2).

R12: We were pleased that the Reviewer appreciated the effort to make recommendations (the reasons for doing so justified in the review). While we have discussed hydrological models earlier within the review, we agree that some reinforcement is needed within the recommendations section. Keeping in mind length and page limits, we have included the suggestion: "(at minimum, estimating average transport distances) " within our recommendation.

Figures and Tables:
Q13: To me, the figures did not seem to be publication quality and could use substantial improvements before publication. I have provided some suggestions for improvement below:

R13: We agree that the figures could be improved and have incorporated the suggestions below.

Q14: Figure 1: I appreciate the introduction of a conceptual diagram to show how various factors may influence eDNA detection. But given the strength of your review in describing the factors in varying aquatic environments, this figure seemed like a missed opportunity. Rather than one diagram alone, could you instead introduce several conceptual diagrams describing eDNA transport in: 1. Freshwater lakes and ponds, 2. Freshwater rivers and streams, and 3. Marine environments? This would make this figure substantially more useful to the community and greatly improve the overall impact of the paper. The color coding for each different “influencer” on the fate of eDNA could be kept so highlight the differences between environments. I also wonder if these could somehow map to the recommendations that the authors discuss?

R14: We appreciate this thoughtful suggestion. We agree that a figure showing specific transport factors would be greatly beneficial to the eDNA community and attempted this by producing a 3-panel figure, as suggested. However, we found that the differences between the 3 systems were not sufficient to change the visual effect, and the figure became inefficient in space use. We have therefore chosen to include the described figure as figure S1.

Q15: Figure 2: I understand the general point of this figure, but it may be more impactful if this again maps back to your review of lentic, lotic, and marine ecosystems. This may also add to your overall recommendations section, but perhaps posing that decay rates are still not well constrained is a powerful open-ended question.

R15: We have updated figure 2 to reflect decay rates in each of lentic, lotic, and marine ecosystems.

Q16: Table 1: several recent studies have shown that hyporheic exchange influences eDNA transport in lotic ecosystems. As an additional suggested data, could you add hydrologic and hydraulic variables to this table?

R16: Done.