Chinchu Mohan[1,2*], Tom Gleeson[2,3*], James S Famiglietti[1,4], Vili Virkki[5], Matti Kummu[5], Miina Porkka[5,6], Lan Wang-Erlandsson[7,8], Xander Huggins[1,2], Dieter Gerten[9,10], Sonja C. Jähnig[10,11]

[1] Global Institute for Water Security, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

[2] Department of Civil Engineering, University of Victoria, Victoria, British Columbia, Canada

[3] School of Earth and Ocean Sciences, University of Victoria, Victoria, British Columbia, Canada

[4] School of Environment and Sustainability, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

[5] Water and Development Research Group, Aalto University, Espoo, Finland

[6] Global Economic Dynamics and the Biosphere, Royal Swedish Academy of Sciences, Stockholm, Sweden

[7] Stockholm Resilience Centre, Stockholm University, Stockholm, Sweden

[8] Bolin Centre for Climate Research, Stockholm University, Stockholm, Sweden

[9] Potsdam Institute for Climate Impact Research (PIK), Member of the Leibniz Association, Potsdam, Germany

[10] Humboldt-Universität zu Berlin, Geography Department and Integrative Research Institute on Transformations of Human–Environment Systems, Berlin, Germany

[11] Leibniz Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, Berlin, Germany

*Correspondence to: Chinchu Mohan (chinchu.mohan@usask.ca), Tom Gleeson (tgleeson@uvic.ca)

ORCID corresponding authors: Chinchu Mohan: 0000-0001-7611-3392; Tom Gleeson: 0000-0001-9493-7707

Key Research Points

- No significant relationship between EF violation and freshwater biodiversity indicators was found at global or ecoregion scale using globally consistent methods and currently available data.

- Several basins show a slight positive correlation between EF violation and biodiversity, which could attribute to the artificial introduction of non-native species.

- A generalized approach that incorporates EF considerations while ignoring the lack of a significant EF-biodiversity relationship at different scales, can

underestimate the stress on the ecosystem at a smaller scale where the actual action is taking place.

- Use of a globally aggregated blue water planetary boundary using biodiversity as a response is deceptive and can potentially impact the hotspot identification and management.

Abstract

The freshwater ecosystems around the world are degrading, such that maintaining environmental flow (EF) in river networks is critical to their preservation. The relationship between streamflow alterations and, respectively, EF violations, and freshwater biodiversity is well established at the scale of stream reaches or small basins ($\sim{<}100$ km²). However, it is unclear if this relationship is robust at larger scales even though there are large-scale initiatives to legalize the EF requirement and EFs have been used in assessing a planetary boundary for freshwater. Therefore, this study intends to evaluate the relationship between EF violation and freshwater biodiversity at globally aggregated scales and for freshwater ecoregions. Four EF violation indices (severity, frequency, probability to shift to violated state, and probability to stay violated) and eight independent freshwater biodiversity indicators (calculated from observed biota data except) were used for correlation analysis. No statistically significant negative relationship between EF violation and freshwater biodiversity was found at global or ecoregion scale. While our results thus suggest that streamflow and EF may not be a main determinant of freshwater biodiversity at large scales, they do not preclude the existence of relationships with more holistic EF methods (e.g., including water temperature, water quality, intermittency, connectivity etc.) or with other biodiversity data or metrics.

Keywords: Environmental flow violation, freshwater biodiversity, Global scale, freshwater ecoregions.

1.Introduction

Water resources are inarguably one of the most important natural resources in the Earth system for sustaining life. Nevertheless, these resources and their associated ecosystems are threatened by human actions [*Bélanger and Pilling*, 2019; *Clausen and York*, 2008; *Charles J Vörösmarty et al.*, 2010; *Wilting et al.*, 2017]. Global freshwater covers up to 0.8% of the total Earth's surface [*Gleick*, 1996], yet it inhabits 6% of all the known species in the world including 40% of total fish diversity and nearly one third of all vertebrates [*Lundberg et al.*, 2000]. Due to the high pressure and multiple uses that meet high species richness in a relatively small area, freshwater ecosystems are more vulnerable to human actions and environmental changes than any other ecosystems [*Dudgeon et al.*, 2006]. The rapid increase in the demand for natural resources is the fundamental cause for freshwater ecosystem degradation [*W Darwall et al.*, 2018]. Anthropogenic climate change [*Allan and Flecker*, 1993; *W R Darwall and Freyhof*, 2016; *IPCC*, 2014; *Knouft and Ficklin*, 2017; *Meyer et al.*, 1999],

overexploitation [*Allan et al.*, 2005], water pollution [*Albert et al.*, 2020; *Dudgeon et al.*, 2006; *Reid et al.*, 2019; *Smith*, 2003], flow alteration [*Nilsson et al.*, 2005; *Charles J Vörösmarty et al.*, 2000], habitat destruction [*Dudgeon*, 2001] and introduction of alien species [*Gozlan et al.*, 2010; *Vitule et al.*, 2009] are some of the manifestations of this increased demand which directly threatens the freshwater ecosystems. In addition, increased water impoundment in large dams and reservoirs has also led to an array of adversities to freshwater ecosystems ranging from habitat destruction to irregular flow alterations [*Bergkamp et al.*, 2000]. This situation is aggravated by increasing pressure on related Earth system functions, such as climate change and nutrient cycles, which are articulated by their respective transgressions in the planetary boundaries framework (Box 1) [*Dudgeon*, 2010]. Freshwater ecosystem processes that were previously governed by natural Earth system facets such as temperature, rainfall, and relief are now increasingly driven by demographic, social, and economic drivers [*Clausen and York*, 2008; *Kabat et al.*, 2004; *Tyson et al.*, 2002; *Vitousek et al.*, 1997; *Charles Joseph Vörösmarty et al.*, 1997]. Freshwater ecosystem health comprises both the biotic factors like biodiversity and the abiotic factors like habitat integrity. As any disruption in the abiotic factors is most likely to be reflected in the biotic status of the freshwater ecosystem, the scope of this paper is confined to the biotic dimension of the freshwater ecosystem (i.e., biodiversity) and not the entire ecosystem health.

There has been an increased recognition in recent decades of the need of maintaining a natural flow regime in streams to sustain healthy ecosystems. [*Holtgrieve et al.*, 2018; *Horne et al.*, 2017; *N L Poff et al.*, 2017; *N L Poff et al.*, 1997; *Tickner et al.*, 2020; *Tonkin et al.*, 2020]. Despite the indispensable role of aquatic biodiversity in maintaining the quality of the system [*W Darwall et al.*, 2018], inclusion of such environmental flow (EF) in water management is often controversial, particularly in regions where freshwater availability is limited and is already a matter of severe competition. These competitions have led to an increasing trend in EF violation (insufficient streamflow than the recommended EF requirement; see section 2.1 for more details) in the past decade both in terms of severity and frequency [*Virkki et al.*, 2021]. This wakeup call has led to several international and national efforts to legalize EF requirements through large-scale EF management schemes [*Arthington and Pusey*, 2003; *B Richter et al.*, 1997; *B D Richter et al.*, 2003]. The Water and Nature Initiative [*WANI*, 2008], the Brisbane declaration [*Declaration*, 2007], and the Global Action Agenda [*Arthington et al.*, 2018] are some of these efforts. Nevertheless, there is a large gap in our understanding of the relationship between EF requirements and biodiversity responses at various spatial and temporal scales. Except for a few [*Domisch et al.*, 2017; *Xenopoulos et al.*, 2005; *Yoshikawa et al.*, 2014], the majority of the studies exploring this relation were conducted at smaller scales and mostly focused on one taxon [*Anderson et al.*, 2006; *Arthington and Pusey*, 2003; *Powell et al.*, 2008]. Thus, there is a significant discrepancy in the scale at which these processes are understood versus the scale at which the policies are set [*Thompson and Lake*, 2010]. Current knowledge of how

the small-scale processes scale up (e.g., validation of large-scale EF hydrologic methods using local data) to a regional or global scale is thus limited, potentially undermining the scientific integrity of existing large scale EF management schemes.

Bunn and Arthington [2002] proposed four guiding principles to substantiate the influence of flow alterations on stream biodiversity: 1) The physical habitat is primarily determined by the flow, which in turn determines the biotic composition, 2) Flow variations directly influenced the evolution of aquatic species, 3) Viability of an aquatic population is determined by the longitudinal and lateral connectivity of the river systems, and 4) Alteration of flow regimes facilitates invasive, exotic species. These four principles and other basin-scale evidence [*Leigh and Datry*, 2017; *Mathers et al.*, 2019; *Sarremejane et al.*, 2020; *Zeiringer et al.*, 2018] suggest that freshwater biodiversity has an inverse relationship with EF violations at all spatial scales. According to this hypothesis, as the EF violation increases, the associated freshwater biodiversity will decrease. Furthermore, the EF-biodiversity relationship is assumed to be scale-independent, meaning that its nature does not change with spatial scale. A graphical representation of this hypothesis is given in Fig. 1. When the hypothesis is valid, a curve fitted against the freshwater biodiversity and EF violation should yield a negative gradient (Fig. 1 a) and the median value of biodiversity (either relative value or absolute value) of all violated basins should be significantly lower than the non-violated counterpart (Fig. 1 b). If either of these conditions are not met, then the hypothesis could be considered invalid.
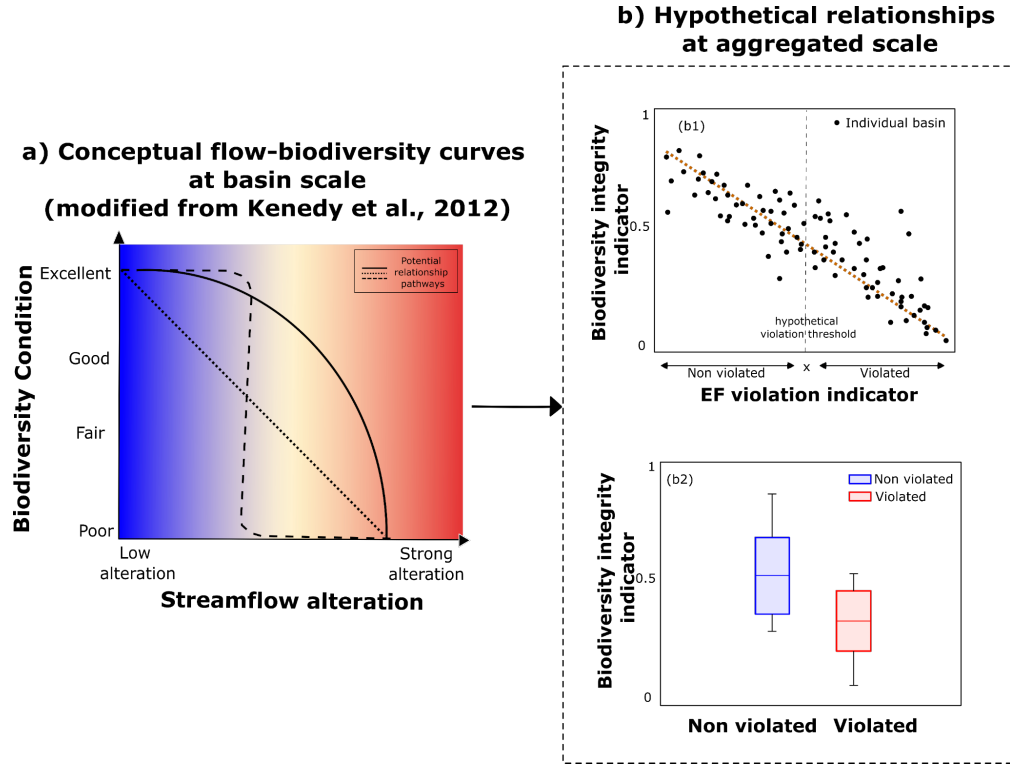
Fig. 1 a) Conceptual flow-biodiversity curves at basin scale modified from [*Kendy et al.*, 2012] and b) hypothetical graphs of the simplest EF-biodiversity relationship (linear) at aggregated scale.

In order to scientifically underpin EF policies, the existing hypothesis of the freshwater biodiversity response to EF violation must be validated. Therefore, in this study, we evaluate the relationship between EF violation and freshwater biodiversity at two different spatial scales (freshwater ecoregion, global), using four EF violation indices (frequency, severity, probability to move to a violated state, and probability to stay violated) and eight freshwater biodiversity indicators describing taxonomic, functional, and phylogenetic dimensions of the biodiversity. The implications of the findings for large-scale water management and the use of the relationship between environmental flows and freshwater biodiversity (hereafter referred to as EF-biodiversity relationship) in the planetary boundary framework (box 1) are also discussed.

Box 1: Introduction to blue water planetary boundary framework
The planetary boundaries framework proposed by *Rockström et al.* [2009] and
further developed by *Steffen et al.* [2015] defines bio geophysical planetary
scale boundaries for Earth system processes that, if violated, can irretrievably
impair the Holocene-like stability. It establishes scientifically determined safe
operating limits for human perturbations, under which humans and other life
forms will coexist in equilibrium without jeopardizing the Earth's resilience.
Nine planetary boundaries were defined to cover all independent significant
Earth system processes. Out of the nine, the freshwater planetary boundary
quantifies the safe limits of the terrestrial hydrosphere [*Gleeson et al.*, 2019;
Gleeson et al., 2020].
The freshwater planetary boundary was originally defined using human water
consumption as the control variable, set at 4000 km³/yr (with an uncertainty
of 4000 to 6000 km³/yr) [*Rockström et al.*, 2009]. *Gerten et al.* [2013]
proposed a bottom-up, spatially explicit quantification of EF violations as part
of the water boundary, while *Gleeson et al.* [2020] subdivided the water
planetary boundary into six sub-boundaries and proposed possible control and
response variables for each, with aquatic biosphere integrity (i.e., EF) as the
potential control variable for a surface water sub-boundary. Quantitative
evaluation of the strength and scalability of the identified control and response
variables is still required.

2.Methodology and Data

The study is carried out at two spatially aggregated scales; 1) global and 2)
ecoregion, for a historic time period of 30 years (1976 - 2005). All the underlying
calculations were done at level 5 HydroBASIN (median basin area = 19,600 km²)
[*Lehner*, 2014; *Lehner and Grill*, 2013] and were aggregated to the corresponding
spatial scale for further analysis. Level 5 HydroBASIN (also referred to as basin
in this paper) was selected as the smallest spatial unit as it is the highest level
of specificity that can be rasterized into a 0.5-degree resolution grid without
significantly reducing the number of sub-basins smaller than a grid cell [*Virkki
et al.*, 2021]. The EF violation indices were calculated using *Virkki et al.* [2021]'s
novel Environmental Flow Envelope (EFE) framework, and the biodiversity was
represented by a combination of relative and absolute value indices.

2.1 Data

2.1.1 Streamflow data   Streamflow data used in the EFE (see section 2.2
for more details) definition were obtained from the Inter-Sectoral Impact Model
Intercomparison Project (ISIMIP) simulation phase 2b outputs of global daily
discharge (available at https://esg.pik-potsdam.de) [*ISIMIP*, 2020]. Monthly
streamflow data (averaged from the daily simulations) for two time periods
were used in this study; 1) for the pre-industrial era (1800 - 1860), which is
considered as the unaltered reference period [*N L Poff et al.*, 1997] and 2) for

the recent time period (1976 - 2005). These monthly streamflow datasets were used to calculate EF violations. For calculating the EF violation indices, the estimated EFEs for each basin were obtained from *Virkki et al.* [2021].

The streamflow data were aggregated to the sub-basin scale according to level 5 HydroBASIN Version 1.0 (https://www.hydrosheds.org/page/hydrobasins) [*Lehner and Grill*, 2013]. The data from ISIMIP 2b is representative of historical land use and other human influences including dams and reservoirs [*Frieler et al.*, 2017]. The maximum discharge cell value within the boundaries of each level 5 HydroBASIN is chosen to represent the outlet discharge value. Any violations within the outlet cell are regarded as indicative of the entire basin, even if conditions can differ in various areas within the level 5 HydroBASIN. See supplementary materials (section S.1) for more details on the datasets used in this study.

2.1.2 Freshwater biodiversity data   In addition to the streamflow data, data on fish and amphibian diversity are also used (Table 1). Freshwater biodiversity was evaluated using eight indices estimated from the observed biota data. The biodiversity indicators were either recalculated or directly obtained from international agencies or the literature. The biodiversity indicators consisted of 6 indicators of relative change in biodiversity and 2 indicators of absolute values of biodiversity.

Absolute biodiversity indicators   The absolute biodiversity indicators consisted of amphibian richness (AmR) and freshwater fish richness (FiR). The richness data for amphibians were obtained from the NASA Socioeconomic Data and Application Center (SEDAC) [*IUCN and CIESIN*, 2015]. The amphibian richness data covering 5918 all known non-extinct amphibian species was an aggregation of the number of species present in each 30 arc second (~1km) grid. The fish richness data was compiled and processed from 1436 published papers, books, grey literature and web-based sources published between 1960 and 2014 [*Tedesco et al.*, 2017]. They cover 3119 basins all over the world and account for 14953 fish species permanently or occasionally inhabiting freshwater systems.

Relative biodiversity indicators   The Relative biodiversity indicators consisted of six freshwater fish facets. Six key facets of freshwater fish - taxonomic, functional, and phylogenetic diversity (TR, FR, PR respectively), as well as dissimilarity of each of the three groups (TD, FD, PD respectively)- were used in this analysis to construct a holistic picture of the state of aquatic biodiversity [*Su et al.*, 2021]. Each facet indicates the change in the corresponding biodiversity component compared to the 18th century (roughly pre-industrial era). The taxonomic facets measure the occurrence of fish in a riverine system. Functional facets are calculated using the morphological characteristics of each species that are linked to feeding and locomotive functions which in turn relates to larger ecosystem functions like food web control and nutrition transport. Phylogenetic facets measure the total length of branches linking all species from the

assemblage on the phylogenetic tree. The richness component of the three categories calculates the diversity among the assemblage whereas the dissimilarity accounts for the difference between each pair of fish assemblage in the same realm. All six fish facets were calculated for the 2465 river basins covering over 10682 fish species all over the world. All six facets are available as a single delta change in time and do not cover multiple timesteps.

Table 1. Details of different data used in this study

Data	Spatial resolution (extend)	Temporal resolution (extend)	Source/Reference
Amphibian richness data	arc-second (global)	Temporal aggregate mostly representing 2013	Observed/Measured data *IUCN and CIESIN* [2015]
Aquatic fish richness data	arc second (3119 drainage basins; ~80% of Earth's land)	Temporal aggregate from data compiled from reports between 1960 and 2014	Observed/Measured data *Tedesco et al.* [2017]
Freshwater fish facets	Basin scale (2465 drainage basins)	Representative of 2015	Derived from observed data *Su et al.* [2021]
EFE	Aggregated to Level 5 HydroBASIN (global)	Monthly (Pre-industrial: 1801-1860)	Model calculated *Virkki et al.* [2021]
Streamflow	Aggregated to Level 5 HydroBASIN (global)	Monthly (Pre-industrial: 1801-1860, Current: 1976-2005)	Model calculated *ISIMIP* [2020]
Basin boundaries	Level 5 HydroBASIN (global)	Not applicable	*Lehner and Grill* [2013]

2.2 Environmental flow violation estimation

The EFE framework proposed by *Virkki et al.* [2021] is used to evaluate EF violations in this study. The EFE framework establishes an envelope of variability constrained by discharge limits beyond which flow in the streams will not meet the freshwater biodiversity needs [*Virkki et al.*, 2021]. EFE uses pre-industrial (1801-1860) stream discharge and pre-industrial EF to establish an upper and lower boundary for EF deviations at monthly time steps. This EFE is used

to define the EF violation at Level 5 HydroBASIN scale. The EF violations were calculated as median ensemble of four Global Hydrological Models (GHM) (H08 [*Hanasaki et al.*, 2018], LPJmL [*Schaphoff et al.*, 2018], PCR-GLOBWB [*Sutanudjaja et al.*, 2018], WaterGAP2 [*Mueller Schmied et al.*, 2016]) and mean ensemble of four Global Circulation Models (GCM) (GFDL-ESM2M [*Dunne et al.*, 2012; *Dunne et al.*, 2013], HadGEM2-ES [*Collins et al.*, 2011; *Martin et al.*, 2011], IPSL-CM5A-LR [*Dufresne et al.*, 2013], MICROC5 [*Watanabe et al.*, 2010]). Moreover, five different EF calculation methods (Smakhtin [*Smakhtin et al.*, 2004], Tennant [*Tennant*, 1976], Q90-Q50 [*Pastor et al.*, 2014], Tessmann [*Tessmann*, 1979] and Variable Monthly Flow [*Pastor et al.*, 2014]) were also used in the EFE derivation [*Virkki et al.*, 2021]. The use of multiple GCMs, GHMs and EF calculation methods in the EFE definition significantly reduces the uncertainty in the violation estimates. Moreover, all the basins with Mean Annual Flow (MAF) < 10 m^3/s were excluded due to high uncertainty in EFE and streamflow estimates [*Gerten et al.*, 2020; *Steffen et al.*, 2015; *Virkki et al.*, 2021]. After this exclusion, a total of 3906 basins were considered for further analysis.

Here we evaluate the EF violation by defining four different EF violation indices: 1) violation severity (S), violation frequency (F), probability to shift to a violated state (P.shift) and probability to stay violated (P.stay). Out of the four EF violation indicators, two (S and F) were a modification from *Virkki et al.* [2021] and the two (P.shift and P.stay) were calculated based on the current EFE deviations from *Virkki et al.* [2021]. P.shift and P.stay were estimated using the partial Hidden Markov Model (HMM) which is used to model a non-observable stochastic process (hidden process), which can only be observed through an observable stochastic process with data sequence [*Rabiner and Juang*, 1986; *Zucchini et al.*, 2017]. HMM helped in estimating the shift and stay probabilities between different states. The state of a basin (violated or non-violated) was identified at an annual time step and the mean probability to shift or remain in that state is calculated.

The detailed definitions of the EF violation indicators are as follows.

1. Violation severity (S): The annual violation severity was calculated as the mean of the magnitude of EF deviation from the EFE lower bound in all the violated months. In this study we considered only the EFE lower bound violations as the upper bound violations were considerably low in the ecologically relevant areas.

2. Violation frequency (F): Frequency of violation is a measure of the proportion of months a basin has violated the EFE lower bound in a year. Frequency is calculated as the percentage of lower bound violated months per year.

3. Probability to shift to a violated state (P.shift): The P.shift is defined in this paper as the probability of a basin to shift to a violated state from a non-violated state. This indicator along with P.stay gives a measure of

9

the stability of violation in each level 5 HydroBASIN. The violated/non-violated state of a basin is calculated annually based on the violations in the low flow months. If a basin violates EFE lower bound for at least three consecutive months during the low flow period (Q<0.4MAF) in a year, then the basin is considered to be in a violated state.

4. Probability to stay violated (P.stay): Once shifted to a violated state, the tendency of a basin to remain in that state or switch to a non-violated state is determined by this indicator. If a basin has a higher P.stay (closer to 1) then the basin continues to remain in the violated state for a longer time before switching to a non-violated state. Whereas, the basins with lower P.stay (closer to 0) tend to remain in the violated state only for a brief period of time. In other words, the number of consecutive violated years is much lower for basins with lower P.stay value.

2.3. Relationship between environmental flow violations and freshwater biodiversity

The relationship between freshwater biodiversity and EF violation was evaluated by aggregating the level 5 HydroBASIN scale values to global level, WWF's Freshwater ecoregions major habitat type scale (results given in SI) [*Abell et al.*, 2008] and G200 freshwater ecoregion level[*Olson and Dinerstein*, 2002]. The G200 freshwater ecoregion is a subset of WWF's freshwater ecoregion that includes only the biodiversity hotspots. Seven freshwater ecoregions in ecologically important regions were studied, and the EF-biodiversity relationship was evaluated separately for each habitat. One of the major challenges in conducting an aggregated evaluation was the discrepancy in the spatial resolution at which the EF violation indices and various biodiversity indicators.

In order to overcome this challenge, two different aggregation/data matching methods were employed; case-1) matching level 5 HydroBASIN data (EF violation indices) to biodiversity data and case-2) matching biodiversity data to level 5 HydroBASIN (See supplementary information (SI); Fig. S2). In the first case every level 5 HydroBASIN (EF violation indices) is matched with the spatially closest biodiversity data point nearest centroid. Whereas in the second case there can be three different scenarios (See SI; Fig. S3): 1) biodiversity basin is smaller than level 5 HydroBASIN; in that case all the biodiversity basins within one level 5 HydroBASIN were matched with the same EF violation value, 2) when biodiversity basin is equal in size to level 5 HydroBASIN; in this case biodiversity basins and level 5 HydroBASIN had a one-to-one match, 3) biodiversity basin is larger than level 5 HydroBASIN. In the last case, two methods were used for data mapping 1) Outlet matching: where each biodiversity basin is mapped with EF violation value from the level 5 HydroBASIN closest to the outlet and 2) Mean matching: each biodiversity basin is mapped with the mean EF violation values of all level 5 HydroBASIN within it. As the results are not very sensitive to the aggregation method, only the results using case 1 (matching level 5 HydroBASIN data to biodiversity data) are discussed in this paper.

3.Results and Interpretations

3.1 Evaluating EF violation drivers and characteristics

The majority of basins face some kind of EF violation (either in terms of severity or frequency or with higher probabilities to shift and/or stay violated) (Fig. 2). Between 1976 and 2005, 20% and 54% of basins, respectively, experienced violation frequency (F) greater than 3 months and severity (S) greater than 20% from the EFE lower bound (normalized violation index $>= 0.25$) (Fig.2 a,b). Additionally, 33% of basins have a higher chance of shifting (P.shift $>=$ 0.5; i.e. 33% basins have over 50% probability to shift to a violated state) to a violated state (Fig.2 c,d). EF violations are very frequent and severe in mostly arid/semi-arid regions such as the Middle East, Iran, Iraq, Pakistan, India, Australia, Sahara, Sub-Saharan Africa, Southern Africa, and the southernmost part of North America. The severity and frequency of EF violations were found to be strongly linked to mean current annual streamflow with an R^2 value of 0.23 and 0.11 respectively (both statistically significant at p value < 0.01) (Fig. 3). On the other hand, regions with higher probability to shift to a violated state (P.shift) were not limited to the low precipitation and low streamflow regions.

Although the majority of regions with high P.shift values were arid or semi-arid, some exceptions included South Eastern Asia (Mekong basin region) and Central South America. When compared against water withdrawal data [*Huang et al.*, 2018], the non-arid regions with higher P.shift also have extremely high water withdrawal in all sectors (agriculture, domestic and industry). This spatial concurrence suggests that human activities, as well as hydroclimatic influences, play a significant role in deciding a region's P.shift. However, once in the violated state, the flow variability regimes in the catchment determine the probability of remaining (P.stay) in the violated state. Catchments with highly variable flow regimes (i.e., receive most of the annual flow as floods; see SI for classification map; Fig. S1) have higher probability to stay violated once shifted whereas catchments with stable flow regimes (year-round steady high baseflow; see SI for classification map; Fig. S1) have a higher tendency to revert back to a non-violated state. An example of this behavior can be seen in the Australian basins. Though, almost all the Australian basins have a very high P.shift, only the highly variable flow regime northern catchments had a higher probability to stay violated. Despite having a very high P.shift, the southern stable catchments swiftly shift back to a non-violated state. Similar behavior can be observed in the Mekong basin as well.
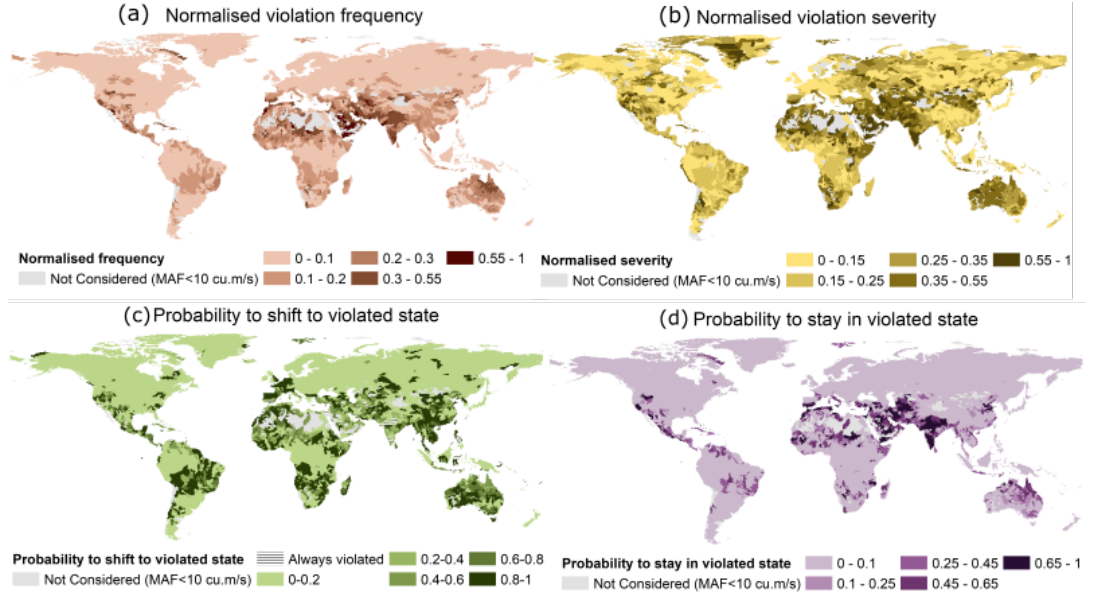
Fig. 2 Four measures of Environmental Flow Envelope (EFE) lower bound violation estimated using ensemble median of four Global hydrological models; a) Normalized frequency of violation, b) Normalized severity of violation, c) Probability to shift to a violated state from a non-violated state and d) Probability to stay violated once shifted to a violated state.
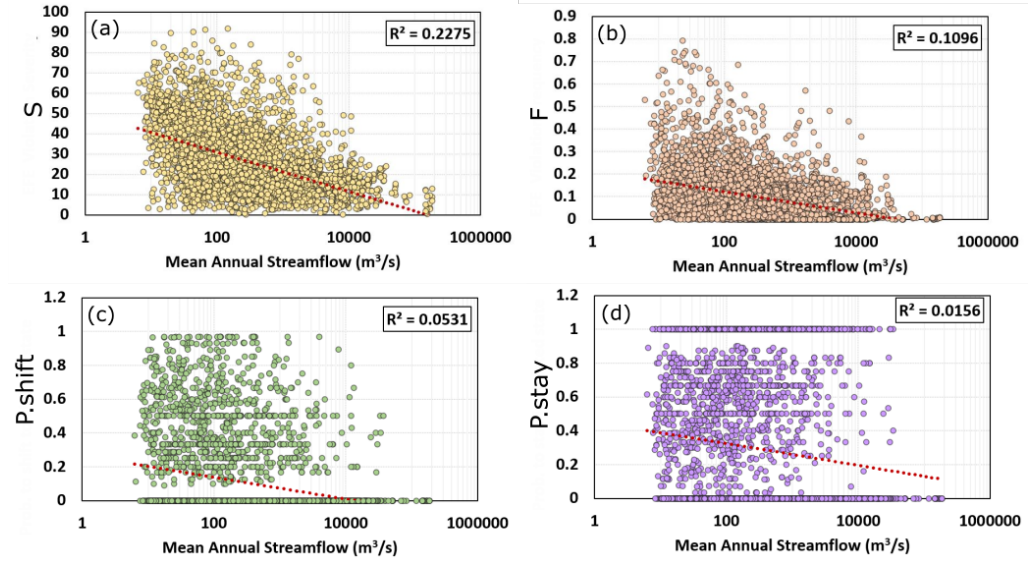

Fig. 3 Scatter between mean annual streamflow and (a) violation frequency, (b) violation severity, (c) probability to shift to violated state and (d) probability

12

to stay in the violated state.

3.2 Relationship between EF violation and freshwater biodiversity

The aggregated analysis was carried out at global and ecoregion level. Multiple aggregation methods (section 2.3) yielded similar results, therefore only the case 1 (level 5 HydroBASIN matched with biodiversity data) results are discussed further (see supplementary material Fig. S4 and S5 for results using other aggregation methods). At global scale, none of the biodiversity indicators correlate (significance of p value <0.05) with any EF violation indices (Fig. 4). The biodiversity indicators were not exhibiting any strong trend in either positive or negative direction. The correlation coefficient value (R value) for the remaining biodiversity indicators ranges only from -0.2 to 0.17 (Fig. 4 b). Surprisingly, all of *Su et al.* [2021]'s fish dissimilarity facets (TD, FD and PD) show slight negative trend whereas the richness facets (TR, FR, and PR) display a slight positive correlation with EF violation, which contradicts the proposed hypothesis. The positive correlation of the richness indicators is attributed to an overall increase in the assemblage in the majority of the basins. Moreover, TR and FiR were showing opposite trends. The positive trend in TR is due to changes with alien species, whereas the FiR describes the current deteriorated state. The increase in the fish assemblage over time was verified using an independent dataset called RivFishTIME (see SI; Fig. S8) [*Comte et al.*, 2021]. The increase in the fish richness facets primarily stems from the introduction of alien species introduced into streams for commercial purposes [*Su et al.*, 2021]. The invasion of alien species can tamper with the existing natural ecosystem equilibrium resulting in the degradation of the overall ecosystem health.

Correlations between EF and biodiversity are generally weak at the scale of G200 freshwater ecoregions as well (see Section 2.2, *Olson and Dinerstein* [2002]). In G200 freshwater ecoregions (see SI; Table S4 for full freshwater ecoregion results) the nature of the EF-biodiversity relationships was highly varying between different ecoregions (Fig 5). In large lakes, large rivers and small lakes, Su et al. fish richness facets were showing a strong and significant positive correlation with most of the EF violation indices. Whereas, in large rivers and xeric basins, AmR and FiR show slight negative trends. However, in the majority of realms, the EF-biodiversity relationship is insignificant (p value >0.05). These results corroborate the above findings that EF violations are not significantly inversely correlated with biodiversity, regardless of realm.
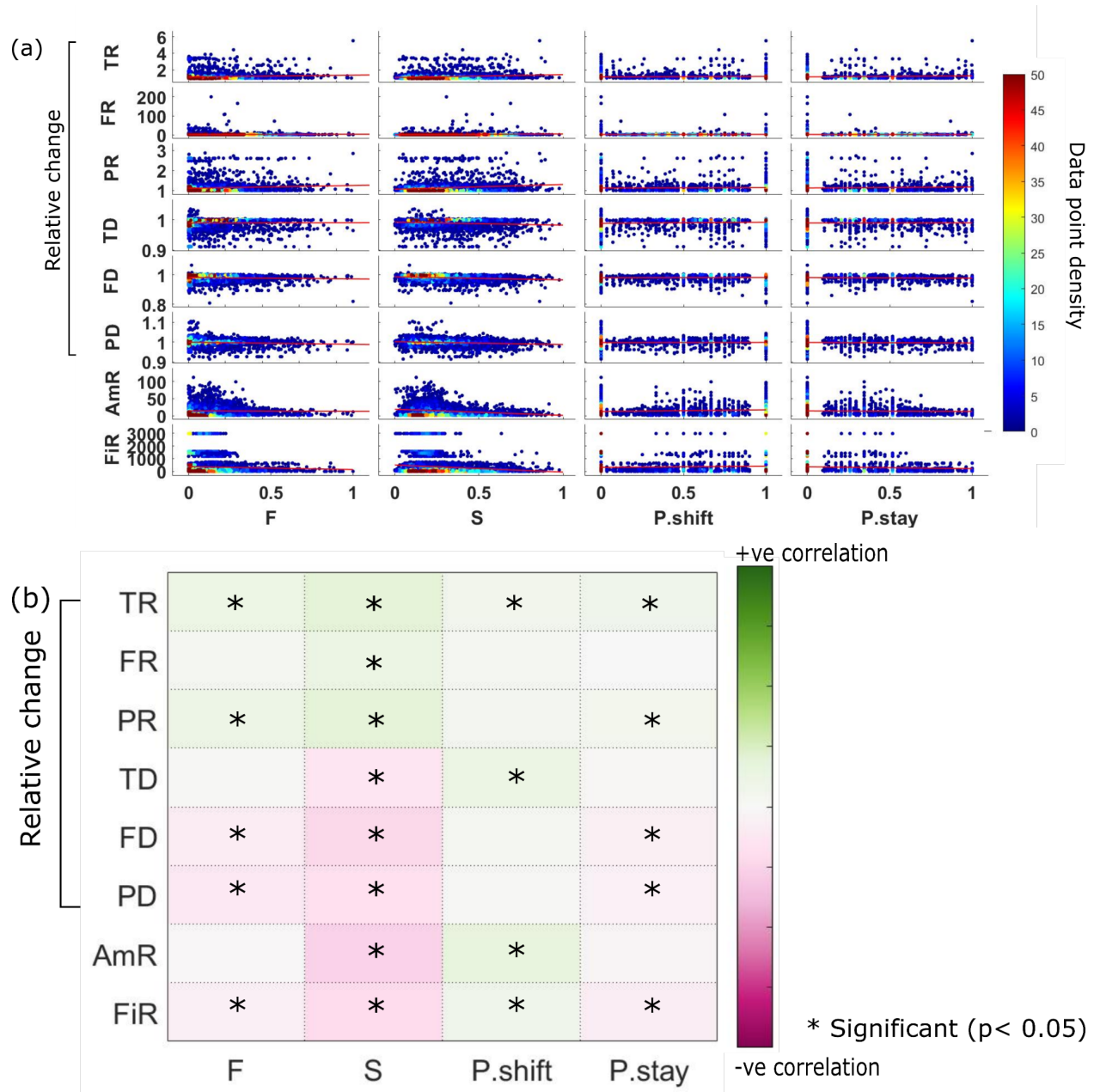
Fig. 4 (a) Scatter between EF violation indices and biodiversity indices with linear fit and corresponding (b) R value and the significance of EF-biodiversity relationships at globally aggregated scale.

Note: This figure represents results from case 1 (level 5 HydroBASIN matched with biodiversity data). The results of other aggregation methods are given in

SI (Fig. S4 and S5).

Abbreviations: AmR-Amphibian richness; FiR-Fish richness; TR-Taxonomic richness; FR-Functional richness; PR-Phylogenetic richness; TD-Taxonomic dissimilarity; FD-Functional dissimilarity; PD-Phylogenetic dissimilarity
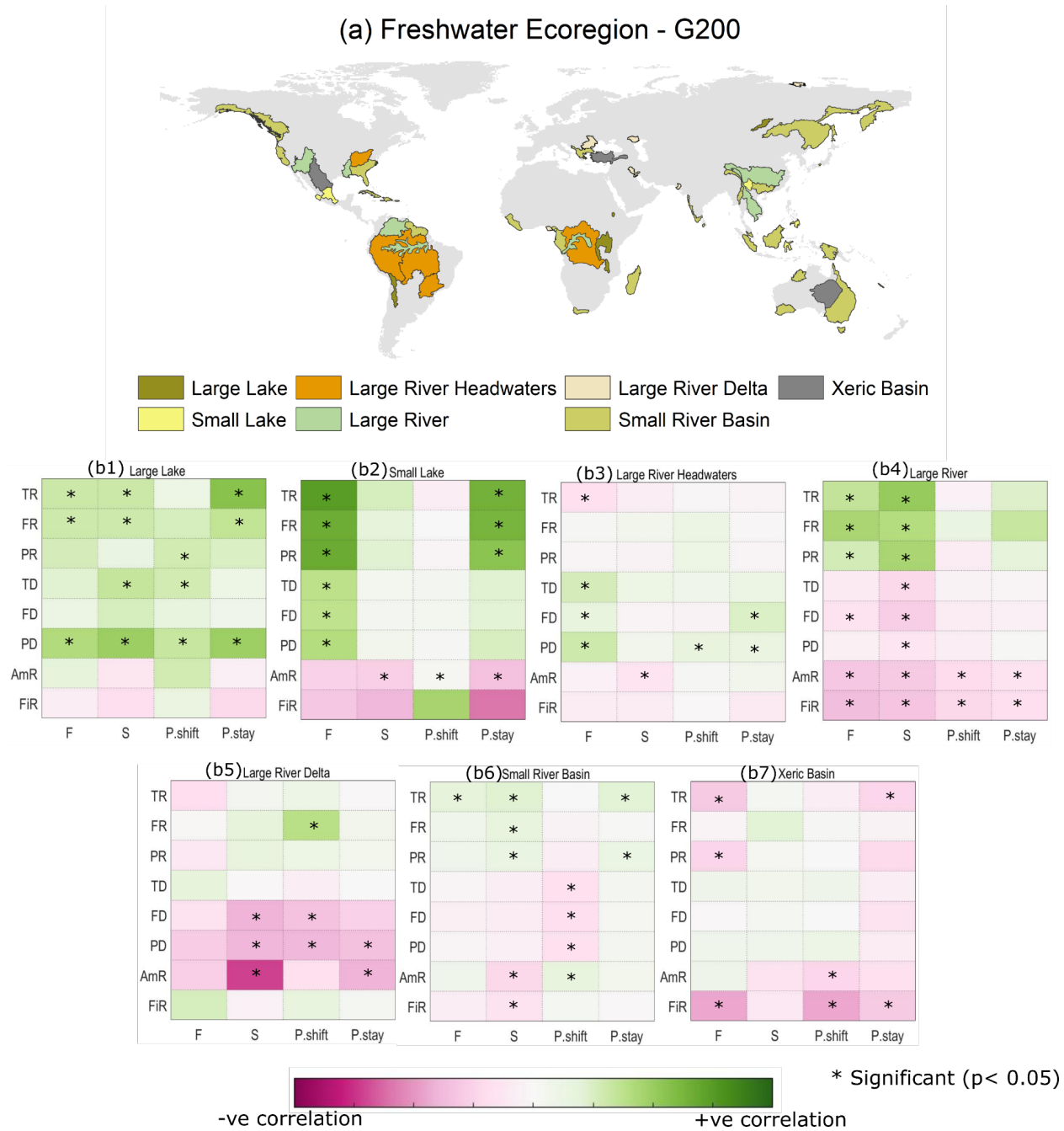
Fig. 5 (a) Spatial distribution of different G200 freshwater ecoregions and (b1-b7) the correlation between EF violation indices and freshwater biodiversity indicators for different G200 freshwater ecoregions.

Note: The results for all the WWF freshwater ecoregions are given in SI (SI section S.5).

4.Discussion

The findings from this study indicate that the EF-biodiversity relationship is poorly correlated when aggregated to global or ecoregion scale. The most likely explanation for the lack of correlation is the overwhelming heterogeneity of the freshwater ecosystems -- with some freshwater species being more susceptible to variations in flow than others [*N L Poff and Zimmerman*, 2010]. Thus, it is implausible to find a generalized response to a common driver. Moreover, when it comes to a larger-scale relationship, several other factors like, climate change [*Davies*, 2010; *N Poff et al.*, 2002], river fragmentation [*Grill et al.*, 2015; *Herrera et al.*, 2020], large-scale habitat degradation [*Moyle and Leidy*, 1992], landscaping/river scaping [*Allan*, 2004], alien species [*Leprieur et al.*, 2008; *Leprieur et al.*, 2009; *Villéger et al.*, 2011] and water pollution [*Brooks et al.*, 2016; *Shesterin*, 2010] can also impact the freshwater ecosystem in multiple ways. Thus, at Earth system level, other interlinked factors potentially confound the impact of EF violation on biodiversity degradation.

4.1 Implications for water management

The lack of strong correlation between EF violation and freshwater biodiversity has profound implications for large-scale water management. A generalized approach that incorporates EF considerations while ignoring the lack of a significant EF-biodiversity relationship at different scales, can underestimate the stress on the ecosystem at a smaller scale where the actual action is taking place. The global hydrological EF methods are often validated using locally calculated EF requirement values [*Pastor et al.*, 2014] with the assumption of adequate scalability in the EF-biodiversity relationship. However, more holistic EF estimation methods combining hydrological, hydraulic, habitat simulation methods, and expert knowledge [*L Poff et al.*, 2010; *Shafroth et al.*, 2010] is essential to ensure a healthy freshwater biodiversity. The policies and decisions taken at various scales need a more dynamic framework, where different dominant drivers of ecosystem degradation can be prioritized based on particular cases. For example, an integrated EF indicator which encompasses quantity, quality and timeliness of water in the streams will be a better hydrologic indicator to evaluate freshwater ecosystem health than an indicator which accounts only for quantity. Moreover, when making water management decisions, care must be given to account for the temporal and spatial heterogeneity in the ecosystem dynamics.

Although there are some coordinated scientific efforts such as ELOHA (Ecological Limits Of Hydrologic Alterations) [*L Poff et al.*, 2010] to provide a holistic framework for EF estimation, its scientific complexity and high implementation cost constrains its use around the world [*B D Richter et al.*, 2012]. For example, several European countries like Romania, Czech Republic, Serbia and

Luxembourg use a national level static method to define minimum environmental flows [*Linnansaari et al.*, 2012]. Similarly, other jurisdictions use the presumptive standards proposed by *B D Richter et al.* [2012] to establish a legal basis for EF protection. These presumptive standards limit hydrologic modifications to a percentage range of natural or historic flow variability. One example of such a case, the North Carolina's Environmental Flow Science Advisory Board uses a presumptive standard of 80-90% of the instantaneous modeled baseline flow as the EF requirement [*NCEFSAB*, 2013]. The limitation of such a practice is the incorrect presumption of uniformity in the EF needs over a larger region. Therefore, we recommend the application of holistic indicators at these large scales (covering all river stretches and tributaries) rather than using simplified hydrologic-only metrics of EF (violation). However, the authors also acknowledge the limits in implementation of a more dynamic EF framework in data limited regions. Programs for more monitoring and data collection and improved, more holistic modelling methods using more/better data need to be implemented in those regions. Thus, applying a holistic framework like ELOHA could be made possible and can capture the heterogeneity in the EF-biodiversity relationship.

4.2 Implications for a water planetary boundary

Inorder to use EF in the water planetary boundary definition, the biodiversity response to a change in EF needs to be proven significant. However, the findings of this paper quantitatively show that the EF-biodiversity relationship is poor at large scale. Moreover, due to the heterogeneity of biodiversity response in space and time, the trend in any aggregate scale is most likely to be relatively rate constant than showing any discernible tipping point (Brook et al., 2013). Considering the higher degree of heterogeneity and lack of strength in the EF-biodiversity relationship, we reconsideration of a water planetary boundary definition and the related response and control variables. Reasons are firstly, freshwater biodiversity does not have pan-regional or "continental-planetary" scale threshold dynamics, and its link with EF violation is inadequate to represent the finer scale variations. Secondly, resource distribution and human impact heterogeneity impel the need of regional boundaries as proposed by *Steffen et al.* [2015]. Thirdly, EF calculation methods used in the current regional/planetary boundary definition are highly restricted to hydrological methods which do not capture the biodiversity status appropriately. A regional boundary transgression can occur even well within planetary-level safe limits [*Brook et al.*, 2013; *Nykvist et al.*, 2017]. For a highly complex biophysical relationship like the EF-biodiversity where multiple shift states are possible, it is nearly impossible to prioritize and manage critical regions without a regional/local boundary. From this we infer that use of a globally aggregated boundary using biodiversity as a response is deceptive and can potentially impact the hotspot identification and management.

4.3 Limitations and ways forward

Despite giving novel insight into the relationship between EF and freshwater biodiversity at different spatial scales, this study has the following limitations:

1) Data scarcity: Even though this study uses state of the art global hydrological models and best available global estimates of EF requirements, freshwater ecological data were limited to amphibians and freshwater fish. Other than these, several other taxa like crayfish, phytoplankton, zooplankton and other benthic invertebrates are also significant in determining the proper functioning of a freshwater ecosystem [*AL-Budeiri*, 2021; *Covich et al.*, 1999; *Domisch et al.*, 2017; *Nyström et al.*, 1996]. However, due to lack of global data, these taxa are not included in this study. To better examine the relationship, global datasets for other freshwater biodiversity metrics are urgently needed.

2) **Discrepancy in data resolution**: The spatial and temporal resolutions at which the EF violation is estimated here, and the biodiversity indicators measured/calculated are inconsistent. The basic spatial measuring unit of the biodiversity is sometimes greater or lesser than the basin size at which EF is measured. This discrepancy could have some impact on the results. However, in this study several resolution matching methods were used to account for this uncertainty. Therefore, more detailed data with better-matching scales are needed to overcome this limitation.

3) **Lack of multi-driver interaction**: In this study, we consider the impact of EF violations on biodiversity as an independent relationship. In reality, this might not be the case. Other drivers of ecosystem degradation like land use change, habitat loss, stream modifications and geographical disconnection can influence the EF-biodiversity relationship. These interactions were outside the scope of this study but should be taken into account in follow up studies.

4) **Simplified representation of human interference with freshwater systems**: The role of humans in impairing the ecosystem balance is represented here based on how human water withdrawals violate hydrologically defined EF. Other human disturbances are thus not accounted for, such as aquatic habitat degradation through change in land use, artificial introduction of nonnative species, and non-point pollution from agriculture. Moreover, this study does not distinguish the climate driven impact on EF violation from the anthropogenic impacts.

5. Conclusion

The relationship between EF violations and freshwater biodiversity is evaluated at globally aggregated levels in this study. No significant relationship between EF violation and freshwater biodiversity indicators was found at global or ecoregion scale using globally consistent methods and currently available data. Relationships may exist at smaller scales and could potentially be identified with more holistic EF methods including multiple factors (e.g., temperature, water

quality, intermittency, connectivity) and finer and more extensive freshwater biodiversity data. The lack of correlation in the EF-biodiversity relationship found in this study suggests that the scientific basis of macro-scale EF policies may not be well-founded, and further implies that the conceptualization of a blue water planetary boundary ought to rest upon a broader set of relationships between hydrological processes and Earth system functioning. At larger scales, the enormous spatial and temporal heterogeneity in EF-biodiversity relationship motivates a holistic estimation of EF grounded on ecosystem dynamics.

Data Availability

The data used in this study are temporarily made available at https://drive.google.com/drive/folders/1dXYByen

The permanent location of the data is to be decided. Any additional data or code will be made available on request.

Author Contribution

CM, TG, JSF devised the conceptual and analysis framework of this study with inputs from MK, MP and VV. VV performed the EFE calculation with help from MK and MP. CM performed the biodiversity data compilation and EF-biodiversity analytical evaluation with help from TG, JSF and XH. CM performed the final analysis and produced the results and visualization shown in the study, discussing together with TG, JSF, XH, MK, MP, VV and LWE. TG, JSF, MK, MP, VV, LWE, XH, DG and SCJ contributed to paper writing and the interpretation of the results. CM took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Compelling Interests

The authors declare no competing interests.

Acknowledgement

Supplementary Information

The supplementary information is submitted separately.

Reference

Abell, R., M. L. Thieme, C. Revenga, M. Bryer, M. Kottelat, N. Bogutskaya, B. Coad, N. Mandrak, S. C. Balderas, and W. Bussing (2008), Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation, *BioScience*, *58*(5), 403-414.

AL-Budeiri, A. S. (2021), The Role Of Zooplankton In The Pelagic Food Webs Of Tropical Lakes, University of Leicester.

Albert, J. S., G. Destouni, S. M. Duke-Sylvester, A. E. Magurran, T. Oberdorff, R. E. Reis, K. O. Winemiller, and W. J. Ripple (2020), Scientists' warning to humanity on the freshwater biodiversity crisis, *Ambio*, 1-10.

Allan, J. D. (2004), Landscapes and riverscapes: the influence of land use on stream ecosystems, *Annu. Rev. Ecol. Evol. Syst.*, *35*, 257-284.

Allan, J. D., and A. S. Flecker (1993), Biodiversity conservation in running waters, *BioScience*, *43*(1), 32-43.

Allan, J. D., R. Abell, Z. Hogan, C. Revenga, B. W. Taylor, R. L. Welcomme, and K. Winemiller (2005), Overfishing of inland waters, *BioScience*, *55*(12), 1041-1051.

Anderson, K. E., A. J. Paul, E. McCauley, L. J. Jackson, J. R. Post, and R. M. Nisbet (2006), Instream flow needs in streams and rivers: the importance of understanding ecological dynamics, *Frontiers in Ecology and the Environment*, *4*(6), 309-318.

Arthington, A. H., and B. J. Pusey (2003), Flow restoration and protection in Australian rivers, *River research and applications*, *19*(5-6), 377-395.

Arthington, A. H., A. Bhaduri, S. E. Bunn, S. E. Jackson, R. E. Tharme, D. Tickner, B. Young, M. Acreman, N. Baker, and S. Capon (2018), The Brisbane declaration and global action agenda on environmental flows (2018), *Frontiers in Environmental Science*, *6*, 45.

Bélanger, J., and D. Pilling (2019), *The state of the world's biodiversity for food and agriculture*, Food and Agriculture Organization of the United Nations (FAO).

Bergkamp, G., M. McCartney, P. Dugan, J. McNeely, and M. Acreman (2000), Dams, ecosystem functions and environmental restoration, *Thematic review II*, *1*, 1-187.

Brook, B. W., E. C. Ellis, M. P. Perring, A. W. Mackay, and L. Blomqvist (2013), Does the terrestrial biosphere have planetary tipping points?, *Trends in ecology & evolution*, *28*(7), 396-401.

Brooks, B. W., J. M. Lazorchak, M. D. Howard, M. V. V. Johnson, S. L. Morton, D. A. Perkins, E. D. Reavie, G. I. Scott, S. A. Smith, and J. A. Steevens (2016), Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems?, *Environmental toxicology and chemistry*, *35*(1), 6-13.

Bunn, S. E., and A. H. Arthington (2002), Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity, *Environmental management*, *30*(4), 492-507.

Clausen, R., and R. York (2008), Global biodiversity decline of marine and freshwater fish: a cross-national analysis of economic, demographic, and ecological influences, *Social Science Research*, *37*(4), 1310-1320.

Collins, W., N. Bellouin, M. Doutriaux-Boucher, N. Gedney, P. Halloran, T. Hinton, J. Hughes, C. Jones, M. Joshi, and S. Liddicoat (2011), Development and evaluation of an Earth-System model–HadGEM2, *Geoscientific Model Development*, *4*(4), 1051-1075.

Comte, L., J. Carvajal-Quintero, P. A. Tedesco, X. Giam, U. Brose, T. Erős, A. F. Filipe, M. J. Fortin, K. Irving, and C. Jacquet (2021), RivFishTIME: A global database of fish time-series to study global change ecology in riverine systems, *Global Ecology and Biogeography*, *30*(1), 38-50.

Covich, A. P., M. A. Palmer, and T. A. Crowl (1999), The role of benthic invertebrate species in freshwater ecosystems: zoobenthic species influence energy flows and nutrient cycling, *BioScience*, *49*(2), 119-127.

Darwall, W., V. Bremerich, A. De Wever, A. I. Dell, J. Freyhof, M. O. Gessner, H. P. Grossart, I. Harrison, K. Irvine, and S. C. Jähnig (2018), The Alliance for Freshwater Life: a global call to unite efforts for freshwater biodiversity science and conservation, *Aquatic Conservation: Marine and Freshwater Ecosystems*, *28*(4), 1015-1022.

Darwall, W. R., and J. Freyhof (2016), Lost fishes, who is counting? The extent of the threat to freshwater fish biodiversity, *Conservation of freshwater fishes*, 1-36.

Davies, P. M. (2010), Climate change implications for river restoration in global biodiversity hotspots, *Restoration Ecology*, *18*(3), 261-268.

Declaration, B. (2007), The Brisbane Declaration: environmental flows are essential for freshwater ecosystem health and human well-being, paper presented at 10th International River Symposium, Brisbane, Australia.

Domisch, S., F. T. Portmann, M. Kuemmerlen, R. B. O'Hara, R. K. Johnson, J. Davy-Bowker, T. Bækken, C. Zamora-Muñoz, M. Sáinz-Bariáin, and N. Bonada (2017), Using streamflow observations to estimate the impact of hydrological regimes and anthropogenic water use on European stream macroinvertebrate occurrences, *Ecohydrology*, *10*(8), e1895.

Dudgeon, D. (2001), Fisheries: pollution and habitat degradation in tropical Asian rivers, *In: Encyclopaedia of Global Environmental Change, Volume 3*.

Dudgeon, D. (2010), Prospects for sustaining freshwater biodiversity in the 21st century: linking ecosystem structure and function, *Current Opinion in Environmental Sustainability*, *2*(5-6), 422-430.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, and M. L. Stiassny (2006), Freshwater biodiversity: importance, threats, status and conservation challenges, *Biological reviews*, *81*(2), 163-182.

Dufresne, J.-L., M.-A. Foujols, S. Denvil, A. Caubel, O. Marti, O. Aumont, Y. Balkanski, S. Bekki, H. Bellenger, and R. Benshila (2013), Climate change projections using the IPSL-CM5 Earth System Model: from CMIP3 to CMIP5, *Climate dynamics*, *40*(9), 2123-2165.

Dunne, J. P., J. G. John, A. J. Adcroft, S. M. Griffies, R. W. Hallberg, E. Shevliakova, R. J. Stouffer, W. Cooke, K. A. Dunne, and M. J. Harrison (2012), GFDL's ESM2 global coupled climate–carbon earth system models. Part I: Physical formulation and baseline simulation characteristics, *Journal of climate*, *25*(19), 6646-6665.

Dunne, J. P., J. G. John, E. Shevliakova, R. J. Stouffer, J. P. Krasting, S. L. Malyshev, P. Milly, L. T. Sentman, A. J. Adcroft, and W. Cooke (2013), GFDL's ESM2 global coupled climate–carbon earth system models. Part II: carbon system formulation and baseline simulation characteristics, *Journal of Climate*, *26*(7), 2247-2267.

Frieler, K., S. Lange, F. Piontek, C. P. Reyer, J. Schewe, L. Warszawski, F. Zhao, L. Chini, S. Denvil, and K. Emanuel (2017), Assessing the impacts of 1.5 C global warming–simulation protocol of the Inter-Sectoral Impact Model Intercomparison Project (ISIMIP2b), *Geoscientific Model Development*, *10*(12), 4321-4345.

Gerten, D., H. Hoff, J. Rockström, J. Jägermeyr, M. Kummu, and A. V. Pastor (2013), Towards a revised planetary boundary for consumptive freshwater use: role of environmental flow requirements, *Current Opinion in Environmental Sustainability*, *5*(6), 551-558.

Gerten, D., V. Heck, J. Jägermeyr, B. L. Bodirsky, I. Fetzer, M. Jalava, M. Kummu, W. Lucht, J. Rockström, and S. Schaphoff (2020), Feeding ten billion people is possible within four terrestrial planetary boundaries, *Nature Sustainability*, *3*(3), 200-208.

Gleeson, T., L. Wang-Erlandsson, M. Porkka, S. Zipper, F. Jaramillo, D. Gerten, I. Fetzer, S. Cornell, L. Piemontese, and L. Gordon (2019), Illuminating water cycle modifications and Earth system resilience in the Anthropocene Water Resources Research, *Water Resources Research*, *56*, 1-24.

Gleeson, T., L. Wang-Erlandsson, S. C. Zipper, M. Porkka, F. Jaramillo, D. Gerten, I. Fetzer, S. E. Cornell, L. Piemontese, and L. J. Gordon (2020), The water planetary boundary: interrogation and revision, *One Earth*, *2*(3), 223-234.

Gleick, P. H. (1996), Water resources, in *Encyclopedia of climate and weather*, edited by S. H. Schneider, pp. 817-823, Oxford University Press, New York, USA.

Gozlan, R. E., J. Britton, I. Cowx, and G. Copp (2010), Current knowledge on non-native freshwater fish introductions, *Journal of fish biology*, *76*(4), 751-786.

Grill, G., B. Lehner, A. E. Lumsdon, G. K. MacDonald, C. Zarfl, and C. R. Liermann (2015), An index-based framework for assessing patterns and trends in river fragmentation and flow regulation by global dams at multiple scales, *Environmental Research Letters*, *10*(1), 015001.

Hanasaki, N., S. Yoshikawa, Y. Pokhrel, and S. Kanae (2018), A global hydrological simulation to specify the sources of water used by humans, *Hydrology and Earth System Sciences*, *22*(1), 789-817.

Herrera, G. A., T. Oberdorff, E. P. Anderson, S. Brosse, F. M. Carvajal-Vallejos, R. G. Frederico, M. Hidalgo, C. Jézéquel, M. Maldonado, and J. A. Maldonado-Ocampo (2020), The combined effects of climate change and river fragmentation on the distribution of Andean Amazon fishes, *Global Change Biology*, *26*(10), 5509-5523.

Holtgrieve, G., M. Arias, A. Ruhi, V. Elliott, S. Nam, P. B. Ngor, T. Räsänen, and J. Sabo (2018), Response to Comments on "Designing river flows to improve food security futures in the Lower Mekong Basin", *Science*, *361*(6398).

Horne, A. C., J. A. Webb, E. O'Donnell, A. H. Arthington, M. McClain, N. Bond, M. Acreman, B. Hart, M. J. Stewardson, and B. Richter (2017), Research priorities to improve future environmental water outcomes, *Frontiers in Environmental Science*, *5*, 89.

Huang, Z., M. Hejazi, X. Li, Q. Tang, C. Vernon, G. Leng, Y. Liu, P. Döll, S. Eisner, and D. Gerten (2018), Reconstruction of global gridded monthly sectoral water withdrawals for 1971–2010 and analysis of their spatiotemporal patterns, *Hydrology and Earth System Sciences*, *22*(4), 2117-2133.

IPCC (2014), *Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change*, Ipcc.

ISIMIP (2020), The Inter-Sectoral Impact Model Intercomparison Project [WWW Document], edited, ISIMIP.

IUCN, I. U. f. C. o. N., and C. f. I. E. S. I. N.-C. U. CIESIN (2015), Gridded Species Distribution: Global Amphibian Richness Grids, 2015 Release, edited, NASA Socioeconomic Data and Applications Center (SEDAC), Palisades, NY.

Kabat, P., M. Claussen, P. A. Dirmeyer, J. H. Gash, L. B. de Guenni, M. Meybeck, R. W. Hutjes, R. A. Pielke Sr, C. J. Vorosmarty, and S. Lütkemeier (2004), *Vegetation, water, humans and the climate: A new perspective on an internactive system*, Springer Science & Business Media.

Kendy, E., C. Apse, and K. Blann (2012), A practical guide to environmental flows for policy and planning, *Nat Conserv*.

Knouft, J. H., and D. L. Ficklin (2017), The potential impacts of climate change on biodiversity in flowing freshwater systems, *Annual Review of Ecology, Evolution, and Systematics*, *48*, 111-133.

Lehner, B. (2014), HydroBASINS Technical Documentation v1.c, World Wildlife Fund and McGill University, Washington DC.

Lehner, B., and G. Grill (2013), Global river hydrography and network routing: baseline data and new approaches to study the world's large river systems, *Hydrological Processes*, *27*(15), 2171-2186.

Leigh, C., and T. Datry (2017), Drying as a primary hydrological determinant of biodiversity in river systems: A broad-scale analysis, *Ecography*, *40*(4), 487-499.

Leprieur, F., O. Beauchard, S. Blanchet, T. Oberdorff, and S. Brosse (2008), Fish invasions in the world's river systems: when natural processes are blurred by human activities, *PLoS Biol*, *6*(2), e28.

Leprieur, F., S. Brosse, E. Garcia-Berthou, T. Oberdorff, J. Olden, and C. Townsend (2009), Scientific uncertainty and the assessment of risks posed by non-native freshwater fishes, *Fish and Fisheries*, *10*(1), 88-97.

Linnansaari, T., W. Monk, D. Baird, and R. Curry (2012), Review of approaches and methods to assess Environmental Flows across Canada and internationally, *DFO Can. Sci. Advis. Sec. Res. Doc*, *39*, 74.

Lundberg, J. G., M. Kottelat, G. R. Smith, M. L. Stiassny, and A. C. Gill (2000), So many fishes, so little time: an overview of recent ichthyological discovery in continental waters, *Annals of the Missouri Botanical Garden*, 26-62.

Martin, G., et al. (2011), The HadGEM2 family of met office unified model climate configurations, *Geoscientific Model Development*, *4*(3), 723-757.

Mathers, K. L., R. Stubbington, D. Leeming, C. Westwood, and J. England (2019), Structural and functional responses of macroinvertebrate assemblages to long-term flow variability at perennial and nonperennial sites, *Ecohydrology*, *12*(6), e2112.

Meyer, J. L., M. J. Sale, P. J. Mulholland, and N. L. Poff (1999), Impacts of climate change on aquatic ecosystem functioning and health 1, *JAWRA Journal of the American Water Resources Association*, *35*(6), 1373-1386.

Moyle, P. B., and R. A. Leidy (1992), Loss of biodiversity in aquatic ecosystems: evidence from fish faunas, in *Conservation biology*, edited, pp. 127-169, Springer.

Mueller Schmied, H., L. Adam, S. Eisner, G. Fink, M. Flörke, H. Kim, T. Oki, F. T. Portmann, R. Reinecke, and C. Riedel (2016), Variations of global and continental water balance components as impacted by climate forcing uncertainty and human water use, *Hydrology and Earth System Sciences*, *20*(7), 2877-2898.

NCEFSAB, N. C. E. F. S. A. B. (2013), Recommendations for Estimating Flows to Maintain Ecological Integrity in Streams and Rivers in North Carolina, North Carolina Department of Environment and Natural Resources.

Nilsson, C., C. A. Reidy, M. Dynesius, and C. Revenga (2005), Fragmentation and flow regulation of the world's large river systems, *Science*, *308*(5720), 405-408.

Nykvist, B., Å. Persson, F. Moberg, L. Persson, S. Cornell, and J. Rockström (2017), National environmental performance on planetary boundaries.

Nyström, P., C. BRÖNMARK, and W. Graneli (1996), Patterns in benthic food webs: a role for omnivorous crayfish?, *Freshwater biology*, *36*(3), 631-646.

Olson, D. M., and E. Dinerstein (2002), The Global 200: Priority ecoregions for global conservation, *Annals of the Missouri Botanical garden*, 199-224.

Pastor, A., F. Ludwig, H. Biemans, H. Hoff, and P. Kabat (2014), Accounting for environmental flow requirements in global water assessments, *Hydrology and Earth System Sciences*, *18*(12), 5041-5059.

Poff, L., B. D. Richter, A. H. Arthington, S. E. Bunn, R. J. Naiman, E. Kendy, M. Acreman, C. Apse, B. P. Bledsoe, and M. C. Freeman (2010), The ecological limits of hydrologic alteration (ELOHA): a new framework for developing regional environmental flow standards, *Freshwater biology*, *55*(1), 147-170.

Poff, N., M. M. Brinson, and J. Day (2002), Aquatic ecosystems and global climate change, *Pew Center on Global Climate Change, Arlington, VA*, *44*, 1-36.

Poff, N. L., and J. K. Zimmerman (2010), Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows, *Freshwater biology*, *55*(1), 194-205.

Poff, N. L., R. E. Tharme, and A. H. Arthington (2017), Evolution of environmental flows assessment science, principles, and methodologies, in *Water for the Environment*, edited, pp. 203-236, Elsevier.

Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr, K. L. Prestegaard, B. D. Richter, R. E. Sparks, and J. C. Stromberg (1997), The natural flow regime, *BioScience*, *47*(11), 769-784.

Powell, S., R. Letcher, and B. Croke (2008), Modelling floodplain inundation for environmental flows: Gwydir wetlands, Australia, *Ecological Modelling*, *211*(3-4), 350-362.

Rabiner, L., and B. Juang (1986), An introduction to hidden Markov models, *ieee assp magazine*, *3*(1), 4-16.

Reid, A. J., A. K. Carlson, I. F. Creed, E. J. Eliason, P. A. Gell, P. T. Johnson, K. A. Kidd, T. J. MacCormack, J. D. Olden, and S. J. Ormerod (2019), Emerg-

ing threats and persistent conservation challenges for freshwater biodiversity, *Biological Reviews*, *94*(3), 849-873.

Richter, B., J. Baumgartner, R. Wigington, and D. Braun (1997), How much water does a river need?, *Freshwater biology*, *37*(1), 231-249.

Richter, B. D., R. Mathews, D. L. Harrison, and R. Wigington (2003), Ecologically sustainable water management: managing river flows for ecological integrity, *Ecological applications*, *13*(1), 206-224.

Richter, B. D., M. Davis, C. Apse, and C. Konrad (2012), A presumptive standard for environmental flow protection, *River Research and Applications*, *28*(8), 1312-1321.

Rockström, J., W. Steffen, K. Noone, Å. Persson, F. S. Chapin III, E. Lambin, T. M. Lenton, M. Scheffer, C. Folke, and H. J. Schellnhuber (2009), Planetary boundaries: exploring the safe operating space for humanity, *Ecology and society*, *14*(2).

Sarremejane, R., J. England, C. E. Sefton, S. Parry, M. Eastman, and R. Stubbington (2020), Local and regional drivers influence how aquatic community diversity, resistance and resilience vary in response to drying, *Oikos*, *129*(12), 1877-1890.

Schaphoff, S., W. v. Bloh, A. Rammig, K. Thonicke, H. Biemans, M. Forkel, D. Gerten, J. Heinke, J. Jägermeyr, and J. Knauer (2018), LPJmL4–a dynamic global vegetation model with managed land–Part 1: Model description, *Geoscientific Model Development*, *11*(4), 1343-1375.

Shafroth, P. B., A. C. Wilcox, D. A. Lytle, J. T. Hickey, D. C. Andersen, V. B. Beauchamp, A. Hautzinger, L. E. McMULLEN, and A. Warner (2010), Ecosystem effects of environmental flows: modelling and experimental floods in a dryland river, *Freshwater Biology*, *55*(1), 68-85.

Shesterin, I. S. (2010), Water pollution and its impact on fish and aquatic invertebrates, *Lysemko C, Squires V, Verhey e WH, Interactions: Food, Agriculture And Environmen t UNESCO Publishing-Eolss Publishers, Oxford, UK*, 59-69.

Smakhtin, V., C. Revenga, and P. Döll (2004), A pilot global assessment of environmental water requirements and scarcity, *Water International*, *29*(3), 307-317.

Smith, V. H. (2003), Eutrophication of freshwater and coastal marine ecosystems a global problem, *Environmental Science and Pollution Research*, *10*(2), 126-139.

Steffen, W., K. Richardson, J. Rockström, S. E. Cornell, I. Fetzer, E. M. Bennett, R. Biggs, S. R. Carpenter, W. De Vries, and C. A. De Wit (2015), Planetary boundaries: Guiding human development on a changing planet, *Science*, *347*(6223).

Su, G., M. Logez, J. Xu, S. Tao, S. Villéger, and S. Brosse (2021), Human impacts on global freshwater fish biodiversity, *Science*, *371*(6531), 835-838.

Sutanudjaja, E. H., R. v. Beek, N. Wanders, Y. Wada, J. H. Bosmans, N. Drost, R. J. Ent, I. E. De Graaf, J. M. Hoch, and K. d. Jong (2018), PCR-GLOBWB 2: a 5 arcmin global hydrological and water resources model, *Geoscientific Model Development*, *11*(6), 2429-2453.

Tedesco, P. A., O. Beauchard, R. Bigorne, S. Blanchet, L. Buisson, L. Conti, J.-F. Cornu, M. S. Dias, G. Grenouillet, and B. Hugueny (2017), A global database on freshwater fish species occurrence in drainage basins, *Scientific data*, *4*(1), 1-6.

Tennant, D. L. (1976), Instream flow regimens for fish, wildlife, recreation and related environmental resources, *Fisheries*, *1*(4), 6-10.

Tessmann, S. A. (1979), *Environmental Use Sector: Reconnaissance Elements of the Western Dakotas Region of South Dakota Study*, Water Resources Institute, South Dakota State University.

Thompson, R. M., and P. S. Lake (2010), Reconciling theory and practise: the role of stream ecology, *River research and applications*, *26*(1), 5-14.

Tickner, D., J. J. Opperman, R. Abell, M. Acreman, A. H. Arthington, S. E. Bunn, S. J. Cooke, J. Dalton, W. Darwall, and G. Edwards (2020), Bending the curve of global freshwater biodiversity loss: an emergency recovery plan, *BioScience*, *70*(4), 330-342.

Tonkin, J. D., J. D. Olden, D. M. Merritt, L. V. Reynolds, J. S. Rogosch, and D. A. Lytle (2020), Designing flow regimes to support entire river ecosystems, *Frontiers in Ecology and the Environment*.

Tyson, P. D., R. Fuchs, C. Fu, L. Lebel, H. Vloemans, E. Odada, J. Perry, W. Steffen, and H. Virji (2002), *Global-regional linkages in the earth system*, Springer Science & Business Media.

Villéger, S., S. Blanchet, O. Beauchard, T. Oberdorff, and S. Brosse (2011), Homogenization patterns of the world's freshwater fish faunas, *Proceedings of the National Academy of Sciences*, *108*(44), 18003-18008.

Virkki, V., et al. (2021), Environmental flow envelopes: quantifying global, ecosystem–threatening streamflow alterations, *Hydrol. Earth Syst. Sci. Discuss.*, *2021*, 1-31.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo (1997), Human domination of Earth's ecosystems, *Science*, *277*(5325), 494-499.

Vitule, J. R. S., C. A. Freire, and D. Simberloff (2009), Introduction of non-native freshwater fish can certainly be bad, *Fish and Fisheries*, *10*(1), 98-108.

Vörösmarty, C. J., R. Wasson, and J. Richey (1997), Modelling the transport and transformation of terrestrial materials to freshwater and coastal ecosystemsworkshop report, International Geosphere Biosphere Programme [Stockholm].

Vörösmarty, C. J., P. Green, J. Salisbury, and R. B. Lammers (2000), Global water resources: vulnerability from climate change and population growth, *science*, *289*(5477), 284-288.

Vörösmarty, C. J., P. B. McIntyre, M. O. Gessner, D. Dudgeon, A. Prusevich, P. Green, S. Glidden, S. E. Bunn, C. A. Sullivan, and C. R. Liermann (2010), Global threats to human water security and river biodiversity, *nature*, *467*(7315), 555-561.

WANI (2008), Water and Nature Initiative (WANI) Project, edited, IUCN

Watanabe, M., T. Suzuki, R. O'ishi, Y. Komuro, S. Watanabe, S. Emori, T. Takemura, M. Chikira, T. Ogura, and M. Sekiguchi (2010), Improved climate simulation by MIROC5: Mean states, variability, and climate sensitivity, *Journal of Climate*, *23*(23), 6312-6335.

Wilting, H. C., A. M. Schipper, M. Bakkenes, J. R. Meijer, and M. A. Huijbregts (2017), Quantifying biodiversity losses due to human consumption: a global-scale footprint analysis, *Environmental science & technology*, *51*(6), 3298-3306.

Xenopoulos, M. A., D. M. Lodge, J. Alcamo, M. Märker, K. Schulze, and D. P. Van Vuuren (2005), Scenarios of freshwater fish extinctions from climate change and water withdrawal, *Global change biology*, *11*(10), 1557-1564.

Yoshikawa, S., A. Yanagawa, Y. Iwasaki, P. Sui, S. Koirala, K. Hirano, A. Khajuria, R. Mahendran, Y. Hirabayashi, and C. Yoshimura (2014), Illustrating a new global-scale approach to estimating potential reduction in fish species richness due to flow alteration, *Hydrology and Earth System Sciences*, *18*(2), 621-630.

Zeiringer, B., C. Seliger, F. Greimel, and S. Schmutz (2018), River hydrology, flow alteration, and environmental flow, in *Riverine ecosystem management*, edited, pp. 67-89, Springer, Cham.

Zucchini, W., I. L. MacDonald, and R. Langrock (2017), *Hidden Markov models for time series: an introduction using R*, CRC press.