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25 **Diatom flora in Mediterranean intermittent streams: serious threat for endangered species**

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36 **ABSTRACT**

37

38 In the context of global environmental changes, Mediterranean rivers are considered highly endangered. Temporal and
39 spatial increases of the dry stretches during the summer lead to the loss of river tridimensional connectivity, which
40 represents a major threat for freshwater biodiversity.

41 In this study, we aimed at exploring the response of diatom communities to summer droughts by analyzing taxonomical
42 composition, specific ecological requirements, ecological guilds and percentages of endangered species. The evolution
43 of diatom communities was monitored under both intermediate and intermittent flows, with traditional and innovative
44 sampling procedures, i.e. collecting diatoms from transects and microhabitats, respectively. Microhabitats differed in
45 terms of water velocity, substrate, isolation and presence of macrophytes.

46 Diatom flora was mainly composed of β -mesasoprobous taxa. We highlighted an increase of species considered as
47 aerophilous and planktonic in sites characterized by intermittent flow. In general, ecological guilds did not respond to
48 hydrological disturbance as expected. Statistical models identified the maintenance of a minimum of 0.20 m/s flow
49 velocity as the main factor influencing the abundance of endangered species. Conversely, flow instability, lentification
50 and habitat fragmentation represented the major threats for endangered species.

51 In conclusion, diatoms can provide useful information to improve river management practices when faced with an
52 increasing water scarcity scenario. Water stability and river habitat heterogeneity strongly favor the presence of
53 endangered diatom species. In the absence of these conditions, isolated pools surrounded by dry riverbed are very
54 important habitats to be preserved, representing the only *refugia* for benthic diatom communities during summer.

55

56 Keywords: Red List, Bacillariophyceae, hydrological instability, pools

57

58

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86 INTRODUCTION

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88 The Mediterranean basin is considered one of the most important biodiversity hotspots worldwide in terms of endemic
89 species, and is considered to be greatly under threat (Cuttelod et al. 2008; Myers et al. 2000). Within this setting,
90 Mediterranean freshwater ecosystems are considered highly endangered, more so than terrestrial ones (Sala et al. 2000),
91 with a potentially huge loss of biodiversity. According to the review published by Dudgeon et al. (2006), the major threats
92 for freshwater biodiversity can be both local (i.e. overexploitation; water pollution; flow modification; destruction and
93 degradation of habitat; exotic species invasion) and global (environmental changes, such as nitrogen deposition; global
94 warming; shifts in precipitation and runoff patterns). Biodiversity conservation and habitat integrity in rivers can be partly
95 guaranteed by maintaining the natural flow stability (Dudgeon et al. 2006). With respect to diatoms, their diversity and
96 species richness in Mediterranean streams are more closely related to hydrological variables than to physical-chemical
97 features (Ros et al. 2009). Mediterranean rivers are naturally characterized by hydrological variations with extreme flows
98 during autumn and winter, with droughts in summer (Pardo and Álvarez 2006). This phenomenon, recently exacerbated
99 by human impact, has led to a temporal and spatial increase of the dry stretches, especially during the summer months.
100 The main consequences of this phenomenon are habitat fragmentation and loss of river tridimensional connectivity
101 (longitudinal, transversal and vertical; Bonada et al. 2006). In this context, residual isolated pools play an essential role
102 for benthic communities in terms of species survival (Ros et al. 2009), and significantly contribute to the recolonization
103 of the stretches after the return of the water (Robson and Matthews 2004).

104 In general, diatoms have developed coping mechanisms in order to confront harsh conditions, and are able to produce
105 different resting forms, namely resting cells, spores and winter stages (McQuoid and Hobson 1996). Spore production
106 requires a vast amount of energy, and cannot be considered, therefore, as a sustainable mechanism to face short-term
107 environmental changes (Round et al. 1990), such as summer droughts in Mediterranean streams. Conversely, the
108 production of resting cells is faster and uses less energy, as no additional silica is required. It has been recently
109 demonstrated that *freshwater diatoms* (as defined in the classification of van Dam et al. 1994) are not able to survive
110 desiccation as vegetative or resistance cells; on the contrary, *terrestrial diatoms* are able to face desiccation as resistance
111 cells and, in some strains, as vegetative cells (Souffreau et al. 2013). In the same study, the authors demonstrated that
112 acclimatization increases the tolerance of diatom strains to desiccation; this demonstrates that droughts can have a stronger
113 negative impact on diatom communities in recently intermittent rivers than in Mediterranean regions.

114 An overall assessment of the diatom biodiversity in Mediterranean rivers is affected by many limitations such as the
115 inconsistent application of taxonomical rules, the lack of historical data and the patchiness of the investigated areas
116 (Tierno de Figueroa et al. 2013). Considering the big void in the literature, the role of the recent studies, which were

117 mainly carried out in Spain (Boix et al. 2010; Ros et al. 2009) and in Portugal (Elias et al. 2015; Novais et al. 2014), is
118 very important. In the scheme of the Water Framework Directive (WFD; European Commission 2000), an important step
119 was carried out by Feio et al. (2014) who defined the Least Disturbed Conditions (LDC) for European Mediterranean
120 rivers. In this context, defining the conservation status of diatom species is an important challenge. Currently, there is
121 only one published Red List, compiled by Lange-Bertalot and Steindorf (1996) for German watercourses. This topic has
122 been widely investigated in several studies carried out in the Alps, where the presence of rare and endangered taxa was
123 shown to be correlated with habitat peculiarity. For instance, almost 50% of the diatom species recorded in the springs of
124 the Adamello-Brenta Nature Park can be considered rare or threatened (Cantonati 1998). In the same way, about 30% of
125 the taxa recorded in lentic habitats of the Maritime Alps Natural Park (mainly springs and peatbogs) can be considered
126 “decreasing” or “endangered” (Falasco and Bona 2011). Conversely, the conservation status of diatom flora in
127 Mediterranean streams was only recently explored by Novais et al. (2014), who highlighted a high proportion of
128 endangered species in permanent and temporary rivers in Portugal and stressed the need to update and complete the
129 diatom Red List with recently described taxa. As indicated by Denys (2000), the abundance of threatened species, as
130 opposed to the number of species itself, can be considered a useful tool for assessing the loss of microhabitat and for
131 evaluating possible deviation from pristine conditions. Therefore, the proposal of creating Red Lists on a scale of more
132 ecologically homogeneous regions, such as hydroecoregions, should be seriously taken in consideration.

133 Given the lack of knowledge on this topic, we focused on the biodiversity status and presence of endangered diatom
134 species in Mediterranean rivers in the Italian peninsula. The aims of our research were i) to investigate the diatom
135 communities in Mediterranean streams in order to provide a baseline knowledge of the flora from both a taxonomical and
136 an ecological point of view; ii) to evaluate diatom biodiversity and the presence of threatened and endangered species
137 under stable (SPRING) and unstable (SUMMER) hydrological conditions.

138 Smucker and Vis (2011) highlighted a significant underestimation of diatom diversity when exclusively collected from
139 epilithic habitats for documenting species distribution and for conservation purposes. Starting from this consideration,
140 here we collected diatoms following two different sampling techniques, namely from transects located in riffles (T) and
141 from microhabitats (MH). These two approaches were chosen in order to obtain the highest diatom diversity for each site;
142 in this way, we were able to gather a significant environmental dataset that was used to better define the ecological
143 preferences of endangered species and, at the same time, to evaluate the effect of habitat heterogeneity and fragmentation
144 on diatom diversity via statistical models. In view of the results obtained, possible methods of management to mitigate
145 the impact of drought are discussed.

146

147 **MATERIALS AND METHODS**

148 *SAMPLING DESIGN*

149 A total of ten Mediterranean streams in Liguria (NW Italy; Fig. 1 and Online resources 1), two in the Apennines N hydro-
150 ecoregion (HER 64) and eight in the Ligurian Alps (HER 122) were selected. To reduce the environmental variability
151 between sites, we only selected stretches characterized by a low anthropogenic pressure. For this purpose, we performed
152 a land use analysis, and chose sampling sites with less than 50% of urban land use calculated in a 100 m buffer (Online
153 resources 1). In addition, we checked historical physical-chemical data provided by the Ligurian Environmental Agency
154 (ARPAL) and we carried out an *in situ* visual characterization of the sampling sites.

155 Five sampling surveys were carried out. The first one was conducted during SPRING (April 2014), under intermediate
156 flow conditions, and involved all ten rivers. This sampling provided a baseline for the knowledge of the diatom flora in
157 the absence of hydrologic disturbance. The other samplings were conducted during SUMMER (late June, July, August
158 and September), when water scarcity characterized part of the stretches to different extents. In these sampling sessions,
159 only five streams out of the initial ten were monitored, namely Argentina, Impero, Merula, Quiliano and Vallecrosia. In
160 order to gain a better knowledge of diatom flora under extreme drought conditions, we decided to focus only on these
161 streams, which have shown the most intermittent character in recent years (historical data provided by the Ligurian
162 Environmental Agency). For each stream, we selected two sampling sites: the first one characterized by permanent flow
163 throughout the year (upstream=UP), and the second one by intermittent flow, with part of the riverbed completely dry
164 during summer (downstream=DW).

165 *PHYSICAL-CHEMICAL PARAMETERS*

166 In each sampling site, we measured: a) the main physical-chemical parameters, i.e. dissolved oxygen (DO) in the water,
167 pH, temperature (TEMP), and conductivity (COND), using a multiparametric probe (Hydrolab mod. Quanta); b) total
168 suspended sediments (TSS) following the Italian standard methods (APAT-IRSA. CNR, 2003); c) flow velocity (VEL),
169 with a current meter (Mod RHCM Idromar) positioned at 0.05 m from the bottom of the riverbed; d) water depth (DEPTH)
170 with a meter tape; e) soluble reactive phosphorous (SRP) and nitrate (N-NO₃) with a LASA 100 spectrophotometer,
171 according to APAT-IRSA CNR standard methods (2003). Environmental features were evaluated *in situ* by visually
172 attributing percentages to: the main substrate composition, macrophytes and algae coverage and checking the presence or
173 absence of shade and connection with the main flow.

174 *DIATOM ANALYSIS*

175 In each site, six epilithic diatom samples were collected and kept separate for the analysis of diatom communities. We
176 followed two different sampling approaches: one sample was collected in accordance with the *transect approach* (T),

177 while the other five samples were collected using the *microhabitat approach* (MH). The T approach followed the standard
178 procedure defined by the European Committee for Standardization (2003). We chose at least five cobbles from the main
179 flow and we collected periphyton by scraping their upper surface using a toothbrush. Considering the MH approach, five
180 microhabitats were selected at each site. Microhabitats were differentiated in terms of current velocity, depth, dominant
181 substrate, presence of macrophytes and shade. When present, isolated pools were preferentially selected. In both cases,
182 we chose to sample only cobbles, in order to reduce the effect of the substrate typology and focus on the influence of the
183 surrounding microhabitat. All diatom samples were preserved in ethanol. Samples were subsequently treated in the
184 laboratory following the standardized method (European Committee for Standardization 2003) by cleaning them with
185 hydrogen peroxide (30%) and HCl. Slides for observation at the light microscope were mounted by means of Naphrax®.
186 Diatom identification was based on several diatom floras and monographies, as well as on recent taxonomic papers (Bey
187 and Ector 2013; Blanco et al. 2010; Ector et al. 2015; Falasco et al. 2013; Hofmann et al. 2011; Krammer 1997 a, b, 2002,
188 2003; Krammer and Lange-Bertalot 1986-1991 a, b; Lange-Bertalot 2001; Lange-Bertalot and Metzeltin 1996; Reichardt
189 1999; Werum and Lange-Bertalot 2004), and at least 400 valves per sample were identified. Diatom communities were
190 analyzed in terms of biodiversity, taxonomical and functional composition of the communities and presence and relative
191 abundance of endangered taxa. The recorded species were classified by means of the OMNIDIA 5.3 software with 2015
192 database, on the basis of their ecological preferences, habitat (Denys 1991), moisture, pH and trophic state (van Dam et
193 al., 1994), and conservation status (Lange-Bertalot and Steindorf 1996).

194 The *Correspondence Analysis* (CA), which is an unconstrained multivariate technique, was applied to the community
195 data in order to visualize the dissimilarities of samples in terms of species composition. Data from SPRING and
196 SUMMER were kept separate. Data in the species matrices were first square root-transformed to achieve a normal
197 distribution. For this analysis, data from the samples collected with the MH approach were merged together. A total of
198 40 samples including 98 species, and 66 samples including 121 species were used for the SPRING and the SUMMER
199 seasons, respectively.

200 In order to understand if there were endangered taxa typical of specific habitats, we performed the Indicator Species
201 Analysis (ISA) on samples collected from all the sampling operations against the following groups: months, rivers,
202 sampling site location, sampling methods, flow velocity, water depth, shade, isolation, dominant substrate, macrophyte
203 presence, algae presence (see Table 3 for further details on group definition).

204

205 *STATISTICAL MODELS*

206 To determine which environmental parameters may favor the presence and abundance of endangered species, we applied
207 Generalized Linear Mixed Models (GLMMs) assuming a Poisson error distribution (Zuur et al. 2009). Two different
208 model structures were tested; the first was the *mesohabitat model*, including month, sampling site, sampling approach
209 and disturbance as fixed effects. We considered samples collected under flow instability (i.e. MH samples from DW
210 during SUMMER) as being disturbed, while all the remaining samples were considered as being collected from
211 undisturbed conditions. The second model was the *microhabitat model*, including flow velocity (categorical variable:
212 group 0 = $v \leq 0.20$ m/s; group 1 = $v > 0.20$ m/s), water depth and macrophyte coverage as fixed effects. We expressed
213 flow velocity as a categorical variable because of the high imbalance towards zero values. Given the spatial dependence
214 of the data, we applied the mixed procedure to include two grouping variables (river and site) as random factors, in order
215 to account for the variation that they introduce into our samples. Before performing GLMMs, data were firstly explored
216 via boxplots to assess the presence of extreme values and avoid unusual observations that may influence the estimated
217 parameters (Zuur et al. 2009). CA was performed with the package *vegan* (Oksanen et al. 2015) and the ISA was
218 performed with the package *indicspecies* (De Caceres and Legendre 2009) in R environment (R Core Team 2015), while
219 GLMMs were performed via the PROC GLIMMIX (SAS software 9.2).

220

221 **RESULTS**

222 *PHYSICAL-CHEMICAL PARAMETERS*

223 Physical-chemical parameters detected during the samplings are shown in Table 1 and in the Online Resource 1.
224 Environmental parameters were comparable between the two sampling seasons, with the exception of minimum-recorded
225 values of DO, which were lower in SUMMER than in SPRING. In UPs, the lowest DO values (27.4%) were reached in
226 the Argentina river, in a shaded lateral pool characterized by silt and coarse particulate organic matter (CPOM) as the
227 main substrates. In DWs, the lowest DO values were generally associated with isolated pools, reaching extreme values of
228 15% and 27%. Nutrient concentrations were low in most of the studied stretches, with SRP levels being contained within
229 the highest quality class in all cases, and nitrates within the second class (Italian Water Legislation D. Leg. 152/2006 and
230 successive ones) in both the intermediate and low flow periods. In accordance with this consideration, chemical
231 parameters were generally below the thresholds proposed by Feio et al. (2014) for the definition of the LDC for European
232 Mediterranean rivers, with the exception of some values for nitrates in DW sites in SPRING and of DO concentration in
233 SUMMER. TSS values were moderate; the highest value (21.17 mg/l) was observed in Varatella (DW), probably due to
234 the presence of outfalls. The pH ranged from circumneutral to alkaline values and reflected the geology that mainly

235 consists of limestone, sedimentary rocks and ophiolites, which dominates the Western part of the region. Conductivity
236 decreased from West to East, following the gradual change in the geological composition.

237

238 *DIATOM ANALYSIS*

239 *Biodiversity*

240 The complete checklist of all the taxa detected in the samples, their ecological requirements, life forms, ecological guilds
241 and conservation status are displayed in the Online Resource 2. A total of 126 diatom samples were analyzed for the
242 SPRING season and a total of 171 taxa belonging to 44 genera were identified. On average, the number of species that
243 composed the communities was comparable in the UP and DW sites (Table 1), as well as the Shannon diversity index,
244 with highest median values in the MH samples of the UPs ($S=2.72$).

245 A total of 240 diatom samples were analyzed for the SUMMER season and a total of 241 taxa belonging to 58 genera
246 were identified. In these samples, species richness was, on average, higher in the UPs than in DWs (Table 1). Regarding
247 biodiversity, Shannon values were higher in the UP than DW sites in June and July, while no substantial differences were
248 observed in August and September, when the median values were comparable. In UPs, MH samples hosted higher
249 biodiversity in June and July (H_{MEDIAN} : June = 3.18; July = 3.25) than T (H_{MEDIAN} : June = 3.02; July = 3.19). Conversely,
250 the MH samples generally showed a negative effect on diatom biodiversity in the sites most subjected to hydrological
251 disturbance i.e. DWs (H_{MEDIAN} : June = 2.60; July = 2.64; August = 3.08). Despite this, we observed some outliers of the
252 Shannon index in DW isolated pools during August ($H_{\text{MAX}} = 4.07$), similar to the results obtained by Ros et al. (2009).

253

254 *Community composition*

255 In all samples, *Achnantheidium minutissimum* was the most abundant and frequent species, followed by *Achnantheidium*
256 *pyrenaicum*. In the Argentina stream, *Achnantheidium delmontii* was also consistently present in both UPs and DWs, with
257 mean relative abundances of 24.4% and 15.3% in T samples, respectively. In the Arrestra stream, in addition to *A.*
258 *minutissimum* and *A. pyrenaicum*, the DW site was characterized by the presence of *Diatoma ehrenbergii* (mean relative
259 abundance of 27.0% in the T sample). Communities in the Impero stream were dominated by *A. minutissimum*, but
260 *Amphora pediculus* also presented high values of relative abundance, especially in the UPs. The Merula stream presented
261 the most unusual flora, namely *Encyonopsis subminuta* and *E. minuta* were found in both UPs and DWs, as well as
262 *Cymbella subtruncata*; in DWs, *Denticula kuetzingii* was often present with an average relative abundance of 10.1% in
263 T samples. The genus *Encyonopsis* was also abundant in the Vallecrosia stream, in particular *E. minuta* and *E. subminuta*.
264 In the Porra stream, the evenness was higher in UPs than DWs; as well as *A. minutissimum*, communities were composed

265 of *Nitzschia inconspicua*. In the UPs, we mainly found sensitive species such as *Cocconeis lineata* and *Achnanthydium*
266 *subatomus*, while in the DWs a large proportion of the community was represented by the tolerant species *Mayamaea*
267 *permitis*. In the Sansobbia stream, 11% of the UP community was characterized by *Nitzschia fonticola*, while *Encyonema*
268 *silesiacum* represented 11% of the DW community. In the Varatella stream, *N. fonticola* also presented high values of
269 relative abundance (22.5%) in UPs, while *Achnanthydium lineare* characterized the DW station (15.0%). In the Quiliano
270 stream, the genus *Fragilaria* (and in particular *F. rumpens*) was frequently recorded, especially in the DW station, where
271 the presence of *Cymbella tropica* was also important. In the Sciusa stream, the genus *Gomphonema* was highly
272 represented in the UP station, while *Cymbella excisa* was abundant in DWs.

273 The CA performed on the SPRING biological dataset (Fig. 2a) revealed a strong dissimilarity between samples collected
274 in different rivers. Moreover, sites located on the negative part of the CA2 axis belonged to streams with higher values of
275 conductivity (>400 $\mu\text{S}/\text{cm}$). Given these results, the importance of mineral content on diatom assemblages collected from
276 Mediterranean streams with comparable nutrient levels was once again confirmed (Sabater et al., 1988; Blanco et al.,
277 2008).

278 Considering the CA performed on the SUMMER biological dataset (Fig. 2b), the site separation driven by stream identity
279 was even more evident, highlighting the peculiarity of the diatom flora in Mediterranean streams.

280

281 *Ecological requirements*

282 In SPRING, diatom communities were mostly composed of β -mesosaprobous species (60% of the total abundance; van
283 Dam et al. 1994), confirming the good water quality revealed from the chemical analyses. In general, 83% of the detected
284 species preferred mean values of salinity and only 9% can be considered as brackish-freshwater taxa (i.e. 500-1000 mg
285 Cl/l or 0.9-1.8 % of salinity; van Dam et al. 1994). The most abundant species belonging to this category were *Navicula*
286 *gregaria* and *Nitzschia inconspicua*. Concerning moisture requirements, 31.6% of the recorded species were classified as
287 mainly occurring in water bodies, but also regularly present on wet and moist places (MOIST=3); while 3.5% were
288 classified as mainly occurring in wet and moist or temporarily dry places (MOIST= 4; van Dam et al. 1994). These taxa
289 were *Adlafia minuscula* and *Geissleria acceptata*. According to the classification of Denys (1991), *A. minutissimum*, one
290 of the most abundant species recorded in this study, should also be considered as commonly recorded in dry subaerial
291 habitats. No strictly terrestrial species were detected. In terms of current velocity, most species (70%) were indifferent to
292 water flow (Denys 1991).

293 There were differences observed in terms of ecological requirements for SUMMER species. In June, diatom communities
294 were mainly composed of β -mesosaprobous taxa. *Achnanthydium minutissimum* and *A. pyrenaicum* dominated the
295 communities, with 70% of total relative abundance in both UP and DW, with no differences between MH and T samples.

296 We observed a higher abundance of species belonging to the genus *Cocconeis* in the UP sites, probably due to a greater
297 presence of aquatic macrophytes. In the DW sites (in particular in MHs), we noted a higher relative abundance of
298 *Denticula kuetzingii* and *Fragilaria pararumpens*, as well as of taxa belonging to the genus *Cymbella*. In July, the growing
299 presence in the Argentina river of *Achnanthydium delmontii* was evident, known for being an invasive species, in
300 accordance with the criteria proposed by Coste and Ector (2000), along with a higher relative abundance of *Amphora*
301 *pediculus* in the UPs. During this sampling session, *D. kuetzingii* was no longer exclusive to the DW sites, but was also
302 recorded in the MH samples of the UP sites. In August, the abundance of *A. minutissimum* was drastically reduced,
303 especially in the UPs, to the same levels as *A. pyrenaicum*, which was almost not recorded in the DWs. There was,
304 however, a general increase of more tolerant species, considered as α -meso-polysaprobous, such as *Eolimna minima*,
305 *Gomphonema parvulum* and *Ulnaria ulna*. In the UP sites, the second most abundant species was *A. pediculus*; moreover,
306 the abundance of *Achnanthydium delmontii* doubled in comparison with the previous sampling session. There was a
307 general increase in the relative abundance of species of the genus *Encyonopsis*, namely *Encyonopsis minuta* and *E.*
308 *subminuta*, compared to the sampling in July. In September, species compositions were similar to those found in August,
309 with slightly lower abundance of α -meso-polysaprobous taxa.

310 Concerning moisture requirements, 30.6% of the species recorded in SUMMER were classified as "MOIST=3".
311 Compared to April, we observed an increase in the number of species classified as mainly occurring in wet and moist or
312 temporarily dry places (MOIST=4; van Dam et al. 1994), representing 4.4% of the total species. Within this category,
313 *Fragilaria alpestris* and *Halamphora montana* were the most frequent and abundant, despite only being found as a few
314 individuals. Only one strictly terrestrial diatom was recorded, namely *Adlafia bryophila*, found in the Vallecrosia UP site,
315 in a slightly shaded MH, characterized by slow flow and 100% filamentous and mat algal riverbed coverage. Species
316 belonging to the MOIST categories 3 and 4 were almost exclusive from the MH samples, but no differences among
317 sampling months were observed.

318 Concerning flow velocity, most of the species were indifferent to water speed. However, we detected three limnophilous
319 taxa, namely *Amphipleura pellucida*, *Cymbella neoleptoceros* and *Diploneis elliptica*. In particular, *C. neoleptoceros*
320 presented the highest percentages in the Impero DW site during September, when the hydrological disturbance was at its
321 maximum.

322 Considering functional traits (Table 1), during SUMMER, colonial taxa were more abundant in the DW sites, where they
323 represented, on average, more than 10% of the communities. No substantial differences were observed between the T and
324 MH samples. This result confirmed the preference of colony-forming diatoms for lentic habitats (Rimet and Bouchez
325 2012) and for unpredictable water flow (Passy 2002). Contrarily to our expectations, *low profile* guild was generally more
326 abundant in the UP than DW sites, while the *high profile* guild was much more abundant in the DW sites. As also observed

327 by Elias et al. (2015), in our research the physical disturbance created by the drought did not increase the relative
328 abundance of the *motile* guild, as we would have expected. Indeed, the *motile* guild was generally more abundant in the
329 UP sites, and more abundant in the MH than in T samples, with the exception of the UP sites in September. Species
330 considered as *adnate* were much more abundant in the UP sites where they preferred the MHs. We observed an opposite
331 trend for the *peduncolate* taxa (both stalked and pad-attached to substrate), which presented a preference for the DW sites.
332 For both UP and DW sites, *peduncolate* taxa were more abundant in the T than MH samples with the exception of DWs
333 in September. The highest peaks in abundance for taxa forming mucous tube colonies were found in the DW sites during
334 the hottest months and generally in the MH samples.

335

336 *Conservation status: Red List species*

337 The percentage of recorded species belonging to different conservation categories is summarized in Table 2. The number
338 of species considered as being endangered per sample was higher in SPRING than in SUMMER, in particular for taxa
339 classified as threatened with extinction. From the results, it is important to note that more than 30% of the recorded species
340 in both SPRING and SUMMER were still not classified in accordance with the Red List. Indications on the statistically
341 significant occurrence of taxa in terms of months, rivers, site location and sampling methods, water velocity and depth,
342 shade, isolation, dominant substrate, macrophyte and algae coverage are shown in Table 3. Throughout the entire the
343 sampling period, we recorded *Didymosphenia geminata* among the “threatened with extinction” taxa. *D. geminata*
344 showed a preference for sites in which macrophytes consistently cover the riverbed (ISA; $p=0.023$). Of the “endangered”
345 species, *Achnanthydium lineare* and *A. gracillimum* were the most abundant and frequent in SPRING, and were mainly
346 present in the Arrestra stream, characterized by high habitat integrity, with peaks in abundance in the MH samples. In
347 particular, a semi-isolated shallow pool and a deeper pool with abundant CPOM sheltered these two species, along with
348 *Achnanthydium exile* that is considered as “decreasing”. During SUMMER, *A. lineare* and *A. gracillimum* were again the
349 most abundant species, with peaks during August and September. In particular, *A. lineare* represented more than 50% of
350 the communities in three samples collected in the UP site of the Quiliano stream, during both August and September. All
351 the samples were collected in shallow pools (flow velocity = 0 m/s and depth < 20cm) shaded by the riparian vegetation.
352 *A. lineare* appeared to be limited by the presence of other benthic algal groups (ISA; $p=0.005$) and preferred naturally
353 shaded (ISA; $p=0.022$) sites. *A. gracillimum* was found in the DW site of the Quiliano stream during September, and was
354 present in all the samples (T and MHs) with the exception of the only isolated pool that was sampled. *A. gracillimum* was
355 recorded in shallow (ISA; $p=0.032$) standing or flowing waters, always connected to the main flow and showed a
356 statistically significant preference for microlithal as the main substrate (ISA; $p=0.006$). Under the same category,
357 *Nitzschia gessneri* was present in the samples of June and July, with a clear preference for the MH samples. This species,

358 not recorded in SPRING, reached peaks in the Merula river, in both the UP and DW sites. In particular, we recorded its
359 presence in an isolated pool with intermittent water presence. *Navicula novaesiberica*, considered as “rare”, was abundant
360 in the Varatella DW site, in a very shallow pool with standing water and high siltation. Among the “probably endangered”
361 taxa, we highlighted *Ulnaria biceps* and *Gomphonema tergestinum* as the most abundant. The former presented the
362 highest abundance during July and September at the DW sites of Vallecrosia (T) and Quiliano (MH) streams, respectively.
363 These samples were collected in shallow (depth ca. 12 cm) flowing (velocity ca. 0.20 m/s) waters with a significant
364 coverage of macroscopic filamentous green algae (from 60 to 100%). The populations were always composed of a few
365 individuals, confirming the observations of Bey and Ector (2013). In the “decreasing” category, we detected 14 species,
366 the most abundant of which were *Gomphonema lateripunctatum* and *Nitzschia tabellaria* that presented peaks in
367 abundance during the warmer months. In particular, the Merula DW site hosted the highest abundance of *G.*
368 *lateripunctatum* in July and September. During July, the species showed peaks in abundance in a pool connected with the
369 main watercourse, presenting standing water and 35 cm of water depth. In September, *G. lateripunctatum* was found in
370 the same stretch as in July, but with a peak in abundance in a MH with flowing water (velocity = 0.13 m/s and depth =
371 11 cm). The preference of *G. lateripunctatum* in the Merula stream can be explained by the fact that it is commonly found
372 in the Mediterranean hydroecoregions with preferences for calcareous streams (Delgado et al. 2013; Gomà et al. 2004).
373 This species was significantly present in pristine sites characterized by microlithal as the main substrate, and its abundance
374 was not limited by isolation from the main flow. *Nitzschia tabellaria*, considered as being characteristic of habitats of
375 high conservation value (Potapova and Charles 2007; Smucker and Vis 2011) was particularly abundant in the UP site of
376 the Argentina stream.

377

378 *STATISTICAL MODELS*

379 Results of the statistical models showed that environmental parameters had a stronger effect on endangered species
380 abundance rather than on their richness. The *mesohabitat model* (Table 4) showed that the sampling month and the
381 sampling method significantly affected the abundance of endangered species, with higher values in April, June and July
382 than in August and September ($p < 0.0055$), and higher values in T than in MH samples ($p = 0.0325$). In April (Fig. 3),
383 the highest abundances were found in MH samples (in both UPs and DWs). Peaks in the abundance of endangered species
384 were observed in the Arrestra stream (UP and DW sites) in two lateral pools connected with the main flow and shaded
385 by the riparian vegetation. During SUMMER (Fig. 4), in the UP sites the highest median values were reached in the MH
386 samples, except in June, in which the median was slightly higher in T samples. In the DW sites, T generally hosted the
387 highest abundance of endangered species. However, if we consider the extreme values, we can observe that peaks of
388 endangered taxa were mainly observed in the MH samples of UP sites, but also in DW sites.

389 Considering the *microhabitat model* (Table 4), significant differences were observed between the two flow velocity
390 categories, with higher values in running waters (group 1) than in standing waters (group 0) ($p = 0.0360$). A negative
391 significant effect of macrophyte coverage was also detected, suggesting that microhabitats with standing waters, normally
392 hosting a high percentage of macrophytes, are less suitable for sheltering endangered species.

393

394 **DISCUSSION**

395 Mediterranean freshwater ecosystems are currently facing a huge species loss, thus calling for evaluation of their
396 biodiversity status (Dudgeon et al. 2006). In this context, diatoms represent a poorly investigated group of freshwater
397 organisms (Novais et al. 2014). In our study, we applied an integrated sampling approach, which allowed us to investigate
398 microhabitats that are usually underrepresented. We highlighted environmental parameters that favor the abundance of
399 endangered taxa and we better defined the ecological preferences of certain threatened species.

400 Firstly, the CA results showed that diatom communities were highly separated at the stream level in terms of community
401 composition, especially during the low flow season. This result is in accordance with Tornés & Ruhí (2013), who observed
402 a higher frequency of idiosyncratic species in hydrologically disturbed rivers, and with Novais et al. (2014), who observed
403 that diatom species in permanent watercourses are also present in temporary watercourses but not vice versa. This
404 highlighted the peculiarity of Mediterranean rivers and underlined the need to redefine the Red List at the
405 hydroecoregional level. Indeed, even if some species are commonly found in other hydroecoregions, they may become
406 rare in the Mediterranean area since they could be relegated to a single watercourse. According to our results, drought in
407 Mediterranean rivers seems to be the main motive for the reduction of the abundance of endangered species. Indeed, as
408 demonstrated by our *mesohabitat model*, we observed a reduction in the abundance of endangered taxa in August and
409 September, and the standard samples, performed in the main stream channel, always hosted the highest abundance of Red
410 List species. The results of the *microhabitat model* also confirmed this trend, as the presence of flowing water (> 0.20
411 m/s) proved to be a determinant parameter for guaranteeing a high abundance of endangered species.

412 In Mediterranean streams, terrestrial species represent key organisms in the recolonization of watercourses following
413 drought. As mentioned previously, Souffreau et al. (2013) observed that only species that tolerate low values of moisture
414 were able to survive desiccation through resting cell formation, sometimes as vegetative forms. In Mediterranean rivers,
415 this strategy would greatly help diatoms to face harsh conditions during the summer months, increasing the survival rate
416 and favoring the recolonization after the return of waters. For this reason, particular importance should be given to the
417 presence of these taxa in rivers characterized by hydrological disturbance, and their inclusion in the Red List as threatened
418 taxa should be considered for temporary rivers. In this research, we recorded only one strictly terrestrial diatom, namely
419 *Adlafia bryophila*

420 When the ecological requirements of endangered species are considered, we can highlight the importance of pool
421 microhabitats, which are normally excluded from standard sampling protocols. *A. lineare* seems to be highly widespread
422 in the temporary streams of the Mediterranean hydroecoregions (Novais et al. 2014), and from our results we confirmed
423 its preference for oligotrophic rivers, in circumneutral to alkaline waters and low-moderate conductivity values (Van de
424 Vijver et al. 2011). *Achnantheidium gracillimum* is considered a sensitive species and is generally found in calcareous
425 rivers with low organic matter and nutrient content (Ponander and Potapova 2007; Hofmann et al. 2011; Bey and Ector
426 2013). In general, both these species were more abundant in shallow pools during both the intermediate and the low flow
427 season. Considering *Nitzschia gessneri*, little information is available on its ecology. We observed that it preferred pool
428 microhabitats and calcareous substrates, without showing high relative abundance, as also observed by Hofmann et al.
429 (2011). Similar preferences were also noted for species belonging to other threatened categories (e.g. *Gomphonema*
430 *tergestinum*, *G. lateripunctatum* and *Navicula novaesiberica*). We can therefore suggest that during the intermediate flow,
431 as well as in those sites characterized by permanent flow all over the year, lentic microhabitats represent suitable and
432 favorable niches in which endangered taxa can be hosted. Therefore, the sampling approach based on microhabitats
433 enhanced the possibility to collect rare and endangered species compared to standard methods, thus contributing to a
434 greater opportunity for increasing the knowledge on their distribution and ecological requirements. Conversely, during
435 the hydrological disturbance, the parts of the river connected with the main flow, where the standard sampling was
436 performed, sheltered the highest number of endangered individuals, while the presence of isolated pools and/or
437 characterized by intermittent flow, negatively affected the presence of threatened taxa. Despite this, the presence of
438 exceptions, represented here by extreme values in the number of endangered individuals during summer, demonstrated
439 the importance of the preservation of aquatic habitats during the dry season.

440 During this study, two species with invasive behavior were recorded, namely *D. geminata* and *A. delmontii*, which both
441 increased in abundance during the summer season. The inclusion of *D. geminata* among the “threatened with extinction”
442 taxa is surprising. This classification is probably derived from the original description of *D. geminata* that considered its
443 diffusion as being limited to mountainous pristine and oligotrophic habitats of the circumboreal regions (Blanco and Ector
444 2009). However, the recent spread of this species all over the world and in different kinds of freshwater habitats (Blanco
445 and Ector 2009; Falasco and Bona 2013), together with the nuisance effect of its blooms, has led us to state that a
446 reconsideration of its conservation status is needed. Concerning *A. delmontii*, this species appeared in France for the first
447 time in 2007, when it was recorded with low percentage relative abundance, and in 2012 it reached more than 60% peaks
448 of abundance (Pérès et al. 2012). To date, the only published records on *A. delmontii* are with respect to its distribution
449 in France. In our study, *A. delmontii* was exclusively collected in the Argentina stream and showed a significant increase

450 of relative abundance from April, when it was absent, to September, when it dominated the communities in some cases
451 reaching almost 70% of relative abundance.

452 In our study, emerging metrics, such as ecological guilds appear not to be reliable response variables for evaluation of
453 this kind of hydrological disturbance, as flow probably plays a secondary role in shaping their relative proportions. Indeed,
454 nutrient content mainly drives diatom functional traits (Larson and Passy 2012; Novais et al. 2014). On the other hand,
455 the percentage of endangered species emerged as a promising and important metric towards quantification of the
456 hydrological disturbance caused by natural and anthropic pressures. Unfortunately, unclear or missing classifications of
457 conservation status for several species still persist, and our work has shown that there is a need to update the Red List.

458

459 **CONCLUSIONS**

460 Diatom communities in Mediterranean rivers shelter a good proportion of species that are considered as threatened at
461 different levels. However, a high percentage of the recorded species is still not classified according to the Red List,
462 highlighting once again the need for its update and extension. Endangered species responded to hydrological disturbance
463 more than functional traits, with the tendency to decrease their abundance with increasing harsh conditions. Sites
464 characterized by permanent water flow throughout the year hosted the highest percentage of endangered species,
465 especially in stretches where heterogeneity is preserved. Thus, the unconventional sampling approach adopted during our
466 research, which involved highly differentiated microhabitats, permitted the recording of a higher number of rare and
467 threatened taxa, which would have been absent if only traditional procedures were followed.

468 Future research on this study area should possibly consider pluriannual samplings in order to account for interannual
469 variability and future trends. In light of our results, diatoms can provide useful information to improve river management
470 practices when faced with an increasing water scarcity scenario. Primarily, the heterogeneity of the river habitat should
471 be preserved and enhanced. This must be carried out in conjunction with the maintenance of flowing waters (with a
472 minimum velocity of 0.20 m/s), which is a key factor for increasing the abundance of threatened taxa. In drought
473 conditions, the maintenance of isolated pools surrounded by dry riverbeds is still very important, as they have to be
474 considered as unique *refugia* for benthic diatom communities.

475

476 **Compliance with Ethical Standards**

477

478 **Conflict of interest: the authors declare that they have no conflicts of interest**

479

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625 **Figure captions**

626 **Fig. 1** Streams and sites locations. Squares represent upstream sites; circles represent downstream sites

627 **Fig. 2** CA representation of diatom communities collected during SPRING (a.) and SUMMER (b.)

628 **Fig. 3** SPRING: relative abundance of endangered species (sum of the categories “threatened with extinction”,
629 endangered”, “probably endangered”, “rare” and “decreasing”) in the up- (UP) and downstream (DW) sites; further
630 distinction between transect (T) and microhabitat (MH) approaches is provided

631 **Fig. 4** SUMMER: relative abundance of endangered species (sum of the categories “threatened with extinction”,
632 endangered”, “probably endangered”, “rare” and “decreasing”) in the up- (UP) and downstream (DW) sites; further
633 distinction between transect (T) and microhabitat (MH) approaches is provided

634 **Tab. 1** Physical-chemical parameters and diatom biological attributes detected during intermediate (SPRING) and low
635 (SUMMER) flow, in both up- and downstream (UP and DW) sites. Mean values and standard deviations are displayed in
636 the table

637 **Tab. 2** Percentages of species belonging to different conservation status. Red List columns refer to conservation status
638 defined in Lange-Bertalot & Steindorf (1996): 1 = threatened with extinction. 2 = severely endangered. 3 = endangered.
639 G = probably endangered. R = rare. V = decreasing. * = at present not considered threatened. ? = not threatened. • =
640 common. Z = not listed. D = data scarce. n° samples = Total number of samples. n° species = Total number of identified
641 species

642 **Tab 3** Results of the Indicator Species Analysis (ISA) on the following groups: sampling MONTH (April, June, July,
643 August, September); RIVER (Argentina, Impero, Merula, Quiliano, Vallecrosia); SITE LOCATION (UP = upstream,
644 DW = downstream); SAMPLING METHOD (T = transect, MH = microhabitat); FLOW VELOCITY (velocity \leq 0.20
645 m/s, velocity $>$ 0.20 m/s); WATER DEPTH (depth \leq 0.25 m, depth $>$ 0.25 m); SHADE (present, absent); ISOLATION
646 (connected to the main channel, isolated habitat); DOMINANT SUBSTRATE (boulders $>$ 40cm diameter, cobbles 40-6
647 cm diameter, pebble and sand $<$ 6 cm diameter); MACROPHYTES (coverage \leq 50%, coverage $>$ 50%); ALGAE
648 (coverage \leq 50%, coverage $>$ 50%). Complete list of the diatom codes is shown in the ESM_2. Significant p values for
649 each species are reported in parenthesis.

650 **Tab 4** Results of the effect of fixed factors in the *mesohabitat* and *microhabitat models* as inferred with GLMMs; results
651 for both the number of Red List taxa (N° of RL taxa) and the abundance of individuals (Abundance of RL taxa) are
652 reported (F = F-value; P = P-value; Est = estimates)

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	SPRING		SUMMER		
	UP	DW	UP	DW	
	mean \pm sd	mean \pm sd	mean \pm sd	mean \pm sd	
physical-chemical parameters					
DO (%)	100.6 \pm 1.25	105.6 \pm 14.14	99.1 \pm 11.37	99.9 \pm 34.10	
pH	8.96 \pm 0.2	8.95 \pm 0.27	8.39 \pm 0.53	8.22 \pm 0.50	
TEMP ($^{\circ}$ C)	13.3 \pm 2.13	15.5 \pm 3.82	20.1 \pm 2.03	21.1 \pm 2.02	
TSS (mg/l)	1.78 \pm 1.27	5.85 \pm 2.61	2.61 \pm 3.65	3.41 \pm 35.09	
COND (μ S/cm)	327 \pm 151	352 \pm 139	400 \pm 160	406 \pm 182	
N-NO ₃ (μ g/l)	750 \pm 309	960 \pm 314	380 \pm 210	400 \pm 913	
SRP (μ g/l)	29 \pm 7	15 \pm 13	10 \pm 13	7 \pm 29	
biological attributes					
ecological guilds	n $^{\circ}$ species	22.8 \pm 5.23	22.3 \pm 6.68	27.6 \pm 6.69	24.9 \pm 6.72
	Shannon Index	2.64 \pm 0.59	2.53 \pm 0.70	3.05 \pm 0.56	2.85 \pm 0.75
	low profile (%)	67.25 \pm 18.57	64.13 \pm 20.56	77.70 \pm 11.25	70.09 \pm 19.79
	high profile (%)	15.00 \pm 11.15	13.72 \pm 9.98	8.15 \pm 6.21	15.73 \pm 11.44
	motile (%)	17.55 \pm 18.27	21.54 \pm 19.26	13.64 \pm 8.64	12.65 \pm 14.35
	planktic (%)	0.20 \pm 0.43	0.61 \pm 1.45	0.51 \pm 1.06	1.53 \pm 6.18
	colonial (%)	11.93 \pm 10.30	11.98 \pm 8.67	6.23 \pm 6.11	11.50 \pm 10.84
	adnate (%)	5.67 \pm 9.19	4.46 \pm 6.00	17.83 \pm 22.16	8.37 \pm 12.86
	pedunculata (%)	70.09 \pm 21.52	70.22 \pm 21.07	66.60 \pm 25.16	76.45 \pm 20.71

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670 Tab. 2

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RED LIST		number of species (relative % of abundance)			
CODE	STATUS	SPRING	TOTAL	SUMMER	TOTAL
1	threatened with extinction	0.60		0.41	
2	severely endangered			0.41	
3	endangered	3.57		4.56	
G	probably endangered	2.98		2.49	
R	rare	0.60		0.41	
V	decreasing	2.98	10.71	5.81	14.11
	at present not considered	23.21		19.50	
*	threatened				
?	not threatened	33.33		29.88	
•	common	1.19	57.74	1.24	50.62
z	not listed	29.17		32.37	
D	data scarce	2.38	31.55	2.90	35.27
	<i>n° samples</i>	126		240	
	<i>n° species</i>	171		241	

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682 Tab. 3

	<i>GROUP DESCRIPTION</i>	taxa (p value)
MONTH	<i>April</i>	ENVE (0.001); GCBC (0.001); GTER (0.001); GOLI (0.001); NLIN (0.001); NGRE (0.001); ADMS (0.001); GMIC (0.001); DEHR (0.002); CAFF (0.001); FCCT (0.001); FSAP (0.003); MCIR (0.003); FPEL (0.001); FARC (0.014); HPDA (0.006); SBKU (0.006); GANT (0.014); NACI (0.022)
	<i>June</i>	GVID (0.021); FDEL (0.043); SANC (0.036)
	<i>July</i>	CNCI (0.007); CALO (0.008); FLAT (0.037)
	<i>August</i>	GRHO (0.001); NIZT (0.024)
	<i>September</i>	DCOF (0.001); SSVE (0.001); CTUM (0.001); SBND (0.024); CNLP (0.010)
RIVER	<i>Argentina</i>	ADPT (0.001); ADMO (0.001); DEHR (0.001); NILA (0.001); NTAB (0.001); DGEM (0.001); FVAU (0.001); NSBN (0.001); ADTH (0.001); DEHT (0.001); ADCT (0.001); ADLA (0.003); FARC (0.010); ESAB (0.044)
	<i>Impero</i>	ADEU (0.001); FRCP (0.001); RABB (0.001); GITA (0.001); DCOF (0.001); CAFF (0.001); RUNI (0.001); SBND (0.001); EOMT (0.001); SSVE (0.013); NMIC (0.049); CNLP (0.012); APAB (0.015); FCCT (0.043); EPRO (0.035)
	<i>Merula</i>	DKUE (0.001); SACU (0.001); CSUT (0.001); GLAT (0.001); NGES (0.001); CEXF (0.001); CDTG (0.001); GCBC (0.001); GVID (0.001); FALP (0.001); NRAD (0.003); EUFL (0.002); CLAE (0.00); GANT (0.018); FDEL (0.040);
	<i>Quiliano</i>	CLNT (0.001); RSIN (0.001); PTLA (0.001); FPRU (0.001); PLFR (0.001); COPL (0.001); NINC (0.001); NIAR (0.001); NCRY (0.001); MPMI (0.001); FRUT (0.001); CTRO (0.001); CPTG (0.001); ADGL (0.001); ADSU (0.001); MVAR (0.001); EULA (0.001); NSPD (0.001); SSEM (0.001); NYCO (0.001); FSAP (0.001); CMEN (0.001); ADMS (0.002); GACU (0.001); CTUM (0.001); CPLA (0.001); GACC (0.001); CNCI (0.005); CALO (0.010); HPDA (0.009); SBKU (0.009); ADTG (0.034); GDEC (0.033); KCLE (0.043)
	<i>Vallecrosia</i>	NVEN (0.001); NCTO (0.001); GPUM (0.001); FMES (0.001); AOVA (0.002); CVUL (0.001); SANC (0.041)
SITE LOCATION	<i>UP</i>	DTEN (0.001); NCTO (0.001); PSBR (0.001); ADAM (0.001); SEBA (0.001); GITA (0.014); EULA (0.015); FMES (0.014); SSEM (0.023); GACU (0.002); NRAD (0.027); NSBN (0.049); APAB (0.021); AOVA (0.021); GANT (0.041); CVUL (0.043); DEHT (0.042)
	<i>DW</i>	NINC (0.015); GLAT (0.006); UBIG (0.001); FPRU (0.001); GPUM (0.001); ADGL (0.001); FRUT (0.009); CAFF (0.024); NPAL (0.005); CTRO (0.014); NYCO (0.026); FCAT (0.009); CNCI (0.028)
SAMPLING METHOD	<i>T</i>	FVAU (0.016); FGRA (0.022); FSAP (0.042); ADLA (0.024); CETG (0.019); NIZT (0.032); SVTL (0.034)
	<i>MH</i>	-
FLOW VELOCITY	<i>V ≤ 0.20 m/s</i>	GCAP (0.011)
	<i>V > 0.20 m/s</i>	DMON (0.001)
WATER DEPTH	<i>depth ≤ 0.25 m</i>	ADGL (0.032)
	<i>depth > 0.25 m</i>	DGEM (0.049); NSBN (0.007); GVID (0.023); ENLB (0.047); ECMT (0.037)

	<i>present</i>	FRCP (0.015)
SHADE	<i>absent</i>	ACLI (0.022); CLNT (0.017); DEHR (0.005); NCTO (0.033); CPTG (0.017); COPL (0.012); ADAM (0.004); GACU (0.001); EULA (0.007); NRAD (0.007); FMES (0.023); SSEM (0.035); FARC (0.010); AOVA (0.037); DMES (0.035); GDEC (0.031); CALO (0.042)
	<i>connected</i>	CLNT (0.045); NGRE (0.032); PTLA (0.049)
ISOLATION	<i>isolated</i>	DKUE (0.001); ESUM (0.001); GLAT (0.001); SACU (0.001); FPDM (0.002); CSUT (0.002); UBIC (0.003); SSTM (0.027); CDTG (0.002); ECES (0.001); ADMO (0.010); GOMP (0.003); GPUM (0.027); NSBN (0.010); EUFL (0.012); FDEL (0.001); CLAE (0.010); DPAR (0.026); CBAM (0.017); EUNO (0.048)
	<i>boulders (> 40cm diameter)</i>	NILA (0.001); CPAR (0.001); SSTM (0.001); DEHR (0.001); NCTO (0.001); DTEN (0.001); ECAE (0.001); FMES (0.001); DVUL (0.002); NVEN (0.001); DGEM (0.001); SEBA (0.001); PSBR (0.003); AOVA (0.001); DEHT (0.001); FGRA (0.003); NSBN (0.006); ADCT (0.001); ADJK (0.013); FARC (0.003); FAUT (0.023); ECMT (0.005); CVUL (0.013); NATG (0.021); ESAB (0.026)
DOMINANT SUBSTRATE	<i>cobbles (40-6 cm diameter)</i>	ADMO (0.002); RABB (0.023); GPUM (0.004); DCOF (0.024)
	<i>pebble and sand (<6 cm diameter)</i>	CLNT (0.001); RSIN (0.001); PTLA (0.001); NCRY (0.001); UBIC (0.001); NGRE (0.002); COPL (0.001); PLFR (0.006); CTRO (0.001); MPMI (0.001); GLAT (0.043); ADSU (0.001); MVAR (0.038); FPRU (0.013); ADGL (0.006); EULA (0.003); FRUT (0.008); ADMS (0.001); FSAP (0.022); CTUM (0.006); CMEN (0.028); GACU (0.004); NSPD (0.011); NREC (0.029); HPDA (0.042); SBKU (0.038)
MACROPHYTES	<i>coverage ≤ 50%</i>	RSIN (0.002); CLNT (0.001); CPTG (0.001); COPL (0.001); FPRU (0.002); FSAP (0.001); DCOF (0.005); MPMI (0.034); GOMP (0.001); NMIC (0.038); CPLA (0.024); ENLB (0.038); HPDA (0.050); SBKU (0.050)
	<i>coverage > 50%</i>	DMON (0.002); DEHR (0.048); GCAP (0.007); FMES (0.002); DGEM (0.023); FGRA (0.010); FAUT (0.042); FCAT (0.011)
ALGAE	<i>coverage ≤ 50%</i>	ACLI (0.005); RSIN (0.001); CLNT (0.001); NIAR (0.003); CPTG (0.001); COPL (0.001); FPRU (0.002); MPMI (0.011); FSAP (0.002); EULA (0.004); DCOF (0.020); GOMP (0.003); GACU (0.008); CPLA (0.036); EUFL (0.048)
	<i>coverage > 50%</i>	DMON (0.001); DEHR (0.018); GCAP (0.004); FMES (0.001); FGRA (0.003); FAUT (0.010); FCAT (0.006); AOVA (0.022); ADCT (0.026); ADLA (0.027)

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692 **Tab. 4**

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MESOHABITAT MODEL					MICROHABITAT MODEL						
Variable	N° of RL taxa		Abundance of RL taxa		Variable	N° of RL taxa			Abundance of RL taxa		
<i>Month</i>	$F_{4,268} = 1.47$	$P = 0.2108$	$F_{4,268} = 9.62$	$P < 0.0001$	<i>Macrophytes</i>	Est = -0.0014	$F_{1,272} = 1.15$	$P = 0.2839$	Est = -0.0061	$F_{1,272} = 5.79$	$P = 0.0163$
<i>Sampling site</i>	$F_{1,268} = 0.72$	$P = 0.3970$	$F_{1,268} = 0.29$	$P = 0.5926$	<i>Flow velocity</i>	Est = 0.1187	$F_{1,272} = 1.55$	$P = 0.2139$	Est = 0.4190	$F_{1,272} = 4.44$	$P = 0.0360$
<i>Sampling method</i>	$F_{1,268} = 0.62$	$P = 0.4307$	$F_{1,268} = 4.62$	$P = 0.0325$	<i>Water depth</i>	Est = 0.0020	$F_{1,272} = 0.53$	$P = 0.4659$	Est = -0.0076	$F_{1,272} = 1.08$	$P = 0.1719$
<i>Disturbance</i>	$F_{1,268} = 0.00$	$P = 0.9494$	$F_{1,268} = 0.09$	$P = 0.7605$							

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