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Functional responses of aquatic macroinvertebrates to flow regulation are shaped by natural flow intermittence in Mediterranean streams

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Biological traits, functional diversity, functional redundancy, hydrologic alteration, intermittent streams

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Abstract

1. Running waters in Mediterranean regions are strongly regulated by dams, which produce significant alterations to natural flow regimes. Climate change will reduce discharge and will increase flow intermittence in Mediterranean streams, which will lead to an intensified flow regulation to meet water demands. Very little is known about how the functional features of aquatic communities vary along combined anthropogenic flow alteration and natural intermittence gradients.

2. As intermittent streams are subjected to natural stress (droughts and flash floods), the flow regime alteration effect may differ from that observed in perennial rivers. Consequently, studies which aim to determine the effects of flow regulation on the functioning of aquatic communities in a global change context are crucial.

3. By applying Linear Mixed-Effect Models and null models to the macroinvertebrate communities from 65 stream sites in the Segura River Basin (SE Spain), we assessed the separate effects of natural flow intermittence and flow regulation, as well as their interaction, on biological traits and functional diversity indices.

4. Natural flow intermittence and flow regulation were mainly associated with loss of taxa with semivoltine or univoltine cycles and more sensitive aquatic stages (i.e., eggs), and with the replacement by taxa with multivoltine cycles and more resistant aquatic stages (i.e., adults). Flow regulation intensified the impact of natural flow intermittence on some biological traits, producing synergistic effects (i.e. decreasing interstitial taxa and tegument breathers and increasing taxa with aquatic adult stages). At the same time, antagonistic (life cycle) and

49   opposing (shredders) interaction effects were also observed. Functional diversity

50   (FD), functional dispersion (FDis) and functional redundancy (FR) underwent a

51   non-random decrease as the flow regime alteration increased, and a significant

52   antagonistic interaction was also found between both stressors for FR.

53 5. In general, flow regulation effects were stronger in perennial than in intermittent

54   streams because natural intermittence imposes a previous eco-evolutionary

55   pressure on aquatic biota by selecting those resistant or recovery traits that

56   confer resilience to anthropogenic flow regime alterations. Thus, the natural

57   flow regime influences the functional sensibility of communities to

58   anthropogenic flow alteration.

1. Introduction

Stream structure and functioning are substantially shaped by flow regimes, which influence the suitability of instream habitats (Benda *et al*., 2004; Lytle & Poff, 2004) and their associated biological communities (Bunn & Arthington, 2002; Death, 2008). Anthropogenic flow regime alterations of natural flow regimes through water abstraction and dams involve changes in the intensity, frequency and seasonal predictability of floods and droughts. These changes can subsequently alter organic matter and sediment transport (Dewson *et al*., 2007; Meador & Carlisle, 2012), with direct effects on riparian and aquatic biota, such as habitat homogenisation (Belmar *et al*., 2013b), changes in indicator taxa and ecological traits (Guareschi *et al*., 2014), reduced diversity (Bruno *et al*. 2016a; Lobera, 2017) and network simplification (Tonkin *et al*., 2018). The ubiquity of flow alteration has led to a recent great interest in understanding aquatic ecosystem response to various types and degrees of flow regime alteration (see reviews by Dewson *et al*., 2007 and Poff & Zimmerman, 2010). This is essential because changes in water availability and demands as a result of global change will intensify dam construction and flow regulation (Milly *et al*., 2005; Chang *et al*., 2018), as well as increase the number of temporary rivers and the severity of flow intermittence (Larned *et al*., 2010).

Mediterranean running waters are naturally subjected to flow extremes, which range from low to no flow during long dry seasons to flash floods during rainy periods (Gasith & Resh, 1999; Bonada *et al*., 2006). In these aquatic ecosystems, flow variability and minimum flow are the major determinants of macroinvertebrate communities (Belmar *et al*., 2013a), which are functionally important for numerous fluvial processes and services, such as biomass production, ecosystem engineering, nutrient cycling and resource processing (e.g. Covich *et al*., 1999; Statzner, 2012). Intermittent rivers

84   support invertebrate species with a variety of physiological, behavioural, morphological

85   and life-history adaptations to survive desiccation (Lytle & Poff, 2004; Datry *et al*.,

86   2014, Stubbington *et al*., 2017). The fauna of temporary watercourses exhibits traits

87   related to resistance and recovery strategies, such as high growth rates, short lifespan,

88   desiccation resistant stages and opportunistic/generalist feeding modes (Williams 2006,

89   Bonada *et al*., 2007b, Stubbington *et al*., 2017), which confer resilience (*sensu* Hodgson

90   *et al*., 2015). Such traits may enhance their tolerance to similar environmental stressors,

91   like those imposed by flow regulation. In fact, both natural intermittence and flow

92   regulation affect water quantity and quality, habitat availability, as well as lateral,

93   longitudinal and vertical connectivity (Bond *et al*., 2008). These impacts can involve

94   changes in aquatic communities (Mulholland *et al*., 1997) and ecosystem functioning

95   (Bruno *et al*., 2016a).

96   Even though the isolated effects of flow intermittence (e.g., Bonada *et al*., 2007b; Bond

97   *et al*., 2008; Belmar *et al*., 2013b; Leigh *et al.*, 2017; Karaouzas *et al.*, 2018) and flow

98   regulation (e.g. Poff & Zimmerman, 2010; Bruno et al. 2014; Ngor *et al*., 2018) on the

99   composition and structure of aquatic communities have been widely addressed, the

100   understanding of how their interactions affect functional features is still limited. Studies

101   in Mediterranean areas, aimed at determining the effects of flow regulation on the

102   structure and functioning of stream communities and their interaction with natural flow

103   intermittence, are essential to anticipate biological responses to stress intensification in

104   a climate change context.

105   The intense development of trait-based approaches has improved the knowledge on the

106   effects of anthropogenic impacts on ecosystem functioning over the last decade

107   (Clapcott *et al*., 2010; Laliberté *et al*., 2010; Mouillot *et al*., 2013; Gagic *et al*., 2015).

108   As the combination of species traits determines their likelihood to overcome

109 disturbances, a non-random taxa selection is expected along environmental gradients

110 and after disturbances (Weiher *et al.*, 2011; Picazo *et al.*, 2012; Mouillot *et al.*, 2013).

111 In addition, trait-based approaches allow functional diversity measures to be estimated

112 (Laliberté & Legendre, 2010) at the community level, such as functional diversity

113 (Tilman *et al.*, 1997), richness, evenness, divergence (Mason *et al.*, 2005, for a review)

114 and redundancy (Rosenfeld, 2002). These indices reflect how an ecosystem operates in

115 terms of niche differentiation, efficiency using resources, resistance and recovery,

116 among others (Petchey & Gaston, 2006). Changes in functional traits caused by

117 environmental stress could involve functional homogenisation (Clavel *et al.*, 2011),

118 with reduced functional diversity (Sonnier *et al.*, 2014; Gutiérrez-Cánovas *et al.*, 2015)

119 and loss of associated ecosystem goods and services (Buisson *et al.*, 2013).

120 Previous studies conducted in Mediterranean rivers have assessed functional changes in

121 aquatic and riparian communities along natural gradients of water salinity and flow

122 intermittence (Suárez *et al.*, 2017), as well as along anthropogenic stress gradients such

123 as agriculture or flow regime alteration (Mellado-Díaz *et al.*, 2008, Gutiérrez-Cánovas

124 *et al.*, 2015, Bruno *et al.*, 2016a,b). These studies found that functional diversity and

125 redundancy measures decreased as environmental stress increased, regardless of

126 whether the origin of such stress was natural or anthropogenic. Given that previous

127 studies in aquatic communities have considered the biological effects of anthropogenic

128 and natural stressors separately, unravelling their interaction effects is crucial to provide

129 reliable measures of the impact of combined anthropogenic and natural stressors for

130 management and conservation. On one side, such understanding could have

131 implications to gain insight into the ecological consequences of the ongoing climate

132 change in rivers by being able to anticipate biological changes in European freshwaters

133 (Bonada *et al.*, 2007a), where climate change models predict increased aridity which

134 will lead to drought intensification and greater flow intermittence (IPCC, 2013). On the

135 other, it constitutes a contribution to the urgent need to evaluate the consequences of the

136 current Mediterranean and global increase in dam construction (Zarfl *et al*., 2015).

137 Our main objective was to assess the effect of anthropogenic flow regime alteration,

138 natural flow intermittence and their interaction on macroinvertebrate trait composition

139 and functional diversity measures, at community level. In the most altered rivers, we

140 expected similar changes to those caused by natural flow intermittence: dominant small-

141 sized taxa, short cycles, multi-voltinism, aquatic adult stages, predators and diapause or

142 dormancy resistance forms, together with lower functional diversity indices due to the

143 functional   simplification   and   homogenisation   of   aquatic   macroinvertebrate

144 communities. Given the resistance and recovery strategies developed by intermittent

145 stream fauna to cope with flow cessation discussed earlier, we hypothesised that natural

146 flow intermittence can moderate the impact of flow regulation. Thus, more antagonistic

147 interaction effects could be expected and functional changes would be more intense in

148 perennial than in intermittent rivers.

149 **2. Methods**

150 *2.1. Study area*

151 The Segura River Basin (18,870 km^2, SE Spain) is an environmentally diverse basin due

152 to human (alteration) and natural (climatic) gradients with a predominately semi-arid

153 climate (see Bruno *et al.*, 2014). The catchment presents a strong climatic and

154 altitudinal gradient from the northwest (NW) to the southeast (SE). Climate ranges from

155 wet (>1,000 mm mean annual precipitation) and cold in the NW mountains (>1,000

156 m.a.s.l.) to semiarid (<350 mm mean annual precipitation) and hot in the SE lowlands.

157 This results in an average yearly precipitation around 362 mm (historical series:

158   1980/81-2005/06; https:\\www.chsegura.es). These conditions determine the existence

159   of eight hydrological types (from large and perennial rivers to ephemeral streams), with

160   differences in flow magnitude and intermittence (Belmar *et al*., 2011). The basin has a

161   high regulatory capacity (770 hm^3, over 90% of natural input) provided by 24 dams and

162   121 weirs, being one of the most regulated river network in Europe (CHS, 2008

163   Grindlay *et al*., 2009, 2011). Irrigation (responsible for 87% of water demands;

164   Grindlay *et al*., 2011) constitutes the main anthropogenic pressure on stream flows.

165   There are two main flow alteration types in the Segura River Basin depending on the

166   river type or natural flow regime (Belmar *et al*., 2013b). Perennial rivers show sudden

167   unpredictable water releases from big dams, which aim to provide water for irrigation in

168   summer. However, natural temporary streams undergo drought intensification due to

169   intense water diversions for agriculture from upstream dams. This is, therefore, a

170   suitable area to study the biological and functional consequences of hydrological

171   stressors in Mediterranean rivers and their interaction due to the variety of natural flow

172   regimes and anthropogenic flow regime alterations.

173   Sixty-five freshwater stream reaches (conductivity < 5,000 µS cm^{-1}) were selected in

174   the basin to ensure large gradients of natural flow intermittence and regulation. The

175   correlation between flow intermittence and hydrologic alteration was tested using

176   Pearson correlation. Each reach was classified according to its degree of flow

177   intermittence (Fig. 1; from "1" or most perennial to "8" or ephemeral; Belmar *et al*.,

178   2011). Hydrologic alteration was assessed using the surface of irrigated land (%),

179   number of dams (count) and regulatory capacity (hm^3) in the drainage area of each

180   sampling site (see Falcone *et al*., 2010a,b and Belmar *et al*., 2013b for details) as these

181   variables are linked to the main flow regime alterations in the Segura Basin (Belmar *et*

182   *al*., 2010). Sites were assigned a score between 0 and 8 for each variable based on their

183 percentile value within the data range. The 1st, 20th, 40th, 60th, 80th, 90th, 95th and 98th

184 percentiles were calculated in "non-zero" values and used as "disturbance thresholds" to

185 assign the corresponding score for each site (Falcone *et al.*, 2010b; Belmar *et al.*,

186 2013b). These irregular thresholds ensure the assignment of outstanding alteration

187 values to the (scarce) most altered locations (Falcone *et al.*, 2010a). Finally, the three

188 scores were summed, which provided a dimensionless index that potentially ranged

189 from 0 (minimum flow alteration) to 24 (maximum flow alteration).

190 *2.2. Macroinvertebrate data*

191 Macroinvertebrates were sampled between 2010 and 2011 in dry-weather months (late

192 spring and early summer). These months corresponded to the season of maximum

193 aquatic invertebrate activity and environmental stress (droughts and flow regulation). A

194 kick-net (500 µm mesh) was used following a multihabitat standardised protocol, with

195 sampling effort proportional to each habitat occurrence (Jáimez-Cuéllar *et al*., 2002).

196 The kick-sample contents were pooled into a unique site-sample and preserved in 96%

197 ethanol. In the laboratory, organisms were identified at genus level, except for dipterans,

198 which were identified at family, sub-family or tribe level in order to achieve a

199 compromise between taxonomic resolution and classification effort. These taxonomic

200 levels are frequently used to analyse trait composition and functional diversity measures

201 (Dolédec *et al.*, 2000, Tachet *et al.*, 2010) due to the general lack of trait databases at

202 species level. To perform analyses, the taxa Hirudinea, Oligochaeta, Ostracoda and

203 Hydracarina were excluded, as trait information is unavailable or mostly incomplete at

204 genus level (see Gayraud *et al.*, 2003; Tachet *et al.*, 2010).

205 The functional features of aquatic macroinvertebrates were characterised using 11

206 biological traits and 63 trait categories (Tachet *et al*., 2010). Traits included maximal

207 potential size, life-cycle duration, potential reproductive cycles per year, aquatic stages,

208 reproduction, dispersal, resistance forms, respiration type, locomotion and substrate

209 relation, food preference and feeding habits. These traits have been pointed out as being

210 responsive to stress intensification in Mediterranean and temperate rivers (Bonada *et al.*,

211 2007b). Each taxon was coded according to its affinity to each trait category using a

212 fuzzy coding approach (Chevenet *et al.*, 1994). The fuzzy coded data were converted

213 into percentages of affinity for each trait category. This procedure standardises the

214 potential differences in the codification scores (i.e., different row sums for each taxon

215 and trait).

216 *2.3 Functional diversity indices*

217 The community-level weighted means of trait values (CWM) were calculated to

218 quantify the proportion of each trait category in each sample. "Taxon × traits" and

219 "taxon × site" matrices were crossed to obtain the CWM matrix with the proportion of

220 each trait character in each sampling site. Based on this trait information, a set of

221 functional diversity indices was calculated: functional dispersion (FDis; *sensu* Mason *et*

222 *al.*, 2005), functional evenness (FEve), functional richness (FRic), functional diversity

223 (measured as Rao's quadratic entropy; FD; Botta-Dukát, 2005) and functional

224 redundancy (FR; Rosenfeld, 2002). Gower dissimilarity matrices (adapted for the fuzzy-

225 coded traits, Pavoine *et al.*, 2009), using all the biological traits, allowed us to compute

226 FDis, FEve and FRic. To calculate FD and FR, only effect traits were considered. Effect

227 traits are those biological features that directly influence a specific function of the

228 ecosystem (e.g. productivity, nutrient cycling) while response traits change according to

229 the abiotic and biotic environment (e.g. climatic conditions and disturbance regime;

230 *sensu* Díaz & Cabido 2001). We selected six effect traits (size, aquatic stage, dispersal,

231 locomotion, food and feeding habits) based on the updated reviews done by Schmera *et*

232 *al.* (2017) and Hevia *et al.* (2017). These traits are related to important processes

associated with ecosystem functions such as energy flow in food webs, nutrient cycling, aeration of sediments and recovery of disturbances (Covich *et al.*, 1999). For example, mobile animal species increase ecosystem resilience by connecting habitats and ecosystems through their movements (Sekercioglu, 2010).

FDis was measured as the abundance-weighted functional dispersion of the functional traits (i.e. functional β-diversity). To quantify this metric for each community, we estimated the weighted mean distance to the weighted community centroid (Laliberté & Legendre, 2010). FEve, which represents the regularity of the distribution and relative abundance of taxa in the functional space for a given community, was calculated by the method of the minimum spanning tree in a functional space based on an all-traits dissimilarity matrix (Villeger *et al*., 2008). FRic was estimated as the hypervolume enclosing the functional space filled by the community (Villeger *et al*., 2008). The number of axes retained to estimate hypervolumes was decided according to the method proposed in Maire *et al*., (2015). This variable was standardised by its maximum, and ranged from 0 to 1. FD is an abundance-weighted metric that measures the mean pairwise dissimilarities of randomly selected taxa in a community as a way to describe the diversity of traits (Rao, 1982). Finally, given the variety of methodologies to estimate FR and the lack of consensus about them (Ricotta *et al*., 2016), the approach that showed better performance in response to natural and anthropogenic disturbances in the study area was used (see Bruno *et al*., 2016b). FR was obtained for each sampling site as the average number of taxa per functional group (Rosenfeld 2002; Laliberté *et al*., 2010). Functional groups were considered sets of organisms with a suite of co-adapted characteristics and similar functional effects on the dominant ecosystem processes that, in comparison to other groups, present differences with clear ecological significance (Díaz & Cabido, 2001; Naiman *et al*., 2005). Taxa were classified into

258   functional groups using a Ward's clustering method based on the effect-trait

259   dissimilarity matrix. Ward's clustering method involves the use of Euclidean distances.

260   Therefore, we ensured that the eigenvectors of a double-centred matrix obtained using a

261   principal component analysis were positive, in order to verify that the Gower effect-trait

262   dissimilarity matrix met this criterion (Laliberté & Legendre, 2010; Bruno *et al.*,

263   2016b). We selected six functional groups based on distinctive functional effect traits

264   through a Non Metric Multidimensional Scaling (NMDS) combined with an

265   environmental fitting procedure done using trait characteristics instead of environmental

266   variables.

267   *2.4 Functional responses to flow regime alteration and natural flow intermittence*

268   The individual and combined effects of flow regime alteration and natural flow

269   intermittence on macroinvertebrate trait composition (defined by CWM) and functional

270   indices were tested by Gaussian linear mixed-effect models (LME) as previously used

271   in similar analyses (e.g., Bruno et al., 2016b). The dependent variables were

272   transformed (square root of arcsine, commonly used with proportions) to fulfil the

273   assumptions of Gaussian models. LMEs included stressor slopes (flow alteration and

274   natural flow intermittence) and the interaction between them as fixed terms, along with

275   a random intercept to account for altitudinal (non-hydrological) variability. Such

276   variability was considered a 6-level factor and represented different elevation ranges

277   (from <200 to >1,000 m.a.s.l., each 200 m). LMEs produce two measures of goodness

278   of fit, the marginal R^2 associated with the fixed effects (those produced by hydrological

279   stressors) and the conditional R^2 that represents also the random effects (caused by

280   environmental variation associated with elevation). These R^2 are less susceptible to

281   problems associated with mixed-effect models than common definitions of R^2

282   (Nakagawa & Schielzeth, 2013). We focused on the former as it is associated with the

283   effects produced by flow alteration and flow intermittence (fixed effects). LMEs were

284   performed on functional indices by a backward-stepwise procedure to retain the model

285   that minimised the Bayesian Information Criterion (BIC) in order to obtain the best

286   model for each functional trait and functional index. As many tests had to be performed

287   for the functional traits, the Benjamini & Hochberg (1995) correction was used to

288   minimise the False Discovery Rate. Model assumptions were checked visually (residual

289   normality and homoscedasticity).

290   In the best-fitting models, we distinguished additive effects when stressors did not

291   interact, but were individually added to each other, and interactive effects when there

292   was a significant interaction term. We followed the criteria proposed by Feld *et al.*

293   (2016) for models obtained from trait-based data along stressor gradients. We

294   considered that an interaction can be antagonistic, which means that the net effect of

295   both is less than the sum of their independent effects, when the individual stressor

296   coefficients take the same sign but the interaction coefficient takes the opposite sign;

297   synergistic, when all the individual and interaction coefficients are of the same sign,

298   thus the interaction effect is stronger than their additive individual effects; and

299   opposing, if individual stressor coefficients have opposite signs no matter the sign of the

300   interaction term's coefficient.

301   A relationship between functional indices and environmental stressors can be found

302   simply because of the underlying taxonomic gradient (Villéger *et al*., 2008). Thus null

303   models were used to assess the non-randomness of the observed trends. For this

304   purpose, we randomly reassigned traits to each taxon (999 runs) by re-examining their

305   relationships with stressors and keeping the same trait combinations, richness gradient

306   and taxon frequency of occurrence. For each simulation, we used the same model and

307   procedure as for the empirical data. We examined the null model's statistical

308 significance using an exact two-tailed test to calculate the probability of the empirical

309 value being significantly ($\alpha = 0.05$) larger or smaller than the simulated distribution.

310 All the functional analyses were performed using the R software (R 3.4.0 version; R

311 Core Team, 2017). Functional diversity indices (including the CWM matrix) were

312 obtained using the function *dbFD* from the package FD (Laliberté & Legendre, 2010;

313 Laliberté & Shipley, 2011). The LMEs were developed using the functions *lmer* and

314 *lme* from the packages *lme4* (Bates *et al*., 2015) and *nlme* (Pinheiro *et al*., 2017),

315 respectively. The NMDS was developed using the functions *metaMDS*, *envfit* and

316 *s.class* from the *vegan* (Oksanen *et al*., 2013) and *ade4* (Dray & Dufour, 2007)

317 packages.

318 **3. Results**

319 3.1 Effects on trait composition

320 One hundred and twenty-four genera (plus 16 higher taxonomic levels) were identified

321 in the study area (Table S1, Supplementary Material). The richest insect Orders were:

322 Coleoptera (33%; 46 out of 140 taxa), Diptera (13%), Ephemeroptera (11%), Hemiptera

323 (11%) Odonata (10%) and Trichoptera (10%). All the considered taxa were classified

324 into six functional groups that represented different life strategies (mainly determined

325 by body size, feeding habits, locomotion and substrate relationships) and ecosystem

326 effects with a clear ecological significance on ecosystem functioning (Table 1; Fig. S1

327 and Fig. S2, Supplementary Material).

328 Flow regime alteration and natural flow intermittence were weakly related. The

329 correlation (Pearson r = -0.53, p-value < 0.01) was statistically significant but showed a

330 moderate value. Such outcome was not enough to discard one of the stressors for

331 analyses, as the correlation coefficient was out of the |0.70| threshold commonly used in

332 ecological studies (e.g. Zuur *et al.*, 2009; Dormann *et al.*, 2013). In addition, the

333 absence of a non-linear relationship was verified (Fig. S3; Supplementary Material).

334 Both stressors significantly increased the proportion of multivoltine taxa and reduced

335 the proportion of univoltine and/or semivoltine ones (Fig. 2; Table S2, Supplementary

336 Material), with an increase in the potential number of cycles per year. Besides, both

337 stressors entailed a statistically significant reduction in the taxa with aquatic eggs and

338 crawlers. Flow alteration also limited the presence of taxa with aquatic active dispersal.

339 Meanwhile, the proportion of taxa with clutches in vegetation, diapause and/or

340 dormancy as resistant forms, and full water swimmers increased significantly with flow

341 intermittence, accompanied by a reduction in interstitial taxa. Natural flow intermittence

342 also produced significant changes in food and feeding habits by increasing the

343 proportion of taxa that preyed on invertebrates and vertebrates, but reducing the

344 relevance of scrapers (Fig. 2; Table S2, Supplementary Material).

345 The interactions of both stressors produced antagonistic, synergistic and opposing

346 effects on some trait categories (Table 2) as the effect of one stressor is conditional on

347 the position along the gradient of the other stressor (Fig. 3). Antagonistic interactions

348 were found in short and long life cycles, as proportions of taxa with shorter life cycles

349 were higher in strongly regulated perennial rivers whereas long life cycles were more

350 frequent in free-flowing perennial rivers or strongly regulated intermittent ones. Positive

351 synergistic effects were found on the proportion of taxa with aquatic adult stages

352 (maximum values in intermittent regulated rivers), but negative synergistic effects

353 (minimum values in intermittent regulated rivers) on the interstitial taxa and tegument

354 breathers (the interaction was significant and with the same sign as the individual

355 coefficients; Table 2). Finally, flow alteration and flow intermittence interaction had an

356   opposing effect on shredder feeding habits, which decreased with flow alteration in

357   perennial rivers but increased in intermittent streams.

358   3.2 Effects on the functional diversity indices

359   All the analysed functional indices, except FEve, significantly decreased with increasing

360   flow alteration, whereas only FR decreased when flow intermittence increased,

361   particularly in free-flowing rivers (Table 3, Fig. 3). The interaction of both stressors

362   resulted in antagonistic effects on functional redundancy (FR) and functional richness

363   (FRic), being the sign of the interaction term opposite to that of each stressor

364   individually. Both indices responded similarly to disturbance, and were the most

365   sensitive indices (the best models explained more than 30% of deviance). Conditional

366   R^2 and marginal R^2 displayed similar values in the best-fitting mixed-effect models for

367   all the functional indices. Thus, the elevation gradient had an inconspicuous influence

368   on the spatial distribution of the functional indices compared with the effect of both

369   stressors.

370   The null models revealed that stressors caused non-random changes in FDis, FD and

371   FR, which were significant ($p < 0.05$) for all the terms of the best-fitting models (Table

372   4). However, null models did not reveal significant effects of the studied factors on

373   FRic, which meant that the observed responses were related to the underlying

374   taxonomic richness gradient.

375   **4. Discussion**

376   The use of trait-oriented functional approaches to evaluate the response of natural

377   communities to the filtering effects of abiotic conditions and ecological interactions is a

378   burgeoning field of study worldwide (Mouillot *et al.*, 2013; Ruhi *et al.*, 2018). It has

379   been especially fruitful in an applied context, in order to understand the response of

communities to anthropogenic impacts such as climate change, habitat loss and land use (e.g. Frainer & McKie, 2015; for stream macroinvertebrates). It has been also used to disentangle the simultaneous effects of natural and anthropogenic disturbances in multi-stressed rivers (e.g. Bruno *et al.*, 2016a,b).

This study disentangles the separate effects of anthropogenic flow regime alteration and natural flow intermittence, as well as their interaction, on macroinvertebrate community functional characteristics in Mediterranean rivers. In general, flow regulation entails a significant reduction in the mean annual discharges and intensified drought conditions (Belmar *et al*., 2013b), which produced some functional patterns common to natural flow intermittence. Firstly, loss of taxa with semivoltine or univoltine cycles and more sensitive aquatic stages (i.e., eggs), and the replacement by taxa with multivoltine cycles and more resistant aquatic stages (i.e., adults). Secondly, reduced functional redundancy, which could jeopardise the ability of aquatic communities to face an intensification of flow intermittence caused by climate change or new anthropogenic disturbances.

We found that natural flow regime influenced the functional sensitivity of communities to human-driven flow alteration, resulting in antagonistic, synergistic and opposing interaction effects. The effects of flow regulation were more intense in perennial streams than in intermittent ones given the relevance of antagonistic effects found between both stressors (considering both trait composition and functional indices). Flow intermittence imposed an eco-evolutionary pressure on aquatic biota by previously selecting those resistant or recovery traits (Bonada & Resh, 2013) that conferred resilience to anthropogenic flow regime alterations.

4.1 Responses of functional traits to flow regulation and natural flow intermittence

404 Most of our predictions about the shifts in life cycle-related functional traits caused by

405 flow regime alteration were confirmed, and common to those produced by flow

406 intermittence: an increase in taxa with multivoltine life cycles and a decrease in the

407 univoltine or semivoltine ones. Multivoltine cycles allow life cycle completion in wet

408 phases by conferring the capacity to recover from floods (Hershkovitz & Gasith, 2013),

409 after rewetting temporary streams (Bonada *et al.*, 2007b) and, in our study, also after

410 dam discharges. Thus, the presence of taxa with short life cycles in intermittent streams

411 confers tolerance to drought intensification as a result of flow regulation, while flow

412 regulation either eliminates or reduces taxa with semivoltine or univoltine cycles in

413 perennial rivers (antagonistic effects on life cycle duration).

414 The increased intensity of both stressors involved a reduction in the taxa with aquatic

415 eggs (the most sensitive stage to floods and dry phases), whose interaction led to an

416 increase in the taxa with aquatic adult stages (synergistic effect), which commonly

417 promotes resilience to flow intermittence (see the review by Stubbington *et al.*, 2017).

418 Adult aquatic stages are more tolerant to aquatic habitat fragmentation as the presence

419 of shrinking pools with harsh environmental conditions (e.g. higher temperatures,

420 mineralisation and distance among pools) are less suitable for earlier life stages

421 (Stubbington *et al*., 2017).

422 Although trait characteristics, such as small size and crawling, promote movement to

423 the hyporheic zone during drying in intermittent rivers (Stubbington *et al*., 2017),

424 reductions in small-sized taxa (0.25-0.5 cm) and crawling locomotion were observed

425 with increased flow alteration and/or flow intermittence. This could be due to different

426 causes in each river type. In perennial rivers, the loss of hyporheic habitat caused by

427 frequent and sudden discharges from dams produced sediment drag and channel

incision (Belmar *et al.*, 2013b). In the intermittent streams, water diversion entails dry channels during long periods below dams and riverbed "terrestrialisation", with loss of interstitial refuges (Belmar *et al.*, 2013b), which negatively affects both interstitial taxa and tegument breathers. In fact, the negative synergistic effect on interstitial taxa found between both stressors was not surprising in our study area, since interstitial taxa are typical of mountain streams with little anthropogenic disturbance, perennial flow and well oxygenated waters (Mellado-Díaz *et al*., 2008) as well as of intermittent alluvial rivers (Vander Vorste *et al.*, 2016).

Natural flow intermittence had some individual effects different from flow regime alteration, such as the increase in taxa with diapause or dormancy resistance forms that promote survival in dry phases (e.g. Gerridae), taxa with clutches in vegetation to avoid dry phases (e.g. some Odonata), full water swimmers that inhabit receding pools and predators (e.g. numerous Dytiscidae). The increase in taxa with diapause matched the expected proliferation of more resistant taxa (Lytle & Poff, 2004; Williams, 2006). However, Filipe *et al*., (2013) stated that organisms with scraper feeding habits, desiccation-resistant eggs and stream drift are common in intermittent streams in Mediterranean regions, and no significant increase was observed in these trait characters. At the same time, flow regime alteration also presented some individual effects not caused by natural intermittence, such as the reduction of aquatic active dispersion and the decrease of crawlers. The differences in the individual effects observed between both stressors may be influenced by the different predictability, duration and spatial patterns of the drying imposed by anthropogenic and natural hydrologic stress, given the importance of such patterns in intermittent and perennial streams (Bogan *et al.*, 2015; Aspin *et al.*, 2018; Sánchez-Montoya *et al.*, 2018).

452 The interaction of natural (flow intermittence) and anthropogenic (flow alteration)

453 stressors may produce opposing effects, like those in shredders, which decreased in

454 regulated perennial rivers but increased in strictly temporary streams with flow

455 alteration. This may be caused by natural differences in riparian areas (i.e., leaf litter

456 quantity and quality) between perennial and intermittent streams that change as a result

457 of the habitat alteration that flow regulation implies (Belmar *et al*., 2013b). Riparian

458 forests are a more important food source in perennial streams (in the absence of

459 alteration) than in semi-arid intermittent streams, where allochthonous organic matter

460 input is more limited (Velasco *et al*., 2003; Hagen *et al*., 2010). However, when the

461 "terrestrialisation" associated with anthropogenic flow regulation in intermittent rivers

462 takes place, particulate organic matter could be available for shredders when flow is re-

463 established.

464 *4.2 Responses of functional indices to flow regulation and natural flow intermittence*

465 As expected, there was a significant decrease in all functional indices (except FEve) in

466 response to stress, with a stronger effect for flow alteration compared to flow

467 intermittence. FDis, FD and FR underwent a non-random (i.e., an effect not attributable

468 to the underlying taxonomic pattern) decrease as flow regime alteration increased, while

469 only FR declined in response to natural flow intermittence and their interaction.

470 Anthropogenic disturbances, such as flow regime alteration, lead to reductions in FRic

471 and FR (Bruno *et al.*, 2016a, Oliveira *et al*., 2018), which can diminish ecosystem

472 resilience to future disturbances (Mouillot *et al*., 2013). On the other hand, intermittent

473 streams have often been characterised by lower FRic and FR values than perennial

474 rivers (Sriever & Lytle, 2006; Suárez *et al*., 2017), a trend that might be especially

475 marked during dry periods due to changes in species traits (e.g. Bêche *et al*., 2006).

476   Functional redundancy was the most sensitive index, as Bruno *et al.* (2016a,b) found

477   when using riparian vegetation. The antagonistic effect found between both stressors in

478   FR reflected a condition in which the adaptation of the aquatic community to natural

479   intermittence mitigated the effect of flow regime alteration, which also occurs with a

480   natural stressor such as water salinity (Suárez *et al*., 2017). Redundancy in temporary

481   streams can reflect the prevalence of taxa with traits that promote resistance and

482   recovery to flow intermittence (Boersma *et al*., 2014), and could confer tolerance to

483   natural and anthropogenic stressors.

484   Our study based on traits at genus level provides interesting results about the interaction

485   effects of both stressors. However, further research on functional traits at specific and

486   intra-specific levels is still recommended to refine functional trait responses, given the

487   uncertainty associated with the use of trait databases (i.e. low availability of traits for

488   analysis and lack of concordance between measured characteristics and published traits;

489   Orlofske & Baird, 2014). Future research is also needed to analyze the response to

490   natural and anthropogenic flow intermittence at larger spatial scales (i.e. among

491   drainage basins, Heino *et al.,* 2013).

492   **5. Conclusions**

493   This study highlights the relevance of considering the interactions of anthropogenic

494   disturbances (flow regulation) with underlying natural stress (flow intermittence) to

495   disentangle the ecological processes that take place in complex environments like

496   Mediterranean rivers, but also in other multi-stressed areas where different

497   environmental stressors interact. Intensification of flow intermittence and flow

498   regulation resulted in similar functional responses of macroinvertebrate communities,

499   like those produced by other natural (e.g. water salinity) and anthropogenic (e.g.

500   agricultural land use) stressors typical of Mediterranean streams. Besides, natural flow

501   intermittence modulated the functional response of communities to anthropogenic flow

502   alteration. Antagonistic, synergistic and opposing interactions were observed between

503   both stressors, thus the effect of flow regulation is conditional to the position along the

504   flow intermittence gradient. In general, the relevance of antagonistic interactions

505   (considering both trait composition and functional indices) means that the effect of flow

506   alteration was stronger in perennial than in intermittent streams, where the aquatic

507   communities are adapted to natural flow intermittence. In addition, flow regulation and

508   intermittence also act synergistically fostering some functional strategies to deal with

509   flow cessation and aquatic connectivity disruption such as resistant stages, but also

510   removing species sensitive to desiccation as interstitial taxa and tegument breathers. The

511   detection of such interactions between stressors is highly relevant for ecosystem

512   management and conservation in Mediterranean streams.

513   The functional approach used in this study and the obtained results could be useful for

514   other climatic areas where flow intermittence is predicted to increase as a result of

515   ongoing climate change, which will lead to more extreme and frequent drought

516   phenomena. In this context, further research to anticipate changes in the stressors

517   studied or the effect of other potential stressors results essential for water management

518   and biomonitoring. More attention must be paid to the specific river type and the

519   functional characteristic monitored when assessing the effects of flow alteration. Given

520   that the ecological effects of human pressures on aquatic communities could be difficult

521   to detect in naturally stressed ecosystems such as intermittent rivers, this sort of studies

522   results of special relevance to disentangle the effects of natural and human pressures, as

523   well as to identify those traits that could be sensitive to each type of stress. This has

524   direct applications to design biomonitoring programs and establish conservation goals.

Supporting information

Additional Supporting Information may be found in the corresponding file.

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Conflict of interest

The authors declare no conflict of interest.

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832   USA.   574   pp.

Tables

834 **Table 1.** Functional group (FG) description based on distinctive functional effect traits, including the number of taxa that compose each group.

FG	Main effect traits shared by the species within each functional group	Taxa
FG1	Big body size, aquatic active dispersion, predators eating living macroinvertebrates	29
FG2	Small body size, aerial active dispersion, piercers eating living invertebrates	11
FG3	Intermediate body size, swimmers, piercers and predators eating living invertebrates	25
FG4	Heterogeneous body size, full water swimmers and crawlers, shredders and scrapers eating living microphytes and microinvertebrates	26
FG5	Heterogeneous body size, crawlers , shredders and scrapers eating a variety of plant sources	26
FG6	Intermediate body size, crawlers and temporarily attached, shredders, deposit and filter feeders eating dead plants, detritus and living microphytes	23

835

836 **Table 2.** Results of the mixed-effect models showing the best-fitting model equation, *p*-values (only
837 significant values, in bold), marginal (R^2m) and conditional (R^2c) goodness of fit for trait characters that
838 presented significant interactions. Pairwise interactions are denoted with an asterisk (A: Flow regime
839 alteration; I: Flow intermittence; 'ns': non-significant). Interaction effect: A, antagonistic; S, synergistic;
840 O, opposing.

Trait	Category	Model equation	A	I	A*I	Effect	R^2m	R^2c
Life cycle	≤ 1 year	y=0.832-0.077A*I	n.s.	n.s.	**0.001**	A	0.269	0.285
duration	> 1 year	y=0.739-0.077A*I	n.s.	n.s.	**0.001**	A	0.269	0.285
Aquatic stages	adult	y=0.438+0.038A*I	n.s.	n.s.	**0.004**	S	0.239	0.239
Respiration	tegument	y=0.540-0.035A*I	n.s.	n.s.	**0.047**	S	0.191	0.191
Locomotion and substrate relation	interstitial	y=0.195-0.037I-0.018A*I	n.s.	**<0.001**	**0.034**	S	0.338	0.338
Feeding habits	shredder	y=0.620+0.053A*I	n.s.	n.s.	**0.045**	O	0.143	0.143

841

842 **Table 3.** Results of the mixed-effect models showing the best-fitting model equation, *p*-values (only
843 significant values, in bold), marginal (R^2m) and conditional (R^2c) goodness of fit for the different
844 functional diversity indices. Pairwise interactions are denoted with an asterisk (A: Flow regime alteration;
845 I: Flow intermittence; 'ns': non-significant). Interaction effect: A, antagonistic; S, synergistic; O,
846 opposing.

847

Response variable	Model equation	A	I	A*I	Effect	R^2m	R^2c
FRic	y=0.42-0.07A+0.04A*I	**< 0.001**	ns	**0.019**	A	0.29	0.32
Fdis	y=0.42-0.022A	**0.002**	ns	ns	ns	0.15	0.15
FEve	ns	ns	ns	ns	ns	ns	ns
FD	y=0.64-0.04A	**0.001**	ns	ns	ns	0.15	0.16
FR	y=1.34-0.21A-0.1I+0.08A*I	**< 0.001**	**0.025**	**0.038**	A	0.35	0.39

848

849 **Table 4.** Null model results (significant *p*-values in bold) for each tested functional index, selected from
850 Table 3 (A: Flow regime alteration. I: Flow intermittence).

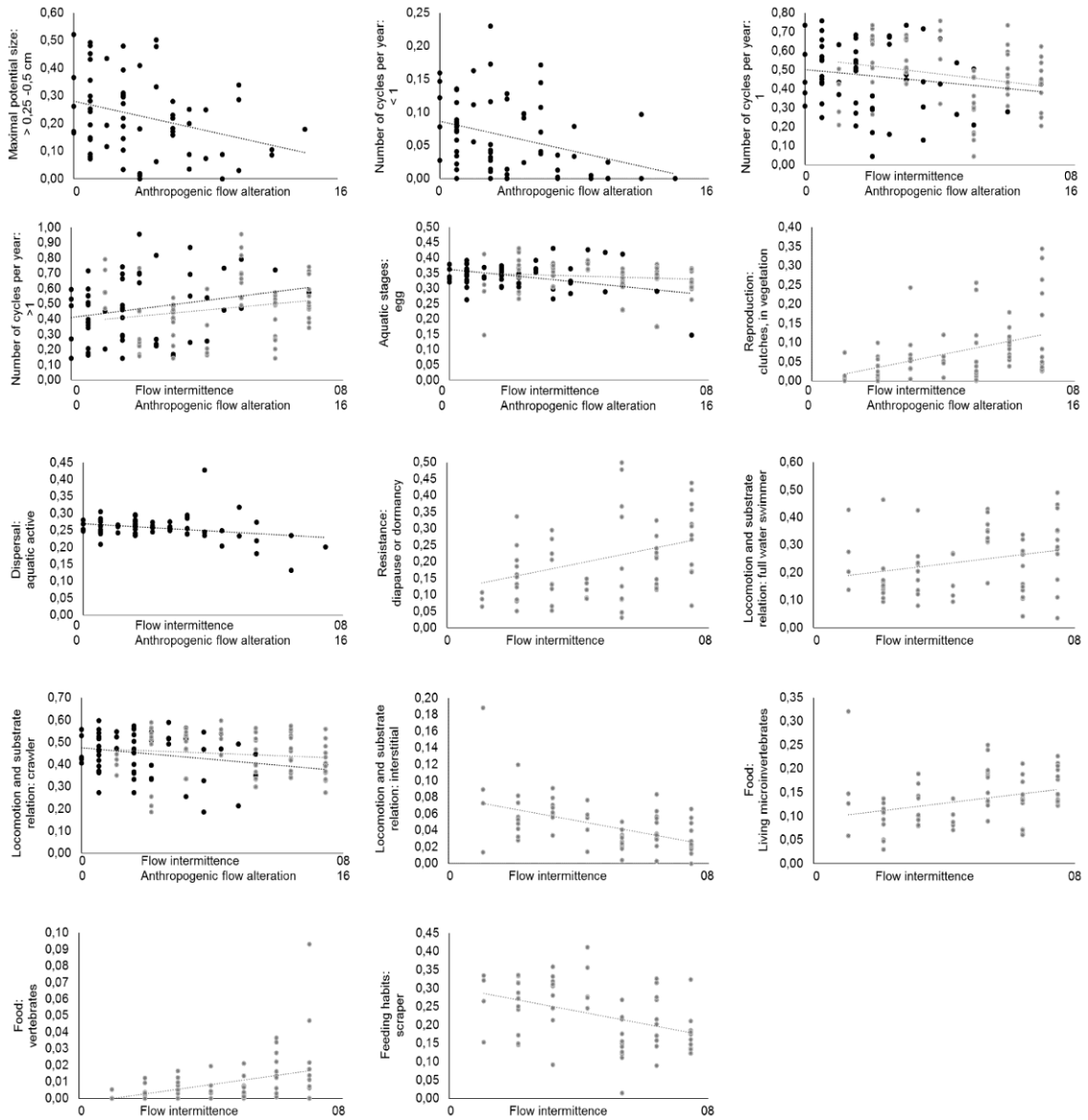|	Functional index			
Stressor	FRic	FDis	FD	FR
***Z* - score**				
Intercept	1.44	42.57	128.14	-40.31
A	-0.74	-6.24	-21.86	6.83
I	-	-	-	-4.33
A*I	-0.26	-	-	-4.55
***P*-value**				
Intercept	0.489	**0.001**	**0.001**	**0.001**
A	0.507	**0.001**	**0.001**	**0.001**
I	-	-	-	**0.001**
A*I	0.889	-	-	**0.003**

Figure captions

852 **Figure 1.** Study area showing the sites (grey points) and dams (black arrows) on the streams and rivers
853 classified according to the flow intermittence gradient (from "1" or perennial to "8" or ephemeral).
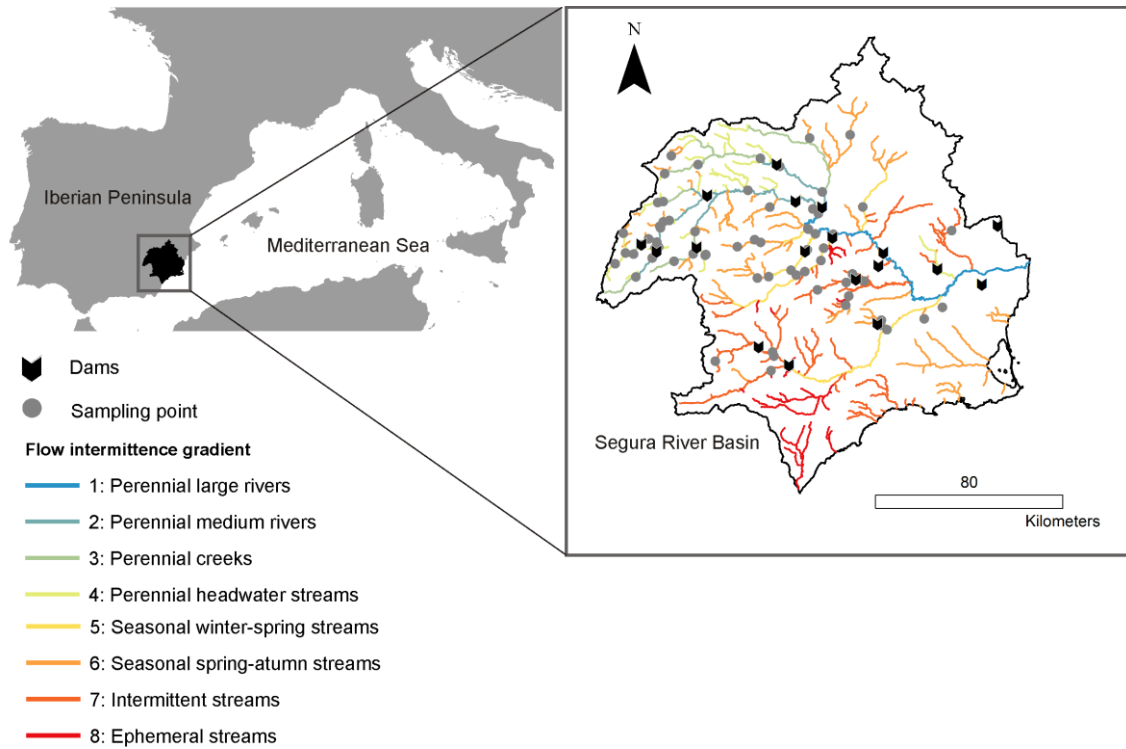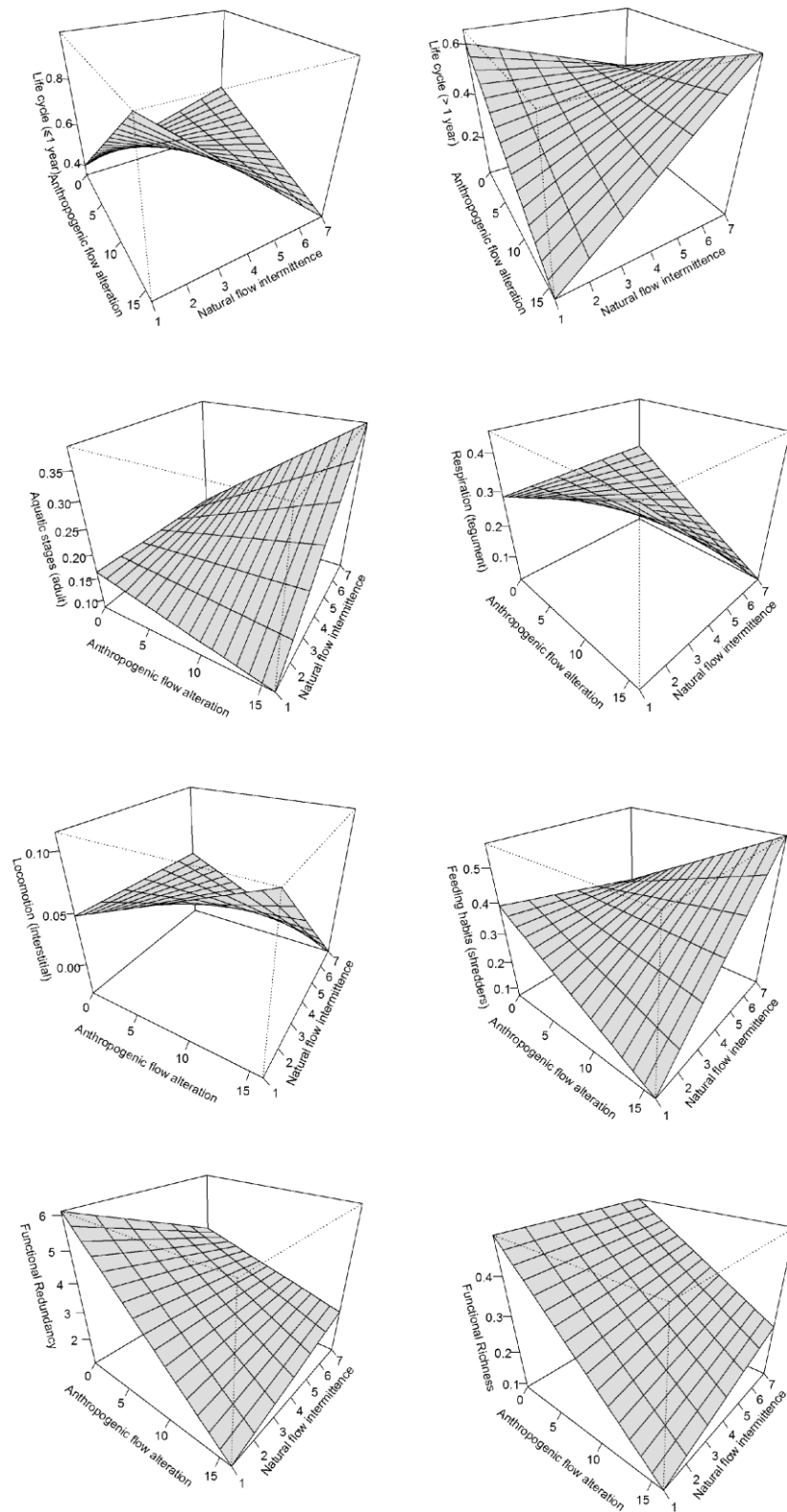

854

855

856 **Figure 2.** Linear plots between flow intermittence (grey) and anthropogenic flow alteration (black) and
857 proportion of each trait character that obtained statistically significant results for individual stressors. The
858 scale of natural stress has been adjusted to be represented together with anthropogenic stress.

859

860

861 **Figure 3.** Three-dimensional plots between flow intermittence, anthropogenic flow alteration and
862 functional characteristics (proportion of each trait character or functional index) that obtained statistically
863 significant results for interaction terms.

864