Natural disturbances may produce misleading bioassessment results: identifying metrics to detect anthropogenic impacts in intermittent rivers

Journal:	<i>Journal of Applied Ecology</i>
Manuscript ID	JAPPL-2019-00725
Manuscript Type:	Research Article
Date Submitted by the Author:	26-Jul-2019
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Key-words:	bioassessment, functional diversity, intermittent rivers and streams, macroinvertebrates, multiple stressors, natural stress, temporary rivers

Title:

Natural disturbances may produce misleading bioassessment results: identifying metrics to detect anthropogenic impacts in intermittent rivers

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26 **Short Running Title:** Natural disturbances produce misleading results in river
27 bioassessment

28
29 **SUMMARY**

- 30 1. Ecosystems experience natural disturbances and anthropogenic impacts that
31 affect biological communities and ecological processes. When natural disturbance
32 modifies anthropogenic impacts, current widely used bioassessment metrics can prevent
33 accurate assessment of biological quality.
- 34 2. Our aim was to assess the ability of biomonitoring metrics to detect
35 anthropogenic impacts at both perennial and intermittent sites, and in the later including
36 both flowing and disconnected pool aquatic phases. Specifically, aquatic
37 macroinvertebrates from 20 rivers were sampled along gradients of natural flow
38 intermittence (natural disturbance) and anthropogenic impacts to investigate their
39 combined effects on widely used river biomonitoring metrics (i.e. taxonomic richness
40 and standard biological indices) and novel functional metrics, including functional
41 redundancy (i.e. the number of taxa contributing similarly to an ecosystem function,
42 here a trophic function) and response diversity (i.e. how functionally similar taxa
43 respond to natural disturbance and anthropogenic impacts).
- 44 3. Only one widely used metric was able to detect anthropogenic impacts in
45 intermittent rivers when used during flowing phase. Several functional metrics also
46 detected anthropogenic impacts regardless of flow intermittence. Besides, functional
47 redundancy of the entire community remained effective even in disconnected pools.
- 48 4. Synthesis and applications. Our results showed that natural flow intermittence
49 can confound river bioassessment, and that a set of new functional metrics could be
50 used as effective alternatives to standard metrics in naturally disturbed intermittent

rivers. Our findings suggest that water managers should incorporate alternative functional metrics in the routine biomonitoring of naturally disturbed rivers.

KEYWORDS

Bioassessment, functional diversity, intermittent rivers and streams, macroinvertebrates, multiple stressors, natural stress, temporary rivers.

INTRODUCTION

Ecosystems experience natural disturbances and anthropogenic impacts that affect biological communities and ecological processes (Turner, Webb, Bowers, & Hastings 2003; Dornelas, 2010). Natural disturbances (e.g. droughts, floods, wildfires) have acted through evolutionary time and have resulted in species adapted to such abiotic stress (Lytle & Poff, 2004; Bowman et al., 2009). In contrast, anthropogenic impacts (e.g. pollution, land use changes, biological invasions, and recently, climate change) are relatively new (<10,000 yr), and may represent novel conditions for biota, impacting both biodiversity and ecosystem functioning (Olden, Poff, Douglas, Douglas, & Fausch 2004; Hooper et al., 2012). Given the predicted consequences of global change, both natural disturbances and anthropogenic impacts may increase in many regions worldwide (Olden, Poff, Douglas, Douglas, & Fausch 2004). Urgent action is thus needed to develop suitable assessment methods and management practices that consider the interaction of both type of impacts (Elliott & Quintino, 2007; Ghazoul, Burivalova, Garcia-Ulloa, & Vigo 2015).

Biomonitoring methods detect anthropogenic impacts using metrics based on aspects of ecosystem structure and function (Bonada, Prat, Resh, & Statzner 2006;

Pereira, Ferrier, Walter, & Al 2013). These metrics underpin biomonitoring programs that aim to halt and reverse biodiversity loss, to maintain and enhance the quality of ecosystems, and to assess the effectiveness of conservation and restoration measures, such as the Water Framework Directive (WFD; European Commission, 2000), the Convention on Biological Diversity (CBD, 2010) or the International Union for Conservation of Nature (IUCN, 2016). Problematically, few current metrics integrate the effects of natural disturbances, which can confound the assessment of anthropogenic impacts and thus lead to inappropriate management actions (Tockner, Pusch, Borchard, & Lorang 2010; Gutiérrez-Cánovas et al., 2019; Pitacco et al., 2019).

Functional metrics based on biological traits have shown to be promising tools for detecting anthropogenic impacts in different types of ecosystems (Laliberté et al., 2010; Statzner & Beche, 2010; Mouillot, Graham, Villéger, Mason, & Bellwood 2013), and to be more accurate for the biomonitoring of naturally disturbed systems (Bruno, Gutiérrez-Cánovas, Velasco, & Sánchez-Fernández 2016a; Belmar et al., 2019).

However, studies assessing the combined effects of natural disturbances and anthropogenic impacts on the functional composition of biological communities remain limited (e.g. Elliott & Quintino, 2007; Mouillot, Graham, Villéger, Mason, & Bellwood 2013; Bruno, Gutiérrez-Cánovas, Sánchez-Fernández, Velasco, & Nilsson 2016b).

Additionally, the prediction capacity of functional metrics might improve when incorporating traits responding to environmental changes (i.e. response traits), and to the effect of such changes on ecosystem functioning (i.e. effect traits) (Suding et al., 2008; Laliberté et al., 2010). Consequently, approaches that more directly link community dynamics to ecosystem functioning are emerging.

Nearly 50% of the current global river network has intermittent flow (Datry et al., 2014). Intermittent rivers, also known as temporary rivers in a broad sense, are dynamic

101 systems that typically shift between flowing, disconnected-pools and dry periods,
102 named hereafter as aquatic phases (Gallart et al., 2012, 2017). Repeated shifts between
103 aquatic phases reorganize biological communities and, therefore, flow intermittence
104 represents a natural disturbance (Lake, 2000; Leigh & Datry, 2017). Intermittent rivers
105 also experience anthropogenic impacts, such as hydrological alterations (flow
106 regulation, surface and/or groundwater extractions), geomorphological impacts (e.g.
107 sediment extraction and physical habitat modifications), pollutants (e.g. heavy metals
108 and nutrient additions) and biological invasions (Chiu, Leigh, Mazor, Cid, & Resh
109 2017). Detection of these impacts at intermittent rivers is challenging because the
110 reliability of standard biomonitoring metrics is generally uncertain for such naturally
111 disturbed systems (Chiu, Leigh, Mazor, Cid, & Resh 2017; Cid et al., 2017). As the
112 global extent of intermittent rivers increases due to climate change and water resource
113 pressures (Döll & Schmied, 2012), understanding the combined effects of natural flow
114 intermittence and anthropogenic impacts on widely used and novel river biomonitoring
115 metrics is needed to underpin reliable biological quality assessments.

116 Our aim was to assess the ability of biomonitoring metrics to detect anthropogenic
117 impacts at both perennial and intermittent sites, and in the later including both flowing
118 and disconnected pool aquatic phases. Specifically, aquatic macroinvertebrates from 20
119 rivers were sampled along gradients of natural flow intermittence (natural disturbance)
120 and anthropogenic impacts to investigate their combined effects on widely used river
121 biomonitoring metrics (i.e. taxonomic richness and standard biological indices) and
122 novel functional metrics based on biological traits. Different types of responses to
123 natural and anthropogenic gradients were used to assess the reliability and calibration
124 requirements of potential metrics to both perennial and intermittent rivers (Fig. 1): (1)
125 single, when a metric responds to anthropogenic impacts but not to natural flow

126 intermittence (Fig. 1a), indicating high reliability without calibration; (2) additive, when
127 the effects of natural flow intermittence add to those of anthropogenic impacts, but
128 effects do not interact (Fig. 1b), indicating that this metric could be reliable after
129 proportional calibration with a constant term; (3) interactive, when the effects of natural
130 flow intermittence reduce or enhance those of anthropogenic impacts, and leads to
131 antagonistic (Fig. 1c), synergistic (Fig. 1d) or opposing (Fig. 1e) interactions (Feld,
132 Segurado, & Gutiérrez-Cánovas 2016). Metrics with these interactive responses could
133 be reliable only after non-proportional calibration (e.g. adapting reference conditions
134 and response gradient to anthropogenic impacts).

135

136 **MATERIALS AND METHODS**

137

138 **Study area**

139 The study was conducted in 20 river sites in the north-eastern Iberian Peninsula (Fig.
140 S1). The area has a mediterranean-climate (i.e. Csa and Csb; Kottek, Grieser, Beck,
141 Rudolf, & Rubel 2006), with high seasonal variability in precipitation and thus
142 streamflow (Bonada & Resh, 2013; Cid et al., 2017). Ten of the sites are intermittent
143 and 10 are perennial. Sites were located in different sub-basins in the Ebro, Júcar and
144 Catalan catchments and experience different degrees of anthropogenic impact and river
145 regime. Sites ranged from 6 to 1,100 m a.s.l. and drained calcareous catchments with
146 discharges ranging from 0 to 417 L·s⁻¹.

147

148 **Macroinvertebrate data**

149 Macroinvertebrates were collected five times per site (i.e. n = 100 samples) at six-week
150 intervals between April and December 2015, to capture the assemblages present during

151 flowing phases and in disconnected pools. In total, 91 samples were taken, because five
152 sites were dry between one or three occasions. Samples were collected using a 250 µm-
153 mesh D-net across all available microhabitats (i.e. different mineral and organic
154 substrates), following the national standard quantitative sampling protocol
155 (MAGRAMA, 2013). Samples were preserved in 4% formaldehyde, and the
156 macroinvertebrates identified to the lowest taxonomic resolution possible, usually
157 genus, but with some Chironomidae and Ceratopogonidae identified to subfamily or
158 tribe (See DATA ACCESSIBILITY). Overall, 194 macroinvertebrate taxa were
159 identified.

160 Macroinvertebrate effect and response traits were characterized. Effect traits
161 describe the potential contribution of macroinvertebrate organisms to ecosystem
162 functioning through the trophic transfer of resources and energy from basal to higher
163 organisms (Suding et al., 2008). Response traits characterize the resistance and
164 resilience of communities to disturbances and, thus, inform on community responses to
165 environmental changes (Suding et al., 2008; McLean et al., 2019). For the effect traits,
166 trophic characteristics of each genus were gathered from the Freshwater Information
167 Platform (<http://www.freshwaterecology.info>) (Moog, 2002; Schmidt-Kloiber &
168 Hering, 2015). Response traits were compiled from Tachet, Richoux, Bournaud, and
169 Usseglio-Polatera (2010) (Table S1). For each genus, traits were assigned using a fuzzy
170 coding approach (i.e. a degree of affinity assigned to each trait category, according to
171 the frequency of occurrence within the genus; Chevenet, Dolédec, & Chessel 1994).
172 For genera without traits or for taxa not identified to genus, averaged data from other
173 genera within the same family or from family-level traits were used. This was the case
174 for 24 taxa identified to genus, as well as for the Chironomidae and Ceratopogonidae

175 identified to subfamily or tribe (See DATA ACCESSIBILITY). Prior to analysis, fuzzy-
176 coded data were converted into percentages of affinity for each trait.

177

178 **Characterisation of flow intermittence and anthropogenic impacts**

179 The degree of flow intermittence at each site was obtained from different descriptors.
180 First, TREHS (Temporary Rivers Ecological and Hydrological Status) software
181 (<http://www.lifetriders.eu/products/trehs-software/>; Gallart et al., 2017) was used to
182 classify the rivers' regime and to identify potential hydrological alterations (Table S2)
183 to differentiate sites affected by natural flow intermittence from those with human-
184 driven flow intermittence. The degree of flow intermittence during the study period (i.e.
185 from April 2015 until December 2015) was calculated from temperature data loggers
186 (UA-002 HOBO), from which is possible to infer the aquatic phases (Gungle, 2006).
187 Two data loggers were installed at each site: one in a riffle, to determine the day flow
188 ceased and resumed, and one in a pool, to identify the day the reach dried completely.
189 Data were used to calculate two descriptors of flow intermittence for each site: the
190 number of days in the disconnected pool phase since last sample was taken (DPI), and
191 the total number of zero-flow days (i.e. disconnected pool or dry riverbed) during the
192 30-week study period (ZF_T).

193 The extent of anthropogenic impacts at each site was measured using the number
194 of impacts in the Mediterranean reference criteria (MRC), which include invasive
195 species, diffuse pollution sources, land use intensity, riparian vegetation, river
196 geomorphology, instream habitat conditions and hydrological alterations (Sánchez-
197 Montoya et al., 2009). To facilitate interpretation, the number of MRC index impacts
198 (i.e. 0 = non-impacted, to 20 = extremely impacted) was used as a descriptor variable
199 (hereafter, number of impacts) (See Table S3 for more details). Physicochemical

parameters of each river were also measured: dissolved oxygen, conductivity, temperature, phosphates, nitrates and Chl-a (Table S2). However, only the number of impacts was used, as it represents a broad spectrum of impacts and was correlated with most of the physicochemical parameters in our dataset (Table S2) and elsewhere (Sánchez-Montoya et al., 2009).

Metrics calculation

Two types of metrics were calculated to characterize biotic responses to flow intermittence and the anthropogenic impacts: widely used and functional. As widely used metrics, taxonomic richness based on invertebrate families (Sfam) or genera (Sgen) and several tolerance-based biological indices used in the Iberian Peninsula were calculated: IBMWP (Alba-Tercedor et al., 2002), IASPT (Jáimez-Cuéllar et al., 2002), and IMMi-T (Munné & Prat, 2009) (Table 1).

For the functional metrics, functional redundancy (i.e. FR; the number of taxa or individuals contributing similarly to an ecosystem function, here a trophic function) and response diversity (i.e. RD; how functionally similar taxa respond to natural disturbance and anthropogenic impacts) (Suding et al., 2008) were calculated (Fig. 2). For FR metrics, using a classification of macroinvertebrates into functional trophic groups (i.e. effect traits; see Appendix S1) was used to obtain the average abundance (ab) and trait richness (i.e. presence-absence; pa) for the entire community and each trophic group: omnivorous (omniv), shredders (shr), predators (pred), grazers (gra), gatherers (gat) and filter feeders (ff) (Fig. 2, Table 1). For RD, traits related to resilience and resistance strategies (i.e. response traits; see Appendix S1) were used to estimate functional richness (FRic, Villéger, Mason, & Mouillot 2008) and functional dispersion (FDis,

224 Laliberté & Legendre, 2010; hereafter, RD) for the whole community and each trophic
225 group, all of them using abundance (ab) measures (Fig. 2, Table 1).

226

227 **Statistical analysis**

228 Two data sets were analysed. One considered all data collected during aquatic phases
229 (i.e. flow and disconnected pools), to identify metrics that respond to anthropogenic
230 impacts independently of flow. The second dataset included only flowing-phase
231 samples, as required in current river biomonitoring protocols. To determine the
232 reliability of each metric to detect anthropogenic impacts across a gradient of flow
233 intermittence, linear mixed-effects models (LME) were fitted with a Gaussian error
234 distribution to model all metric responses. Before analyses, to reduce distribution
235 skewness and improve linearity, a square-root-transformation was applied to Sfam,
236 Sgen and IBMWP, and a log- or square-root-transformation was applied to almost all
237 functional metrics. Predictor variables (i.e. the number of impacts, DPi and ZF_T) were
238 standardised to mean=0 and SD=1 to allow for model coefficient comparison.

239 For the all aquatic-phases dataset, LMEs were fitted using the number of impacts,
240 DPi and ZF_T and their pairwise interactions as fixed factors (i.e. DPi × number of
241 impacts, and ZF_T × number of impacts). For the flowing-phase dataset, the same
242 analysis was performed but excluding DPi. All models included site as a random factor
243 to account for the non-independence of samples collected from the same location. A
244 multi-model inference approach was adopted to quantify predictor's standardised effect
245 sizes and significance (See Appendix S2 for more details).

246 For each metric, the combined effect type was determined using the regression
247 coefficient sign (+ or -) and p-value of individual predictors and their interactions (Feld,
248 Segurado, & Gutiérrez-Cánovas 2016). Under this framework, a non-significant

249 interaction term and one significant predictor coefficient indicate a single effect,
250 whereas two or more significant single predictor coefficients indicate an additive effect.
251 Interactive (antagonistic, synergistic or opposing) effects were identified by: a
252 significant ($p < 0.05$) or potential (p-value between 0.05 and 0.25) interaction and a
253 single interaction explaining $rc^2 \geq 5\%$ of variance. Negative coefficients for individual
254 predictors with a positive interaction coefficient indicated an antagonistic effect.
255 Negative individual predictor and interaction coefficients indicated a synergistic effect.
256 Different coefficient signs between individual predictors indicated an opposing effect.
257 Effective metrics should be responsive to anthropogenic impacts (i.e. explaining a high
258 percentage of total variance and $r^2m > 0.20$), and show a similar pattern across a
259 gradient of flow intermittence, which is reflected by non-interactive responses.
260 For all predictors (i.e. number of impacts, ZF_T and DPi), null models were used to
261 determine whether observed functional metric responses to anthropogenic impacts
262 reflected taxonomic variation (i.e. the probability of finding more or fewer abundance or
263 trait categories due to the number of taxa; see Appendix S3).

264 All analyses were conducted in R version 3.4.1 (R Core Team, 2015), using the
265 packages “lme4” (Bates, Maechler, Bolker, & Walker 2015), “MuMIn” (Barto’n, 2017)
266 and “variancePartition” (Hoffman & Schadt, 2016). The code and functions used to run
267 these analyses are available at DATA ACCESSIBILITY.

268

269 RESULTS

270 Overall, metrics decreased with increasing the number of impacts and with flow
271 intermittence predictors (i.e. DPi and ZF_T) (Table 2 and Table 3). However, responses
272 varied between metrics and between datasets (i.e. flowing-phase and all aquatic-phases).

273

274 **Widely used metrics**

275 All widely used metrics were negatively related to the number of impacts, explaining
 276 >50% of total variance (Table S4 and Table S5). A single effect of the number of
 277 impacts on flowing-phase dataset (but not at the all aquatic-phases dataset) was found
 278 for IBMWP (Fig. 3h, Table 3). For other widely used metrics, the most common effect
 279 was the antagonistic (Fig. 3, Table 2 and Table 3). All widely used metrics from both
 280 datasets were identified as responsive metrics ($r^2m > 0.20$), with r^2m values ranging
 281 from 0.39 to 0.54 for the all aquatic-phases dataset (Table 2) and from 0.24 to 0.43 for
 282 flowing-phase samples (Table 3). According to the criteria in Fig. 1, IBMWP is reliable
 283 for flowing-phase assessments in both perennial and intermittent rivers (i.e. universal
 284 metric; Table 4), whereas other widely used metrics would require calibration before
 285 use in intermittent rivers.

287 **New functional metrics**

288 The number of impacts explained >50% of the variance for most functional metrics but,
 289 in some cases, ZF_T, DPi and their interactions with the number of impacts explained a
 290 similar percentage (Table S4 and Table S5). A single effect of the number of impacts
 291 was observed for several FR and RD metrics (Table 2 and Table 3): FR.ab, FR.shr.ab,
 292 RD.gra.ab, RD.gat.ab and RD.ff.ab for the all aquatic-phases dataset, and FR.ab,
 293 FR.shr.pa, FR.ff.pa, RD.shr.ab, RD.gra.ab, RD.gat.ab and RD.ff.ab for the flowing-
 294 phase dataset. Among them, the most responsive metrics ($r^2m > 0.20$) were FR.ab (r^2m
 295 =0.23) and RD.ff.ab (r^2m =0.29) from the all aquatic-phases dataset, and FR.ff.pa (r^2m
 296 =0.33) from the flowing-phase dataset (Table 4). Following our criteria (Fig. 1), FR.ab,
 297 FR.ff.pa and RD.ff.ab represent universal metrics (Table 4), with FR.ab and RD.ff.ab

298 reliable for samples collected in both flowing and disconnected pool phases, and
299 FR.ff.pa reliable only for flowing-phase samples.

300 For other functional metrics showing significant responses to predictors, the
301 number of impacts and ZF_T resulted in interactive effects. For the all aquatic-phases
302 dataset, nine metrics showed an antagonistic response and one synergistic (Table 2),
303 whereas for the flowing-phase dataset, all interactive metrics showed an antagonistic
304 response (Table 3). We also observed interactions between the number of impacts and
305 DPi resulting in four antagonistic, one synergistic and one opposing effect on the all
306 aquatic-phases metrics (Table 2). Among them, several functional metrics were
307 identified as responsive metrics ($r^2_m > 0.20$), with r^2_m values ranging from 0.20 to 0.39
308 in the all aquatic-phases dataset, and from 0.24 to 0.45 in the flowing-phase dataset
309 (Table 4). According to our criteria (Fig. 1), these metrics could be reliable in
310 intermittent rivers after non-proportional calibration.

311 Null models indicated that all predictor terms had non-random effects on FR.pa,
312 FR.ab and FR.omniv.pa models including all aquatic phases (Table S6) and, thus,
313 functional metric responses reflected anthropogenic impacts and/or flow intermittence,
314 not taxonomic variation. For the flowing-phase dataset, several metrics had non-random
315 effects on all model terms (i.e. FR.ab, FR.omniv.pa, FR.pred.pa, FR.omniv.ab,
316 FR.pred.ab and FRic models; Table S7), and null models indicated that the number of
317 impacts term had non-random effects on all models except for FR.shr.ab and FR.ff.ab
318 (Table S7).

319

320 **DISCUSSION**

321 Our results showed that natural disturbances (i.e. flow intermittence) can confound
322 biological quality assessments by altering metric responses to anthropogenic impacts.

323 Most of our widely used and new functional metrics decreased with increasing
324 anthropogenic impacts, but showed an interactive antagonistic response with flow
325 intermittence. Thus, most metrics would not accurately indicate the biological quality of
326 intermittent rivers, and would require calibration (Fig. 1c). However, the widely used
327 IBMWP index and few functional metrics could provide reliable biological assessments
328 in intermittent rivers when applied during the flowing phase, and the functional
329 redundancy based on the whole community abundance (FR.ab) remained effective even
330 in disconnected pools.

331 Compared to perennial rivers, aquatic communities in intermittent rivers typically
332 support fewer taxa and a higher proportion of generalists, due to environmental
333 harshness (Bogan et al., 2017; Soria, Leigh, Datry, Bini, & Bonada 2017). As a result,
334 the usefulness of many widely used biomonitoring metrics based on taxon diversity is
335 limited, especially when using standard values obtained from perennial rivers (Prat et
336 al., 2014; Chiu, Leigh, Mazor, Cid, & Resh 2017). Similar limitations of widely used
337 metrics have been observed in other naturally disturbed ecosystems such as when using
338 riparian plant communities as indicators in intermittent rivers (Bruno, Gutiérrez-
339 Cánovas, Sánchez-Fernández, Velasco, & Nilsson 2016b) or when using invertebrates
340 in estuaries (Elliott & Quintino, 2007) or saline rivers (Gutiérrez-Cánovas et al., 2019).
341 Nonetheless, our results suggested that one widely used metric, the IBMWP index,
342 could be applied in intermittent rivers during flowing phases. This aligns with other
343 studies in mediterranean-climate rivers (e.g. Munné & Prat, 2011; Prat et al., 2014;
344 Mazor, Stein, Ode, & Schiff 2014), which suggest that widely used metrics may work at
345 intermittent sites if flowing phases are sufficiently long and predictable (Gallart et al.,
346 2012, 2017). For example, Munné and Prat (2011) found no differences for IBMWP
347 between perennial and intermittent rivers during wet years, as flow permanence in

intermittent rivers was high. However, during dry years, IBMWP values varied considerably even if samples were collected during flowing phases (Munné & Prat, 2011). Thus, the wide spatial and temporal hydrological variability within intermittent rivers (e.g. different dry phase duration at different locations at different years) could produce misleading bioassessment results in most cases (Mazor, Purcell, & Resh 2009; Chessman, Jones, Searle, Growns, & Pearson 2010; Papastergiadou & Manolaki, 2012), especially in less predictable, seasonal climate types (Tonkin, Bogan, Bonada, Rios-Touma, & Lytle 2017). Hence, to avoid misleading biological quality assessments in intermittent rivers, either existing metrics need to be recalibrated (e.g. by incorporating long-term variability in these metrics) or new metrics, as those presented here, require development (Munné & Prat, 2011; Stubbington et al., 2018).

Most of our new functional metrics responded differently at perennial and intermittent sites, as evidenced by antagonistic effects. Our results align with recent studies considering flow intermittence and salinity as natural disturbances combined with other anthropogenic impacts (Belmar et al., 2019; Gutiérrez-Cánovas et al., 2019). The fact that intermittent rivers often showed a weaker reduction in functional metrics may reflect strong trait filtering in naturally disturbed ecosystems (Mouillot, Graham, Villéger, Mason, & Bellwood 2013). Moreover, traits enabling species' persistence in certain naturally disturbed environments may contribute to be co-tolerance of other disturbances (Vinebrooke et al., 2004). In intermittent rivers, resistance and resilience traits that enable species to persist in highly variable flow conditions might also enable them to persist under certain anthropogenic impacts (Bonada & Resh, 2013; Belmar et al., 2019). For example, macroinvertebrates with mechanisms for tolerating low dissolved oxygen concentrations may in turn be indicative of either flow intermittence or organic pollution (Stubbington et al., 2017). Thus, metrics that indicate adaptations to

373 flow intermittence could be used for biomonitoring anthropogenic impacts only if they
374 are calibrated differently for perennial and intermittent rivers.

375 Our results also identified functional metrics with similar responses to
376 anthropogenic impacts in perennial and intermittent rivers (i.e. single effect), indicating
377 their ability to detect impacts regardless of flow intermittence. The most responsive
378 metrics during flowing phases were functional redundancy based on the whole
379 community abundance (FR.ab) and the number of filter feeder's presence-absence
380 (FR.ff.pa), as well as abundance-weighted response diversity of filter-feeders
381 (RD.ff.ab), with FR.ab also responsive in disconnected pools. Thus, for example, the
382 decrease in RD.ff.ab with increasing anthropogenic impacts indicates a reduction in
383 resistance and/or resilience traits (i.e. response traits) within this functional trophic
384 group. The decrease in FR.ff.pa also evidenced taxonomic homogenization within this
385 functional trophic group as the number of anthropogenic impacts increased (Laliberté et
386 al., 2010; Gagic et al., 2015). Our results thus indicate that metrics derived from
387 functional trophic groups, which are not sensitive to natural disturbances, could act as
388 universal metrics in naturally disturbed ecosystems such as intermittent rivers.

389 By using the response-effect trait approach, functional metrics may provide
390 detailed information on environmental changes and their effects on ecosystem
391 functioning (Suding et al., 2008; Laliberté et al., 2010; Mouillot, Graham, Villéger,
392 Mason, & Bellwood 2013). Response diversity (RD) metrics might help to differentiate
393 community responses to both natural disturbances and anthropogenic impacts, whilst
394 functional redundancy (FR) metrics based on effect traits might help to anticipate the
395 effects of that change on ecosystem functioning (Suding et al., 2008). For example, the
396 decrease in FR for filter feeder taxa (FR.ff.pa) might affect organic matter processing
397 and thus change ecosystem functioning (Bogan et al., 2017). As a consequence,

community structure might also be less resistant to environmental changes and therefore increase the vulnerability to future anthropogenic impacts (Gutiérrez-Cánovas, Sánchez-Fernandez, Velasco, Millán, & Bonada 2015; McLean et al., 2019). Similarly, the distinct decrease of FR and RD metrics in the predator functional trophic group (i.e. FR.pred.pa, FR.pred.ab and RD.pred.ab) in intermittent rivers could signal potential effects of anthropogenic impacts on predation rates, and inform about cascade effects stemming from reduced predator abundance (Hooper et al., 2012; Rodríguez-Lozano, Verkaik, Rieradevall, & Prat 2015).

Given the different predictability, duration and spatial patterns of the aquatic phases in intermittent rivers (Bonada & Resh, 2013; Leigh & Datry, 2017), our proposed functional metrics could contribute to improved bioassessment of these rivers. Even though the widely used IBMWP index detected anthropogenic impacts in intermittent rivers during flowing phases, this needs to be considered with caution as its reliability may vary between wet and dry years (Munné & Prat, 2011). Compared to widely used metrics, the functional redundancy based on whole community abundance (FR.ab) could be even applied during disconnected pools. Besides, functional metrics are more accurate in detecting ecosystem degradation and may represent a step forward in the management of naturally disturbed ecosystems (e.g. Bruno, Gutiérrez-Cánovas, Sánchez-Fernández, Velasco, & Nilsson 2016b; Belmar et al., 2019). These metrics might enable prediction of biological responses to intensifying anthropogenic impacts in a climate change context (Belmar et al., 2019). Therefore, water managers should start incorporating alternative functional metrics in the routine biomonitoring of naturally disturbed rivers. Future research should test such metrics in datasets encompassing multi-year time periods, across different climates, and for specific anthropogenic impacts, as well as on other taxonomic groups.

423

424 **AUTHORS' CONTRIBUTIONS**

425 MS, CG-C and NC conceived the ideas and designed the methodology, assisted by NB.
426 NC, DV, GB, PF and PR-L collected the data; RA identified the macroinvertebrate data;
427 MS and CG-C analysed the data. MS, CG-C, NB and NC led the writing of the
428 manuscript and FG, JL, NP, PL contributed to the drafts. All authors gave final approval
429 for submission.

430

431 **ACKNOWLEDGEMENTS**

432 The study was supported by the LIFE+ TRivers (LIFE13 ENV/ES/000341) project. We
433 acknowledge the contribution of FEHM lab members and students who participated in
434 fieldwork. CG-C was supported by a “Juan de la Cierva-Formación” research contract
435 (MINECO, FJCI-2015-25785). We especially thank Rachel Stubbington for her
436 supervision and useful suggestions that greatly improved the manuscript.

437

438 **DATA ACCESSIBILITY**

439 Data will be available in the Dryad Digital Repository.

440

441 **REFERENCE S**

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669

670 **FIGURES AND TABLES**

671 **Figure 1.** Potential effects of natural disturbances and anthropogenic impacts on a
672 biomonitoring metric and the associated reliability for its use in bioassessment, with a
673 special focus on intermittent rivers. Colours indicate communities not affected (blue)
674 and affected (orange) by natural disturbance.

675

676 **Figure 2.** Analytical framework for the functional metrics used in this study (i.e.
677 functional redundancy, FR, and response diversity, RD). For FR functional trophic
678 groups were used as effect traits. For RD biological traits related to resilience and
679 resistance strategies were used as response traits (Table S1). Both FR and RD are
680 represented as the average abundance (ab) or trait richness (i.e. presence-absence; pa)
681 for each functional group. Circle size is proportional to abundance. Black circles:
682 species present; white circles: species absent; grey circles: species not included in the
683 functional effect group of the example (i.e. shredders). PCoA: Principal Coordinate
684 Analysis.

685

686 **Figure 3.** Plots showing the individual response of the five widely used metrics from
687 the all aquatic-phases dataset (Sfam, Sgen, IBMWP, IASPT and IMMi-T; a to e,
688 respectively; Table 1) and the flowing-phase dataset (f to j) along an anthropogenic
689 impacts gradient (N° impacts). Full circles represent perennial rivers and empty circles
690 intermittent ones. Fitted values for LMEs models are represented as blue lines for
691 perennial rivers and as orange lines for intermittent rivers. All responses were
692 antagonistic except for h, for which a single effect was observed.

693

Type and description of effect		Reliability of the metric in bioassessment	Metric designation
(a) Single No effect of natural disturbance: the effect of anthropogenic impacts is independent of the effect of natural disturbance.		The metric is reliable and can be used both in systems affected and not affected by natural disturbance (e.g. perennial and intermittent rivers). No calibration needed.	Universal
(b) Additive The effect of natural disturbance adds to the effect of anthropogenic impacts, but they do not interact.		The metric could be reliable in ecological systems affected by natural disturbance only after proportional calibration with a constant term.	Flexible
(c) Antagonistic Interactive. The effect of natural disturbance reduces the effect of anthropogenic impacts.		The metric could be reliable in ecological systems affected by natural disturbance only after non-proportional calibration (e.g. adapting reference conditions and response gradient to anthropogenic impacts).	Specific
(d) Synergistic Interactive. The effect of natural disturbance enhances the effect of anthropogenic impacts.
(e) Opposing Interactive. The effect of natural disturbance is the opposite to that of anthropogenic impacts.

694

695 Figure 1 (Soria et al.)

Figure 2 (Soria et al.)

Figure 3 (Soria et al.)

729 **TABLES**

730 **Table 1.** Community metrics used in this study. Functional redundancy (FR) and
731 response diversity (RD) metrics were calculated using effect and response traits,
732 respectively. *Type of data:* FR and RD were calculated as the average abundance (ab)
733 and/or richness (i.e. presence-absence; pa) for each functional trophic group and for the
734 entire community. *Omnivorous* taxa had mixed feeding habits, including a variety of
735 shredding, grazing and gathering affinities. See methods for further details.

Type of metric	Metric	Description	Type of data
<i>Widely used</i>	Sfam	Taxon richness at the family level	pa
	Sgen	Taxon richness at the genus level	pa
	IBMWP	Iberian Biological Monitoring Working Party (Alba-Tercedor et al., 2002)	pa
	IASPT	Iberian Average Score Per Taxon (Jáimez-Cuéllar et al., 2002)	pa
	IMMi-T	Multimetric index for Mediterranean rivers (Munné & Prat, 2009)	pa
Functional redundancy	FR	Functional redundancy of the entire community	pa, ab
	FR.omniv	Functional redundancy of the omnivorous (omniv) functional group	pa, ab
	FR.shr	Functional redundancy of the shredder (shr) functional group	pa, ab
	FR.pred	Functional redundancy of the predator (pred) functional group	pa, ab
	FR.gra	Functional redundancy of the grazer (gra) functional group	pa, ab
	FR.gat	Functional redundancy of the gatherer (gat) functional group	pa, ab
	FR.ff	Functional redundancy of the filterers (ff) functional group	pa, ab
Response diversity	RD	Response diversity of the entire community	ab
	RD.omniv	Response diversity of the omnivorous (omniv) functional group	ab
	RD.shr	Response diversity of the shredders (shr) functional group	ab
	RD.pred	Response diversity of the predators (pred) functional group	ab
	RD.gra	Response diversity of the grazers (gra) functional group	ab
	RD.gat	Response diversity of the gatherers (gat) functional group	ab
	RD.ff	Response diversity of the filterers (ff) functional group	ab
	FRic	Functional richness of the response traits	ab

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Table 2. LME results from the all aquatic-phases dataset. R^2m : marginal goodness of fit. R^2c : conditional goodness of fit. (-): not selected in the top model. ns: non-significant effect. Significant p-values (< 0.05) are indicated as * for all variables. Potential p-values (i.e. between 0.05 and 0.25) are also indicated for interactions as (.). N° impacts: number of impacts. DP_i: number of days in the disconnected pool phase since last sample was taken. ZF_T: total number of zero flow days. See Table 1 for further details about metrics.

	Intercept	N° impacts	ZF _T	DP _i	N° impacts × ZF _T	N° impacts × DP _i	R^2m	R^2c	Effect type: N° impacts × ZF _T	Effect type: N° impacts × DP _i
Sfam	5.488	-0.105 *	-2.725	-0.003	0.262 (.)	-	0.39	0.77	antagonistic	single
Sgen	6.392	-0.135 *	-3.529	-0.006	0.355 (.)	-0.0001	0.45	0.75	antagonistic	single
IBMWP	0.973	-0.024 *	-0.379	-0.002	0.056 (.)	0.0001	0.31	0.82	antagonistic	single
IASPT	0.982	-0.032 *	-0.762	-0.004 *	0.083 (.)	0.0003	0.44	0.73	antagonistic	additive
IMMiT	1.045	-0.045 *	-1.116 *	-0.005 *	0.126 *	0.0002	0.54	0.86	antagonistic	additive
FR.pa	1.316	-0.006	-0.265	0.001	-0.03 (.)	-0.0002	0.22	0.22	synergistic	ns
FR.ab	3.949	0.064 *	1.481	-0.005	0.187	-	0.23	0.47	single	single
FR.omniv.pa	1.45	-0.085 *	-2.332 *	-0.004	0.278 *	-	0.32	0.62	antagonistic	single
FR.shr.pa	1.093	-0.043 *	-0.272	-0.01	-	0.001 (.)	0.23	0.36	single	antagonistic
FR.pred.pa	3.913	-0.092 *	-3.085 *	0.001	0.244 (.)	-	0.39	0.65	antagonistic	single
FR.gra.pa	1.671	-0.016	-0.161	-0.005 *	-	0.0004	0.10	0.57	ns	single
FR.gat.pa	9.262	-0.274 *	-7.735 *	-0.02	0.92 *	-0.004 (.)	0.34	0.56	antagonistic	synergistic
FR.ff.pa	1.492	-0.023	-0.914	-0.005	0.099 (.)	0.001 (.)	0.13	0.28	antagonistic	antagonistic
FR.omniv.ab	4.476	-0.328 *	-5.028	-0.033	1.498 *	-0.003	0.33	0.64	antagonistic	single
FR.shr.ab	2.097	-0.083 *	-0.171	-0.01	-	-	0.09	0.26	single	single
FR.pred.ab	5.261	-0.133 *	-3.665	0.004	0.471 *	-	0.2	0.39	antagonistic	single

	Intercept	N° impacts	ZF _T	DPi	N° impacts × ZF _T	N° impacts x DP _i	R ² _m	R ² _c	Effect type: N° impacts x ZF _T	Effect type: N° impacts x DPi
FR.gra.ab	5.307	-0.103	-1.879	-0.014	0.761 *	0.001	0.15	0.42	antagonistic	ns
FR.gat.ab	6.592	0.06	1.242	-0.016	0.292	-0.001	0.06	0.43	ns	ns
FR.ff.ab	4.616	-0.069	-3.565	-0.044	0.307	0.009 *	0.15	0.19	ns	antagonistic
RD.ab	1.101	-0.002 *	-0.037 *	0	0.006 *	-0.00002	0.36	0.36	antagonistic	single
RD.omniv.ab	0.059	-0.003	-0.012	0	-	-	0.03	0.08	ns	ns
RD.shr.ab	0.111	-0.008 *	-0.005	-0.002	-	0.0002 (.)	0.17	0.23	single	antagonistic
RD.pred.ab	0.465	-0.004	-0.043	0.001	-	-0.0004 *	0.33	0.33	ns	opposing
RD.gra.ab	0.452	-0.007 *	-0.145	0	0.005	-	0.11	0.1	single	single
RD.gat.ab	0.156	-0.005 *	0.01	0	-	-	0.16	0.16	single	single
RD.ff.ab	0.287	-0.016 *	-0.37	0.001	0.028	-	0.29	0.45	single	single
FRic	0.235	-0.011 *	-0.194	0	0.023 (.)	-	0.35	0.71	antagonistic	single

Table 3. Results of LMEs from the dataset including only the flowing phase. See Table 2 for further details.

	Intercept	N° impacts	ZF _T	N° impacts x ZF _T	R ² _m	R ² _c	Effect type
Sfam	5.467	-0.104 *	-2.441	0.258 (.)	0.36	0.71	antagonistic
Sgen	42.014	-1.546 *	-45.606 *	4.187 *	0.43	0.65	antagonistic
IBMWP	0.97	-0.023 *	-0.15	-	0.24	0.82	single
IASPT	0.965	-0.03 *	-0.674	0.083 (.)	0.32	0.71	antagonistic
IMMiT	0.997	-0.039 *	-1.058 *	0.126 *	0.40	0.87	antagonistic
FR.pa	1.303	-0.005	-0.307 *	-0.021	0.14	0.14	single
FR.ab	3.988	0.073 *	1.699	0.141	0.20	0.51	single
FR.omniv.pa	3.56	-0.209 *	-4.54	0.662 (.)	0.25	0.47	antagonistic
FR.shr.pa	1.13	-0.043 *	-0.283	-	0.15	0.21	single
FR.pred.pa	3.975	-0.101 *	-3.662 *	0.294 *	0.41	0.56	antagonistic
FR.gra.pa	1.705	-0.019	-0.001	-	0.04	0.5	ns
FR.gat.pa	3.094	-0.058 *	-1.532 *	0.195 *	0.38	0.58	antagonistic
FR.ff.pa	1.584	-0.025 *	-1.078	0.05	0.33	0.42	single
FR.omniv.ab	4.473	-0.346 *	-5.472	1.922 *	0.45	0.76	antagonistic
FR.shr.ab	1.944	-0.066	0.995	-	0.03	0.35	ns
FR.pred.ab	5.199	-0.133 *	-2.482	0.376 (.)	0.24	0.45	antagonistic
FR.gra.ab	5.231	-0.099 *	-0.199	0.600 *	0.07	0.25	antagonistic
FR.gat.ab	6.637	0.072	1.519	-	0.05	0.53	ns
FR.ff.ab	4.576	-0.035	-3.027	-	0.04	0.07	ns
RD.ab	0.211	-0.005 *	-0.026	0.008 (.)	0.43	0.43	antagonistic
RD.omniv.ab	0.063	-0.003	-0.015	-	0.03	0.03	ns
RD.shr.ab	0.123	-0.009 *	0.016	-	0.15	0.18	single
RD.pred.ab	0.463	-0.004	-0.017	0.022 (.)	0.06	0.06	antagonistic
RD.gra.ab	0.448	-0.007 *	-0.223	0.017	0.08	0.08	single
RD.gat.ab	0.155	-0.005 *	0.011	-	0.18	0.25	single
RD.ff.ab	0.101	-0.007 *	-0.118	0.011	0.28	0.39	single
FRic	0.239	-0.011 *	-0.197	0.027 (.)	0.37	0.63	antagonistic

Table 4. Selection of the most responsive metrics ($r^2_m > 0.20$) in detecting anthropogenic impacts in perennial and intermittent rivers. See Figure 1 for details of metric designation.

Metric designation	Type of metric	Valid for both flowing and disconnected pool conditions?	Metric
Universal (i.e. no calibration needed)	Functional	YES	FR.ab
		NO, only valid during flowing phase	FR.ff.pa RD.ff.ab
	Widely used	NO, only valid during flowing phase	IBMWP
Specific (i.e. non-proportional calibration; adapting reference conditions and response gradient to anthropogenic impacts)	Functional	YES	FR.pa
			FR.omniv.pa
			FR.shr.pa
			FR.pred.pa
			FR.gat.pa
			FR.omniv.ab
			FR.pred.ab
			RD.ab
			RD.pred.ab
			FRic
	Widely used	YES	Sfam
			Sgen
			IASPT
			IMMi-T

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Distribution of the 20 sampling sites in northeast of the Iberian Peninsula along Catalan internal basins, Ebro basins and Júcar River Basins.

Table S1. Categories of biological traits used as response traits grouped by resistance and resilience traits, and the rationale of the response obtained from Lytle & Poff, 2004; Bogan et al., 2017; Stubbington et al., 2017. Selected categories of traits were: asexual reproduction, with resistance forms (i.e. diapause or dormancy, cocoons), aerial respiration (i.e. spiracle, hydrostatic vesicle), flier and burrower (i.e. epibenthic) or interstitial (i.e. endobenthic) locomotion and substrate relation, less than a year life cycle duration, more than one reproduction cycles per year, aerial active and aquatic passive (i.e. drift) dissemination.

TYPE OF TRAIT	TRAIT	Category	Rationale
Resistance trait	Resistance forms	With resistance forms (i.e. diapause or dormancy, cocoons)	When flow is disconnected increased resistance against droughts may be favoured.
	Respiration	Aerial (i.e. spiracle, hydrostatic vesicle)	Increased oxygen deficit may favour air breathers.
	Locomotion and substrate relation	Flier Burrower (epibenthic) and interstitial (endobenthic)	When flow is disconnected, strategies to escape may be favoured. For adults, it would facilities recolonization over larger areas. Drying promotes movement into the hyporheic zone or other refuges (e.g. moist sediment under rocks, detritus patches, shallow depressions beneath woody debris).
Resilience trait	Life cycle duration	<1 year	Allows life cycle completion and faster recolonization. Synchronized emergence.
	Potential number of reproduction cycles per year	>1 cycles	Adult females more likely to survive long enough to lay eggs when flow resumes.
	Reproduction	Asexual	It promotes survival during dry periods.
	Dispersal	Aerial active Aquatic passive (drift)	Flow cessation favours flying. For adults, it facilities recolonization over larger areas and from distant perennial refuges (pools). Especially for intermittent rivers with natural long dry periods. It is the primary source of invertebrate colonization when flow resumes. Drift is also known to increase with chemical disturbances.

Table S2. Both natural and observed hydrological regimes (HR) of each river were calculated by TREHS software (Gallart et al., 2017), and * indicates a hydrological impact. Physicochemical parameters (dissolved O₂, conductivity, T^a, PO₄, NO₃, Chl-a) of each river indicating the maximum and minimum values and their standard deviation. Rivers are listed from 1 to 20, see Table S3 for further details.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
HR	Natural	PR	PR	PR	PR	PR	IR	IR	IR	IR	PR	PR	IR	PR	IR	IR	IR	PR	PR	IR	IR
	Observed	PR	PR	PR	PR	PR	IR*	IR	IR	IR*	IR*	IR*	IR	PR*	IR	IR*	IR*	PR*	PR*	IR	IR*
O₂ (mg/l)	max	12	10.3	10	18.2	11.6	11	11.1	10.9	11.3	9.8	11.9	10.8	9.2	9.5	6.5	10.1	11.2	10	8.6	11
	min	6.2	6.4	7.4	7.6	6.5	7.2	8.7	2.4	3.7	2.6	4	1.7	4.3	3	1.8	1	5.2	3.3	4.5	1.5
	SD	2.2	1.5	1.3	4.1	2.1	1.6	1.3	4.1	3.2	5.1	3.1	4.3	2	4.6	1.9	3.7	2.7	2.4	2.1	4
Cond (μS/cm)	max	523	598	665	377.7	471	2152	418.6	635	1393	498.4	1437	420.1	552	613	2970	943	503	1063	1034	626
	min	293.8	374	404.3	225.6	313.9	1488	319.5	251.1	556	420	595	302.7	386	554	2195	688	399.7	478.1	422.1	153.3
	SD	87	83	112	66	62	241	54	154	331	55.4	309	50	63	42	320	98	42	251	326	200
T^a (°C)	max	23.7	25.5	20.3	20.8	19.9	34.7	29.1	26.2	21.5	16.5	29.1	21.7	23.7	13.8	26.2	25.6	20.6	21.3	18.6	22
	min	4.7	8.5	10.9	3.2	7.3	15.5	7.9	7.5	7.8	15.5	9	7.2	6.7	12	11.3	12.7	12.9	12.9	8.8	12.9
	SD	7	6	4	7	5	7	11	7	5	0.7	8	6	6	1	6	5	3	3	5	4
PO₄ (mg/l)	max	1.4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0	0.1	0.1	0.1	0.1	2.6	5.4	0.1	1.5	0.1	0.1
	min	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	SD	0.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0.8	2	0	0.4	0	0
NO₃ (mg/l)	max	2.5	2.5	2.5	2.5	2.5	73.4	5.3	2.5	2.5	7.5	20	2.5	10.9	5.8	51.4	60.6	17.9	38.9	9.2	9.7
	min	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	SD	0	0	0	0	0	23	2	0	0	3.5	7	0	3	2	19	26	8	13	4	3
Chl-a (mg/l)	max	18.1	18.1	19.3	40.3	58.9	106.7	3.8	16.2	30.9	11.7	110.4	6.3	25.4	7	185	51.8	53.1	44.6	45.6	22.1
	min	0.1	0.9	2.7	7.5	3	28.7	0.1	3	15.4	5	3.2	1.2	2.6	1.6	26.7	13.2	2.9	10.8	5	6.8
	SD	7	7	6	13	23	29	2	5	6	4.7	46	3	9	4	59	17	25	14	21	7

Table S3. The degree of anthropogenic impacts of each site was measured considering the number of impacts occurring at each river location through the Mediterranean reference criteria (MRC) (Sánchez-Montoya et al., 2009). The MRC index includes information on several types of impacts, such as invasive species, diffuse sources of pollution, land use intensity, riparian vegetation, the morphology of the river and their habitat conditions, and their hydrological alterations and regulations (Sánchez-Montoya et al., 2009). The information for each site was obtained from the list of pressures and impacts of the three different River Basin water authorities included in our study combined with field observations (e.g. detection of invasive species) and, in the case of hydrological alterations, from the TREHS software. Rivers are listed from 1 to 20: Aigua d'Ora (1), Algars (2), Brugent (3), Cabriel (4), Canaletes (5), Carraixet (6), Cèrvol (7), Daró (8), Glorieta (9), Guadazaón (10), Matarranya (11), Monlleó (12), Onyar (13), Pineda (14), Poyo (15), San Miguel (16), Sènia (17), Torrent del Puig (18), Talamanca (19) and Tossa (20).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A) Riparian vegetation zone	A1) Cover and composition appropriate for the type and geographical location of the river	1	1	1	1	1	0	1	1	1	0	0	1	1	1	0	0	0	0	0	0
	A2) Lateral connectivity between river and riparian corridor is maintained (i.e. no cultivation and significant impervious area in riparian zone)	1	1	1	1	1	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0
B) Introduced sp. pollution	B1) No significant impairment by exotic plant or animal species on autochthonous species	1	1	0	0	1	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0
	B2) No dumping of urban effluents	1	1	1	1	1	0	1	1	1	0	0	1	1	1	0	0	1	0	1	0
	B3) No dumping of industrial effluents	1	1	1	1	1	0	1	1	1	1	1	1	0	1	0	0	1	0	1	1
	B4) No irrigation return channel for floodwater farming	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
C) Diffuse sources of	C1) Dry land farming < 20% of drainage area (i.e. cereal, vineyard and tree crops as olive) and not connected to riparian vegetation zone	1	1	1	1	1	0	1	1	1	0	0	1	0	1	0	0	0	0	1	1

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
pollution and land uses	C2) Intensive irrigated farming < 3% in drainage area (i.e. rice field, irrigated vineyard and others irrigated fruit tress) and not connected to riparian vegetation zone	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1
	C3) Urban use < 0.7% in drained area	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	1	1	0	1	0
	C4) Burnt vegetation < 7% in drainage area at least seven years ago and not connected to riparian vegetation zone	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	C5) No evidence of intensive use of grazing	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
	C6) Natural land uses > 80% in drainage area	1	1	1	1	1	0	1	1	1	0	0	1	0	1	0	0	0	0	1	0
D) River morphology and habitat conditions	D1) Representative diversity of substrate materials appropriate for the type	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1
	D2) No canalisation (i.e. stream bottoms and stream margins must not be fixed)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	D3) No transversal structures “dams” (i.e. no retention of sediments)	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	1	0	1	1	1
	D4) No sand or gravel extraction	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
E) Hydrological alteration and regulation	E1) No water diversion for irrigation or other purpose	1	1	1	1	1	0	1	1	0	0	0	1	0	1	0	1	0	0	0	0
	E2) No alterations of the natural hydrograph and discharge regime (e.g. reservoirs, hydroelectric)	1	1	1	1	1	1	1	1	0	0	0	1	0	1	0	0	0	0	1	0
	E3) No effect of inter-basin water transfer	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	E4) Near natural level of groundwater (i.e. aquifer not affected by over-exploitation)	1	1	1	1	1	0	1	1	1	0	0	1	1	1	0	0	0	0	1	1
Total MRC		20	20	19	19	20	8	20	19	18	9	8	19	14	19	7	10	8	9	17	12
Total number of impacts (calculated as the inverse of MRC)		0	0	1	1	0	12	0	1	2	11	12	0	6	1	13	10	7	11	3	8

Table S4. Percentage of the variance explained by N° impacts, DPi and ZF_T and by their interactions for each metric using the dataset including all aquatic-phases. N° impacts: number of impacts. DPi: the number of days in the disconnected pool phase since last sample was taken. ZF_T: total number of zero flow days.

Response	N° impacts	ZF _T	DPi	N° impacts x ZF _T	N° impacts x DPi	Residuals	R ² m
Sfam	53	36	0	11	0	0.21	0.39
Sgen	53	29	2	16	0	0.23	0.45
IBMWP	67	18	6	9	0	0.16	0.31
IASPT	49	27	11	11	2	0.26	0.44
IMMiT	49	16	14	20	1	0.14	0.54
FR.pa	33	51	10	5	1	0.76	0.22
FR.ab	56	33	5	6	0	0.49	0.23
FR.omniv.pa	56	12	6	25	1	0.35	0.32
FR.shr.pa	57	4	29	1	9	0.60	0.23
FR.pred.pa	38	49	0	14	1	0.33	0.39
FR.gra.pa	29	14	43	7	7	0.39	0.10
FR.gat.pa	49	13	9	26	3	0.42	0.34
FR.ff.pa	20	55	6	6	13	0.68	0.13
FR.omniv.ab	23	5	17	54	1	0.34	0.33
FR.shr.ab	78	0	21	0	1	0.71	0.09
FR.pred.ab	53	16	0	29	2	0.54	0.2
FR.gra.ab	0	13	13	73	1	0.54	0.15
FR.gat.ab	32	11	32	22	3	0.53	0.06
FR.ff.ab	0	33	1	7	59	0.76	0.15
RD.ab	69	0	6	22	3	0.65	0.36
RD.omniv.ab	74	0	0	1	25	0.87	0.03
RD.shr.ab	50	0	27	6	17	0.73	0.17
RD.pred.ab	39	3	19	0	39	0.69	0.33
RD.gra.ab	54	25	7	7	7	0.89	0.11
RD.gat.ab	82	0	6	6	6	0.82	0.16
RD.ff.ab	54	35	3	4	4	0.50	0.29
FRic	65	22	0	10	3	0.27	0.35

Table S5. Percentage of the variance explained by N° impacts and ZF_T, and by their interactions metric using the flowing-phase dataset. N° impacts: number of impacts. ZF_T: total number of zero flow days.

Response	N° impacts	ZF _T	N° impacts x ZF _T	Residuals	R ² _m
Sfam	69	23	8	0.26	0.36
Sgen	65	22	13	0.33	0.43
IBMWP	83	9	8	0.15	0.24
IASPT	71	19	10	0.26	0.32
IMMiT	74	11	15	0.12	0.40
FR.pa	29	65	6	0.84	0.14
FR.ab	67	28	5	0.45	0.20
FR.omniv.pa	73	14	13	0.48	0.25
FR.shr.pa	97	3	0	0.69	0.15
FR.pred.pa	53	34	13	0.41	0.41
FR.gra.pa	91	2	7	0.40	0.04
FR.gat.pa	67	11	22	0.39	0.38
FR.ff.pa	47	50	3	0.65	0.33
FR.omniv.ab	37	7	56	0.22	0.45
FR.shr.ab	85	15	0	0.58	0.03
FR.pred.ab	77	12	11	0.48	0.24
FR.gra.ab	29	10	61	0.69	0.07
FR.gat.ab	74	14	12	0.42	0.05
FR.ff.ab	18	82	0	0.88	0.04
RD.ab	94	0	6	0.57	0.43
RD.omniv.ab	100	0	0	0.93	0.03
RD.shr.ab	91	1	8	0.79	0.15
RD.pred.ab	50	5	45	0.91	0.06
RD.gra.ab	49	44	7	0.90	0.08
RD.gat.ab	92	0	8	0.71	0.18
RD.ff.ab	82	15	3	0.58	0.28
FRic	81	11	8	0.33	0.37

Table S6. Null model results showing z-score values for each functional metric from the dataset including all aquatic-phases. Significant p-values (< 0.05) are indicated in bold. N° impacts: number of impacts. DP_i: the number of days in the disconnected pool phase since last sample was taken. ZF_T: total number of zero flow days.

	N° impacts	ZF _T	N° impacts x ZF _T	DP _i	N° impacts x DP _i
FR.pa	-	-3.11	3.18	-2.18	1.95
FR.ab	-2.22	-	-	2.25	2.34
FR.omniv.pa	-2.22	-1.74	-	1.99	-2.20
FR.shr.pa	-	-1.59	-	-1.79	-1.77
FR.pred.pa	1.35	-	-1.26	1.35	-1.22
FR.gra.pa	1.17	1.20	-	-	1.27
FR.gat.pa	-	-1.05	-	-1.08	-
FR.ff.pa	-	0.86	0.88	-0.85	-
FR.omniv.ab	0.88	-0.72	-	-	-0.88
FR.shr.ab	0.82	-0.77	-	-	-
FR.pred.ab	0.80	0.75	-0.74	-0.75	-0.74
FR.gra.ab	-0.66	0.74	-	-	0.74
FR.gat.ab	-0.51	-0.61	-0.66	-	0.69
FR.ff.ab	-	-	-0.52	-0.49	-
RD.ab	0.38	-	-0.43	0.37	-
RD.omniv.ab	-0.27	0.47	0.35	-0.27	-0.34
RD.shr.ab	-0.34	-	-0.31	-	-0.26
RD.pred.ab	-	0.28	-0.25	0.34	-0.21
RD.gra.ab	0.22	-0.09	-	0.23	0.25
RD.gat.ab	-0.08	-	-0.26	0.17	-0.20
RD.ff.ab	0.09	-0.10	-	0.10	-
FRic	-0.07	-	0.04	-	-

Table S7. Null model results showing z-score values for each functional metric from the flowing-phase dataset. Significant p-values (< 0.05) are indicated in bold. N° impacts: number of impacts. ZF_T: total number of zero flow days.

	N° impacts	ZF _T	N° impacts x ZF _T
FR.pa	-2.99	1.65	-3.19
FR.ab	58.74	-17.97	25.38
FR.omniv.pa	-4.08	-1.82	5.73
FR.shr.pa	-3.23	-0.91	1.01
FR.pred.pa	-10.55	-6.25	7.56
FR.gra.pa	-2.88	-0.98	1.74
FR.gat.pa	-3.06	-1.53	2.18
FR.ff.pa	-2.13	-1.2	1.11
FR.omniv.ab	-4.08	-1.82	5.73
FR.shr.ab	-1.52	-0.79	0.99
FR.pred.ab	-3.98	-4.16	4.87
FR.gra.ab	-1.8	-1.31	2.6
FR.gat.ab	1.85	-1.25	-0.23
FR.ff.ab	-1.13	-1.24	0.8
RD.ab	-5.1	-1.33	2.04
RD.omniv.ab	-2.45	-0.46	0.56
RD.shr.ab	-3.13	0.04	-0.38
RD.pred.ab	-3.9	-1.3	2.81
RD.gra.ab	-2.69	-1.04	1.23
RD.gat.ab	-3.36	-0.7	1.42
RD.ff.ab	-2.89	-0.87	0.97
FRic	-13.34	-5.27	6.83

Appendix S1. Details on functional metrics estimation:

To quantify functional redundancy metrics (FR), a Gower effect trait taxon-by-taxon dissimilarity matrix was derived from the original taxon-by-effect-trait matrix to produce a classification dendrogram for macroinvertebrates, based on Ward's clustering method (e.g. Laliberté et al., 2010; Bruno, Gutiérrez-Cánovas, Velasco, & Sánchez-Fernández 2016a). After visual inspection of the dendrogram and assessing groups' coherence, six functional trophic groups representing different trophic roles were defined: omnivorous (omniv), shredders (shr), predators (pred), grazers (gra), gatherers (gat) and filter feeders (ff). Omnivorous had mixed feeding habits, including a variety of shredding, grazing and gathering affinities. FR for each sampling site was calculated as the average abundance (ab) or traits richness (i.e. presence-absence; pa) for each functional trophic group as well as for the entire community (i.e. total abundance or richness divided by the number of occurring functional trophic groups) (Laliberté et al., 2010) (Table 1). To obtain response diversity metrics (RD), a second Gower taxon-by-taxon dissimilarity matrix based on response traits was calculated to build a functional response space through a Principal Coordinate Analysis (PCoA). The five most explanatory response trait axes (explained variance= 56.4 %; mean squared deviation=0.005) were retained, according to the method proposed in Maire, Grenouillet, Brosse and Villéger (2015). As a measure of Functional Richness of the response traits (FRic, Villéger, Mason, & Mouillot 2008), the ratio between the hyper volume filled by each community in the functional response space and that filled by all the taxa occurring within the study area was estimated, ranging 0 to 1. Also, the functional dispersion of the entire community (FDis, Laliberté & Legendre, 2010) was estimated, which accounted for the mean distance of each taxon to the mean weighted centroid in the functional effect space (i.e. unweighted, presence-absence as RD.pa, and

abundance-weighted as RD.ab). Similarly, RD for each of the six functional trophic groups was quantified considering only the taxa from each group present at each community. In the latter case, only the abundance weighted index was used (e.g. RD.shr.ab, RD.ff.ab; see Table 1).

Appendix S2. Data analysis methods:

A multi-model inference approach to quantify predictor's standardised effect sizes and significance was adopted. This statistical technique ranks all the models generated using all the possible combination of predictors using Akaike's Information Criterion (AIC). Top models differing in no more than two AIC units ($\Delta AIC \leq 2$) from the model ranked first (i.e. with minimum AIC) were selected. To conduct the model averaging a natural average method was adopted. For each LME model, two measures of goodness-of-fit were estimated: marginal goodness-of-fit (r^2_m) indicates the variance explained only by the fixed factors, while conditional goodness-of-fit (r^2_c) shows the variance accounted by both fixed and random terms. All models were validated by visually checking their residuals for normality and homocedasticity. When heteroscedasticity was observed, a model term was added to LME to account for residual heterogeneity. This occurred for FR.shr.pa, RD.shr.ab and RD.ff.ab metrics from both datasets, for FR.omniv.pa from the all aquatic-phases, and for FR.gra.pa, FR.ff.pa and FR.omniv.ab from the flowing-phase dataset.

Appendix S3. Null models conducted in functional metrics:

To confirm that functional patterns were not simply a consequence of the underlying taxonomic variation, a distribution of 999 null coefficient values was produced for each empirical model term following this procedure: 1) randomly re-assigning effect

(trophic) and response trait combinations to each taxon on each trait table, 2) re-estimating functional trophic groups and metrics for each sample and 3) re-examining its relationship with full LMEs including the number of impacts, DP_i and ZF_T and their pairwise interactions as fixed factors. Multi-model inference was not used to avoid problems due to potentially different subsets of selected predictors. Given that trait randomisation was performed on the trait tables, the overall species frequency and the species richness for each plot were kept unmodified in the null models. Whether the empirical coefficients differed from the null model distributions was examined by using an exact two-tailed test to calculate the probability (i.e. occurrence of values greater or lower than the null coefficient values / null model runs+1) that the observed value was significantly larger or smaller than the simulated distribution at $\alpha = 0.05$. Then, when a model parameter was not significantly different from the null model, the functional shift was assumed as indistinguishable from changes due to taxonomic variation. Otherwise, when a model parameter was significantly lower than the null model, we assume that either habitat filtering, respectively, is explaining the functional changes, whose decline would be greater than expected by changes in taxonomic variation.