

1 Partial decoupling between exotic fish and habitat
2 constraints remains evident in late invasion stages

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4 Marco Milardi¹, Anna Gavioli^{1,*}, Giuseppe Castaldelli¹, Janne Soininen²

5 1 - University of Ferrara, Department of Life Sciences and Biotechnology, via Luigi Borsari 46,
6 44121, Ferrara, Italy.

7 2 - University of Helsinki, Department of Geosciences and Geography, PO Box 64, FI-00014,
8 Helsinki, Finland.

9 * Corresponding author: gvlanna@unife.it, +39 3472737393

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21 **Abstract**

22 We investigated the relationships between exotic freshwater fish invasions, environmental
23 factors and ecofunctional diversity in streams. We used data from 335 stream sites belonging
24 to 105 watersheds and 3 basins in Italy to test whether the exotic species invasion was
25 dominated by species with generalist traits and whether the environment-ecofunctional trait
26 relationships of exotic and native species would differ from each other. We also tested the
27 hypothesis that ecofunctional uniqueness patterns between exotic and native species would
28 be substantially different. We found that generalist traits were widespread in nearly all areas
29 where exotic species occurred, but not all generalist traits were equally abundant in exotic
30 communities. Only temperature tolerants, low oxygen tolerants and eurytopic traits were
31 typically more dominant in exotic communities than native ones, suggesting that not all
32 generalist traits are equally important in the invasion process and that more complex
33 mechanisms of trait selection could take place. Environment-ecofunctional trait relationships
34 of exotic and native species partly differed both in direction and magnitude, suggesting that
35 invasion dynamics could partly decouple environment and biotic communities, but also that
36 this decoupling might decrease at later invasion stages. Finally, site and trait ecofunctional
37 uniqueness differed between exotic and native species and exotic species ecofunctional
38 diversity hotspots were located in human-disturbed areas, suggesting that human disturbance
39 might play a strong role in invasion patterns. We advocate for a wider use of ecofunctional
40 approaches in conservation studies in the future, as they could be a key to understand
41 complex ecological processes such as exotic invasions.

42 **Keywords:** biodiversity conservation, alien species, species diversity, functional diversity,
43 ecofunctional uniqueness, native species

44 **Introduction**

45 The study of geographical distribution of species and spatial variation of biodiversity on Earth
46 has a long history. Recently, hundreds of studies have underlined the importance of biodiversity
47 for ecosystem functioning and resilience to different pressures (e.g. Hooper et al. 2005), as well as
48 its general worldwide decline (Wiens 2016). Much effort has been devoted to investigate the
49 dispersal and evolutionary processes driving taxonomical biodiversity in the past (see e.g. Herrera
50 1995). However, the need for a shift from taxonomical to trait-based approaches in biodiversity
51 research has been acknowledged for over two decades, because of the close linkage between
52 environment and ecological traits, as well as their fast response to environmental changes (see
53 e.g. Poff et al. 2006; Violle et al. 2014). As a consequence, recent works shifted their attention
54 towards functional diversity, exploring its spatial patterns and dynamics.

55 Functional diversity quantifies the distinctive combination of morphological, biochemical,
56 physiological, structural, phenological or behavioral traits that characterizes living communities
57 and has been advocated to measure ecosystem functioning more reliably than taxonomical
58 diversity (Cadotte et al. 2011). Ecofunctional diversity, a subset of functional diversity, identifies
59 the combination of ecological traits in communities, selected by environmental conditions and
60 habitat availability (Poff and Ward 1990). Habitat diversity, geography, land use, soil or water
61 chemistry might all play a role in determining which ecofunctional traits occur in a community
62 (Comte et al. 2016). In this perspective, the habitat selects which traits prevail in a given
63 environment, ultimately linking habitat diversity with species ecofunctional diversity. This linkage
64 has been explored to infer ecological quality from ecofunctional diversity of biotic communities
65 (Milardi and Castaldelli 2018; Mouillot et al. 2013; Pont et al. 2006; Schmutz et al. 2007), but
66 exotic species invasions have been recognized as a potential confounding factor for this linkage.

67 Exotic species introduction is a global phenomenon, and one of the main causes of biodiversity
68 loss (Leprieur et al. 2008; Sala et al. 2000) and community homogenization in space and time (e.g.
69 Rahel, 2000). Lot of attention has been devoted to investigate the consequences of exotic invasions
70 on taxonomical diversity at different geographical scales (Sax and Gaines 2003). A common
71 notion in invasion biology suggests that successful invaders in environmentally degraded areas
72 could be favored by their tolerance to a broad range of environmental conditions and their
73 generalist adaptations (Lurgi et al. 2014; Marvier et al. 2004; Milardi et al. 2018b). If most invaders
74 are generalists, invasion dynamics would override environmental drivers behind species and trait
75 distributions and cause a peculiar ecofunctional diversity distribution for exotic communities,
76 compared to that of native ones which had a longer environment-selection time and thus clear
77 environment-trait relationships. However, such difference between exotic and native communities
78 could decrease with time, as exotic species become more and more selected by environmental
79 constraints when time after invasion events passes.

80 The overall ecofunctional structure and distribution of fish has been poorly studied, so far,
81 and similarly little research exists on the impact of exotic invasions on the ecofunctional
82 structure of Mediterranean freshwater fish (Colin et al. 2018; Villéger et al. 2013). As in many
83 other geographical areas, exotic invasions are one of the main factors causing biodiversity loss,
84 but this is particularly true in the Mediterranean region and its freshwater ecosystems (Crivelli
85 1995; Cuttelod et al. 2009; Médail and Quézel 1999). Only recently, an ecofunctional
86 characterization of native and exotic fish species has been defined for some areas (Milardi and
87 Castaldelli 2018), enabling further research on the linkages between biological invasions,
88 ecofunctional diversity and the environment.

89 In this study, we focused on the northern Italian peninsula, previously highlighted as a good
90 area to investigate the outcomes of freshwater fish invasions (Castaldelli et al. 2013; Gavioli et
91 al. 2018; Gavioli et al. 2019; Lanzoni et al. 2018; Milardi et al. 2018a). We used a spatially-
92 comprehensive dataset, including several river basins, to explore the spatial distribution of
93 riverine freshwater fish ecofunctional traits and investigate the relationships between
94 environmental factors and ecofunctional diversity of native and exotic species. We tested the
95 hypothesis (H₁) that the invasion process would be largely led by less-specialized species, able to
96 adapt to a wide range of environmental conditions (i.e. generalist species). Under this hypothesis,
97 we expected that generalist ecofunctional traits would be widespread and dominant in exotic
98 species communities of invaded areas, as generalist species are usually leading the invasion
99 process (e.g. Evangelista et al. 2008). However, we expected this dominance to decrease at late-
100 invasion stages. We also tested the hypothesis (H₂) that the environment-ecofunctional traits
101 relationships of exotic and native species would differ. In particular, we expected that the
102 relationships would be overall weaker for exotic species, as native species traits have been
103 selected from the regional species pool by environmental factors over a longer time than the
104 residence time of exotic species. We ultimately investigated the hypothesis (H₃) that
105 ecofunctional traits of exotic and native species would have different spatial patterns (i.e.
106 between upland and lowland streams), because lowland areas were highlighted as an invasion
107 hotspot (Lanzoni et al.2018; Milardi et al. 2018a). Finally, our results would help understanding
108 the factors shaping the ecofunctional diversity of fish communities under exotic invasions, one of
109 the major threats to native fish conservation worldwide.

110

111 **Materials & Methods**

112 *Study area*

113 We focused our investigation on northern Italy (Fig. 1), an area hosting more than 17 million
114 inhabitants and impacted by agricultural activities and livestock farming. The study region has a
115 Mediterranean continental climate, with an annual average precipitation of 1036 mm and a mean
116 temperature of 12° C. Within this area lies the largest river basin in Italy, the Po River basin (71,000
117 km²), of which we included the Po River itself (in all its course), the Oglio River (one of the most
118 important left tributaries of the Po River) and the right tributaries in the Emilia-Romagna region.
119 We also included two additional groups of rivers outside of this basin: the Brenta River (north-
120 east of the Po River basin) and watercourses of the Emilia-Romagna (south of the Po River basin).
121 Overall, a total of 335 sampling sites (i.e. 335 stretches of 105 watercourses) were included in this
122 study, covering heterogeneous habitats within river ecosystem (e.g. riffles, pools), and different
123 altitudinal zones and environmental conditions (Fig. 1).

124 In the uplands, organic material originating from villages, small towns and livestock farms is
125 the main source of river pollution. Conversely, a high degree of urbanization and intensive
126 agriculture characterize the lowland rivers, where high nutrient loads have led to eutrophication
127 (Castaldelli et al. 2013). To support agricultural irrigation, a complex network of drainage canals
128 has been established in the lowlands; this system is completely human regulated with hydrological
129 management directed to both irrigation supply and drainage (Castaldelli et al. 2013; Milardi et al.
130 2017).

131 *Data collection*

132 Fish data were collected within monitoring programs of the Emilia-Romagna region (Regione
133 Emilia Romagna 2008), the Padova Province (Provincia di Padova 2010), the Po River (Autorita'

134 di bacino del Po 2008) and the Oglio River (Consorzio dell'Oglio 2016). The monitoring programs
135 were carried out separately, in different years, over a relatively long-term period (1999–2010).

136 Fish sampling was performed by electrofishing, adapting the standard national monitoring
137 guidelines to the particular conditions of each watercourse, and using direct current at 400–600 V
138 and 4–5 A (Backiel and Welcomme 1980; Reynolds 1996). River stretches were sampled once,
139 during daylight, in an upstream zigzag direction, ensuring that the range of present macrohabitats
140 of each site was fully surveyed (Hankin and Reeves 1988). Electrofishing is considered the best
141 quantitative method for fish sampling in shallow waters, up to a maximum of 1 m (Zalewski and
142 Cowx 1990) but its efficacy may be low in deeper waters, with big and mobile specimens, or with
143 high conductivity. Such special conditions occurred in almost all the lower stretches of rivers and
144 in the canals of the lowlands. For this reason, at these sites, electrofishing was immediately
145 followed up with a standard set of trammel nets (with variable mesh size from 90 to 5 mm), with
146 the support of professional fishermen (Backiel and Welcomme 1980). In order to allow
147 comparisons between sites (i.e. river stretches), the sampling effort at each sampling site was
148 standardized according to the national fish monitoring guidelines (APAT 2007).

149 Fish species were classified according to Kottelat and Freyhof (2007), taking into account
150 recent taxonomic determinations and common names as listed in FishBase (Froese and Pauly
151 2017). Each species was categorized as native or exotic: a species was considered as native when
152 naturally present in Italian watercourses and as exotic when human-introduced (IUCN Comitato
153 Italiano 2019; Milardi et al. 2018a), irrespective of the time elapsed since the introduction (see
154 Supplementary Table 1 for further details on introduction dates). Hybrid specimens or uncertain
155 species were excluded from this study in order to avoid taxonomic asymmetries.

156 Abundance of each species was expressed with Moyle classes (Moyle and Nichols 1973)
157 ranging from 1 (lower abundance, 1-2 individuals per site) to 5 (higher abundance, more than 50
158 individuals per site). However, numerical abundance classes tend to overestimate the ecological
159 significance of small-bodied species and underestimate that of large-bodied ones. A weight was
160 thus assigned to each species based on their average size published in the literature (1=small body
161 up to ~150 g; 2=medium body ~150–400 g; 3=large body over ~400 g) and multiplied by Moyle
162 abundance classes, in order to obtain a more ecologically-balanced representation of the
163 community, closer to relative biomasses (i.e. body-mass-corrected abundances, hereafter referred
164 to simply as abundances, Milardi and Castaldelli 2018).

165 Water physicochemical sampling was performed with standard methods in temporal and
166 spatial proximity with the fish sampling, by different Regional Environmental Protection Agencies
167 (ARPAs, in Italian) for the Po, the Brenta and the rivers in the Emilia-Romagna region. The Oglio
168 River Water Authority carried out the water sampling in the Oglio River. Eight physicochemical
169 variables were monitored: water temperature (T ; °C), electrical conductivity (EC ; $\mu\text{S cm}^{-1}$),
170 chemical oxygen demand (COD ; $\text{O}_2 \text{ mg l}^{-1}$), biological oxygen demand (BOD_5 ; $\text{O}_2 \text{ mg l}^{-1}$), total
171 suspended solids (TSS ; mg l^{-1}), total phosphorus (P ; mg l^{-1}), ammonia (NH_4^+ ; mg l^{-1}) and nitrate
172 nitrogen (NO_3^- ; mg l^{-1}). The geographical position (longitude – $Long$, latitude – Lat) and the
173 altitude (Alt) were also considered.

174 *Fish ecofunctional traits*

175 In order to investigate the ecofunctional composition of fish communities, five different
176 ecological functions were taken into account: feeding, reproduction, migration, tolerance and
177 habitat use. These functions were included because of their ecological significance and close
178 relationship with the environment (Noble et al., 2007; Milardi and Castaldelli, 2018). Within these

179 ecological functions, all fish species were classified in guilds, each representing an ecofunctional
180 trait and therefore referred as such hereafter (Table 1, see also Noble et al. (2007)).

181 Ecological functions, guilds and classification for most species in this study were taken from
182 Milardi and Castaldelli (2018), where all available information was used to identify appropriate
183 guilds for each species. Guilds were mutually exclusive (i.e. each species could be assigned to
184 only one guild within each ecological function) and species were classified according to their
185 prevalent adult characteristics (see also Table 1 in Milardi and Castaldelli 2018 for a more detailed
186 explanation), thus partly discounting ontogenetic shifts (e.g. in diet or habitat) and ecological
187 plasticity. The same methodology was applied to classify euryhaline species that were not included
188 in the previous work (see also Supplementary Table 1).

189 *Spatial distribution of exotic species generalist traits*

190 We focused our analysis on generalist ecofunctional traits, with the aim to verify their spatial
191 and community dominance within the exotic communities, therefore selecting all areas with exotic
192 species presence. Additionally the patterns observed for exotic communities were compared with
193 those found in native communities over the same areas, to investigate any differences in dominance
194 of generalist traits.

195 Inside each ecological functions, the ecological guilds indicative of no preference for, or no
196 adaptation to, specific environmental conditions (i.e. generalist traits) were selected (ecological
197 function – ecological guild): Feeding – Generalists (feeding on any source), Reproduction –
198 Polyphils (spawning on any substrate), Tolerance – Low oxygen tolerants and High temperature
199 tolerants (not selective for oxygen or temperature), Habitat use – Eurytopics (adapted to a wide
200 range of current velocities) and Wide range of turbidity conditions (*idem* for turbidity).

201 To assess the spatial distribution of generalist ecofunctional traits, the number of sites in which
202 generalist traits were detected was expressed as a share (percentage) of the total sites with exotic
203 species presence (Table 2). To assess the community dominance of generalist ecofunctional traits,
204 the share (percentage) of these traits in the communities was calculated using the abundances of
205 all species (exotic and native) possessing each generalist trait (Table 2). Both distribution and
206 dominance were also calculated separately for native and exotic communities, at each site (Table
207 2).

208 The community dominance of generalist traits of exotic and native species communities was
209 also investigated in the area where exotic and native species distributions overlapped, testing the
210 null hypothesis that exotic and native communities would not differ in generalist trait dominance.
211 Differences in generalist trait dominance were tested using the paired t-test (paired-t, a parametric
212 paired test) and the Wilcoxon signed-rank test (Wilcoxon, a non-parametric paired test), which
213 investigate differences in the mean and median, respectively. Testing was performed using the
214 PAST 3.06 software (Hammer et al. 2001).

215 The ArcGIS software (ESRI 2011) was used to map the spatial distribution of ecofunctional
216 traits in exotic communities, and to investigate the spatial autocorrelation of each generalist trait,
217 using the *Spatial Autocorrelation (Global Moran's I)* tool. The null hypothesis of the Global
218 Moran's I analysis is that the attribute being analyzed is randomly distributed among the features
219 in the study area. Being an inferential statistic, positive (and significant) values of the Global
220 Moran's I indicate spatial clustering of a generalist trait.

221 *Exotic and native species environment-ecofunctional traits relationships*

222 Land cover data were obtained from the CORINE database (2012,
223 <https://www.eea.europa.eu/data-and-maps/data/copernicus-land-monitoring-service-corine>). In

224 the lowlands, where estimation of watershed areas is more difficult due to low slopes and human-
225 regulated flow, the land cover of the whole river basin or of the administrative province was used.
226 CORINE land cover classes were merged in five categories based on the main land use in order to
227 better describe the study area: urban use (*Urban*), agricultural use (*Agri*), forest (*Forest*), other
228 natural area (*OtherNat*), freshwater (*Fresh*) and brackish water (*Brack*). Land cover was expressed
229 as the share of each of these categories in the watershed of each site.

230 Relationships between geographical variables, land use features, water physico-chemical
231 variables and fish ecofunctional traits were explored using the distance-based Redundancy
232 Analysis (db-RDA, Legendre and Anderson 1999). This analysis is normally used to analyze
233 species-by-site data, but here it was used to analyze trait-by-site data (i.e. the total body-size-
234 corrected abundance of all species with a certain ecological trait at each site). One of the
235 advantages of db-RDA is that it can be used with any non-Euclidean distance measures (Legendre
236 and Anderson 1999). Therefore, taking into account the work of de Bello et al. (2013), Gower
237 distance measures were used because of their better suitability in detecting changes in
238 ecofunctional diversity along environmental gradients. Linear dependencies between variables
239 were assessed through the Variance Inflation Factors (VIF) and variables showing high collinearity
240 ($VIF > 10$) were removed from the analysis (Borcard et al. 2011). Explained variance in db-RDA
241 was reported through adjusted r^2 values (correct constrained variance).

242 Matrices of traits-by-site data were Hellinger transformed (Legendre and De Cáceres 2013;
243 Legendre and Gallagher 2001) to standardize variations among both species and community size.
244 Environmental variables expressed as percentages were arcsine transformed, while all the other
245 ones were log-transformed.

246 These analyses were performed in R software version 3.4.3 (R Core Team 2017): db-RDA
247 analysis used the *capscale* function within the ‘vegan’ R package (Oksanen et al. 2017) on all
248 species, and also separately for native and exotic species.

249 *Ecofunctional uniqueness patterns of native and exotic species*

250 Stream order for each site was calculated using a Digital Elevation Model (DEM)
251 (<http://www.sinanet.isprambiente.it/it/sia-ispra/download-mais/dem20/view>), resampled into 10
252 m pixel size to harmonize it. Flow direction and accumulation, as well as the watershed of each
253 sampling site, were calculated based on the DEM layer. For the entire river network generated by
254 flow accumulation, stream order was derived with the Strahler method (Strahler 1957). This
255 procedure was reliable for upland streams, but it was less so in the lowland, possibly due to the
256 fact that flow direction and magnitude in the lowlands are not always natural because of human
257 intervention. The stream order was thus manually checked and revised when necessary in lowland
258 rivers and streams. Rivers were grouped into four classes based on stream order: class 1 (Strahler
259 stream order 1 and 2), class 2 (stream order 3 and 4), class 3 (stream order 5 and 6) and class 4
260 (stream order > 6). As drainage and irrigation canals could not be assigned into any natural class,
261 a separate class called “Canals” was created. Canals are man-made environments, usually
262 characterized by low habitat heterogeneity and controlled hydrology, located in the lowlands
263 southwest of the Po River, near its delta.

264 The ecofunctional uniqueness of communities across sites was investigated with the Local
265 Contribution to Beta Diversity (LCBD) and Species Contribution to Beta Diversity (SCBD)
266 approaches, developed by Legendre and De Cáceres (2013), accounting for stream order. These
267 analyses were originally developed to calculate the total beta diversity from the total variance of a
268 site, partitioning the total variance into local contributions to beta diversity (i.e. LCBD), and

269 species contributions to beta diversity (i.e. SCBD) across the area, respectively. However, for the
270 first time, the same approach was applied here to the ecofunctional diversity of communities: high
271 values of LCBD indicate a unique ecofunctional composition of the local community (arising
272 from a combination of low ecofunctional diversity, low species richness and a rare distribution in
273 the sites) and low values of SCBD highlight those ecofunctional traits that are the most unique in
274 the data (arising from the uniqueness of traits and their rare distribution in the dataset).

275 Uniqueness analyses were performed in R software version 3.4.3 (R Core Team 2017)
276 through the ‘vegan’ R package (Oksanen et al. 2017) and the ‘adespatial’ R package (Dray et al.
277 2016) on all species, and also separately for native and exotic species.

278

279 **Results**

280 A total of 59 fish species were detected in the study area; of these 37 were native and 22
281 were exotic species. Each of these species was assigned to guilds within ecological functions, as
282 defined in Table 1 (see also Supplementary Table 1).

283 *Spatial distribution of exotic species` generalist traits*

284 Exotic species were rather widespread in the area (209 out of 335 sites, 62.4% of the total)
285 and occurred mostly in the lowlands. Exotic species occasionally reached rather high abundances
286 (abundance ≥ 40 in 12 sites, Fig. 2a, and constituting 100% of the fish community in 9 sites).

287 Overall, generalist traits were overall widely distributed in the area occupied by exotics, but not
288 all traits were equally dominant in the exotic communities. The three most widespread generalist
289 traits were low oxygen tolerance, high temperature tolerance and eurytopic adaptation to current
290 velocity (98.1%, 97.6% and 94.7% of the sites, respectively, Fig. 2d, e and f, Table 2). The least

291 widespread traits were polyphily and adaptation to a wide range of turbidity conditions (31.1%
292 and 79.4% of the sites, respectively, Fig. 2c and g).

293 Generalist feeder traits were relatively widespread (80.9% of the sites, Fig. 2b), but were a
294 minor component of the community (18.6% of the total abundance, Table 2), on average.

295 Polyphil traits dominance was also very low, totaling 4.9% of the overall abundance, on average.

296 Generalist traits were relatively slightly less widespread within the native species communities
297 present in the same area, with the exception of adaptations to a wide range of turbidity conditions
298 (94.3% of the sites, Table 2). However, rather surprisingly, some generalist traits were in general
299 more dominant in native communities than exotic ones (H1). Generalists feeders, polyphils and
300 species adapted to a wide range of turbidity were all more dominant in native communities than
301 exotic ones, while high temperature tolerants, low oxygen tolerants and eurytopics and were less
302 dominant (Table 2).

303 Differences in dominance of generalist traits between native and exotic communities were
304 statistically significant for adaptations to a wide range of turbidity, high temperature tolerance
305 and low oxygen tolerance (paired-t $P < 0.01$, Wilcoxon $P < 0.01$, for all these traits), as well as
306 for polyphily and eurytopic adaptation to current velocity (paired-t $P < 0.05$, Wilcoxon $P < 0.05$),
307 but not for generalist feeders (paired-t $P = 0.19$, Wilcoxon $P = 0.85$). All generalist traits showed
308 a significant spatial clustering (Global Moran's $I > 0$; P -values < 0.001 , for all traits), indicating
309 spatial autocorrelation.

310 *Exotic and native species environment-ecofunctional traits relationships*

311 Environmental variables did not show collinearity ($VIF < 10$) and they were thus all included
312 in the db-RDA analysis. The total amount of ecofunctional trait variance in the communities
313 explained by db-RDA was 41.90% (Fig. 3a). The first axis explained 30.13% of the variance and

314 the second axis explained 4.36% of the variance (Fig. 3a). Most ecofunctional traits were related
315 to environmental features (e.g. rheophile (Rhe), low oxygen intolerant (OxInt) and clear water
316 (CW) adapted species at higher elevations and vice versa), but some traits (e.g. herbivore (H) or
317 parasite (Pa) feeding) did not clearly relate with environmental variables (Fig. 3a). Overall, the
318 environmental factors most strongly related with ecofunctional traits were altitude, temperature
319 and stream order class, which are all closely linked to each other and influenced both native and
320 exotic species. The db-RDA analysis conducted exclusively on native species (50.12% of
321 variance explained) showed a clear distribution of ecofunctional traits (Fig. 3b, the first axis
322 explained the 29.43% of the variance and the second axis explained the 11.63% of the variance),
323 with clear distinctions related to environmental features, similar to those found in the general
324 analysis. Exotic species had an overall lower linkage with environmental variables (35.46% of
325 variance explained) (Fig. 3c, the first axis explained the 15.23% of the variance and the second
326 axis explained the 7.77% of the variance). In general, these three analyses revealed similarities
327 along the first ordination axis in terms of the key environmental variables (altitude, forests,
328 temperature), whereas results differed clearly more for the second axis in terms of which
329 environmental variables drove trait composition.

330 *Ecofunctional uniqueness patterns of native and exotic species*

331 According to the LCBD analysis, the degree of uniqueness of the communities'
332 ecofunctional composition per each stream order class showed a clear decreasing pattern with
333 increasing stream order class (Fig. 4a). Communities in lower stream orders (in the uplands) had
334 high values of ecofunctional uniqueness, but communities in canals also showed a surprisingly
335 high degree of uniqueness compared with other lowland sites (Fig. 4a). According to the SCBD
336 analysis, high ecofunctional uniqueness was associated with clear water adaptation, intermediate

337 migration and low oxygen tolerance traits (Fig. 4b). Herbivorous, planktivorous and parasitic
338 feeding traits were associated with low uniqueness (Fig. 4b). The econfunctional uniqueness of
339 native communities (LCBD analysis) followed a similar pattern than that underlined in overall
340 communities (Fig. 4c), but a comparison with exotic species (Fig. 4e) revealed that the latter
341 contributed the least to uniqueness in higher stream orders and canals. Agreeing with our
342 hypothesis H₃, uniqueness patterns differed between native and exotic species. Among native
343 species, lithophilic, low oxygen tolerant and clear water traits contributed the most to uniqueness
344 according to the SCBD analysis (Fig. 4d), while planktivory, pelagic spawning and parasitism
345 contributed the least. Among exotic species, piscivory, phytophily and benthivory were the traits
346 that contributed the most to uniqueness according to the SCBD analysis (Fig. 4f), while low
347 oxygen tolerance, herbivory and pelagic spawning contributed the least.

348

349 **Discussion**

350 Our results partly agreed with our initial hypothesis (H₁) that generalist traits were
351 widespread in nearly all areas with exotic species presence, but not all generalist traits were
352 equally abundant in the exotic communities. Moreover, only temperature tolerants, low oxygen
353 tolerants and eurytopic traits were typically more dominant in exotic communities than native
354 ones, suggesting that not all generalist traits are equally determinant in the invasion process and
355 that more complex mechanisms of trait selection could be in place. Our analyses underlined
356 strong linkages between the environment and ecofunctional traits for both native and exotic
357 species but, as we hypothesized (H₂), environmental variables explained overall a lower
358 proportion of exotic species traits than of native traits. This suggests that the linkage between
359 exotic traits and environmental factors was less evident, i.e. that the environment explained less

360 the trait composition of exotic species than that of native species, even at the late-invasion stage.
361 This suggests that invasion dynamics could partly override habitat selectivity resulting in a
362 partial uncoupling of environment and biotic communities, and that these effects last long.
363 Finally, we found some differences in the ecofunctional uniqueness patterns of native and exotic
364 species and their respective ecofunctional traits, being in line with our third hypothesis (H₃).
365 Exotic species ecofunctional diversity hotspots were common in human-disturbed areas,
366 suggesting that human disturbance might play a strong role in invasion patterns.

367 *Spatial distribution of exotic species` generalist traits*

368 It was clear from our results that exotic species presence was relatively low in upland areas,
369 likely halted by natural gradients (e.g. temperature and habitat factors) or perhaps by physical
370 dispersal barriers (Dynesius and Nilsson 1994; Tockner and Stanford 2002). On the contrary,
371 exotic species were widespread in the lowlands, therefore still exerting a significant propagule
372 pressure in the less-invaded areas in the foothills (Lockwood et al. 2005; Simberloff 2009).
373 Generalist traits were widespread in exotic communities of the lowlands, but not all traits were
374 equally represented. Furthermore, generalist trait distribution was not the result of a random
375 process, but different selection processes operate on different ecological functions (H₁). Some
376 traits related to broad tolerance and flexible habitat use (temperature tolerance, low oxygen
377 tolerance and adaptation to a wide range of water current conditions) were dominant among
378 exotic communities (and more so than in native communities). However, reproductive polyphily
379 and adaptation to a wide range of turbidity were more dominant in native communities over the
380 same area, indicating that perhaps not all generalist traits are equally relevant to determine
381 invasion success. The timeline of invasion processes could partly explain this complex result:
382 early invasion phases could be spearheaded by true generalists, but on the long run more

383 specialized species can also be introduced and spread in the area, overriding some of the overall
384 invasion patterns (Nagelkerke et al. 2018). As this area is in a late invasion stage (Milardi et al.
385 2018a), what we observe could be the result of the complex interactions between species-specific
386 introduction timing and mechanisms of dispersal and interaction with native species, as well as
387 the overall outcome of longer-term environmental selection of exotic communities. These
388 mechanisms could not be fully disentangled through our analysis, and should be further
389 investigated in future studies, also considering that the spatial scale covered could influence the
390 results (Taylor et al. 2019).

391 *Exotic and native species environment-ecofunctional traits relationships*

392 Our results confirmed that there are differences in how the environmental variables influence
393 the distribution of traits for native and exotic species (H₂). The fact that native species
394 ecofunctional traits are more clearly coupled with the environment confirms our initial
395 hypothesis of a longer selection time. This tighter coupling of environmental niches was found in
396 other taxa (Marks and Lechowicz 2005), but very few studies have dealt with this aspect in fish
397 communities, so far (but see e.g. Buckwalter et al. 2018). Ecological theory predicts that exotic
398 species should be equally influenced and selected by the environment, eventually, but evidently
399 this selection could take a much longer time than that elapsed in our study area (Lambrinos
400 2004). The lower influence of the environment on exotic species ecofunctional traits suggests
401 that invasion dynamics might still play a role in the observed ecofunctional distributions (Strayer
402 et al. 2006). Among invasion dynamics, the introduction of new species through fisheries
403 stocking practices and active dispersal by anglers could confound spatial distribution patterns
404 and their linkage with the environment, for both exotic and native species (Hesthagen and
405 Sandlund 2007; Vörösmarty et al. 2010). Moreover, invasion processes are not driven by abiotic

406 factors only, and the invasion of one species could facilitate the invasions of other species
407 (Simberloff and Von Holle 1999), a mechanism previously suggested to be at play in this area
408 (Lanzoni et al. 2018; Milardi et al. 2018a). Our results confirmed that exotic species are less
409 affected by habitat filtering than native species, even at late-invasion stages.

410 *Ecofunctional uniqueness patterns of native and exotic species*

411 Our results (H₃) revealed a low ecofunctional trait diversity in mountain streams, which
412 typically have lower habitat complexity and resource availability but higher habitat quality than
413 rivers in the lowlands (Aschonitis et al. 2018; Bouska 2018). This could also be linked to a lower
414 taxonomical diversity in these areas (Gavioli et al. 2019) as species number is known to affect
415 uniqueness measures (Legendre 2014). Conversely, ecofunctional diversity was relatively higher
416 in higher stream orders and in the lowlands, with the exception of canals, which had a lower
417 ecofunctional diversity than natural rivers in the same areas. Canals should have low-
418 heterogeneity habitats, due to their artificial nature, which can partly explain this result.
419 However, our analysis highlighted that native species low ecofunctional diversity played a strong
420 role in shaping this uniqueness pattern. Previous studies have underlined how freshwater fish
421 invasions have caused a severe decrease of native taxonomical diversity at the local level,
422 especially in canals (Castaldelli et al. 2013; Milardi et al. 2018b) and low species richness could
423 be at the root of the low native ecofunctional diversity seen in canals. On the contrary, exotic
424 ecofunctional diversity was highest in the lowlands and particularly in canals, which could be
425 caused by a high number of ecofunctionally diverse exotic species. Canals in this area are indeed
426 hotspots of exotic species diversity (Lanzoni et al. 2018) and host the highest number of exotic
427 species in our dataset. This is somewhat counterintuitive, given the canals' simplified habitats,
428 but could be explained by the higher degree of human disturbance. In the studied canal network,

429 human intervention continuously causes redistribution of fish species between different canals,
430 thus likely increasing the spread and colonization of exotic species (Castaldelli et al. 2013). Our
431 results suggested that exotic invasions might contribute to shaping the spatial patterns of
432 ecofunctional diversity in fish communities.

433 Clear water, rheophilic, intermediate migration, phytophilyc, lithophilyc and piscivorous
434 species seem to contribute the most to the overall ecofunctional uniqueness of fish communities
435 in the area, being typical traits of native species and most likely belonging to species with
436 intermediate site occupancy. However, perhaps more interesting are those ecofunctional traits
437 which are rarest in the dataset, as they highlight the presence of ecofunctionally unique and
438 relatively rare native marine species (e.g. planktivores), temporarily entering estuarine and
439 upstream freshwater areas. Rare ecofunctional traits also highlight the presence of hard-to-detect
440 exotic species (e.g. herbivores, such as grass carp, *Ctenopharyngodon idella*), which were found
441 only in canals during standard monitoring, but are also present elsewhere in the Po River basin
442 (Milardi et al. 2017; Milardi et al. 2015). Further investigations are needed to unravel the exact
443 contribution of species richness, ecofunctional diversity and its spatial distribution on the
444 ecofunctional uniqueness of communities, as well as the relationship between ecofunctional
445 uniqueness and trait diversity.

446 **Conclusions**

447 Our results underlined how the decoupling of the environment-ecofunctional trait
448 relationships of exotic species is still detectable in late invasions stages, and how invasions might
449 affect the ecofunctional diversity patterns of fish communities. However, other factors
450 potentially affecting functional diversity (e.g. climate change and habitat loss) should also be

451 investigated, as they might provide useful insights on the final outcome of exotic and native
452 species interactions.

453 Our results also highlighted the importance of extending the analysis from taxonomical to
454 functional diversity, when attempting to address the complexity of species interactions in
455 invaded communities (see e.g. Colin et al. 2018; Godoy 2019; Trivellone et al. 2014). We thus
456 advocate for a wider use of ecofunctional approaches in the future to evaluate the consequences
457 of exotic species invasions (Griffiths and Harris 2010; Loiola et al. 2018; Schlaepfer et al. 2011),
458 and consequently when drafting conservation and management plans aimed at preserving native
459 biodiversity from exotic invasions.

460

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632 **Tables**

633

634 Table 1 – Ecological functions and guilds (and their abbreviations) considered for each species in
 635 this study. Edited from Milardi and Castaldelli (2018).

Ecological function	Guild	Abbreviation	Description
Feeding	Planktivores	P	Plankton feeders
	Herbivores	H	Vegetation feeders
	Benthivores	B	Bottom feeders
	Invertivores	I	Invertebrate feeders
	Piscivores	Pi	Fish feeders
	Parasite	Pa	Ematophages
	Generalists	G	Unspecialized feeding
Reproduction	Lithophils	Lp	Spawning on stones or gravel
	Phytophils	Pp	Spawning on submerged vegetation
	Phytolithophils	PL	Spawning on stones or vegetation
	Psammophils	PS	Spawning on sand or mud
	Ostracophils	O	Spawning in molluscs
	Pelagophils or live breeding	LB	Pelagic spawners or live spawners
	Polyphils	Pps	Generalist spawners
	Sea spawning	Sea	Saltwater spawners
Migration	Short	S	Within or close to the site
	Intermediate	M	Up and downstream or into flooded areas
	Long	L	Anadromous and catadromous species
Tolerance	Low oxygen tolerants	OxT	Tolerance/intolerance to low oxygen (indicatively below 3 ppm)
	Low oxygen intolerants	OxInt	
	High temperature tolerants	HTT	Tolerance/intolerance to high temperature (indicatively above 20 °C)
	High temperature intolerants	HTInt	
Habitat use	Rheophiles	Rhe	Preferring fast flowing water
	Limnophiles	Lim	Preferring slow or no current
	Eurytopics	Eur	Having no preference on current velocity
	Clear water	CW	Clear water adapted
	Turbid water	TW	Turbid water adapted
	Wide range of conditions	WR	Adapted to a wide range of water turbidity

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639

640 Table 2 – Distribution and average abundance of generalist ecofunctional traits in the area
 641 where native and exotic species distribution overlapped

Ecological Function	Trait	Exotic community		Native community	
		Distribution (% sites with trait presence)	Average dominance (% of fish community)	Distribution (% sites with trait presence)	Average dominance (% of fish community)
Feeding	Generalists	80.9	18.6	67.0	22
Reproduction	Polyphils	31.1	4.9	29.2	8.3
Tolerance	Low oxygen tolerants	98.1	95.9	84.2	53.3
	High temperature tolerants	97.6	94	84.2	49.4
Habitat use	Eurytopics	94.7	71	91.4	65.7
	Wide range of turbidity	79.4	25.3	94.3	71.9

642

643

644 **Figure legends**

645

646 Figure 1 – Distribution of the 335 sampling sites used in this study (green dots), the watercourses
647 and the three main basins considered in northern Italy.

648

649 Figure 2 – Spatial distribution of exotic abundances in the study area (a) and respective
650 abundances of generalist traits within different ecological functions. Feeding – Generalists (b),
651 Reproduction – Polyphils (c), Tolerance – Low oxygen tolerants (d) and High temperature
652 tolerants (e), Habitat use – Eurytopics (f) and Wide range of turbidity conditions (g).

653

654 Figure 3 – Plot of distance-based RDA of all species (a), native species (b) and exotic species (c)
655 fish ecofunctional traits composition (colored labels) using geographical variables, land cover
656 classes, water physico-chemical variables as explanatory variables (black labels). Fish
657 ecofunctional traits abbreviations are given in Table 1.

658

659 Figure 4 – Ecofunctional uniqueness of fish communities along a stream order gradient based on
660 Local Contribution to Beta Diversity, LCBD (a) and contribution of each fish guild to
661 uniqueness based on Species Contribution to Beta Diversity, SCBD (b). Uniqueness patterns
662 were also analyzed separately for native (LCBD (c), SCBD (d)) and exotic (LCBD (e), SCBD
663 (f)) species. Guild abbreviations are given in Table 1.

664