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1 **Efficiency of invertebrate-based bioassessment for evaluating the ecological status of**
2 **streams along a gradient of flow intermittence**

3

4

5 Julie Crabot^{1,2}, Sylvain Dolédec³, Maxence Forcellini¹, Thibault Datry¹

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7 ¹ INRAE, UR RiverLy, Centre de Lyon-Villeurbanne, France

8 ² Université Clermont Auvergne, CNRS, GEOLAB, F-63000 Clermont-Ferrand, France

9 ³ Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR 5023 LEHNA, F-69622,

10 Villeurbanne, France

11

12 **Corresponding author:** julie.crabot@gmx.fr

13 **Abstract**

14 Intermittent rivers and ephemeral streams (IRES) represent the majority of the global river
15 network, support high biodiversity and provide multiple ecosystem services. However, they
16 are being degraded at alarming rates, and it may be questioned whether current invertebrate-
17 based biomonitoring protocols, which were designed for rivers and streams that flow all year
18 round, are also appropriate for assessing the ecological status of IRES. Here, we test for the
19 first time the efficiency of the French invertebrate-based biomonitoring indicators to assess
20 the ecological status of intermittent rivers downstream six sewage treatment plants along a
21 gradient of flow intermittence (i.e. the percentage of a year without flow at a given site). We
22 found that the effects of drying confounded with that of sewage and in most cases, current
23 biomonitoring metrics did not distinguish between the effect of sewage and those of drying.
24 Except for the taxonomic richness of invertebrates resistant to drying, all metrics decreased
25 linearly with increasing flow intermittence. In addition, the efficiency for detecting sewage
26 effects was lower at intermittent reaches compared to perennial reaches. Last, we identified
27 biological traits responding to flow intermittence, but not to sewage effluents, and we further
28 discuss the implication for traits-based monitoring. If current biomonitoring protocols
29 calibrated for perennial rivers could still be applied to reaches prone to weak flow
30 intermittence, alternatives are urgently needed for integrating intermittent rivers into current
31 biomonitoring national programs.

32

33 **Keywords**

34 Traits-based approaches; I2M2; biomonitoring; ecological status; drought; temporary rivers

35 **1. Introduction**

36 In the Anthropocene, water management relies on biomonitoring approaches to assess the
37 responses of aquatic ecosystems to the multiple anthropogenic stressors they are facing (Reid
38 et al., 2019). Accordingly, international legislations such as the Water Framework Directive
39 in Europe (European Commission, 2000) or the Clean Water Act in the USA (Environmental
40 Law Institute, 2012) call for biomonitoring to assess ecological quality of rivers and streams.
41 The use of biological signatures to assess human impairment in streams has a long tradition in
42 freshwater ecology (e.g. reviews in Dolédec and Statzner, 2010 and Friberg et al., 2011) and
43 benthic macroinvertebrates are one of the most widely used aquatic organisms for achieving
44 such assessments (see e.g. Bonada et al., 2006; Rosenberg and Resh, 1993). To date, current
45 biomonitoring approaches are based almost exclusively on aquatic communities collected
46 from perennial rivers and streams (Reyjol et al., 2014; Seaman et al., 2016; Stubbington et al.,
47 2018) and suggested alternatives for intermittent and ephemeral rivers remain rare and are not
48 integrated into national efforts (Leigh et al., 2013; Prat et al., 2014; Steward et al., 2018).
49 Intermittent rivers and ephemeral streams, hereafter IRES, are defined as every rivers and
50 streams that cease to flow and/or dry at some points in space and time (Datry et al., 2014).
51 Many authors have questioned the suitability of current biomonitoring approaches in IRES
52 (Mazor et al., 2014; Munné and Prat, 2011; Prat et al., 2014; Reyjol et al., 2014; Steward et
53 al., 2018; Stubbington et al., 2018; Wilding et al., 2018). However, only a few studies tested
54 the applicability of the current macroinvertebrate-based biomonitoring approaches for
55 intermittent rivers. For example, Morais et al. (2004) showed that the impact of a sewage
56 treatment plant was not detectable in an intermittent stream in Portugal. In addition, a review
57 by Stubbington et al. (2018) suggested that current biomonitoring approaches needed to be
58 refined to be efficient for IRES. Recently, a global quantification of the prevalence of non-
59 perennial rivers indicates that IRES comprise the majority of the river channels on Earth and

60 might represent the norm rather than the exception (Messenger et al., 2021). Such a shift in
61 paradigm thus calls for assessing urgently whether current biomonitoring approaches can
62 apply to IRES and if not, the limitations in their use or interpretation can be identified and
63 corrected to accurately reflect the ecological status of the river networks facing the
64 Anthropocene.

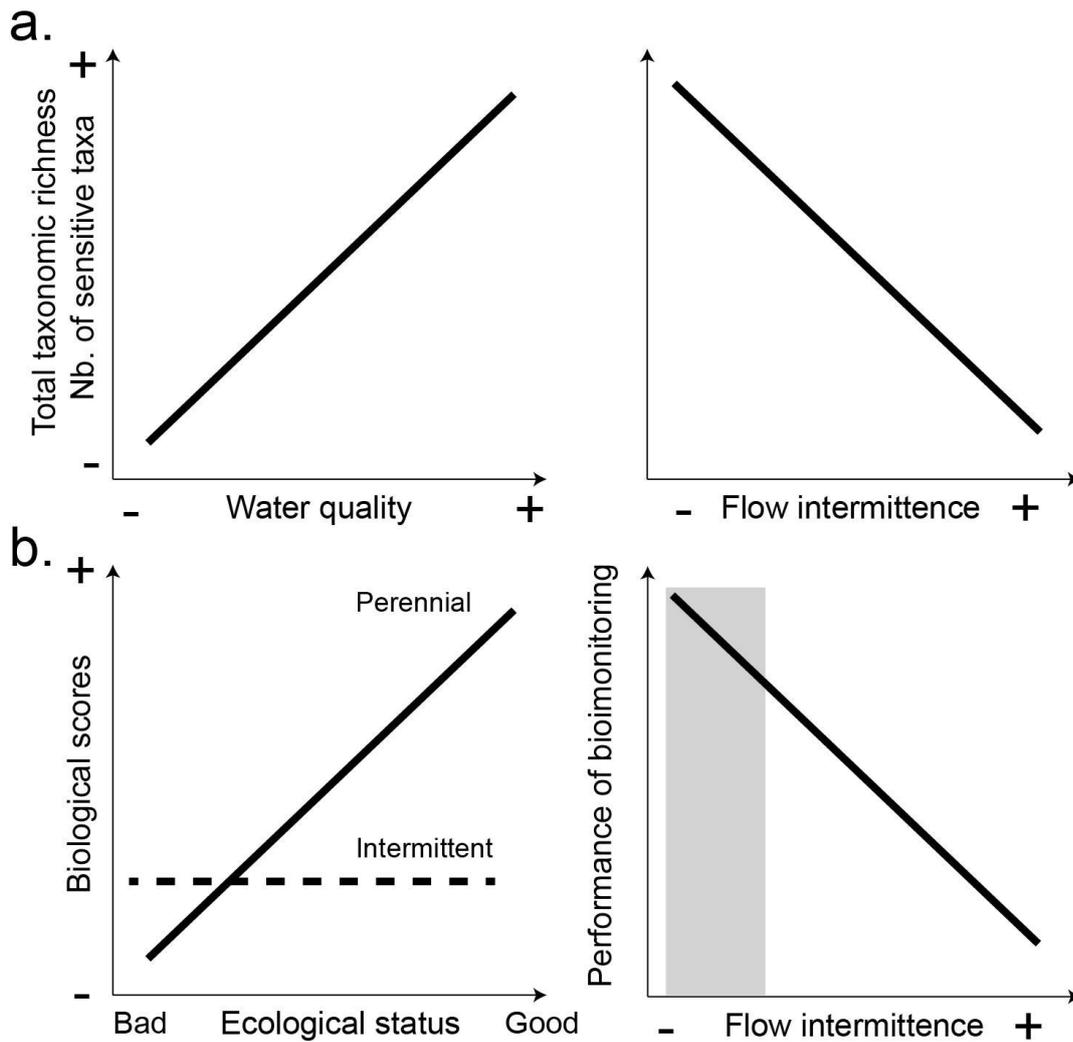
65 Both short- and long-term effects of drying on aquatic communities are relatively well
66 understood due to the exponential number of dedicated studies through the past decade (e.g.
67 Busch et al., 2020; Leigh et al., 2016). Across climatic and biogeographic zones, aquatic
68 alpha-diversity (i.e. the local taxonomic richness at a given location) consistently decreases
69 when annual flow intermittence (i.e. the percentage of a year without flow at a given site)
70 increases (Belmar et al., 2019; Datry et al., 2014; England et al., 2019; Gauthier et al., 2020).
71 Immediately upon drying, taxa without adaptations to cope with desiccation are eliminated
72 locally due to death or migration towards perennial refuges within the landscape (Bogan et al.,
73 2015; Datry et al., 2014). The longer the dry event, the smaller the remaining pool of resistant
74 taxa on the surface (Bogan et al., 2013; Datry, 2012; Pařil et al., 2019) and subsurface (Storey
75 and Quinn, 2013; Stubbington et al., 2016; Stubbington and Datry, 2013) sediments. Upon
76 rewetting, the arrival of species with high dispersal abilities from refuges also promotes
77 community recovery (Bogan et al., 2015; Datry et al., 2014). With increased drying duration,
78 the distance to refuges tends to increase, thus limiting the recolonization of previously dry
79 sites (Cañedo-Argüelles et al., 2015; Crabot et al., 2020; Gauthier et al., 2020; Sarremejane et
80 al., 2017b). As a result, legacy effects of drying occur even a long time after rewetting and
81 local taxonomic diversity is generally much lower than that of adjacent perennial reaches
82 (Crabot et al., 2021b; Datry, 2012; Gauthier et al., 2020; Soria et al., 2017). Previous studies
83 reported that such taxa loss at intermittent sites was associated with a lower functional
84 diversity compared to perennial sites (B-Béres et al., 2019; Schriever et al., 2015). However,

85 because biological trait combinations can be expressed by many taxa or by only a few taxa in
86 a local community, functional diversity response to flow intermittence might sometimes differ
87 from the taxonomic patterns. As such, flow intermittence might reduce taxonomic diversity
88 between intermittent and perennial reaches more importantly than functional diversity (Crabot
89 et al., 2021a; Vander Vorste et al., 2016).

90 The effects of water quality degradation may confound with the natural responses of
91 macroinvertebrate communities to drying (Soria et al., 2020). A large majority of the taxa
92 used as indicators, i.e. with high sensitivity to water quality degradation such as
93 Ephemeroptera, Plecoptera, Trichoptera (EPT) are those that are also primarily affected by
94 drying. For example, the reduction of lateral hydrological connectivity associated with
95 drought leads to a decline in EPT richness (Bonada et al., 2006b). Moreover, the number of
96 EPT taxa differs between permanent and intermittent reference streams (Bogan et al., 2013;
97 Datry, 2012; Straka et al., 2019). In contrast, some taxa known to be resistant to desiccation
98 and widespread in IRES, such as Odonata, Coleoptera and Heteroptera (OCH), but also
99 Oligochaeta and Diptera (Bonada et al., 2006b; Datry, 2012) are more tolerant to different
100 types of water quality degradation, including water pollution (Chang et al., 2014). For
101 example, looking at the 38 indicators taxa used in the French IBG-DCE for their sensitivity to
102 water quality impairment, the only Coleoptera considered are Elmidae and are given a
103 sensitivity of 2 (out of a maximal sensitivity of 9; AFNOR, 2004). Neither Heteroptera nor
104 Odonata are considered in this list. Consequently, we expect a poor success of current
105 biomonitoring approaches in the context of flow intermittence (Kalogianni et al., 2017; Soria
106 et al., 2020; Stubbington et al., 2018) since both the number of taxa and taxa showing
107 sensitivity to water quality degradation should decrease with increasing water quality
108 degradation and flow intermittence (**Fig. 1a**). In turn, the absence of relationships between the
109 measured biological scores and the ecological status may occur in case of flow intermittence,

110 contrary to what is observed in perennial rivers (Munné et al., 2021; Soria et al., 2020) (**Fig.**
 111 **1b**). However, as the responses to flow intermittence are known to be linear (e.g. Datry et al.
 112 2014), an effective operational space for weakly intermittent rivers and streams could allow
 113 using current biomonitoring approaches (**Fig. 1b**).

114



115

116

117 **Figure 1.** Conceptualized responses of communities to water quality degradation and flow
 118 intermittence (a.) and expected biomonitoring scores and performance in case of perennial
 119 and intermittent flow regimes (b.). In grey, the area that could represent an operational space
 120 along which biomonitoring approaches developed for perennial rivers could be applicable to
 121 intermittent rivers.

122 In this study, we quantified the structural and functional community responses to six point-
123 source of sewage along with a gradient of flow intermittence (0 to 60% within a year). We
124 tested the hypothesis that current macroinvertebrate-based biomonitoring protocols were not
125 able to infer the ecological status of naturally intermittent rivers. This is because the effect of
126 drying on communities confounds with those of sewage pollution. Therefore, we first
127 expected that impacted sites (downstream of effluents) located in intermittent reaches would
128 have similar communities to that observed upstream of effluents. Thus, biomonitoring scores
129 would not differ between downstream and upstream sites in the context of flow intermittence,
130 contrary to perennial reaches. Second, we expected the capacity of current macroinvertebrate-
131 based biomonitoring protocols to decrease with increasing flow intermittence, as taxonomic
132 and functional richness are likely to decrease linearly with flow intermittence. Third, we
133 anticipated that some traits should be favored by flow intermittence and some by water
134 quality degradation. To unravel the underlying mechanisms, we identified how biotic and
135 functional traits groups varied with flow intermittence and which one warranted further study
136 over the long-term for establishing robust indicators of intermittent rivers quality.

137

138 **2. Materials and methods**

139 *2.1. Study sites*

140 The Albarine River is naturally intermittent draining a 313km² catchment located in the
141 temperate eastern France (**Fig. 2a**). The upper section of the Albarine flows for 45 km on a
142 karstic plateau and through gorges, where several reaches are intermittent due to karstic
143 infiltrations, mostly occurring during the summer periods (Gauthier et al., 2020). Then, the
144 Albarine flows over a 15-km long alluvial plain to finally end in the Ain River, a tributary of
145 the upper Rhône. Surface water continuously infiltrates into the underneath aquifer due to its
146 alluvial plain underlain by thick (>36 m) and highly permeable (from $2 \cdot 10^{-2}$ to $7 \cdot 10^{-2}$ m s⁻¹)

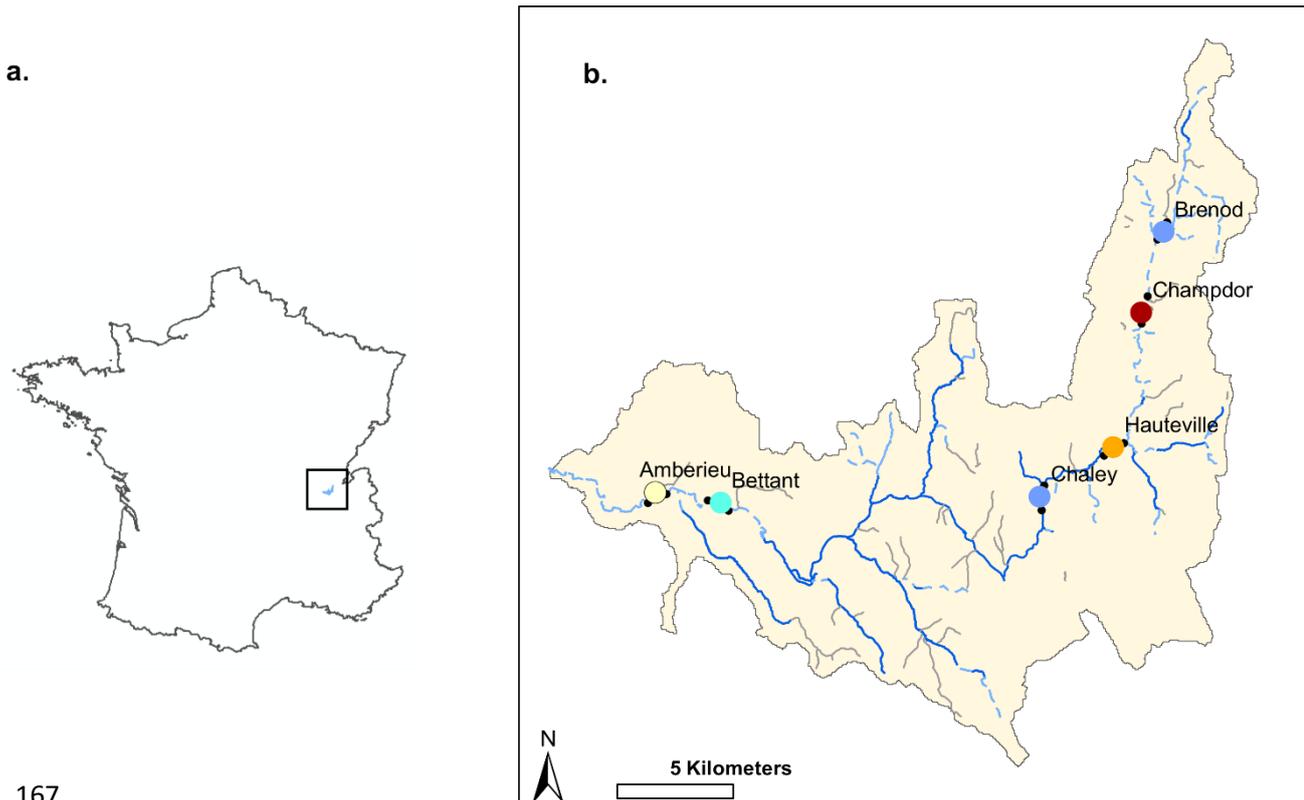
147 sediments and because the river is generally perched 1-14 m above the regional aquifer water
148 table along this section (Datry, 2012; Larned et al., 2011). As a result, this section dries
149 completely during summer, when rainfall cannot compensate for transmission losses into the
150 aquifer (Datry, 2012; Gauthier et al., 2020; Larned et al., 2010). Estimated surface water
151 losses to the aquifer range from 0.85 to 2.5 m³ s⁻¹ (Datry, 2012).

152 In the upper section, depending on the winter snowpack and spring rainfall, drying can occur
153 throughout spring and summer, following a very patchy spatial pattern with high temporal
154 dynamism. Drying events start downstream of the lower sections during spring, at the
155 confluence with the Ain River, and the drying front progressively moves upstream throughout
156 the summer period (Datry, 2012). Flow resumption along the entire river occurs generally at
157 late autumn/early winter (Datry, 2012; Larned et al., 2011). Consequently, annual flow can
158 range between 0 and 60% along the Albarine River (Datry et al., 2011; Larned et al., 2010).

159

160 *2.2. Sampling design*

161 We selected 6 reaches along the Albarine course, each under the impact of a sewage treatment
162 plant, whose effluents are directly released in the river, and considering the overall gradient of
163 flow intermittence (hereafter FI) ranging from 0 to 60 % (**Fig. 2b**). At each reach, we selected
164 a site located between 50 and 100m downstream from the effluent release point and an
165 upstream site located between 50 and 200m upstream from the effluent release point (**Fig.**
166 **2b**).



167

168

169 **Figure 2.** Location of the catchment in France (a), and location of the six sewage treatment
 170 plants on the Albarine (b). The color of the circles indicates the flow intermittence at each
 171 reach (from dark blue for 0% FI to red for 60% FI) and the small black dots indicate the
 172 samplings upstream and downstream of the sewage effluents. Continuous lines represent the
 173 perennial sections of the rivers and the dotted lines represent the intermittent sections. Grey
 174 section are unknown.

175

176 *2.3. Flow intermittence estimates*

177 Flow intermittence was quantified at the 3 reaches located in the upper section using photo-
 178 traps (Bushnell Trophycam®) setup for recording one photo every day at midnight and
 179 midday and left in place for 2 years (January 2017 until December 2018; Gauthier et al.,
 180 2020). For the 3 reaches selected in the lower section, FI was modelled for each reaches using
 181 the ELFMOD model (Larned et al., 2011). This model associated flow measurements at rated
 182 water-level recorders at each end of the 45-km alluvial section to flow state (flowing or dry)
 183 and flow rate (point source discharge measures) at all points between the recorders. The
 184 model was calibrated using data from gauging runs between October 2003 and January 2016,

185 and validated using data collected outside of the calibration period. The empirical model was
186 used to reconstruct a 29-yr record of daily flows along the river (Rupp et al., 2008). We
187 subsequently tested the accuracy and precision of model-based estimates of FI using monthly
188 observations of flow made between October 2011 and June 2019 at the selected sampling
189 sites. Modelled FI at each reach was calculated by averaging annual estimates of flow
190 intermittence over the record. Observed FI was highly correlated with modelled FI ($r = 0.95$,
191 $p < 0.001$). The FI took into account only flowing and dry phases because the Albarine River
192 does not present phases of isolated pools, as it is often the case for alluvial rivers with porous
193 streambed sediments (Vander Vorste et al., 2016).

194

195 *2.4. Physical and chemical measurements*

196 The physical and chemical parameters up and downstream effluent releases were sampled at
197 the six reaches during spring (17 April 2018) and fall (19 December 2018) i.e., prior to and
198 after the summer drying period. Water samples and analytical measurements were carried out
199 by the CARSO laboratory (<https://www.groupecarso.com/en/>) that routinely measures the
200 physical and chemical parameters of water bodies within the WFD context. At each location
201 of each reach, 4L of surface water was collected and brought back to the laboratory within 6h
202 to measure the following physical and chemical parameters according to the national French
203 standards (Rodier et al., 2016): water temperature ($^{\circ}\text{C}$), pH, specific conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$),
204 dissolved oxygen ($\text{mg}\cdot\text{L}^{-1}$), total phosphorus ($\text{mg}\cdot\text{L}^{-1}$, NF EN ISO 9308-3), suspended solid
205 ($\text{mg}\cdot\text{L}^{-1}$, NF EN 872), total organic carbon ($\text{mg}\cdot\text{L}^{-1}$, NF EN 872), dissolved organic carbon
206 ($\text{mg}\cdot\text{L}^{-1}$, NF EN 1484), biochemical demand in oxygen for 5 days ($\text{mg}\cdot\text{L}^{-1}$, NF EN 1899-2),
207 chemical oxygen demand ($\text{mg}\cdot\text{L}^{-1}$, ISO 15705), Kjeldahl nitrogen ($\text{mg}\cdot\text{L}^{-1}$, NF EN 25663),
208 ammonium ($\text{mg}\cdot\text{L}^{-1}$, NF T90-015-2), nitrates ($\text{mg}\cdot\text{L}^{-1}$, NF EN ISO 10304-1), orthophosphates
209 ($\text{mg}\cdot\text{L}^{-1}$, NF EN ISO 68) and nitrites ($\text{mg}\cdot\text{L}^{-1}$, NF EN ISO 13395). In addition, organic

210 pollution was measured using the density of *Escherichia coli* (MPN.10 ml⁻¹, NF EN ISO
211 9308-3).

212

213 2.5. Benthic macroinvertebrates

214 2.5.1. Sampling

215 Benthic macroinvertebrates were sampled up and downstream effluent releases by the
216 Eurofins Hydrobiology Consulting Company in spring (16-18th April 2018) and fall (17-19th
217 December 2018). Macroinvertebrate sampling was carried out following the NF T90-333
218 standards (AFNOR, 2016). Briefly, this consisted in the following steps. First, each sampling
219 site was first delineated by estimating the percentage of habitats (e.g. pool, riffles, run),
220 percentage of substrates (e.g., sand, gravel, cobbles) and percentage of velocity ranges (e.g.
221 slow ≤ 0.5 m/s, fast > 0.5 m/s) over a length of approximately 15 times the mean wetted
222 width. This allowed estimating the variety and relative abundance of habitats separated into
223 marginal ($\leq 5\%$ of the overall sampled location area) and dominant ($> 5\%$ of the overall
224 sampled location area) substrates. Then, 12 samples were collected up and downstream
225 effluent releases at each reach using a Surber sample (1/20 m², 500 μ m mesh size) and they
226 were separated into 3 phases: 4 samples into the marginal habitats, 4 samples into the
227 dominant habitats taken in decreasing order of carrying capacity (from the more biogenic –
228 bryophytes, to the last biogenic – artificial uniform surfaces), and 4 complementary samples
229 into the dominant habitats considering their relative proportion over the sampling site.
230 Samples were washed in the field with the coarsest material (sediment, wood) cleaned with a
231 brush and put back in the river. All macroinvertebrates were preserved in 96% ethanol and
232 brought back to the laboratory within the day. Finally, all organisms of each sample were
233 counted and identified to the lowest practical taxonomic level. Most insects and all mollusks

234 were identified to genus or species levels, and crustaceans, annelids and mites to family, order
235 or genus levels following the French standard XP T90-388 (AFNOR, 2010).

236 *2.5.2. Biological indices*

237 Two biotic indexes are currently implemented at the national scale to assess stream
238 impairment in France: the so-called French biotic index (abbreviated IBG-DCE for “Indice
239 Biologique Global Directive Cadre sur l’Eau”; AFNOR, 2016, 2010, 2004) and the French
240 invertebrate multimetric index (abbreviated I2M2 for “Indice Invertébrés Multi-Métrique”;
241 Mondy et al., 2012). The IBG-DCE enables quantifying the impairment of the water
242 chemistry and the hydromorphological characteristics of a reach by considering the taxonomic
243 richness (TR, grouped into classes ranging from 1 to 14) and the sensitivity of 38 specific
244 indicator taxa (distributed into 9 indicator groups or IG) to water quality impairment. IBG-
245 DCE is computed as: $TR + IG - 1$ (with IBG-DCE >20 set to 20). IBG-DCE values varies
246 between 1 (low quality) and 20 (high quality). The categories of ecological status for the IBG-
247 DCE are bad (note of 0-5), poor (5-9), fair (9-12), good (12-14) and high (14-20). The I2M2
248 is made up of 3 elementary metrics considering the sensitivity to anthropogenic disturbance:
249 Average Score Per Taxon or ASPT, relative abundance of ovoviviparous and multivoltine
250 taxa in the assemblage; and 2 metrics describing the taxonomic assemblage structure:
251 Shannon diversity and taxonomic richness. The I2M2 is compatible with the requirements of
252 the Water Framework Directive, as it considers the stream reach typology, includes the
253 calculation of a deviation from a reference state and is expressed in Ecological Quality Ratio
254 from 1 (assemblage conforming to the reference) to 0 (completely disturbed). According to
255 the classification rules for the Albarine, the categories of ecological status for the I2M2 values
256 are bad (<0.118), poor (0.118-0.236), fair (0.236-0.354), good (0.354-0.605) and high
257 (>0.605). It should be finally noted that IBG-DCE invertebrates uses the family level as the
258 finest level of identification whereas for I2M2 it is the genus.

259 *2.5.3 Biological traits*

260 The biological traits were extracted from the European freshwater invertebrate database
261 (Tachet et al., 2010). We considered 10 traits: maximal size, life span, number of generations
262 per year, types of reproduction, forms of resistance, types of respiration, dispersal, types of
263 locomotion, food and feeding habits. In this database, traits are divided into categories and are
264 quantified using fuzzy coding (Chevenet et al., 1994), which consists in giving an affinity
265 score to a given trait category, between 0 and 3 (maximal size, life span, number of
266 generations per year, types of reproduction, forms of resistance, types of respiration, dispersal,
267 types of locomotion) or 0 and 5 (food and feeding habits), for each taxon. Fuzzy coding
268 enables the compilation of information from various sources and accounts for within-taxon
269 variations.

270 *2.6. Data analysis*

271 To quantify the effects of the sewage treatment plants, we compared the concentrations in
272 nutrients and associated parameters between downstream and upstream effluent releases. For
273 each physical and chemical parameter, we calculated the difference between the downstream
274 and upstream values at each reach and each season. Positive values indicated a higher
275 degradation caused by sewage effluents. For each physical and chemical parameter, we tested
276 if the mean difference was significantly greater than zero using unilateral paired t-tests. To
277 assess the efficiency of the overall family richness, IBG-DCE and I2M2 at detecting water
278 quality degradation, we used a mixed-effect model with sewage (up or downstream effluent
279 releases), season (spring vs. fall) and their interaction as fixed effects, and reaches as random
280 effects. The significance of mixed-effect models was evaluated by a comparison with its
281 associated null model using likelihood-ratio tests. All mixed-effect models were computed
282 with the function ‘lmer’ of package lme4 (Bates et al., 2019). We checked whether the
283 random effects were significant using the function ‘rand’ of package lmerTest (Kuznetsova et

284 al., 2020). We performed four separate analyses considering overall, EPT, OCH, and
285 Gastropods, Oligochaeta, and Diptera (GOLD) richness. These groups were chosen because
286 they are known to respond differently to FI (e.g. Bonada et al., 2006b; Datry et al., 2014).

287

288 We tested our first hypothesis on the negative influence of FI on family richness, IBG-DCE
289 and I2M2 by performing a multiple linear regression with FI and season (spring vs. fall) as
290 explanatory variables.

291

292 To test our second hypothesis that the severity of flow intermittence would impact the
293 efficiency of pollution detection, we ran the same tests only on sites upstream effluent
294 releases. We also computed the differences (hereafter delta) in family richness, IBG-DCE and
295 I2M2 between up and downstream effluent releases at each reach and each sampling date. If
296 sewage effects were detected, we would expect positive delta values for OCH and GOLD taxa
297 richness, but negative delta values for the overall family richness, EPT richness, and for the
298 IBG-DCE and I2M2 metrics. We assessed the effect of FI on these delta values with a
299 multiple linear regression, using FI and season (spring vs. fall) as explanatory variables.

300

301 To further analyze how intermittence influenced our ability to detect the effects of sewage on
302 biota, we calculated the absolute difference in abundance between up and downstream
303 effluent releases for each taxon. These delta-abundances were log-transformed to reduce the
304 disparities between taxa. We performed a Principal Component Analysis (PCA) on the log-
305 delta abundance values using the 'dudi.pca' function of package ade4 (Dray et al., 2007;
306 Thioulouse et al., 2018). The effect of flow regime (FR; perennial vs. intermittent) and season
307 on the taxon delta-abundances was assessed by running Monte-Carlo tests on the proportion
308 of variance explained between groups (with groups determined either by FR or by season;

309 Dolédec and Chessel, 1987) using the functions ‘bca’ and ‘randtest’ of package ade4. These
310 patterns were further tested by a two-way ANOVA on the mean log-delta abundance values
311 of all taxa for each reach and each season, with FR, season and their interaction as
312 explanatory variables.

313

314 Finally, to test our third hypothesis on the influence of sewage and FI on the trait combination
315 in communities, we calculated the Community Weighted Mean (CWM) array using the
316 ‘functcomp’ function of package FD (Laliberté et al., 2014). Then, we computed a two-way
317 ANOVA on the CWM values of each trait category, using sewage (up and downstream
318 effluent releases), FR (intermittent vs. perennial) and their interaction as explanatory
319 variables.

320 All analyses were performed using the R software (R Core Team, 2021).

321 **3. Results**

322 *3.1. Flow and physical and chemical characteristics of the studied reaches*

323 Our dataset comprised two perennial reaches and four intermittent reaches, the latter drying
324 from 30% to 60% of the time within a year (Table 1). The difference between up and
325 downstream effluent releases values for all physical and chemical parameters were strictly
326 positive in 64% of all reaches and seasons. The average difference was positive for total
327 organic carbon, dissolved organic carbon, total phosphorus and orthophosphate (Appendix A).
328 This indicates that the sewage treatment plants altered the water quality of the stream. In
329 contrast, the average differences between up and downstream effluent releases values were
330 not influenced by the flow regime (Appendix B).

331

Reaches	Distance to the source (km)	FR	FI (%)
Brenod	7.2	Perennial	0
Champdor	11.0	Intermittent	60
Hauteville	19.3	Intermittent	50
Chaley	23.7	Perennial	0
Bettant	74.5	Intermittent	30
Amberieu en Bugey	51.6	Intermittent	40

332

333 **Table 1.** Hydrological characteristics of each sampled reach. FR stands for ‘flow regime’, FI
334 stands for ‘flow intermittence’.

335

336 *3.2. Biological indices*

337 A weak difference between up and downstream effluent releases values occurred only for the
338 GOLD richness (about 1 taxa) (Table 2). In contrast, all biological metrics decreased strongly
339 with increasing FI except the OCH and GOLD taxa richness (Table 3, Fig. 3). This holds
340 when considering samples made only upstream effluent releases for GOLD taxa richness,
341 which was also influenced by season (Tables 2, 3). Except for OCH taxa richness, the delta
342 values between up and downstream effluent releases did not respond to the FI gradient (Table
343 3). We found a higher number of OCH taxa down than upstream effluent releases for the more
344 intermittent reaches. Last, all variables were lower in fall compared to spring (Table 2).

345

346 A two-way ANOVA with FR (intermittent or perennial) and season (spring or fall) as factors
347 indicated that two delta values responded to season (Table 4, Fig. 4). For instance, GOLD
348 taxa were more numerous up than downstream effluent releases during spring (delta value: -
349 1.20 ± 2.17) and it was the opposite during fall (1.67 ± 1.21). The IBG-DCE was slightly higher

Variable	Degradation					Season					Interaction		
	Mean upstream	Mean downstream	df	Chi2	p-value	Mean spring	Mean fall	df	Chi2	p-value	df	Chi2	p-value
Family richness	25(±8)	27(±7)	2	0.61	0.738	30(±6)	22(±7)	2	10.12	0.006 **	1	0.04	0.838
EPT richness	6(±2)	5(±3)	2	4.57	0.102	7(±2)	4(±2)	2	15.39	0.000 ***	1	0.63	0.427
OCH richness	4(±2)	5(±3)	2	1.88	0.391	5(±3)	3(±2)	2	7.224	0.027 *	1	0.03	0.861
GOLD richness	9(±1)	9(±2)	2	8.21	0.016 *	9(±1)	9(±2)	2	7.751	0.021 *	1	7.22	0.007 **
IBG-DCE	13.3(±3.4)	13.5(±3.3)	2	1.22	0.544	14.7(±2.2)	12.0(±3.8)	2	10.39	0.006 **	1	1.19	0.276
I2M2	0.50(±0)	0.57(±0.15)	2	5.33	0.070	0.58(±0.15)	0.48(±0.16)	2	8.851	0.012 *	1	0.04	0.838

350

351 **Table 2.** Results of the mixed-effect models performed on the family richness for all taxa, EPT, OCH and GOLD, and biological indices IBG-
352 DCE and I2M2, as response variables, sewage (up vs. downstream effluent releases), season (spring vs fall) and their interaction as fixed effects,
353 and reaches as random effects. The random effect was significant for all variables except GOLD richness. EPT stands for Ephemeroptera,
354 Plecoptera, Trichoptera; OCH for Odonata, Coleoptera, Heteroptera; GOLD for Gastropods, Oligochaeta and Diptera. ‘df’ stands for degrees of
355 freedom.

	Estimate	All sites					Reference sites only					Delta impacted-reference				
		Estimate	SD	t value	p-value		Estimate	SD	t value	p-value		Estimate	SD	t value	p-value	
Family richness	Intercept	35	2	18.93	<0.001	***	37	2	15.75	<0.001	***	-2	3	-0.79	0.447	
	FI	-19	4	-4.45	<0.001	***	-26	5	-5.02	0.001	**	14	7	2.06	0.069	
	Fall	-7	2	-3.74	0.001	**	-7	2	-2.90	0.018	*	-2	3	-0.52	0.614	
EPT richness	Intercept	9	1	14.33	<0.001	***	9	1	11.03	<0.001	***	-1	1	-0.94	0.371	
	FI	-7	1	-4.70	<0.001	***	-7	2	-3.51	0.007	**	0	2	0.00	1.000	
	Fall	-2	1	-3.74	0.001	**	-2	1	-2.32	0.046	*	-1	1	-1.36	0.208	
OCH richness	Intercept	6	1	6.98	<0.001	***	6	1	9.74	<0.001	***	-1	1	-0.67	0.521	
	FI	-2	2	-1.27	0.219		-5	1	-3.66	0.005	**	6	2	2.42	0.039	*
	Fall	-2	1	-2.13	0.045	*	-2	1	-2.74	0.023	*	0	1	-0.31	0.763	
GOLD richness	Intercept	9	1	14.67	<0.001	***	9	1	12.78	<0.001	***	-1	1	-0.50	0.626	
	FI	-1	1	-0.97	0.345		-1	2	-0.57	0.581		-1	2	-0.48	0.640	
	Fall	0	1	0.62	0.541		-1	1	-1.32	0.219		3	1	2.40	0.040	*
IBGN	Intercept	17.7	0.7	24.15	<0.001	***	17.5	1.3	13.58	<0.001	***	0.62	0.88	0.70	0.500	
	FI	-10.1	1.7	-6.09	<0.001	***	-10.5	2.9	-3.61	0.006	**	0.16	1.99	0.08	0.939	
	Fall	-2.8	0.8	-3.54	0.002	**	-2.0	1.3	-1.49	0.170		-2.17	0.92	-2.36	0.043	
I2M2	Intercept	0.72	0.04	17.36	<0.001	***	0.79	0.05	14.61	<0.001	***	-0.116	0.054	-2.13	0.062	
	FI	-0.46	0.09	-4.91	<0.001	***	-0.55	0.12	-4.56	0.001	**	0.147	0.122	1.20	0.261	
	Fall	-0.10	0.04	-2.35	0.029	*	-0.10	0.06	-1.78	0.108		-0.045	0.057	-0.80	0.446	

356

357

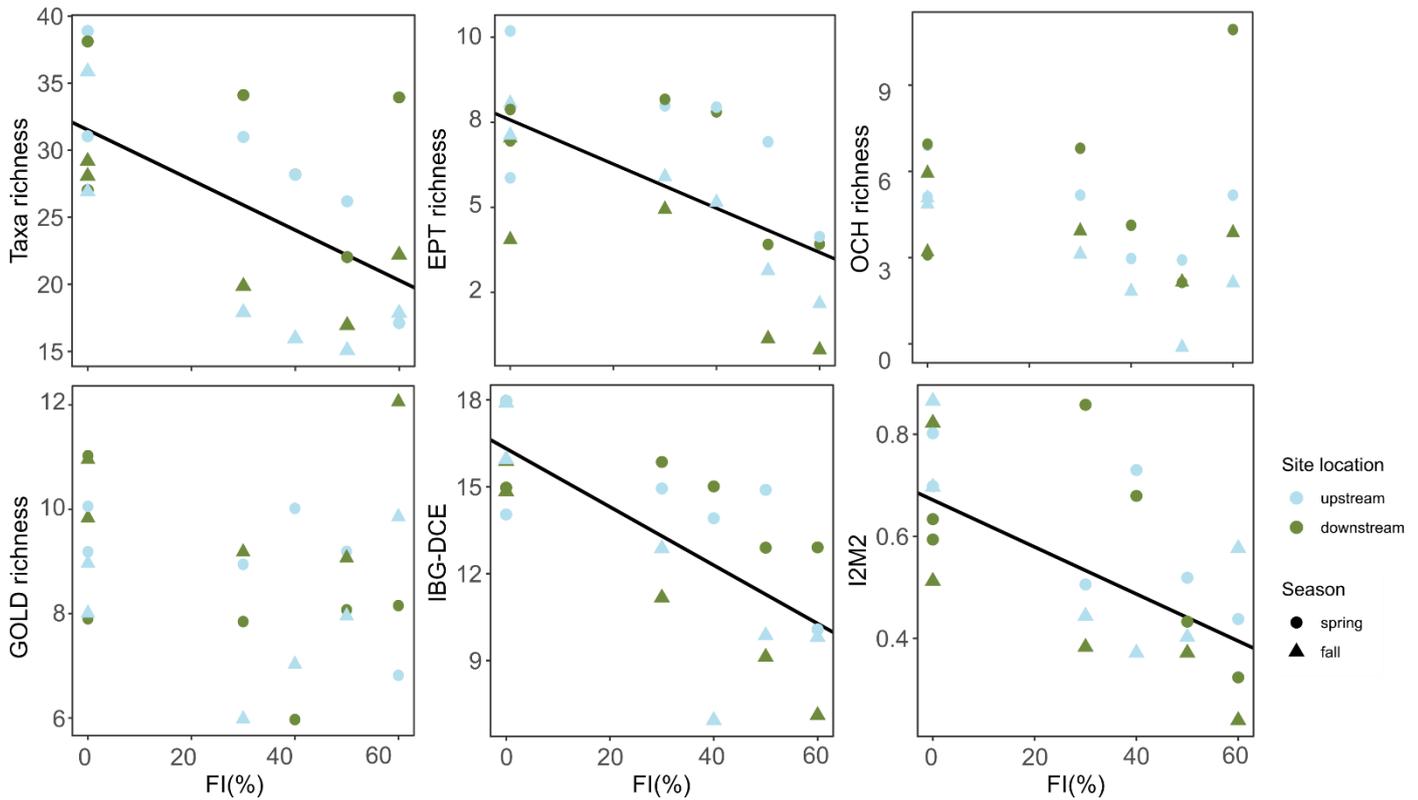
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Table 3. Results of the linear regression performed on the taxonomic richness for all taxa, EPT, OCH and GOLD, and indicators IBG-DCE and I2M2 as response variables, FI, and season (spring vs. fall) as explanatory variables. All models were computed using all sites, upstream effluent releases only, and on delta values between up and downstream effluent releases. EPT stands for Ephemeroptera, Plecoptera, Trichoptera; OCH for Odonata, Coleoptera, Heteroptera; GOLD for Gastropods, Oligochaeta and Diptera.



362

363

364 **Figure 3.** Macroinvertebrate family richness for the overall, EPT, OCH and GOLD taxa, and
 365 for biological indices (IBG-DCE and I2M2) along a gradient of flow intermittence (noted FI).
 366 The lines represent the significant fits of the mixed models. Circles and triangles represent
 367 spring and fall samples respectively, and blue and green colors stand for sites upstream and
 368 downstream releases respectively. EPT stands for Ephemeroptera, Plecoptera, Trichoptera;
 369 OCH for Odonata, Coleoptera, Heteroptera; GOLD for Gastropods, Oligochaeta and Diptera.

370

371 up than downstream effluent releases during spring (0.67 ± 1.63) and greatly lower during fall
 372 (-1.80 ± 1.30). Delta I2M2 values were the only values that responded to FR, with I2M2 being
 373 always higher up than downstream effluent releases. Such a difference was higher at perennial
 374 (-0.160 ± 0.052) than intermittent reaches (-0.033 ± 0.067). In addition, the I2M2 values
 375 classified the intermittent reaches up and downstream effluent releases in “good” ecological
 376 status, whereas perennial reaches were classified as “high”, upstream effluent releases, and
 377 “good”, downstream effluent releases.

378

379 Four out of the 6 delta values did not respond to FI nor to FR: overall family richness, EPT
 380 and GOLD family richness, and IBG-DCE (Table 4, Fig. 4). The overall delta family richness
 381 equaled ~ zero or was even greater than zero (on average 0 ± 4 families), suggesting that
 382 family richness was sometimes greater downstream than upstream effluent releases (Table 4,
 383 Fig. 4). The delta EPT values were low (-1 ± 1 taxa), indicating no response of this group to
 384 sewage.

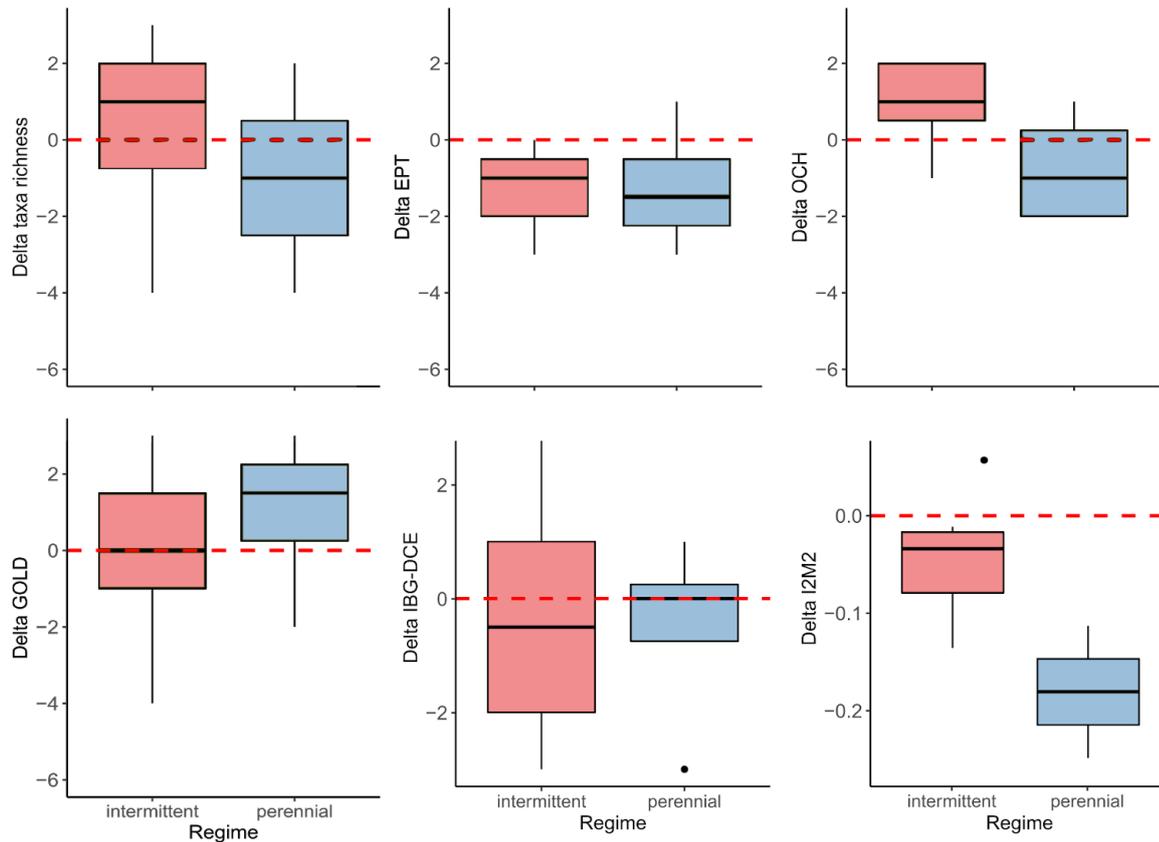
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Delta value	Effect	Df	Mean Sq	F-value	P	
Taxa richness	FR	1	33	2.52	0.156	
	Season	1	3	0.27	0.623	
	FR:Season	1	4	0.32	0.589	
	Residuals	7	13			
EPT richness	FR	1	0	0.00	0.968	
	Season	1	2	1.06	0.337	
	FR:Season	1	1	0.33	0.585	
	Residuals	7	2			
OCH richness	FR	1	8	3.92	0.088	
	Season	1	1	0.42	0.590	
	FR:Season	1	0	0.00	0.964	
	Residuals	7	2			
GOLD richness	FR	1	3	0.85	0.388	
	Season	1	24	7.86	0.026	*
	FR:Season	1	1	0.47	0.514	
	Residuals	7	21			
IBGN	FR	1	0.0	0.01	0.948	
	Season	1	16.6	5.89	0.046	*
	FR:Season	1	0.4	0.13	0.733	
	Residuals	7	2.8			
I2M2	FR	1	0.04	9.25	0.019	*
	Season	1	0.00	0.04	0.851	
	FR:Season	1	0.00	0.82	0.396	
	Residuals	7	0.00			

386

387 **Table 4.** Two-way ANOVA results showing the effects of season, flow regime (FR) and their
 388 interaction on the delta values (difference between up and downstream effluent releases) of
 389 the overall, EPT, OCH and GOLD family richness, and biological indices (IBG-DCE and
 390 I2M2). EPT stands for Ephemeroptera, Plecoptera, Trichoptera; OCH for Odonata,
 391 Coleoptera, Heteroptera; GOLD for Gastropods, Oligochaeta and Diptera.

392



393

394 **Figure 4.** Delta (difference between up and downstream effluent releases) for the overall,
 395 EPT, OCH and GOLD family richness and biological indicators (IBG-DCE and I2M2) in
 396 intermittent (red) and perennial (blue) reaches. The red dashed line stands for no effect of
 397 sewages. EPT stands for Ephemeroptera, Plecoptera, Trichoptera; OCH for Odonata,
 398 Coleoptera, Heteroptera; GOLD for Gastropods, Oligochaeta and Diptera.

399

400 3.3. Structural responses

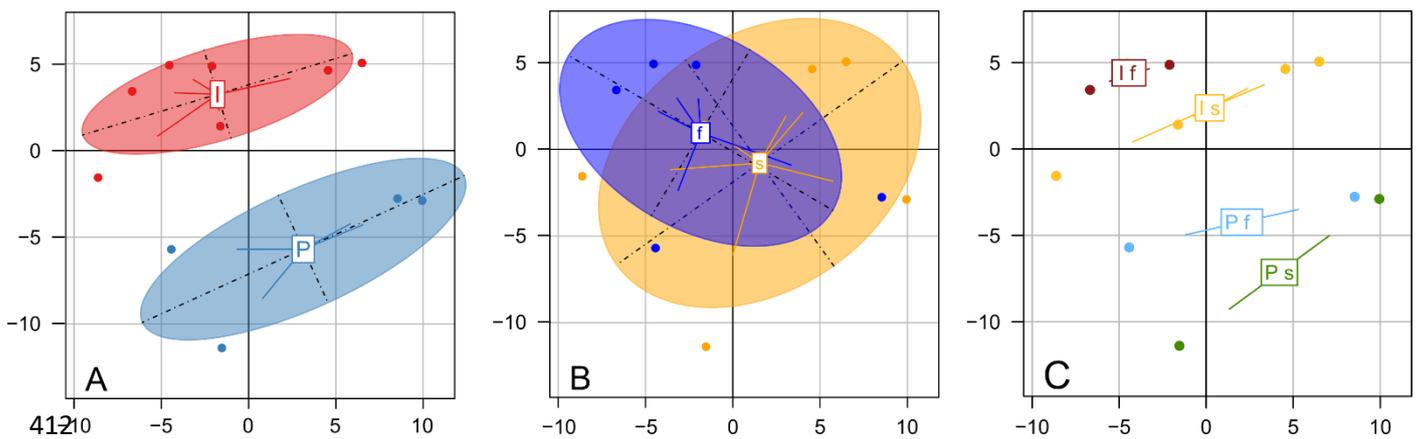
401 The first-two axes of a PCA performed on the delta log-abundances of taxa explained 55.2%
 402 of the total variance. The respective between-groups variance equaled 22.4%, $p=0.011$ for FR
 403 (Fig. 5A), 11.2%, $p=0.281$ for the season (Fig. 5B) and 40.5%, $p=0.049$ for their combination
 404 (Fig. 5C). The difference in abundances between up and downstream effluent releases were
 405 lower intermittent reaches compared to perennial reaches (0.57 ± 0.24 and 1.03 ± 0.22
 406 respectively, Table 5). These differences were also lower in fall compared to spring
 407 (0.58 ± 0.34 and 0.87 ± 0.26 respectively, Table 5).

	Df	Sum Sq	Mean Sq	F	P
FR	1	0.56	0.56	22.34	0.002 **
Season	1	0.28	0.28	11.11	0.013 *
FR:Season	1	0.03	0.03	1.02	0.347
Residuals	7	0.18	0.03		

408

409 **Table 5.** Results of a two-way ANOVA performed on the overall log-delta abundances
 410 (difference between up and downstream effluent releases).

411



413 **Figure 5.** Projection of the samples upon the first two axes of a PCA performed on the log-
 414 delta abundances (absolute difference between up and downstream effluent releases) grouped
 415 by FR (A), season (B), and both (C). I and P stands for “intermittent” and “perennial”
 416 respectively, f and s stands for “fall” and “spring” respectively.

417

418 3.4. Functional responses

419 There was no difference in affinity for any trait category between up and downstream effluent
 420 releases (Appendix C), whereas affinity differed between intermittent and perennial reaches
 421 for 11 trait categories belonging to six different traits. Perennial reaches were characterized by
 422 higher proportions of organisms with long life span, using ovoviviparity and passive aquatic
 423 dispersal. They were also characterized by lower proportions of organisms that were
 424 univoltine, with aerial active dispersal, resistance forms, using cemented clutches and feeding
 425 on living macrophytes compared to intermittent reaches (Appendix C). The affinity of only
 426 one trait category differed between up and downstream effluent releases according to the FR:

427 at intermittent reaches, the proportion of organisms consuming dead plants (>1mm) was
428 higher upstream than downstream effluent releases at intermittent reaches and the opposite
429 was true at perennial reaches.

430 431 **4. Discussion**

432 In this study, we quantified the structural and functional responses to point-source sewage
433 effluents along a significant gradient of flow intermittence (FI; from 0 to 60%). As expected,
434 the effect of drying confounded with that of water quality degradation and in most cases,
435 current biomonitoring metrics did not distinguish between the effect of sewage and those
436 linked to drying. As all community metrics decreased linearly with increased FI, our second
437 prediction was partly validated and current biomonitoring approaches could be appropriately
438 applied to reaches prone to weak flow intermittence. Last, we identified six biological traits
439 responding to FI but not to water quality degradation. Confirming previous suggestions
440 (Morais et al., 2004; Soria et al., 2020; Stubbington et al., 2018), these results validate that
441 current biomonitoring approaches are unsuitable for application in intermittent rivers. If
442 intermittent rivers with very short (i.e. < 30 days per year) periods of drying could still be
443 monitored as their perennial counterparts, alternative biomonitoring approaches are needed
444 for intermittent rivers with longer periods of drying (i.e. > 30 days per year), along with
445 additional work to define a safe operational space for the application of a standardized
446 biomonitoring approaches in intermittent rivers (Gallart et al., 2017; Munné et al., 2021).

447 448 *4.1. Current macroinvertebrate-based biomonitoring tools are not suitable in intermittent* 449 *rivers*

450 Our overarching hypothesis that the ecological status inferred by macroinvertebrate-based
451 biomonitoring protocols is poorly related to water quality of naturally intermittent rivers was
452 largely supported through several lines of circumstantial evidence. First, taxonomic richness

453 was not different up and downstream effluent releases, and the difference in abundances
454 between up and downstream effluent releases were lower at intermittent reaches compared to
455 perennial ones. Consequently, and not surprisingly, IBG-DCE did not differ between up and
456 downstream effluent releases at intermittent reaches. The I2M2 scores were slightly lower
457 downstream effluent releases, but this was largely due to the differences detected in perennial
458 reaches, and not intermittent ones.

459

460 We found no differences between up and downstream effluent releases in terms of the relative
461 presence of any trait category tested here. The two trait categories considered in the I2M2,
462 namely multivoltinism and ovoviviparity, were selected for this biomonitoring tool because
463 they allowed a good trade-off between the discrimination efficiency, the specificity and the
464 stability of the metric, and because these trait categories were known to respond to changes in
465 water quality or habitat degradation (Mondy et al., 2012). Indeed, a high number of
466 reproductive cycles is a resilience strategy in ecosystems prone to disturbance (Townsend and
467 Hildrew, 1994). Ovoviviparity might prevent high egg mortality rate as they are protected
468 from harsh conditions (e.g. Dolédec and Statzner, 2008; Piscart et al., 2006), and it has been
469 shown to occur prominently during dry seasons (Bêche et al., 2006). However, for these two
470 trait categories we found no difference in their proportions between up and downstream
471 effluent releases. Voltinism varied between perennial and intermittent reaches but we did not
472 observe higher multivoltinism at intermittent reaches, although it is sometimes considered as
473 a strategy for aquatic macroinvertebrates to persist in the context of flow intermittence
474 (Bonada et al., 2007; Stubbington et al., 2017). The negative relationships between
475 ovoviviparity and FI is likely due to ovoviviparous (or partially ovoviviparous) taxa collected
476 that are highly dependent on flow permanence (e.g. *Gammarus* and *Pisidium*). Hence, using
477 ovoviviparity in biomonitoring methods in intermittent rivers might lead to unexpected
478 patterns. In contrast, water quality degradation and drying had combined effects on the

479 proportion of organisms that feed on dead plants, which were scarce (8 taxa per sample) up
480 and downstream effluent releases of intermittent reaches, but were found in higher
481 proportions upstream effluent releases at perennial reaches (14 taxa per sample). The lack of
482 trait responses to water quality degradation suggests that even when accounting for biological
483 traits, current biomonitoring metrics (i.e. I2M2) are poorly discriminant in intermittent rivers,
484 partly due to a certain degree in the functional redundancy observed in aquatic
485 macroinvertebrate communities from naturally intermittent rivers due to a strong
486 environmental filtering (Crabot et al., 2021a). While previous work highlighted that the I2M2
487 was more responsive to chemical water conditions compared to hydromorphological
488 alterations (Corneil et al., 2018), our study suggests that drying is an extreme case of flow
489 regime alteration which can hinder the detection of the effect of sewage.

490

491 Previous studies have suggested that applying current biomonitoring approaches in a context
492 of flow intermittence can be challenging (e.g. Cid et al., 2020; Datry et al., 2014; Morais et
493 al., 2004; Reyjol et al., 2014; Steward et al., 2012; Stubbington et al., 2018). To our
494 knowledge, this is the first time that a dedicated study confirms this challenging issue using
495 macroinvertebrate-based metrics along a gradient of flow intermittence. More than half of the
496 worldwide river network is prone to flow intermittence (Messenger et al., 2021), being as high
497 as 35% in France (Beaufort et al., 2018; Snelder et al., 2013), with obviously profound
498 implications in terms of management in the context of the Water Framework Directive in
499 Europe. Many water bodies can be considered as in poor or mediocre state while they are in
500 fact in a good ecological status. For example, in our study, the abundance of indicator species
501 including species from several EPT families such as Odontoceridae (Trichoptera),
502 Chloroperlidae and Capniidae (Plecoptera) decreased with pollution in perennial reaches but
503 not in intermittent reaches.

504 *4.2. Defining an operational spaces for assessing the ecological status of intermittent rivers*

505 Our second prediction was only partly supported, which suggests that identifying thresholds

506 in FI for shifting from current macroinvertebrate-based biomonitoring approaches to some

507 alternative is not straightforward. Confirming previous studies carried out in other intermittent

508 rivers (e.g. Crabot et al., 2021a; Datry et al., 2014; Piano et al., 2020), we found a decrease in

509 taxonomic richness, the number of EPT taxa, and the two French biomonitoring indices with

510 increasing FI. However, such relationships were significant only upstream effluent releases;

511 we found a linear effect as shown by others (e.g. Datry et al., 2014) but our limited number of

512 reaches (n=6) may hinder the identification of tipping-points in those relationships. This

513 indicates thresholds in FI may be difficult to identify and suggests that defining safe

514 operational spaces for applying current macroinvertebrate-based biomonitoring approaches in

515 intermittent rivers may be a difficult task. By the way, downstream effluent releases, the

516 combined effects of sewage and FI hindered these relationships.

517

518 We also found a strong temporal variability in community responses to FI and sewage,

519 corroborating the view of intermittent rivers as being highly dynamic (Datry et al., 2016;

520 Sarremejane et al., 2017a), which makes the assessment of the ecological status of these

521 prevalent ecosystems even more complex. The delta values in taxonomic richness, number of

522 OCH taxa and the IBG-DCE were higher in fall, after the dry season compared to the spring

523 pre-drying season, in accordance with previous results showing difference in biomonitoring

524 scores between seasons on intermittent streams in reference conditions (Munné and Prat,

525 2011). This is most likely due to legacy effects of drying events on aquatic communities and

526 processes, as recovery upon rewetting can take several months when observed (e.g., Datry,

527 2012; Piano et al., 2020). However, even in spring, before the dry season, the persisting effect

528 of drying was still too strong to allow detection of the effects of sewage effluents.

529 Nevertheless, in some cases, the organic pollution was dramatic, with as high as 9 mg/L of
530 DOC and up to 31133 MPN/100mL for *E. coli* (respectively twice and 26 times higher than
531 the values found upstream effluent releases of the same reach). But even in this case, the
532 responses of community and biomonitoring metrics were not different downstream compared
533 to upstream the effluent release due to the drying regime of the reach. These results suggest
534 that when applied before the drying season, current macroinvertebrate-based biomonitoring
535 approaches are poorly applicable to naturally intermittent rivers, which echoes some recent
536 calls to develop alternative approaches (e.g. Cid et al., 2020; Stubbington et al., 2018), either
537 focusing on aquatic biotic groups adapted to desiccation (e.g. using biological traits, Soria et
538 al., 2020), terrestrial and semi-aquatic organisms (e.g. riparian arthropods, Steward et al.,
539 2018) or including regional processes to set up dynamic references (Cid et al., 2020).

540

541 For those bioindicators based on a reference condition such as the I2M2 (Mondy et al., 2012),
542 a first step could be to include new references in the current classification that would be
543 representative of the effect of flow intermittence across ecoregions. Another path would be to
544 better take into account the temporal dynamic of these ecosystems (Cid et al., 2020).

545 Confirming previous observations, our results highlighted the high temporal variation of
546 communities at reaches prone to drying and its influence on biomonitoring methods (Crabot
547 et al., 2020; Leigh et al., 2019; Munné and Prat, 2011). As such, future models built to
548 describe reference conditions at intermittent reaches should include replicates of biotic and
549 abiotic samples at different seasons to capture the temporal variability of community
550 composition and avoid underestimating the diversity at these locations (Cid et al., 2020;
551 Sarremejane et al., 2017a). This could be especially important in Mediterranean headwater
552 streams with regular phases of stagnant disconnected pools, which greatly differ from flowing
553 and dry phases (Bonada et al., 2020; Gallart et al., 2017). This may be challenging because

554 the ideal biomonitoring tools should be designed according to 12 major criteria among with
555 the implementation comprising: low costs for sampling and sorting (field approaches), simple
556 sampling protocol and low cost for taxa identifications (no specialists in taxonomy required)
557 (Bonada et al., 2006a). Therefore, assessing the ecological status of intermittent rivers will
558 surely involve a shift in paradigm.

559

560 Many formerly perennial rivers are becoming intermittent (Döll and Schmied, 2012;
561 Messenger et al., 2021), raising the question whether current biomonitoring tools would also
562 fail in such novel-ecosystems (*sensu* Hobbs et al., 2013). Whereas we are unaware of
563 empirical data that could help to address this key question, our results suggest that current
564 macroinvertebrate-based approaches would be even less adequate in artificially intermittent
565 rivers, at least during the first times following the change in flow regime. This is because in
566 such contexts, a dramatic decrease in the number of species is expected, along with cascading
567 effects on functional traits due to poor functional redundancy (Aspin et al., 2019; Crabot et
568 al., 2021a). The key question is to determine the trajectory of communities in newly-
569 intermittent rivers, which remain unknown (Crabot et al., 2021b). If community trajectories
570 converge gradually towards those from naturally intermittent rivers, our results indicate
571 substantial improvements will be needed to accurately assess the ecological status of rivers
572 and streams prone to drying. If these trajectories take a different path, we are today unable to
573 foresee which biomonitoring tools will be needed and call for a research agenda taking into
574 account the alterations of flow regimes and appearance of hybrid ecosystems (*sensu* Hobbs et
575 al., 2013) in the Anthropocene era.

576

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587 **Author contributions**

588 **Julie Crabot**: Formal analysis, Writing – Original Draft. **Sylvain Dolédec**: Formal analysis,
589 Writing – Original Draft. **Maxence Forcellini**: Formal analysis. **Thibault Datry**:
590 Conceptualization, Methodology, Resources, Writing – Original Draft.

591 **Competing interest**

592 The authors have no competing interest to declare.

593 **Data availability**

594 Data will be available on Dryad at the temporary location:

595 <https://doi.org/10.5061/dryad.kpr4xh5w>

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