

1 Poor correlation between large-scale environmental flow 2 violations and freshwater biodiversity: implications for water 3 resource management and water planetary boundary

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21 Key Research Points

- 22 ● No significant relationship between environmental flow (EF) violation and freshwater
23 biodiversity indicators was found at global or ecoregion scales using globally consistent
24 methods and currently available data.
- 25 ● Several basins show a slight positive correlation between EF violation and biodiversity
26 indicators, which could be attributed to the artificial introduction of non-native species.
- 27 ● A generalized approach that incorporates EF considerations but ignores the lack of a
28 significant EF-biodiversity relationship at large scales can underestimate the stress on the
29 ecosystem at smaller scales which correspond with eco-hydrological processes that
30 determine ecological impacts from EF violation.

- 31 • Use of a globally aggregated blue water planetary boundary using biodiversity-based
32 response variables is deceptive

33 **Abstract**

34 The freshwater ecosystems around the world are degrading, such that maintaining
35 environmental flow (EF) in river networks is critical to their preservation. The relationship
36 between streamflow alterations and, respectively, EF violations, and freshwater biodiversity is
37 well established at the scale of stream reaches or small basins ($\sim < 100 \text{ km}^2$). However, it is unclear
38 if this relationship is robust at larger scales even though there are large-scale initiatives to legalize
39 the EF requirement. Moreover, EFs have been used in assessing a planetary boundary for
40 freshwater. Therefore, this study intends to carry out an exploratory evaluation of the
41 relationship between EF violation and freshwater biodiversity at globally aggregated scales and
42 for freshwater ecoregions. Four EF violation indices (severity, frequency, probability to shift to
43 violated state, and probability to stay violated) and seven independent freshwater biodiversity
44 indicators (calculated from observed biota data) were used for correlation analysis. No
45 statistically significant negative relationship between EF violation and freshwater biodiversity
46 was found at global or ecoregion scales. While our results thus suggest that streamflow and EF
47 may not be an only determinant of freshwater biodiversity at large scales, they do not preclude
48 the existence of relationships at smaller scales or with more holistic EF methods (e.g., including
49 water temperature, water quality, intermittency, connectivity etc.) or with other biodiversity
50 data or metrics.

51

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

54 **1. Introduction**

55 Water resources are inarguably one of the most important natural resources in the Earth system
56 for sustaining life. Nevertheless, these resources and their associated ecosystems are threatened
57 by human actions (Bélanger and Pilling, 2019; Clausen and York, 2008; Vörösmarty et al., 2010;

58 Wilting et al., 2017). Global freshwater covers up to 0.8% of the total Earth's surface (Gleick,
59 1996) and inhabits 6% of all the known species in the world including 40% of total fish diversity
60 and nearly one third of all vertebrates (Lundberg et al., 2000). Since freshwater ecosystems have
61 high species richness in a relatively small area and are exposed to a high level of pressure, they
62 are more vulnerable to environmental change and human actions than any other ecosystems
63 (Dudgeon et al., 2006). The rapid increase in the demand for natural resources is the fundamental
64 cause for freshwater ecosystem degradation (Darwall et al., 2018). Anthropogenic climate
65 change (Allan and Flecker, 1993; Darwall and Freyhof, 2016; Knouft and Ficklin, 2017; Meyer et
66 al., 1999), overexploitation (Allan et al., 2005), water pollution (Albert et al., 2021; Dudgeon et
67 al., 2006; Reid et al., 2019; Smith, 2003), flow alteration (Nilsson et al., 2005; Vorosmarty et al.,
68 2000), habitat destruction (Dudgeon, 2001) and introduction of alien species (Gozlan et al., 2010;
69 Vitule et al., 2009) are some of the manifestations of this increased demand which directly
70 threatens the freshwater ecosystems. In addition, increased water impoundment in large dams
71 and reservoirs has also led to an array of adversities to freshwater ecosystems ranging from
72 habitat destruction to irregular flow alterations (Bergkamp et al., 2000). This situation is
73 aggravated by increasing pressure on related Earth system functions, such as climate change and
74 nutrient cycles, which are articulated by their respective transgressions in the planetary
75 boundaries framework (Box 1) (Dudgeon, 2010). Freshwater ecosystem processes that were
76 previously governed by natural Earth system facets such as temperature, rainfall, and relief are
77 now increasingly driven by demographic, social, and economic drivers (Clausen and York, 2008;
78 Kabat et al., 2004; Tyson et al., 2002; Vitousek et al., 1997; Vörösmarty et al., 1997). Freshwater
79 ecosystem health comprises both biotic factors like biodiversity and abiotic factors like habitat
80 integrity. As any disruption in the abiotic factors is most likely to be reflected in the biotic status
81 of the freshwater ecosystem, the scope of this paper is confined to the biotic dimension of the
82 freshwater ecosystem (i.e., biodiversity) and not the health of the entire ecosystem.

83

84 There has been an increased recognition in recent decades for the need of maintaining a natural
85 flow regime in streams to sustain healthy ecosystems. (Horne et al., 2017; Poff et al., 1997, 2017;
86 Tickner et al., 2020; Tonkin et al., 2021). Despite the indispensable role of aquatic biodiversity in

87 maintaining the quality of the system (Darwall et al., 2018), inclusion of such environmental flow
88 (EF) in water management is often controversial, particularly in regions where freshwater
89 availability is limited and is already a matter of severe competition. These competitions have led
90 to an increasing trend in EF violation (insufficient streamflow than the recommended EF
91 requirement; see section 2.1 for more details) in the past decade both in terms of severity and
92 frequency (Virkki et al., 2022). This wakeup call has led to several international and national
93 efforts to legalize EF requirements through large-scale EF management schemes (Arthington and
94 Pusey, 2003; Richter et al., 1997, 2003). The Water and Nature Initiative (Smith and Cartin, 2011),
95 the Brisbane declaration (Declaration, 2007), and the Global Action Agenda (Arthington et al.,
96 2018) are some of these efforts. Nevertheless, there is a large gap in our understanding of the
97 relationship between EF requirements and biodiversity responses at various spatial and temporal
98 scales. Except for a few (Domisch et al., 2017; Xenopoulos et al., 2005; Yoshikawa et al., 2014),
99 the majority of the studies exploring this relation were conducted at smaller scales (Anderson et
100 al., 2006; Arthington and Pusey, 2003; Powell et al., 2008). Thus, there is a significant discrepancy
101 in the scale at which these processes are understood versus the scale at which the policies are
102 set (Thompson and Lake, 2010). Current knowledge of how the small-scale processes scale up
103 (e.g., validation of large-scale EF hydrologic methods using local data) to a regional or global scale
104 is thus limited, potentially undermining the scientific integrity of existing large-scale EF
105 management schemes.

106

107 In order to scientifically underpin large scale EF policies, the existing assumption of the inverse
108 relationship between freshwater biodiversity response and EF violation must be tested at
109 regional and global scales (see Supplementary information S1 for more details). Therefore, in this
110 study, we evaluate the relationship between EF violation and freshwater biodiversity at two
111 different spatial scales (freshwater ecoregion, global) using four EF violation indices (frequency,
112 severity, probability to move to a violated state, and probability to stay violated) and seven
113 freshwater biodiversity indicators describing taxonomic, functional, and phylogenetic
114 dimensions of the biodiversity. The paper is not intended to be a definitive test on the
115 relationship between EF violation and aquatic biodiversity. It is rather intended to be an

116 exploratory analysis of the idea of conducting more detailed evaluations of the EF-biodiversity
117 relationship before formulating large scale EF management policies. The implications of the
118 findings for large-scale water management and the use of the relationship between
119 environmental flows and freshwater biodiversity (hereafter referred to as EF-biodiversity
120 relationship) in the planetary boundary framework (box 1) are also discussed.
121

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 biogeophysical planetary scale boundaries for Earth system processes that, if violated, can irretrievably impair the Holocene-like stability of Earth system. The framework establishes scientifically determined safe operating limits for human perturbations through control and response variable relationships, 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., 2020a, b).

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. (2020b) 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.

122 **2.Methodology and Data**

123 The study is carried out at two spatially aggregated scales; 1) global and 2) ecoregion, for a
124 historic time period of 30 years (1976 - 2005). All the underlying calculations were done at level
125 5 HydroBASIN (median basin area = 19,600 km²) (Lehner and Grill, 2013) and were aggregated to
126 the corresponding spatial scale for further analysis. Level 5 HydroBASIN (also referred to as basin
127 in this paper) was selected as the smallest spatial unit as it is the highest level of specificity that
128 can be rasterized into a 0.5-degree resolution grid without significantly reducing the number of
129 sub-basins smaller than a grid cell (Virkki et al., 2022). The EF violation indices were calculated
130 using Virkki et al. (2022)'s novel Environmental Flow Envelope (EFE) framework, and biodiversity
131 was represented by a combination of relative and absolute value indices.

132

133 **2.1 Data**

134 *2.1.1 Streamflow data*

135 Streamflow data used in the EFE (see section 2.2 for more details) definition were obtained from
136 the Inter-Sectoral Impact Model Intercomparison Project (ISIMIP) simulation phase 2b outputs
137 of global daily discharge (available at <https://esg.pik-potsdam.de>) (Warszawski et al., 2014).
138 Monthly streamflow data (averaged from the daily simulations) for two time periods were used
139 in this study; 1) for the pre-industrial era (1800 - 1860), which is considered as the unaltered
140 reference period (Poff et al., 1997) and 2) for the recent time period (1976 - 2005). These monthly
141 streamflow datasets were used to calculate EF violations. For calculating the EF violation indices,
142 the estimated EFEs for each basin were obtained from Virkki et al. (2022). A total of 4 Global
143 Hydrological Models (GHM) (H08 (Hanasaki et al., 2018), LPJmL (Schaphoff et al., 2018), PCR-
144 GLOBWB (Sutanudjaja et al., 2018), WaterGAP2 (Müller Schmied et al., 2016)) were used to
145 obtain the monthly streamflow data. Each GHM was forced with four different Global Circulation
146 Models (GCM) outputs (GFDL-ESM2M (Dunne et al., 2012), HadGEM2-ES (Collins et al., 2011;
147 Bellouin et al., 2011), IPSL-CM5A-LR (Dufresne et al., 2013), MICROC5 (Watanabe et al., 2010)).
148 All the GHM outputs used in this study are extensively validated and evaluated in several previous
149 studies (e.g. (Zaherpour et al., 2018; Gädeke et al., 2020). Moreover, as part of the ISIMIP impact

150 model intercomparison activity, all the GCM climate input data were bias corrected using
151 compiled reference datasets covering the entire globe at 0.5 deg resolution (Frieler et al., 2017).
152 Additionally the GHM outputs are also validated using historical data to better fit reality (Frieler
153 et al., 2017). Therefore, no additional validation of the data is done in this study.

154
155 The streamflow data were aggregated to the sub-basin scale according to level 5 HydroBASIN
156 Version 1.0 (<https://www.hydrosheds.org/page/hydrobasins>) (Lehner and Grill, 2013). The data
157 from ISIMIP 2b is representative of historical land use and other human influences including dams
158 and reservoirs (Frieler et al., 2017). The maximum discharge cell value within the boundaries of
159 each level 5 HydroBASIN is chosen to represent the outlet discharge value. Any violations within
160 the outlet cell are regarded as indicative of the entire basin, even if conditions can differ in various
161 areas within the level 5 HydroBASIN. As the spatial resolution of the study is level 5 HydroBASIN
162 to allow a global analysis, we accept a certain homogenization of the local scale characteristics.
163 See supplementary materials (see Supplementary information S.2) for more details on the
164 datasets used in this study.

165

166 *2.1.2 Freshwater biodiversity data*

167 In addition to the streamflow data, data on fish diversity were also used in this study (Table 1).
168 Freshwater biodiversity was evaluated using seven indices estimated from the observed biota
169 data. The biodiversity indicators were obtained from international agencies or the literature. The
170 biodiversity indicators consisted of six indices of relative change in biodiversity and one index of
171 absolute values of biodiversity.

172 *a) Absolute biodiversity indicator*

173 The absolute biodiversity indicator consisted of freshwater fish richness (FiR). The fish richness
174 data was compiled and processed from 1436 published papers, books, grey literature and web-
175 based sources published between 1960 and 2014 (Tedesco et al., 2017). They cover 3119 basins
176 all over the world and account for 14953 fish species permanently or occasionally inhabiting
177 freshwater systems.

178 *b) Relative biodiversity indicators*

179 The Relative biodiversity indicators consisted of six freshwater fish facets. Six key facets of
 180 freshwater fish - taxonomic, functional, and phylogenetic diversity (TR, FR, PR respectively), as
 181 well as dissimilarity of each of the three groups (TD, FD, PD respectively)- were used in this
 182 analysis to construct a holistic picture of the state of aquatic biodiversity (Su et al., 2021). Each
 183 facet indicates the change in the corresponding biodiversity component compared to the 18th
 184 century (roughly pre-industrial era). The taxonomic facets measure the occurrence of fish in a
 185 riverine system. Functional facets are calculated using the morphological characteristics of each
 186 species that are linked to feeding and locomotive functions which in turn relates to larger
 187 ecosystem functions like food web control and nutrition transport. Phylogenetic facets measure
 188 the total length of branches linking all species from the assemblage on the phylogenetic tree. The
 189 richness component of the three categories calculates the diversity among the assemblage
 190 whereas the dissimilarity accounts for the difference between each pair of fish assemblage in one
 191 biogeographical realm. All six fish facets were calculated for the 2465 river basins covering over
 192 10682 fish species all over the world. All six facets are available as a single delta change in time
 193 and do not cover multiple timesteps.

194

195 Table 1. Details of different data used in this study

Data	Spatial resolution (extent)	Temporal resolution (extent)	Source/Reference
Aquatic fish richness data	30 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 (change compared to preindustrial era)	Derived from observed data Su et al. (2021)
EFE	Aggregated to Level 5 HydroBASIN	Monthly (Pre-industrial: 1801-1860)	Model calculated

	(global)		Virkki et al. (2022)
Streamflow	Aggregated to Level 5 HydroBASIN (global)	Monthly (Pre-industrial: 1801-1860, Current: 1976-2005)	Model calculated Warszawski et al. (2014)
Basin boundaries	Level 5 HydroBASIN (global)	Not applicable	Lehner and Grill (2013)

196

197 **2.2 Environmental flow violation estimation**

198 The EFE framework proposed by Virkki et al. (2022) is used to evaluate EF violations in this study.
 199 The EFE framework establishes an envelope of variability constrained by discharge limits beyond
 200 which flow in the streams may not meet the freshwater biodiversity needs (Virkki et al., 2022).
 201 EFE uses pre-industrial (1801-1860) stream discharge to establish an upper and lower boundary
 202 for EF deviations at monthly time steps. This EFE is used to define the EF violation at Level 5
 203 HydroBASIN scale. The EF violations were calculated as median ensemble of four Global
 204 Hydrological Models (GHM) (H08, LPJmL, PCR-GLOBWB, WaterGAP2) and mean ensemble of four
 205 Global Circulation Models (GCM) (GFDL-ESM2M, HadGEM2-ES, IPSL-CM5A-LR, MICROC5).
 206 Moreover, five different EF calculation methods (Smakhtin (Smakhtin et al., 2004), Tennant
 207 (Tennant, 1976), Q90-Q50 (Pastor et al., 2014), Tessmann (Tessmann, 1979) and Variable
 208 Monthly Flow (Pastor et al., 2014)) were also used in the EFE derivation (see Supplementary
 209 Information, Table S3 for more information on EF methods) (Virkki et al., 2022). This approach
 210 addresses the uncertainty related to the outputs of models and may eliminate the largest model-
 211 related extremes that might cause results to be distorted (Virkki et al., 2022). In spite of the
 212 uncertainty in hydrological estimates generated by different models, a simple ensemble matrix
 213 often produces acceptable discharge and therefore also EF estimates at larger scales because the
 214 bias of the individual models is removed (Zaherpour et al., 2018). Moreover, all the basins with
 215 Mean Annual Flow (MAF) < 10 m³/s were excluded due to high uncertainty in EFE and streamflow
 216 estimates (Gleeson et al., 2020a; Steffen et al., 2015; Virkki et al., 2022). After this exclusion, a
 217 total of 3906 basins were considered for further analysis.

218

219 Here we evaluate the EF violation by defining four different EF violation indices: 1) violation
220 severity (S), violation frequency (F), probability to shift to a violated state (P.shift) and probability
221 to stay violated (P.stay). Out of the four EF violation indicators, two (S and F) were a modification
222 from Virkki et al. (2022) and the two (P.shift and P.stay) were calculated based on the current
223 EFE deviations from Virkki et al. (2022). P.shift and P.stay measures the likelihood of a given year
224 to shift or stay in a violated state. The state of a basin (violated or non-violated) was identified at
225 an annual time step and the mean probability to shift or remain in that state is calculated.

226

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

228

229 1) Violation severity (S): The annual violation severity was calculated as the absolute mean
230 of the magnitude of EF deviation from the EFE lower or upper bound in all the violated
231 months. The normalized value of S is used in this study.

232 2) Violation frequency (F): Frequency of violation is a measure of the proportion of months
233 a basin has violated the EFE lower or upper bound in a year. Frequency is calculated as
234 the percentage of violated months per year. The normalized value of F is used in this
235 study.

236 3) Probability to shift to a violated state (P.shift): The P.shift is defined in this paper as the
237 probability of a basin to shift to a violated state from a non-violated state (Eq. 1). This
238 indicator along with P.stay gives a measure of the stability of violation in each level 5
239 HydroBASIN. The violated/non-violated state of a basin is calculated annually based on
240 the violations in the low flow months. If a basin violates EFE lower or upper bound for at
241 least three consecutive months during the low flow period ($Q < 0.4MAF$) in a year, then
242 the basin is considered to be in a violated state.

243
$$P.shift = \frac{\text{number of years shifted to violated state (i.e. year } i \text{ is violated and year } i-1 \text{ is not)}}{\text{total number of years}} \quad (1)$$

244

245 4) Probability to stay violated (P.stay): Once shifted to a violated state, the tendency of a
246 basin to remain in that state or switch to a non-violated state is determined by this
247 indicator. If a basin has a higher P.stay (closer to 1) then the basin continues to remain in
248 the violated state for a longer time before switching to a non-violated state (Eq 2).
249 Whereas, the basins with lower P.stay (closer to 0) tend to remain in the violated state
250 only for a brief period of time. In other words, the number of consecutive violated years
251 is much lower for basins with lower P.stay value.

$$252 \quad P.stay = \frac{\text{number of violated years with at least one consecutive year violated}}{\text{total number of violated years}} \quad (2)$$

253

254 **2.3. Relationship between environmental flow violations and freshwater biodiversity**

255 The relationship between freshwater biodiversity and EF violation was evaluated by aggregating
256 the level 5 HydroBASIN scale values to global level, WWF's Freshwater ecoregions major habitat
257 type scale (results given in SI) (Abell et al., 2008) and G200 freshwater ecoregion level (Olson and
258 Dinerstein, 2002). The G200 freshwater ecoregion is a subset of WWF's freshwater ecoregion
259 that includes only the biodiversity hotspots. Seven freshwater ecoregions in ecologically
260 important regions were studied, and the EF-biodiversity relationship was evaluated separately
261 for each ecoregion type. Aggregating to major ecoregion types accounts for some data's
262 natural/spatial variability, in addition to using an analysis of global data.

263

264 One of the major challenges in conducting an aggregated evaluation was the discrepancy in the
265 spatial resolution at which the EF violation indices and various biodiversity indicators and the loss
266 of heterogeneity. Aggregation of any scale will lead to some level of homogenization of the data.
267 A reach-by-reach evaluation will be an ideal solution to capture all the heterogeneity. However,
268 this is not very practical for a global study due to data and computational limitations. Therefore,
269 to partially address this challenge, two different aggregation/data matching methods were
270 employed; case-1) matching level 5 HydroBASIN data (EF violation indices) to biodiversity data
271 and case-2) matching biodiversity data to level 5 HydroBASIN (See supplementary information
272 (SI); Section S5). In the first case every level 5 HydroBASIN (EF violation indices) is matched with

273 the biodiversity data point nearest centroid. Whereas in the second case there can be three
274 different scenarios (See SI; Fig. S4): 1) biodiversity basin is smaller than level 5 HydroBASIN; in
275 that case all the biodiversity basins within one level 5 HydroBASIN were matched with the same
276 EF violation value, 2) when biodiversity basin is equal in size to level 5 HydroBASIN; in this case
277 biodiversity basins and level 5 HydroBASIN had a one-to-one match, 3) biodiversity basin is larger
278 than level 5 HydroBASIN. In the last case, two methods were used for data mapping 1) Outlet
279 matching: where each biodiversity basin is mapped with EF violation value from the level 5
280 HydroBASIN closest to the outlet and 2) Mean matching: each biodiversity basin is mapped with
281 the mean EF violation values of all level 5 HydroBASIN within it. Data matching methods were
282 employed to partially understand the uncertainty due to scale discrepancy between datasets. As
283 the results are insensitive to the aggregation method, only the results using case 1 (matching
284 level 5 HydroBASIN data to biodiversity data) are discussed in this paper.

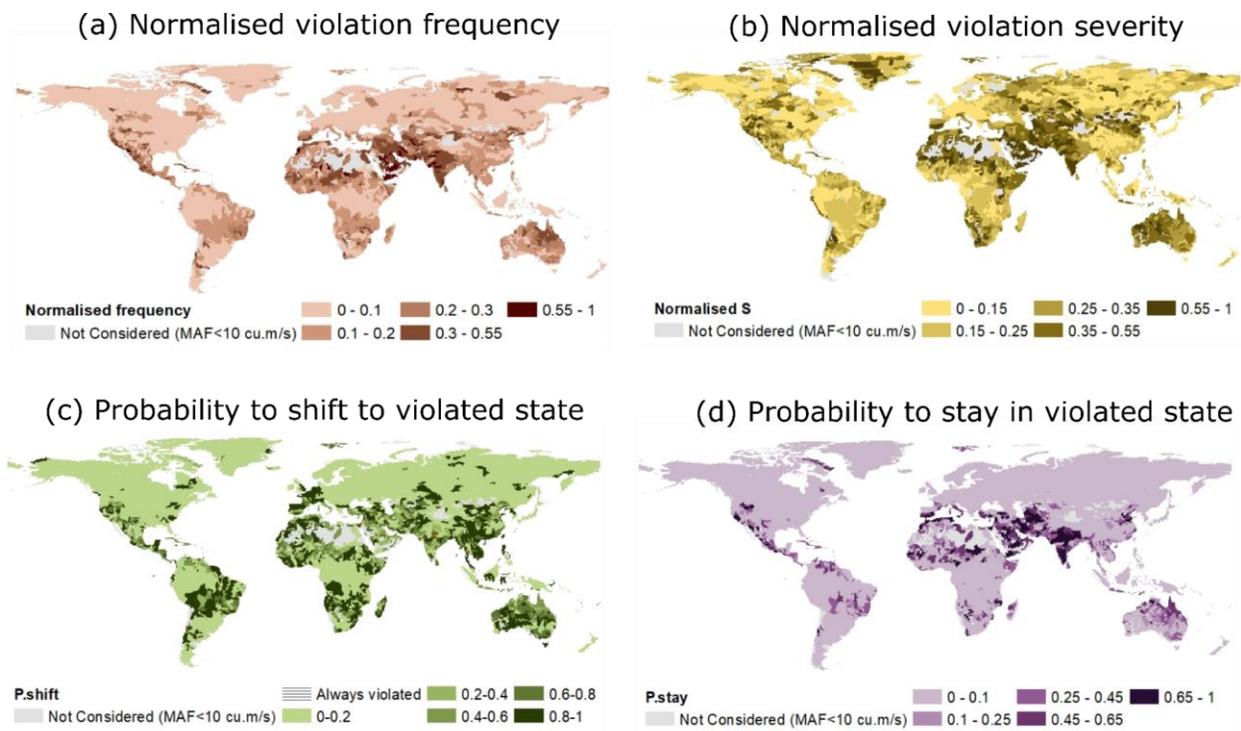
285 **3.Results and Interpretations**

286 **3.1 Evaluating EF violation drivers and characteristics**

287 The majority of basins face some kind of EF violation (either in terms of severity or frequency or
288 with higher probabilities to shift and/or stay violated) (Fig. 1). Between 1976 and 2005, 17% and
289 45% of basins, respectively, experienced violation frequency (F) greater than 3 months/year and
290 severity (S) greater than 20% from the EFE lower or upper bound (normalized violation index \geq
291 0.25) (Fig.2 a,b). Additionally, 33% of basins have a higher chance of shifting (P.shift \geq 0.5; i.e.
292 33% basins have over 50% probability to shift to a violated state) to a violated state (Fig.2 c,d).
293 EF violations are very frequent and severe in mostly arid/semi-arid regions such as the Middle
294 East, Iran, Iraq, Pakistan, India, Australia, Sahara, Sub-Saharan Africa, Southern Africa, and the
295 southernmost part of North America. On the other hand, regions with higher probability to shift
296 to a violated state (P.shift) were not limited to the low precipitation and low streamflow regions.
297
298 Although the majority of regions with high P.shift values were arid or semi-arid, some exceptions
299 included South Eastern Asia and Central South America. The non-arid regions with higher P.shift
300 also have extremely high water withdrawal in all sectors (agriculture, domestic and industry).

301 This spatial concurrence suggests that human activities, as well as hydroclimatic influences, play
 302 a significant role in deciding a region's P.shift. However, once in the violated state, the flow
 303 variability regimes in the catchment determine the probability of remaining (P.stay) in the
 304 violated state. Catchments with highly variable flow regimes (i.e., receive most of the annual flow
 305 as floods; see SI for classification map; Fig. S2) have higher probability to stay violated once
 306 shifted whereas catchments with stable flow regimes (year-round steady high baseflow) have a
 307 higher tendency to revert back to a non-violated state. An example of this behavior can be seen
 308 in the Australian basins. Though, almost all the Australian basins have a very high P.shift, only the
 309 highly variable flow regime northern catchments had a higher probability to stay violated.
 310 Despite having a very high P.shift, the southern stable catchments swiftly shift back to a non-
 311 violated state.

312



313

314 Fig. 1 Four measures of Environmental Flow Envelope (EFE) lower or upper bound violation
 315 estimated using ensemble median of four Global hydrological models; a) Normalized frequency
 316 of violation, b) Normalized severity of violation, c) Probability to shift to a violated state from a
 317 non-violated state and d) Probability to stay violated once shifted to a violated state.

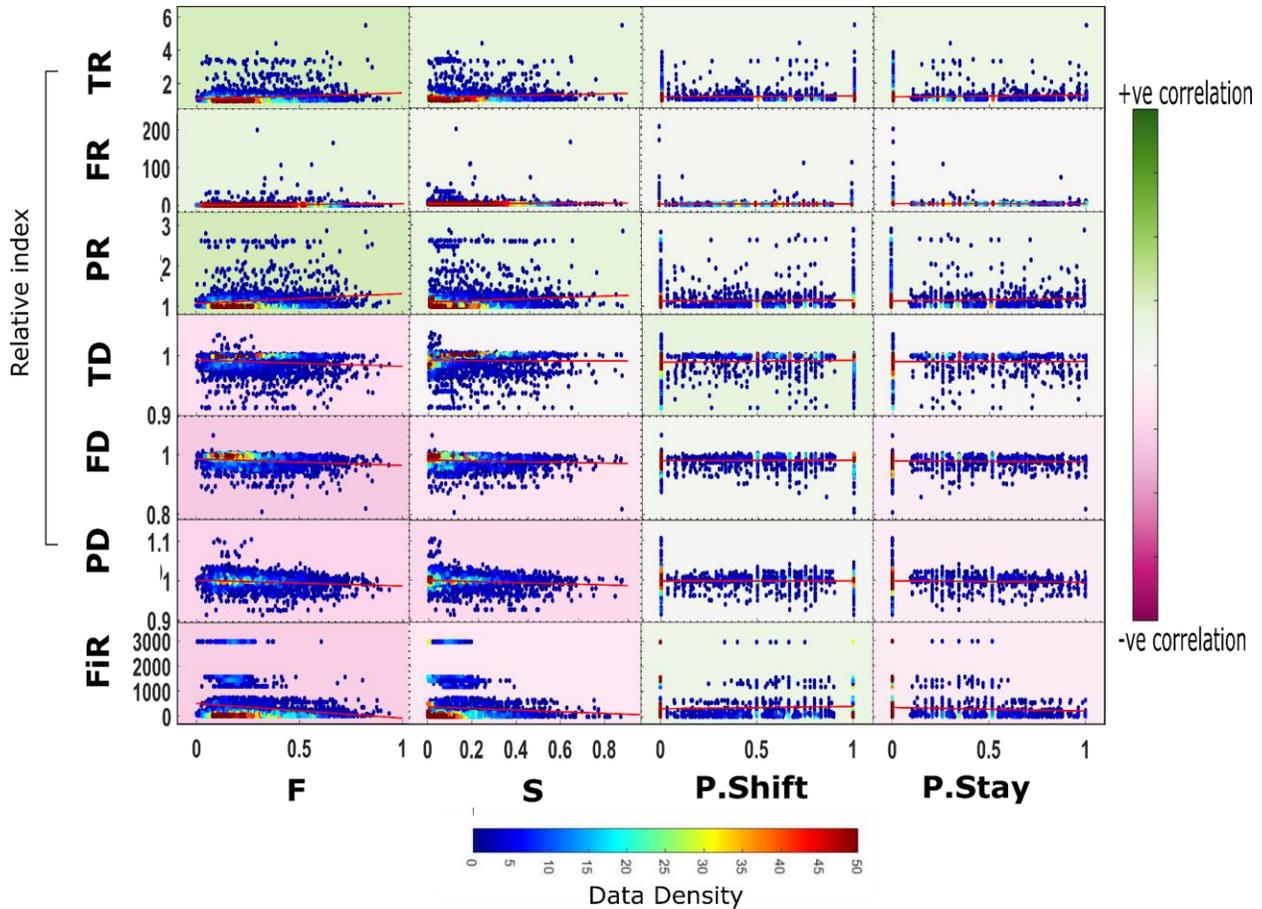
318 **3.2 Relationship between EF violation and freshwater biodiversity**

319 The aggregated analysis was carried out at global and ecoregion scales. Multiple aggregation
320 methods (section 2.3) yielded similar results, therefore only the case 1 (level 5 HydroBASIN
321 matched with biodiversity data) results are discussed further (see supplementary material Fig. S5
322 and S6 for results using other aggregation methods). At the global scale, none of the biodiversity
323 indicators correlated (significance of p value <0.05) with any EF violation indices (Fig. 2). The
324 biodiversity indicators were not exhibiting any strong trend in either positive or negative
325 direction. The correlation coefficient value (R value) for the remaining biodiversity indicators
326 ranges only from -0.2 to 0.17 (Fig. 2 b). The three fish dissimilarity facets (TD, FD and PD) show
327 slight negative trend whereas the richness facets (TR, FR, and PR) display a slight positive
328 correlation with EF violation. The positive correlation of the richness indicators is attributed to
329 an overall increase in the assemblage in the majority of the basins despite the increase in EF
330 violation. Moreover, (relative) TR and (absolute) FiR were showing opposite trends. The positive
331 trend in TR could be attributed to changes involving non native species, whereas the FiR describes
332 the current deteriorated state. The increase in the fish assemblage over time was verified using
333 an independent dataset RivFishTIME (see SI; Fig. S8) (Comte et al., 2021). The increase in the fish
334 richness facets primarily stems from the introduction of alien species introduced into streams for
335 commercial purposes (Su et al., 2021). The invasion of alien species can tamper with the existing
336 natural ecosystem equilibrium resulting in further degradation of the overall ecosystem health.

337
338 Correlations between EF and biodiversity are generally weak at the scale of G200 freshwater
339 ecoregions as well (see Section 2.2, (Olson and Dinerstein, 2002)). In G200 freshwater ecoregions
340 (see SI; Table S5 for full freshwater ecoregion results) the nature of the EF-biodiversity
341 relationships was highly varying between different ecoregions (Fig 3). In large lakes, large rivers
342 and small lakes, Su et al. (2021) fish richness facets were showing a strong and significant positive
343 correlation with most of the EF violation indices. Whereas, in large rivers, large river deltas and
344 xeric basins, the dissimilarity indices, FiR show negative trends. However, in the majority of
345 ecoregions, the EF-biodiversity relationship is insignificant (p value >0.05). These results

346 corroborate the above findings that EF violations are not significantly inversely correlated with
 347 biodiversity, regardless of ecoregions with the current dataset.

348



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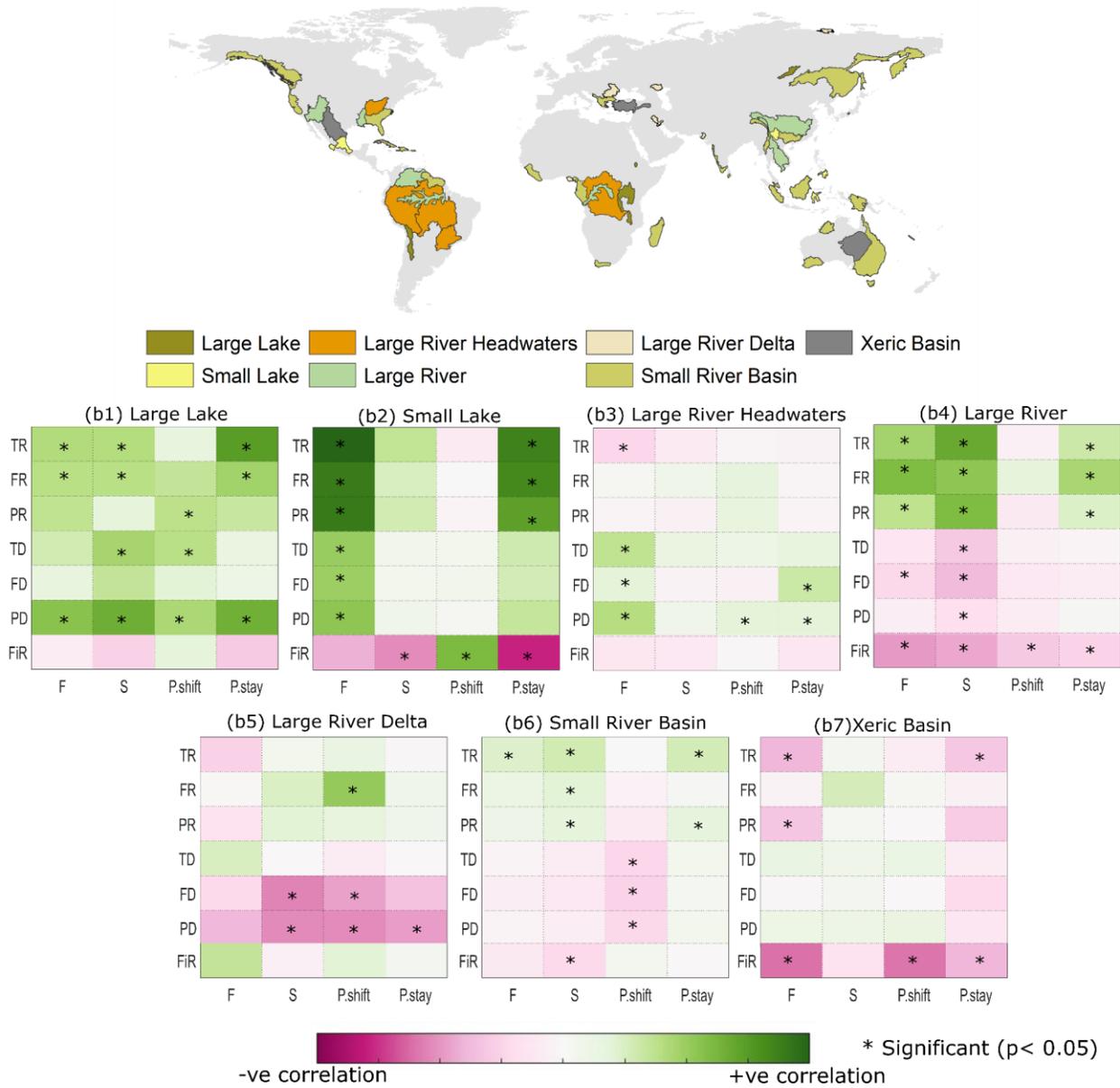
350 Fig. 2 Scatter between EF violation indices and biodiversity indices with linear fit and
 351 corresponding R value at globally aggregated scale.

352 Note: This figure represents results from case 1 (level 5 HydroBASIN matched with biodiversity data). The results of
 353 other aggregation methods are given in SI (Fig. S4 and S5).

354 Abbreviations: FiR-Fish richness; TR-Taxonomic richness; FR-Functional richness; PR-Phylogenetic richness; TD-
 355 Taxonomic dissimilarity; FD-Functional dissimilarity; PD-Phylogenetic dissimilarity

356

(a) Freshwater Ecoregion - G200



357

358 Fig.3 (a) Spatial distribution of different G200 freshwater ecoregions and (b1-b7) the correlation

359 between EF violation indices and freshwater biodiversity indicators for different G200

360 freshwater ecoregions.

361 Note: The results for all the WWF freshwater ecoregions are given in SI (SI section S.7).

362 **4.Discussion**

363 The findings from this study indicate that the EF-biodiversity relationship is poorly correlated at
364 global or ecoregion scales with currently available data and methods. The most likely explanation
365 for the lack of correlation is the overwhelming heterogeneity of the freshwater ecosystems - e.g.
366 with some freshwater species being more susceptible to variations in flow than others (Poff and
367 Zimmerman, 2010) - which is not adequately represented in the used spatial resolution (level 5
368 Hydrobasin). Moreover, when it comes to a larger-scale relationship, several other factors like
369 climate change (Davies, 2010; Poff et al., 2002), river fragmentation (Grill et al., 2015; Herrera-R
370 et al., 2020), large-scale habitat degradation (Moyle and Leidy, 1992), landscaping/river scaping
371 (Allan et al., 2005), alien species (Leprieur et al., 2008, 2009; Villéger et al., 2011) and water
372 pollution (Brooks et al., 2016; Shesterin, 2010) can also impact the freshwater ecosystem in
373 multiple ways. Thus, at Earth system level, other interlinked factors potentially confound the
374 impact of EF violation on biodiversity degradation.

375

376 **4.1 Implications for water management**

377 The lack of correlation between EF violation and freshwater biodiversity has implications for
378 large-scale water management. A generalized large scale EF approach can underestimate the
379 stress on the ecosystem at a smaller scale where the actual action is taking place. It is undeniable
380 that adequate flow is essential for maintaining freshwater ecosystems. Nonetheless, the current
381 generalized EF estimation methods need further refinement to adequately capture this
382 importance. The global hydrological EF methods are often validated using locally calculated EF
383 requirement values (Pastor et al., 2014) with the assumption of adequate scalability in the EF-
384 biodiversity relationship. However, more holistic EF estimation methods combining hydrological,
385 hydraulic, habitat simulation methods, and expert knowledge (Poff and Zimmerman, 2010;
386 Shafroth et al., 2010) are essential to ensure a healthy freshwater biodiversity. The policies and
387 decisions taken at various scales need a more dynamic framework, where different dominant
388 drivers of ecosystem degradation can be prioritized based on particular cases. For instance, an
389 integrated EF indicator which encompasses quantity, quality and timeliness of water in the
390 streams will be a better hydrologic indicator to evaluate freshwater ecosystem health than an

391 indicator which accounts only for quantity. Moreover, when making water management
392 decisions, care must be given to account for the temporal and spatial heterogeneity in the
393 ecosystem dynamics.

394

395 Although there are some coordinated scientific efforts such as ELOHA (Ecological Limits Of
396 Hydrologic Alterations) (Poff et al., 2010) to provide a holistic framework for EF estimation, its
397 scientific complexity and high implementation cost constrains its use around the world (Richter
398 et al., 2012). For example, several European countries like Romania, Czech Republic, Serbia and
399 Luxembourg use a national level static method to define minimum environmental flows
400 (Linnansaari et al., 2012). Similarly, other jurisdictions use the presumptive standards proposed
401 by Richter et al. (2012) to establish a legal basis for EF protection. These presumptive standards
402 limit hydrologic modifications to a percentage range of natural or historic flow variability. One
403 example of such a case, the North Carolina's Environmental Flow Science Advisory Board uses a
404 presumptive standard of 80-90% of the instantaneous modeled baseline flow as the EF
405 requirement (NCEFSAB, 2013). The limitation of such a practice is the incorrect presumption of
406 uniformity in the EF needs over a larger region. Therefore, we recommend the application of
407 holistic indicators at these large scales (covering all river stretches and tributaries) rather than
408 using simplified hydrologic-only metrics of EF (violation). However, the authors also acknowledge
409 the limits in implementation of a more dynamic EF framework in data limited regions. Programs
410 for more monitoring and data collection and improved, more holistic modeling methods using
411 more/better data need to be implemented in those regions. Thus, applying a holistic framework
412 like ELOHA could be made possible and can capture the heterogeneity in the EF-biodiversity
413 relationship.

414

415 **4.2 Implications for a water planetary boundary**

416 The current rationale in using EF in the water planetary boundary relationship is based on the
417 assumption of its universal relationship with freshwater biodiversity. However, with the currently
418 available data and methods the findings for EF-biodiversity relationship are inconclusive.
419 Moreover, due to the heterogeneity of biodiversity response over time and space, the trend in

420 any aggregate scale is likely to remain relatively constant instead of showing any discernible
421 tipping point (Brook et al., 2013). We suggest that to reconsider the use of environmental flows
422 in defining water planetary boundaries, given the higher degree of heterogeneity and lack of
423 strength in the ecosystem function-biodiversity relationship. Some of the potential reasons for
424 the reconsideration are, firstly, freshwater biodiversity may not have pan-regional or
425 "continental-planetary" scale threshold dynamics, and its link with EF violation might be
426 inadequate to represent the finer scale variations. Secondly, resource distribution and human
427 impact heterogeneity suggest the need for regional boundaries as proposed by Steffen et al.
428 (2015). Thirdly, EF calculation methods used in the current regional/planetary boundary
429 definition are highly restricted to hydrological methods which may not be adequate to capture
430 the biodiversity status. A regional boundary transgression can occur even well within planetary-
431 level safe limits (Brook et al., 2013; Nykvist et al., 2017). Therefore, for a highly complex
432 biophysical relationship like the EF-biodiversity where multiple shift states are possible, it is very
433 difficult to prioritize and manage critical regions without a regional/local boundary.

434

435 **4.3 Limitations and ways forward**

436 1) **Data scarcity:** Even though this study uses state of the art global hydrological models and best
437 available global estimates of EF requirements, freshwater ecological data were limited to
438 freshwater fish. Other than these, several other taxa like crayfish and other benthic
439 invertebrates, phytoplankton, or zooplankton are also significant in determining the proper
440 functioning of a freshwater ecosystem (AL-Budeiri, 2021; Domisch et al., 2017; Nyström et al.,
441 1996). However, due to lack of global data, these taxa are not included in this study. To better
442 examine the relationship, global datasets for other freshwater biodiversity metrics are urgently
443 needed.

444

445 2) **Discrepancy in data resolution:** The spatial and temporal resolutions at which the EF violation
446 is estimated here, and the biodiversity indicators measured/calculated are inconsistent. The
447 basic spatial measuring unit of the biodiversity is sometimes greater or lesser than the basin size
448 at which EF is measured. This discrepancy could have some impact on the results. However, in

449 this study several resolution matching methods were used to account for this uncertainty.
450 Therefore, more detailed data with better-matching scales are needed to overcome this
451 limitation.

452

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

458

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

465

466 5) **Exclusion of impact of dams:** The dams are indeed a large contributing factor to the results
467 uncertainty. The dam regulated rivers may have a significantly different effect on biodiversity
468 compared to free-flowing rivers. The ISIMIP data used to calculate EF violations considers the
469 effects of large dams on streamflow. However, in order to explicitly isolate the effects of dams in
470 this analysis from other drivers, the information on dam operation schemes for each sub-basin
471 would be necessary and this would require a paper on its own. Therefore, the effects of the dams
472 are incorporated in this study but are not explicitly analyzed separately from other drivers.

473 **5. Summary and Conclusion**

474 The relationship between EF violations and freshwater biodiversity is evaluated at globally
475 aggregated levels in this study. No significant relationship between EF violation and freshwater
476 biodiversity indicators was found at global or ecoregion scale using globally consistent methods

477 and currently available data. Relationships may exist at smaller scales and could potentially be
478 identified with more holistic EF methods including multiple factors (e.g., temperature, water
479 quality, intermittency, connectivity) and more extensive freshwater biodiversity data.

480

481 The paper is not intended to be a definitive test on the relationship between EF and aquatic
482 biodiversity but more to be an exploratory analysis to tests a widely used but rarely verified
483 assumption on the relationship at global and ecoregion scale. The lack of correlation in the EF-
484 biodiversity relationship found in this study suggests to take particular care when developing
485 macro-scale EF policies (regional and above), and further implies that the conceptualization of a
486 blue water planetary boundary ought to rest upon a broader set of relationships between
487 hydrological processes and Earth system functioning. At larger scales, the enormous spatial and
488 temporal heterogeneity in EF-biodiversity relationship motivates a holistic estimation of EF
489 grounded on ecosystem dynamics.

490 **Data Availability**

491 The data used in this study are temporarily made available at
492 https://drive.google.com/drive/folders/1dXYByen5fcUqCQI3R4E0baCorpMwqN_q?usp=sharing
493 The permanent location of the data is to be decided. Any additional data or code will be made
494 available on request.

495 **Author Contribution**

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

504 **Compelling Interests**

505 The authors declare no competing interests.

506 **Acknowledgement**

507 Authors acknowledge various funds that made this research possible. CM received funding from
508 Canada First Research Excellence Fund (CFRE); MK received funding from Academy of Finland
509 funded project WATVUL (grant no. 317320), Academy of Finland funded project TREFORM (grant
510 no. 339834), and European Research Council (ERC) under the European Union’s Horizon 2020
511 research and innovation programme (grant agreement No. 819202). VV received funding from
512 Aalto University School of Engineering Doctoral Programme and European Research Council
513 (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant
514 agreement No. 819202). SCJ acknowledges funding through the Leibniz Association for the
515 project Freshwater Megafauna Futures.

516 **Supplementary Information**

517 The supplementary information is submitted separately.

518

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