

Detection of terrestrial mammals using environmental DNA during heavy rainfall events and associated influencing factors

ABSTRACT

- I'm confused at the beginning of this abstract. You've introduced the idea of monitoring terrestrial vertebrates then immediately segway into aquatic eDNA. I think there's something missing there to connect terrestrial with aquatic.
- GF/D and GF/F don't need to be introduced here, it's a bit distracting with arbitrary abbreviations that may not mean much to the reader.
- It might be worth an additional sentence on how you calculated or estimated the assumed point of entry into the river, since this is one of the key findings.

INTRODUCTION

- Line 41: after "present in environmental samples (e.g., sea and soil)", it may be useful to add "via the introduction of biological material shed from an organism (e.g., hair, skin cells, fecal matter)."
- Line 42: remove "the" before "target DNA" for grammatical accuracy.
- Line 49: flowers should be "flowers" here I think if I'm understanding the sentence correctly.
 - Now that I'm rereading this sentence, I think that this is supposed to be "flowers, surface, soil,"?
- Line 53-54: Please enhance this sentence as it seems incomplete.
- Line 54-56: What did this study find?
- Paragraph 2 of the introduction needs a bit of refinement - I appreciate the structure of introducing other work regarding aquatic detection of terrestrial animals, but the ideas and statements are disjointed.
- Line 60: What is "low river utilization rate"? That may be useful if reworded or defined.
- Line 62: "degradation" may be better than "removal" here.
- Line 68-70: please review correct grammar and punctuation here.
- Lines 76-77: it feels like there should be an ending to this sentence saying "... highly turbid water samples, which likely contain organic substances which may degrade genetic material."
- Line 78-80: this is unfortunately untrue, there are many studies that have been in turbid environments (Holmes et al., 2024, Egeter et al., 2018, Ip et al., 2021, Kumar et al., 2022, Turba et al., 2023, many estuarine-based eDNA work, etc...).
- Lines 80-81: again, untrue. It's been published that filter pore size cannot be generalized across all studies - for some species or environments (including waterbodies with high primary productivity), larger pore sizes are preferred (Jo et al., 2020, Deiner et al., 2018).
- Line 88: The Nucleospin (Macherey-Nagel) extraction kit is directly intended to provide a centrifugation-based method for obtaining eDNA from natural environments - including those with high sediment loads/inhibition.
- Line 94: "has been used" would be correct rather than "was used" here.

- Line 105: It would help to start this sentence with a justification word(s): “Therefore, in this study,” “To investigate this, we used”, “The aim of this study was to use dPCR to...”
- Line 107: please add a “,” after *B. taurus* in this sentence.
- Line 109: Beware of calling one method “superior” to another, qPCR may be better for certain applications especially if working with a constrained budget.
- Please add a concluding sentence to the last paragraph of the introduction which contextualizes the importance of this study.

MATERIALS AND METHODS

- Line 120: please modify this to “southwestern Japan: the Mizunashi, Oka, and Tagami Rivers as well as the irrigation canal connected to the latter...”
- Line 122-129: Why was this land use mapping done? What is the purpose? This would be nice to preface prior to diving into the specifics of the land classification efforts. Also, a period is missing on line 129.
- Line 134: How is this a low abundance species if the study focused on “widely distributed” *B. taurus*?
- Line 134: There should not be a period between “abundance” and “And”. It should be one sentence here.
- Line 135: How many cattle in each shed? This is important for the outcome(s) of this study.
- Line 159: It’s unclear if 4 replicate water samples mean 4 x 1L of river water or 1L consists of 4 samples?
- Were lids used for the eDNA sampling buckets/containers?
- Line 183-184: I recommend removing the end of this sentence as it’s not a fully supported finding across eDNA studies.
- Line 191: Please remove the comma between “filtering” and “to prevent”.
- Line 194-196: I’m confused at this statement - can you elaborate or remove this?
- Line 204: How were the lab work benches cleaned? Ethanol/bleach? UV sterilization? Both?
- Line 222: Can you please include the primer/probe sequences and their fragment length(s) here?
- Line 226: What was the starting concentration of the dilution series?
- Line 243: Why were these PCR volumes so much higher than the ones on line 233-236?
- Line 247-249: Nice!!!
- Lines 252-254: This is information that should stick to the introduction section. The methods part begins at the end of line 254.
- Lines 259-261: Does this refer to where the cattle houses are? How many were utilized/modeled in this study?

RESULTS

- It would help the non-specialist readers more if you changed the terminology throughout the results to “small” and “large” filters (or something along those lines) when describing results for GF/D and GF/F.
- Line 347: Please include a range of values in parenthesis after “extremely low”.

- Line 348-350: This information is more appropriate for the discussion section.
- Lines 352-355: This information (and info above in the “Measurements of basic water quality parameters” would be easier for readers to interpret in a Table (or 2) - perhaps you could make one summary table for all of the river-specific information/statistics that are written throughout the results section.
 - This could also include info from the methods regarding the rivers: Lines 129-132, 143-147, 262-265.
- Line 358: A comma is not needed between “traces” and “collected”.

DISCUSSION

- To avoid using “thus” two times in the first paragraph, you could change one to “therefore”.
- Line 382: It doesn’t quite make sense to say “the source of the target terrestrial eDNA is terrestrial”, can you modify this?
- Lines 383-387: It’s not standard to refer to figures/tables in the discussion section of a paper.
- Line 391-394: This was not tested in the current study...
- Line 394: Please fix the grammar “this may why”.
- Have you considered a time lag following the rainfall may also influence terrestrial DNA being found in a waterbody? Maybe 1 day post rainfall is actually more effective than during the rain event.
- Line 440: Please add “these sources include, but are not limited to,”.
- Lines 440-451: The ideas introduced here are seemingly random and do not support this story. I understand that you’re leading to the fact that there is stochasticity in the effectiveness of various sources of terrestrial-based eDNA, but I’m not convinced by the way this is written.
- Line 465: Please fix the capitalization of Sampling.
- Line 475: Please add “which highlight the advantage...”
- Line 482: Since “catchment” is used throughout the discussion section, it’d be beneficial to define this earlier either in the introduction or M&M because it’s an important lake-based term that not all readers would understand the significance of.
- It might be best to end the conclusion on a positive note rather than a recommendation for future work. Just a suggestion.

FIGURES/TABLES

- Please increase the size and resolution of Figure 1.
- Please fix typos in the description of Figure 1.
- The caption for Figure 2 describes water quality parameters but these are not referenced or written anywhere in the Fig 2 diagram.
- Please increase the resolution of Figure 3.
- I suggest using the same font across figures/tables.
- Please add definitions for σ and μ in the caption for Table 3.
- Please use the same number of digits after the decimal point throughout the manuscript.