Authors sincerely thank Davy Vanham for his constructive comments which have helped to improve the article. We address each comment in turn below.

Note: All line numbers in responses correspond to the revised manuscript.

**Comment 3.1:** Here they put as aim (lines 107-108) “In order to scientifically underpin large scale EF policies, the existing assumption of the inverse relationship between freshwater biodiversity response and EF violation must be tested regional and global scales” However, in this paper I see a mismatch between the analysis conducted on the one hand and the interpretation/discussion on the other hand. The authors only look at environmental flows. They acknowledge that the violation of environmental flows influences aquatic biodiversity (eg lines 84-86). As they discuss in lines 368-374, other factors influence aquatic biodiversity: climate change, river fragmentation, large-scale habitat degradation, landscaping/river scaping, alien species introduction and water pollution. They however do not account for these factors in their analysis.

Response 3.1: The primary motivation for conducting this study is due to the fact that the majority of the methods used to estimate EF operates at a coarser (global) scale with an underlying assumption that the proportion of flow allocated directly impacts the ecosystem health. However, what this study is doing is reevaluating this assumption. As raised in the discussion of this paper, a holistic approach including bio-geo-hydro-physical approach is necessary to ensure the proper functioning of associated ecosystems. Authors agree with the commenter that it is necessary to evaluate the influence of non-hydrologic factors on aquatic ecosystem wellbeing. Moreover, the climate change impact is indirectly taken into account in the EFE analysis (Virkki et al., 2022). Moreover, we have also included the aspect of other confounding environmental factors that might strongly influence the result in the discussion/limitation section (see lines 500-504)

**Comment 3.2:** I do not follow the reasoning in section 41. It is clear that there
are many different EF methodologies, and that more holistic EF estimation methods are required for water management. But this has been expressed by many (recent studies), such as https://doi.org/10.1016/S2542-5196(21)00234-5 or many others. It is not the authors of this study who prove that with their analysis. I doubt whether for more holistic EF methods better correlations can be found, as long as the many other factors are not taken into account.

Response 3.2: By ‘holistic approach’, authors mean the inclusion of non-hydrologic factors. Through this study, we try to promote a more inclusive approach to estimating the flow requirements for freshwater ecosystems. This idea is supported in this paper by quantitatively evaluating whether environmental flow is the only or key driver of aquatic biodiversity. Authors are in complete agreement with the commenter that a holistic approach that is not limited to the quantity of water in the streams is a better alternative to conventional EF methodologies. This message is emphasized throughout the entire paper acknowledging the literature before.

Comment 3.3: I also do not see why this analysis has implications for a water planetary boundary (section 42). What you only show is that global assessments, due to data restrictions and assumptions, lead to quite some uncertainty. But that does not mean the current bottom up methodology using EFs would be lacking, it just means the boundary has a wide uncertainty range. EF do provide a meaningful boundary for freshwater biodiversity. That is why it is used in SDG indicator 642, a very significant upgrade from the millennium goal on water scarcity. You actually have a methodology that has global monitoring obligations for UN member states, thereby making it directly policy relevant. Due to the fact that you do not account for the other factors affecting aquatic biodiversity, and therefore do NOT prove inconsistency in “…universal relationship with freshwater biodiversity” (line 416-418) I do not see any justification for the statement “We suggest that to reconsider the use of environmental flows in defining water planetary boundaries” (line 421-422).

Response 3.3: There are several studies proposing environmental flow transgressions as a potential control variable for defining the safe operating space for a freshwater planetary boundary (Steffen et al., 2015; Gerten et al., 2013). However, these assumed relationships between streamflow and aquatic biodiversity have not been studied at global or large regional scales. Therefore, as mentioned in the previous response, this study aids in testing a widely used but unverified assumption on the relationship between environmental flow and aquatic biodiversity at the global and ecoregion scales.

Comment 3.4: To conclude, I recommend that the authors re-evaluate their section 4, as well as conclusions, abstract and title. As an example, for the key research points (with in capital letters recommendations):

- No significant relationship between environmental flow (EF) violation and freshwater biodiversity indicators was found at global or ecoregion scales using globally consistent methods and currently available data, WHEN NOT ACCOUNTING FOR OTHER FACTORS AFFECTING FRESHWATER BIODIVERSITY
- Several basins show a slight positive correlation between EF violation and biodiversity indicators, which could be attributed to the artificial introduction of non-native species. HOW IS THE INFLUENCE OF FACTOR NON-NATIVE SPECIES PROVEN? WHAT WITH THE OTHER FACTORS?
A generalized approach that incorporates EF considerations but ignores the lack of a significant EF-biodiversity relationship at large scales can underestimate the stress on the ecosystem at smaller scales which correspond with eco-hydrological processes that determine ecological impacts from EF violation. NOT CLEAR, AS YOU DO NOT ACCOUNT FOR OTHER FACTORS. ALSO, THESE OTHER FACTORS ARE ESSENTIAL FROM LOCAL TO GLOBAL SCALE, THEY WILL DETERMINE AT ALL SCALES THE CORRELATION between environmental flow (EF) violation and freshwater biodiversity. WHAT YOU PROBABLY MEAN IS WHAT GLOBAL MODELS ARE ABLE TO CAPTURE. But then, future data availability will only improve making multi-regression assessments

Response 3.4: Necessary changes are made in the manuscript

Comment 3.5: Lines 363-374: again, poor correlation by ignoring these other factors. Lines 364-368: no, the other factors are determining. Line 368: no, not only for larger-scale relations, also on a local level. The sudden introduction of a point source pollution can plummet aquatic biodiversity on a very small scale, and therefore also on this small scale, even with very detailed data availability, the other factors need to be accounted for when looking at correlations

Response 3.5: Please refer to response 3.2

Comment 3.6: Ps I also think that putting a title like "Poor correlation between large-scale environmental flow violations and freshwater biodiversity", is not helpful for implementing EFs in the field or policy agendas. As said, its inclusion in SDG indicator 642 is a major advancement and international success. Your title could be misused for not acting on preserving or rehabilitating EFs. When not put in context, some could use it as a slogan not to act on EFs.

Response 3.6: The paper is not intended to be a definitive test to disprove the relationship between EF and aquatic biodiversity. It is intended to be an exploratory analysis to identify the validity of the relation. We do not, in any way, intend to disregard the importance of flow, but instead, our aim is to estimate the usability of large-scale generalized EF estimation methods by evaluating their relationship to aquatic biodiversity indicators. The single negative result is not a final say but it is a call for conducting more studies on existing generalized and well-applied methods.

We acknowledge the risk of reporting a non-correlation between EF and biodiversity. To avoid the risk of misjudgment by the readers, we have strengthened the discussion that our findings are only applicable at global or ecoregion scale and with currently available data. At a scale smaller than this, several studies have already proved the importance of flow for maintaining ecosystem services. The authors, however, think it is more appropriate to keep the title unchanged to be upfront, simple and honest about the findings.

Necessary changes are made in the abstract and conclusion section to minimize the chances of miscommunication of the intended purpose of the paper.
Please also note the supplement to this comment: