

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

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Abstract

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

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

Introduction

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

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

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

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

In this study, we focused on the northern Italian peninsula, previously highlighted as a good area to investigate the outcomes of freshwater fish invasions (Castaldelli et al. 2013; Gavioli et al. 2018; Gavioli et al. 2019; Lanzoni et al. 2018; Milardi et al. 2018a). We used a spatially-comprehensive dataset, including several river basins, to explore the spatial distribution of riverine freshwater fish ecofunctional traits and investigate the relationships between environmental factors and ecofunctional diversity of native and exotic species. We tested the hypothesis (H_1) that the invasion process would be largely led by less-specialized species, able to adapt to a wide range of environmental conditions (i.e. generalist species). Under this hypothesis, we expected that generalist ecofunctional traits would be widespread and dominant in exotic species communities of invaded areas, as generalist species are usually leading the invasion process (e.g. Evangelista et al. 2008). However, we expected this dominance to decrease at late-invasion stages. We also tested the hypothesis (H_2) that the environment-ecofunctional traits relationships of exotic and native species would differ. In particular, we expected that the relationships would be overall weaker for exotic species, as native species traits have been selected from the regional species pool by environmental factors over a longer time than the residence time of exotic species. We ultimately investigated the hypothesis (H_3) that ecofunctional traits of exotic and native species would have different spatial patterns (i.e. between upland and lowland streams), because lowland areas were highlighted as an invasion hotspot (Lanzoni et al. 2018; Milardi et al. 2018a). Finally, our results would help understanding the factors shaping the ecofunctional diversity of fish communities under exotic invasions, one of the major threats to native fish conservation worldwide.

Materials & Methods

Study area

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

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

Data collection

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

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

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

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

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

Water physicochemical sampling was performed with standard methods in temporal and spatial proximity with the fish sampling, by different Regional Environmental Protection Agencies (ARPAs, in Italian) for the Po, the Brenta and the rivers in the Emilia-Romagna region. The Oglio River Water Authority carried out the water sampling in the Oglio River. Eight physicochemical variables were monitored: water temperature (T ; °C), electrical conductivity (EC ; $\mu S\ cm^{-1}$), chemical oxygen demand (COD ; $O_2\ mg\ l^{-1}$), biological oxygen demand (BOD_5 ; $O_2\ mg\ l^{-1}$), total suspended solids (TSS ; $mg\ l^{-1}$), total phosphorus (P ; $mg\ l^{-1}$), ammonia (NH_4^+ ; $mg\ l^{-1}$) and nitrate nitrogen (NO_3^- ; $mg\ l^{-1}$). The geographical position (longitude – $Long$, latitude – Lat) and the altitude (Alt) were also considered.

Fish ecofunctional traits

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

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

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

Spatial distribution of exotic species generalist traits

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

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

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

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

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

Exotic and native species environment-ecofunctional traits relationships

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

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

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

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

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

Ecofunctional uniqueness patterns of native and exotic species

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

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

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

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

Results

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

Spatial distribution of exotic species` generalist traits

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

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

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

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

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

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

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

Exotic and native species environment-ecofunctional traits relationships

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

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

Ecofunctional uniqueness patterns of native and exotic species

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

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

Discussion

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

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

Spatial distribution of exotic species` generalist traits

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

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

Exotic and native species environment-ecofunctional traits relationships

Our results confirmed that there are differences in how the environmental variables influence the distribution of traits for native and exotic species (H_2). The fact that native species ecofunctional traits are more clearly coupled with the environment confirms our initial hypothesis of a longer selection time. This tighter coupling of environmental niches was found in other taxa (Marks and Lechowicz 2005), but very few studies have dealt with this aspect in fish communities, so far (but see e.g. Buckwalter et al. 2018). Ecological theory predicts that exotic species should be equally influenced and selected by the environment, eventually, but evidently this selection could take a much longer time than that elapsed in our study area (Lambrinos 2004). The lower influence of the environment on exotic species ecofunctional traits suggests that invasion dynamics might still play a role in the observed ecofunctional distributions (Strayer et al. 2006). Among invasion dynamics, the introduction of new species through fisheries stocking practices and active dispersal by anglers could confound spatial distribution patterns and their linkage with the environment, for both exotic and native species (Hesthagen and Sandlund 2007; Vörösmarty et al. 2010). Moreover, invasion processes are not driven by abiotic

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

Ecofunctional uniqueness patterns of native and exotic species

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

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

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

Conclusions

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

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

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

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632 **Tables**

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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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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 |

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Figure legends

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

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

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

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