Dear Dr Ip,

Your manuscript, "Fish from the sky: Airborne eDNA tracks aquatic life", has now been assessed.

We invite you to revise your paper, carefully addressing the comments from the reviewers and the editor. Please ensure the results are accurately reported, any overstated conclusions are rewritten and the limitations of the work fully explained. When your revision is ready, please submit the updated manuscript and a point-by-point response. This will help us move to a swift decision.

Editor Comments:

"The manuscript should be corrected following the revieres' suggestions and comments. There are some questions raised by the reviewers. The manuscript should be corrected and improved also following these questions. The title should be modified to better address the applied methodology and the obtained results, with a scientific focus."

-Luca Fontanesi

Editor

Scientific Reports

We recommend submitting all revisions within the mentioned deadline.

If you need more time, please contact us and include your submission ID.

Kind regards,

Amrin Sayyed

Assistant Editor

Scientific Reports

Support contact: srep@nature.com

Submission ID: 72d97ba1-ab4c-4648-af26-da49efbfac58

Reviewer 1

In the presented work, the authors used eDNA-based analyses in both water and air, to estimate salmon abundance based on traces left at the air-water interface. Combining insights of four samplers, three kinds of filters and an open tray of deionized water, they found a solid return of airborne eDNA reflecting salmonid presence. The study in its current form provides an innovative piece of evidence on the potential of airborne DNA for aquatic biomonitoring. In order to move toward publication, I do have some remaining remarks to be resolved, especially regarding very limited information provided (or lacking whatsoever) on exiting knowledge gaps in the introduction, as well as on part of the used methods. More detailed remarks provided as follows:

In its current version, the review is made rather difficult as no line numbers are provided, so difficult to refer to the concerned part of the text.

In general, I found the introduction somewhat shortcoming on the existing literature and/or knowledge gaps and hypotheses.

The number of studies on airborne eDNA detecting aquatic species as non-target taxa seems (based on the text and citing included in the introduction), rather limited and it would be interesting to include a small reflecting/review result as a starting point for this paper.

Included an example from Tournayre et al., 2025 which reported the occurrence of aquatic species in air eDNA samples, but the authors emphasized that the underlying mechanisms responsible for these detections remain unclear, suggesting multiple, non-exclusive scenarios including prey DNA and soil re-suspension.

In the third paragraph of the introduction, it is mentioned that evaporation, bubble-burst aerosolization from riffles and splashes, leaping fish, churning substrates, etc. all influence the transferral of eDNA from water to the aerial environment. I wonder on the impact of for example river dynamics on the detection limits of these particles. Is this already known? Or is it a research gap? Suggestion to slightly expand this paragraph with the relevant insights or expectations, especially as the study used eDNA results to contrast to fish abundance (so going beyond presence/absence).

To our best knowledge this is the first study to demonstrate the transferal of aquatic eDNA across mediums (water to air) so no previous knowledge on this topic can be found elsewhere. Additionally, the scope of the paper was to prove that eDNA genetic material is transferable between mediums and that genetic material is kept intact to the quality that can be captured, amplified and sequenced, rather than focusing on the variables that can influence this relationship. However we have added and acknowledged the knowledge gap on environmental variables effect on cross-medium transferability of eDNA and called in for more studies to further investigate this relationship.

Regarding ‘we collected and measured eDNA concentrations in paired water and passive air samples over a six-week peak migration period, with visual fish counts from hatchery staff.’.. I wonder if by the way the study was designed, the correlation between airborne eDNA and fish counts is not a reflection of the used method: if fish are quantified by visual surface fish counts, then the eDNA found at the surface is perhaps expected to reflect exactly those individuals, but usually counts are also extrapolated to the studied water body. However, it now reads as the eDNA reflecting all fish present in the studied water body. Moreover, the study was performed on a species that is very surface active during spawning periods, and I wonder how these results can be generalized towards other time periods, study species and study systems. All aspects that are currently lacking in either or both of the introduction and discussion of the study, therewith limiting the application potential of the presented work. See for example ‘While this study centers on salmon, its implications extend broadly, proving that aquatic eDNA in the air is quantifiable and potentially species rich.’ In the last paragraph of the introduction, yet no insights or expectations are given in introduction nor discussion of the text.

We thank the reviewer for this comment. While visual counting can include a variety of inferences including the one pointed by the reviewer, in this case they represent the accumulated the number of individual fish at the hatchery gate, hence were let in after being counted. Technically there is no Coho salmon in the river other than the one that are allowed to be passed through the gates and hence counted by the hatchery staff. Because of this caveat all the Coho salmon eDNA (or at least the vast majority of it) should be generated by the fish (which is observed) that at the river damn (hatchery). Due to this caveat we are able to model the visual observation and the eDNA concentration as a derivative of the X (fish density) at time *t* since they both should reflect the same amount of individuals, given their respective bias (φ of the Negative Binomial distribution for the visual observations, and ω for the eDNA molecules which is a draw from a Normal distribution).

The fourth and fifth paragraph of the introduction already include results of the study and reflection, which should be kept for the respective results and discussion sections. Specifically for example ‘By comparing detection sensitivity, temporal patterns, and quantitative performance of these four collectors against water eDNA concentrations and visual counts, we show that—even at roughly 25,000-times dilution—airborne eDNA can reliably track real-world aquatic population dynamics. Our findings provide the first evidence that aquatic genetic material can indeed transfer from water to air under natural conditions, and extends further with quantitative demonstrations that reflect actual population dynamics with just passive air samplers….’. I think the introduction would benefit a slight rephrasing of this section towards hypotheses and objectives rather than an already presented conclusion of the work.

Parts of this paragraph were incorporated in discussion and the majority of parts got deleted.

Regarding the statement: ‘This advancement re-imagines ecological monitoring as one that bridges water and air to build more resilient, comprehensive surveillance networks in a rapidly changing world.’. I found it a rather strong statement, which following the previous comments is difficult to assess as the application potential isn’t detailed.

We have deleted this paragraph in accordance with other comments.

2.1 Field sampling: can you include details on this stream: river discharge rate? Habitat characteristics? Any details on the hydrological background of the stream?

@Aden can you please add 1 sentence about the details on this stream: river discharge rate

Was visual fish count as performed by the hatchery staff standardized over effort somehow?

Yes, the effort was standardized by the number of days elapsed between consecutive gate openings (E) in equation 1. We have added more clarity on the manuscript (line xxx) by stating ‘and let E denote the counting effort (measured as the elapsed number of days between consecutive gate openings; Et = ∆t; hence Et=1 = 0).’

Regarding ‘Throughout our nine sampling events, weather conditions ranged from clear, calm days to periods of rainfall; detailed records of air temperature, wind speed, relative humidity, and precipitation are provided in Supplementary Material 1.’ Is this expected to have impact on the detection? On which detection? Why? Something to include in the introduction?

Thank you for the reviewer pointing this out. We initially thought these environmental factors could have impact on the binding of eDNA onto the filters, but we have too few data points to draw any meaningful conclusions. For this reason, we chose to provide detailed environmental records in the Supplementary Material (Table S2) as contextual information. This allows readers working with similar data or sample types to evaluate potential influences more thoroughly and may facilitate future comparative studies.

At this point, a lot of details are provided on the airborne DNA sampling, yet is it rather unclear on how the water sample was obtained. Four different air filter methods are repeated several times, but it seems also river water was collected, but how? This information on water sampling is provided at the end of this section indeed, yet it seems odd to just take water at on time and location, whereas in many studied a cross-sectional sampling is advised, or a larger extend of the river is sampled, reflecting better the present fish communities. So a clear motivation of the chosen water sampling method is required.

We acknowledge that many studies employ cross-sectional or spatially extensive water sampling to better capture community-level diversity. In our case, only one water sample was included in this study for two reasons. First, although multiple water samples were collected during fieldwork, these were allocated to other projects (orthogonal omics endpoints and were not analyzed with the same assay used in this study) and therefore could not be incorporated here. Second, there is already substantial knowledge from previous research on how water eDNA quantities behave in relation to biological inference (Shelton et al., 2023; Guri et al., 2024). Building on this existing understanding, we are confident that a single water sample was sufficient for our specific aim of providing a point of comparison for the airborne eDNA data, rather than attempting a comprehensive survey of fish communities. Because community composition was outside the scope of this study, we focused instead on testing the aerosol factor. Importantly, one water sample was collected at each of six sampling events (i.e., we have 6 replicated observations for estimating the aerosolization factor), which we consider sufficient for estimating the aerosolization factor from water to air.

A qPCR assay was used targeting a CYTB fragment of coho salmon: no reference is made to an earlier published validation of this assay. If this was not published before, and to assess the use of this assay, some details are needed (main text and/or supplementary) on the in silico and in situ specificity of the assay.

To address the reviewer’s concern, we have now included Supplementary Figure S6, which shows the standard curves and detection probabilities, demonstrating that the assay performance is robust and reliable.

Regarding the visual observation model: it is stated that the aerosolization factor is calculated including water eDNA measurements, but water was only sampled on one location and once per sampling event? I believe the use of the different factors in this model needs to be made more clear, and/or will benefit the combined resolution of previous comment throughout the methods.

We thank the reviewer for highlighting this important point. As noted above, a single water sample was collected in parallel with the air samples during each of the six sampling events, resulting in replication across six independent time points. While only one water sample was taken per event, the repeated pairing with airborne samples provides six independent estimates of the aerosolization factor (η).

Regarding: ‘We model the upstream migration of coho salmon (Oncorhynchus kisutch) as arising from the inferred unknown true density of fish.’ How was survey/count effort considered?

As mentioned on the comment above the count effort was explicitly incorporated into the model through the variable *E*, which represents the elapsed number of days between successive gate openings. This variable captures the effort associated with each count, as longer intervals allow for greater accumulation of fish prior to enumeration which is different from the counting bias and uncertainty. For example, if 200 fish are observed after 5 days (λ), the inferred true daily accumulation rate X is 200/5 = 40 fish/day at time *t*. In our model λ is a draw from a Negative Binomial with a fixed overdispersion parameter (from literature), which accounts for counting bias and uncertainty. Thus, while the mean is 200 fish/effort (hence fish/E), the distribution allows for plausible values around this mean (e.g., 100–300 fish/E), with probabilities decreasing further from the central estimate (200 fish/E).

Regarding: ‘As individuals move upriver, fish accumulate in a holding area immediately downstream of the hatchery river dam.’ Were any considerations included in the exact choice of both air and water sampling locations?

We wanted to capture the highest quantity of eDNA released from Coho salmon so the choice of air and water sampling locations were selected to be the closest to the fish accumulation. For clarity we added in parenthesis ‘(our water and air sampling location)’ right after ‘in a holding area immediately downstream of the hatchery river dam’.

Regarding: ‘At discrete times t,..’ which were?

We added the sampling times by stating ‘across six weekly sampling points from October 17th to November 21st' after at discrete times t

In the Visual observation model, a fixed overdispersion parameter is used, yet ideally this is estimated from the data or via a prior distribution. In this same model, independence between timepoints is assumed, yet this may not hold if fish arrivals are temporally autocorrelated (which you hint at given certain migration patterns: see also ‘Coho salmon migration occurs not as a single continuous event, but rather as a series of distinct burst peaks from mid-October through late November, where the peaks are highly likely to be connected with environmental factors such as water temperature and discharge.’).

We appreciate the reviewer’s insightful comment. In our case, it is not possible to estimate the overdispersion parameter directly from the data, since we have only one observation per time point. Nonetheless, as mentioned above, we have imported the overdispersion value from literature which provides a biologically informed prior assumption for variance of fish counting.

With respect to temporal autocorrelation, we agree that fish arrivals are known to be autocorrelated. However, our study design treats each sampling event (visual counts, water eDNA, and airborne eDNA) as an independent paired observation. We do not attempt to model temporal dynamics or infer relationships across time points. Instead, our focus is on comparing and linking different observation methods at each discrete sampling event. For this reason, while temporal autocorrelation is an important feature of salmon migration generally, it falls outside the scope of our current objectives.

Regarding: molecular process model, specifically eDNA concentration (copies/L) as estimated to be present in the sampling area, or as retrieved from qPCR analyses? Hence, since the absolute concentrations are used, perhaps include info on LOD and LOQ of the (new) assay. Furthermore, the model assumes an instantaneous and proportional relationship between fish abundance and eDNA concentration, ignoring eDNA emission and decay rates, transport dynamics, dilution, … these simplified assumptions might affect accuracy, especially in lotic systems. Many recent studies have been published on exactly these facets of the ‘ecology of eDNA in lotic systems’, and are currently lacking in both the text (insights, hypotheses), as well as in the used methods..

We thank the reviewer for this detailed and constructive comment. Concerning the suggestion to include LOD and LOQ values, we note that these metrics are derived through a frequentist framework and are therefore not directly applicable within our joint Bayesian modeling approach. Instead, our model inherently accounts for detection uncertainty in a probabilistic manner (see Guri et al., 2024, *Quantifying the Detection Sensitivity and Precision of qPCR and ddPCR Mechanisms for eDNA Samples*).

Regarding to the relationship between fish abundance and eDNA concentration, we rely on existing studies demonstrating that this relationship is proportional mainly because the biological and environmental factors such as shedding, decay rates, and dilution are constants while sampling effort can also be considered constant if left unchanged (or accounted for) thus can be encapsulated into a parameter (such as ω here). For further details on this parameter please see Guri et. al., 2024 - *Predicting trawl catches using environmental DNA*, where the equivalent parameter θ was applied.

In the model conditions (2.4), no model checking or validation (posterior predictive checks, cross-validation etc) is mentioned, neither is any info on priors. this could heavily impact posterior estimates, especially for latent variables. Also many of the equations have assumptions that currently lack testing or motivation. For example, air eDNA model (4) assumes slope = 1 and only varies intercept η, which could be too rigid: actual water-to-air transfer may vary non-linearly. Equations 6-10 assume a shared standard curve between different sample types, which could be a problem if such relationships (impacvt of inhibitors) differ across air and water samples, in this regard, specific intercepts or random effects associated with the filter type may provide a solution.

We thank the reviewer for raising these important points. Information on model validation was included: we used the Gelman–Rubin statistic to assess model convergence, and details on all priors and their distributions are provided in Table S1. We have now also added to Section 2.4 that posterior predictive checks were conducted, with results presented in the SI Appendix (Figure S5).

Regarding equation 4, we agree that this relationship might be too rigid but the reasoning for such relationship is twofold. First, the water–air relationship is modeled in log-space (multiplicative effects), and we have no evidence to suggest that the aerosolization factor behaves differently at high versus low DNA concentrations. That is to say that, for example, if 100000 copies/L in water that are transferred into 1000 copies/day/cm2 in air (aerosolization factor 100:1) then proportionally, 1000 copies/L or 10 copies/L in water should correspond to 10 and 0.1 copies/day/cm2 in air, respectively. Thus, we expect scaling to remain linear in log-space, justifying the fixed slope and not change from 100:1 to 80:1 on high or low DNA concentrations. Second, due to identifiability issues between the slope and ω, we can’t learn both parameters especially due to low paired sample number between air and water (six), thus the model will yield more estimates when we fix the relationship with a slope of 1.

Regarding the comment on equation 6-10, similar to the reviewer comment, one can think that genomic DNA or gblocks should not have a similar standard curve as the water samples because they arise from two distinct processes, one from tissue or lab while the other from an environmental sample in water. It is important to understand that the standard curve is modeling the processes (or biases) that arise on the qPCR machine (for example plate to plate variation is high and accounted; see Figure S6) and not the inhibition which could occur both in air and/or water. With simple words equation 6-10 is accounting for the bias of the qPCR machine reading the quantity given that the DNA is present and amplifiable. Processes of inhibition are happening a step prior to this, blocking the DNA for being amplifiable. While we haven’t accounted specifically for this process in our model, such erroneous factor would appear in equation 4 or 5 in parameters η, τ and ρ (Table 1) for each filter specifically. For instance, if gelatin filters consistently yield stronger inhibition than PTFE filters, this would be reflected in η; inhibition tied to atmospheric conditions or day-to-day variation would be reflected in τ; and sporadic inhibition would be reflected in ρ.

Results: While ω is precise, the biological assumptions behind it (e.g., eDNA emission rate is constant across individuals (varying in size?) and time) are strong and should be stated more clearly. Hence, see also earlier comment on the methods section regarding inclusion of emission and decay.

We thank the reviewer for this valuable comment. At the individual level, we do not have data on body size and therefore cannot directly measure or estimate how shedding varies with size. However, when eDNA is used to infer population size (this study), individual-level variation of size or shedding rate becomes embedded within the statistical distribution of ω. While we acknowledge that such variation exists at individual level, at the population scale these differences are expected to average out, allowing them to be represented by a single parameter (ω).

The remainder of my feedback of the results is highly similar to the feedback provided to the methods: missing assumption checks and validation mostly.

Discussion:

While the mechanisms are plausible, no direct measurements of wind speed, humidity, or air particle movement were taken or tested for. These environmental mediators are invoked but not analysed. So either analyses or text should be rephrased accordingly.

Rephrased the paragraph to account for the fact that analyzing the effect of environmental conditions was outside the scope of this study, but we state precaution for future work to be considerate. ‘Although quantifying these effects was beyond the scope of this study, they -- together with hydrological variables -- should be considered when evaluating how passive samplers capture transient pulses of biological activity.’ was added.

The claim of a “real ecological signal” would be stronger with replication across streams or species, currently it's based on a single system, which limits generalizability.

We think this term might be brought out of context. Here the word ‘real’ was intended in a comparative sense from other studies who discard such information as possible contamination. Although we agree that having more robust and conclusive statements would require investigating more systems, we can’t neglect the facts presented in this study that air eDNA is tracing changes of real ecological abundances. More philosophically, because we don’t know everything the eDNA aerosolization (multiple streams, how environmental variables affect it etc.) that doesn’t mean that we can’t say anything at all.

Regarding ‘sampler design shapes signal detection’: the interpretation is insightful, yet a lot is assumed and inferred from patterns, not directly tested.

As this point is presented in the discussion section, our intention is to outline plausible scenarios to help interpret the results. If we had data to back our plausible interpretations, we would have included them in Results and not in Discussion. We can only lay plausible interpretation in discussion in concordance with other studies.

In regards to ‘air is dilute water’: do you mean ‘diluted’ water? Typo? + •Some statements about degradation rates are speculative or extrapolated from (a very limited number of) aquatic studies, whereas airborne DNA decay might differ substantially. More reflection in this section is needed, and see also some remarks made in the methods to include existing insights from literature in the sampling design / modelling.

More caution has been inserted with calls for further study on airborne eDNA decay rates and longevity.

You could also mention enclosed vs. open filter housing designs (not just vertical vs. horizontal orientation) as another way to mitigate degradation or rain exposure.

Reviewer 2

Thank you for giving me the opportunity to review this important and interesting manuscript. Here, the authors introduce a novel way to monitor Coho salmon in a spawning ground using passive airborne eDNA detection and compare this against traditional methods (aquatic eDNA sampling and visual fish counts). The authors built joint statistical models to make estimations about airborne eDNA’s origin, transport, dispersal/dilution, fate, and state, which are crucial for informing future studies of airborne eDNA in similar systems. By collecting airborne eDNA alongside traditional methods, the authors explore fish abundance through multiple complementary approaches. They estimate how Coho salmon eDNA concentrations differ between water and air samples, quantifying the dilution effect across mediums, and introduce droplet and particle-based eDNA transfer through the atmosphere as an effective and novel way to monitor aquatic species, a finding which will be of broad interest to the aquatic biomonitoring community.

The concept behind this paper is both exciting and novel, and the authors have identified a compelling knowledge gap in the eDNA literature. However, in some places, statements would benefit from added nuance to avoid potential overgeneralization. I’ve included specific suggestions below to help strengthen the interpretation and clarity of the findings. Additionally, I have suggested some language changes and structural edits to improve clarity. First, I’ve provided general feedback, then more granular feedback with specific suggestions for editorial changes. With these revisions, I believe the manuscript will be well-positioned for publication.

General feedback:

At several points throughout the manuscript, the authors draw the conclusion that their research shows that aquatic species can be biomonitored with airborne eDNA. I don’t dispute this claim, and I think it’s an exciting finding. However, its broad applicability across diverse environmental contexts requires further investigation. The data collected here supports this conclusion for the specific context the question was studied in, i.e. with Coho salmon in a riverine system. As the authors rightly point out, Coho salmon are very large fish that frequently leap from the water, spawn in selective river stretches, decay in large numbers around river shores, and have strong pulses in abundance corresponding to spawning. However, there are many aquatic organisms that do not do this, and so the discussion of the findings and their significance should be nuanced accordingly. For example, would one expect that airborne eDNA from other organisms (e.g., macrobenthic invertebrates), or from other systems (e.g., lentic systems), would relate to aquatic eDNA concentration in the same way as it does for Coho salmon? The answer is ultimately unknown due to the lack of data (though I suspect that the dilution factor would differ in different systems). That said, this study paves the way for other researchers looking to answer those sorts of questions, and this can also be emphasized in the discussion of this work’s significance and future research directions.

Thank you. We appreciate the comment and have addressed it accordingly.

Specific Feedback:

Introduction:

1 - “Over the past decade, environmental DNA (eDNA) has up ended our paradigm for biodiversity assessment by detecting and quantifying organisms through the genetic material they shed into their surroundings.” – For grammatical correctness, change “up ended” to “upended”. Also - Environmental DNA does not directly quantify organisms; instead, it quantifies DNA to infer organism presence/absence and uses DNA concentration (or relative read abundance in metabarcoding studies) as a proxy to hint at organism abundance. This should be rephrased to something more nuanced, e.g. "by detecting and quantifying extra-organismal DNA, the genetic material shed by organisms into their surroundings." Remember also that in the context of microbes, eDNA samples often contain entire organisms (as the authors mention when introducing gelatin filters that retain culturable microbes). Consider expanding on this slightly here.

The suggested changes are now included with the new sentence stating ‘Over the past decade, environmental DNA (eDNA) has upended our paradigm for biodiversity assessment by detecting and quantifying extra-organismal DNA through the genetic material they shed into their surroundings which can thereafter used to infer organismal abundance.’

2 - “Such integrated approach” should be changed to “Such integrated approaches” for grammatical correctness.

Changed

Methods:

3- Field sampling - To improve ecological context and reproducibility, please also include the province/state(s) and country that the sampling locations are located in.

We added the city and the state and the sentence is now as follows:

We conducted this study near Seattle, Washington USA, in Issaquah Creek, a salmon spawning stream, outside of the Issaquah Salmon Hatchery

4 – What does “water samples were collected by hand” mean in this context? If surface samples were collected in sterilized bottles, it would be good to include that information here.

We clarified the sentence by adding ‘by pulling 3L of water from the river, using sterile water bottles’

5 - “Supplementary Material 1”, which should contain environmental metadata, is referenced in the text but missing from the submission. This information is essential for interpreting eDNA results and aligns with best practices outlined in Nicholson et al. (2020) https://doi.org/10.1002/edn3.81

Add the environmental metadata xxx

Figure 2:

6 - I found the y axis label in Figure 2 somewhat confusing. “Χ – Fish density (fish/day)” makes it look like this axis is representing a function subtracting fish density from Χ. I believe that the authors are trying to convey that the variable Χ represents fish density in fish/day. If so, please use a different axis label, for example: “Χ: Fish density (count/day)” or simply “Fish density (count/day)”. For clarity, consider just stating “Fish Density” with units and leaving out the variable name X – you can explain that this value is represented by ‘X’ in the text and equations.

We agree that this can lead to confusion hence ‘X’ has been removed from the labels.

Results:

7 - “These results provide compelling proof-of-concept evidence that genetic material from aquatic organisms can be recovered from the atmosphere without requiring active airflow systems, thereby demonstrating the viability of fully passive sampling approaches for detecting airborne aquatic eDNA under field conditions.” – This sentence feels somewhat out of place in the results and would be better situated in the discussion. It also seems like an overgeneralization of the results – see general feedback. This statement should be refined to something that preserves technical accuracy while still appreciative of the implications – something along the lines of “These results provide compelling proof-of-concept evidence that genetic material from salmonids can be recovered from the atmosphere around riverine systems without requiring active filtration, thereby demonstrating the viability of fully passive sampling approaches for detecting airborne aquatic eDNA in these environments.”

We agree with reviewer’s comment and moved the sentence to the discussion section.

8 - The first paragraph in section 3.2 is only two sentences – consider expanding the paragraph by adding a third sentence, or merging this paragraph with another for grammatical correctness.

The paragraphs were merged together for increasing the readability of the section.

Discussion:

9 – “Perhaps most striking result from our work” should be changed to “Perhaps the most striking result from our work” for grammatical correctness.

Changed

10 - “makes it viable for remote headwaters, steep mountain channels, urban stormwater networks, and contaminated waters where sampling is unsafe” – Consider also discussing what passive airborne eDNA collection could look like in winter months in colder climates – when the passive filtration device freezes, how could that impact eDNA retention? This would be good to consider in future studies, since one of the benefits of eDNA studies is that they can be conducted in winter months in colder climates.

We rephrased the paragraph for incorporating previous comments and yielded the way that further studies should investigate the effect of environmental variables such as temperature, seasonality etc.

11 - “In an era of intensifying droughts, floods and public-health risks such as bacterial outbreaks in stagnant waters, airborne eDNA offers resilient pathways for rapid invasive-species alerts, real-time disease surveillance in flood-prone wetlands and non-invasive population censuses in protected spawning grounds.” – I find this very exciting, but I think this statement needs to be more carefully nuanced. By simply changing to “airborne eDNA may offer”, the authors can communicate that this is an area identified for future research, and not something they are trying to prove with the data generated in this study.

This comment has been accounted and changed respectively with the previous comment.

12 – “Our study begins to chart a portion of airborne eDNA ecology’s” – change “ecology’s” to “ecology” for grammatical correctness.

Changed

13 - “By demonstrating that genetic signals from fish and other aquatic life routinely escape into and can be captured from the air, we open a new paradigm for ecological monitoring.” - This feels like an overgeneralization and overstatement of the results. Here, the authors have only demonstrated that genetic signals from Coho salmon escape the aquatic systems, not necessarily other aquatic life, though it is likely that the phenomenon does extend to other aquatic organisms as well. To add appropriate nuance while still emphasizing the novelty and excitement of the findings, I recommend rephrasing to something like “By demonstrating that genetic signals from Coho salmon routinely escape into and can be captured from the air in predictable ways, we open new paradigm for ecological monitoring.” This way, the statement is specific about research findings while emphasizing the novelty and importance of the results in the broader context.

The paragraph has been revised to account for this and previous comments accordingly. In short, we have stated that this study demonstrated that eDNA from Coho salmon was found in air but to which rate this is extendable to other organisms is unknown. Additionally we added that if the underlying process is driven by physical processes such as bubble bursting and evaporation, the migration of eDNA from water to air shouldn’t be exclusive to fish but if the underlying process is driven by biological behaviors such as jumping and splashing then then the eDNA transferability may be restricted to organisms that interact directly with other media.

14 - “This advance promises transformative applications from invasive species alerts in drought-stricken reservoirs to pathogen surveillance in flood-prone wetlands” – Again, this is not directly shown by this data. It suggests that these things might be possible, but I think that “promises” is too strong a word choice. Consider something like “This advance supports the development of transformative applications.”

This sentence has been removed.

15 - “Rainfall also introduces a trade-off for the tray: heavy rain can dilute the accumulated eDNA but may also scour additional airborne or splash-borne DNA into the water.” - This is interesting and has implications for how the results are interpreted. How can the authors be sure that the DNA is from their target system if rainwater could be scouring DNA from other locations due to movement of clouds via wind? Discussing this briefly would strengthen the discussion and add more nuance to the interpretation of results.

The paragraph has been revised, and we toned down the cause and effect of atmospheric variables by stating that these are plausible factors that can affect the integrity of DNA. However we have stated that these are untestable within our work.

References:

16 – Reference #3: italicize species name Triturus cristatus

Done

17 – Throughout reference list, format journal title names consistently – capitalization differs throughout.

Done

18 – Reference [55] is missing journal and publication info – I’ll defer to the editor on how to handle referencing of unpublished works but wanted to flag this.

Done

Closing remarks:

Thank you again for allowing me to review this interesting manuscript. I applaud the authors on the work’s novelty, and I am excited to see the new biomonitoring strategies that come from this work. I trust that the comments I provided will be helpful in the preparation of the manuscript for publication, and I with the authors and editorial staff all the best in their future endeavours.