Science Of The Total Environment
June 2018, Volume 625 Pages 861-871
https://doi.org/10.1016/j.scitotenv.2017.12.316
https://archimer.ifremer.fr/doc/00626/73785/

Functional diversity measures revealed impacts of non-native species and habitat degradation on species-poor freshwater fish assemblages

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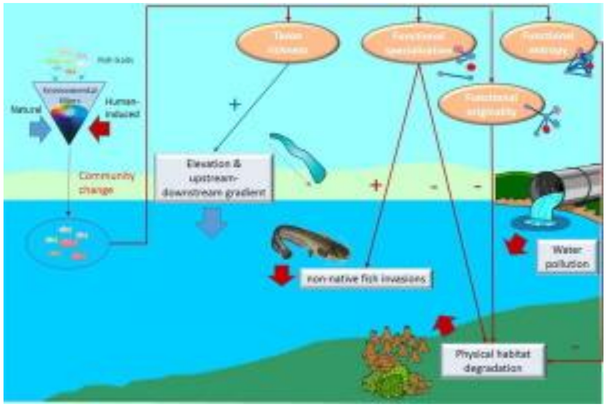
Abstract :

Trail-based ecology has been developed for decades lo infer ecosystem responses to stressors based on the functional structure of communities, yet its value in species-poor systems is largely unknown. Here, we used an extensive clataset in a Spanish region highly prone to non-native fish invasions (15 catchments, N 389 sites) to assess for the first time how species-poor communities respond to large-scale environmental gradients using a taxonomic and functional trait-based approach in riverine fish. We examined total species richness and three functional trait-based indices available when many sites have <= 3 species (specialization, FSpe; onginaliy, FOri and entropy, FEnt). We assessed the responses of these taxonomic and functional indices along gradients of altitude, water pollution, physical habitat degradation and non-native fish biomass. Whilst species richness was relatively sensitive to spatial effects, functional diversity indices were responsive across natural and anthropogenic gradients. All four diversity measures declined with altitude but this decline was modulated by physical habitat degradation (richness, FSpe and FEnt) and the non-native total fish biomass ratio (FSpe and FOri) in ways that varied between indices. Furthermore, FSpe and FOri were significantly correlated with Total Nitrogen. Non-native fish were a major component of the taxonomic and functional structure of fish communities, raising concerns about potential misdiagnosis between invaded and environmentally-degraded river reaches. Such misdiagnosis was evident in a regional fish index widely used in official monitoring programs. We recommend the application of FSpe and FOri to extensive clatasets from monitoring programs in order to generate valuable cross-system information about the impacts of non-native species and habitat degradation, even in species-poor systems. Scoring non-native species apart from habitat degradation in the indices used to determine ecosystem health is essential to develop better management strategies.

Graphical abstract



Keywords : Fish assemblages, Human disturbance, Functional diversity, Mediterranean rivers, Non-native species, Biomonitoring

1. Introduction

Biodiversity loss is occurring at unprecedented rates on Earth, and freshwater ecosystems are a prime example (Strayer and Dudgeon, 2010; Tittensor et al., 2014). The EU's Water Framework directive has been highly influential in Europe to take conservation actions on major threats to freshwater ecosystems, mainly water pollution and hydromorphological alterations (EU Commission, 2003). However, effective management strategies can only be developed with a good knowledge of how multiple impacts affect aquatic biota, including biological invasions (Thomsen et al., 2014).

The loss of sensitive species is a well-known response of aquatic communities to stress (Kolkwitz and Marsoon, 1909; Friberg et al., 2011), and the basis of the myriad of taxonomic-based procedures developed, hereafter referred to as indices of biotic quality (IBQs), to assess the health status of rivers (Birk et al., 2012; Karr, 1981). Despite being widely adopted by resource managers, many criticisms have arisen from their use (Friberg et al., 2011; Jackson et al., 2016). Among the most important is that the extensive use of IBQs may have limited our ability to develop theory on how aquatic assemblages respond to stress. Ecological niche theory states that habitat acts as 'filter' selecting those species with the best set of traits for a given condition (Chase and Leibold, 2003). That is, communities geographically distant can differ in species composition but have similar trait combinations (e.g. Bonada et al., 2007).

Towards predicting the response of communities, functional ecology has been developing in recent decades (Petchey and Gaston 2006; Statzner et al., 2001) including functional diversity (FD) measurements based on species' functional traits, i.e. attributes of organisms linked to their response to environment or their role in ecosystem processes (Maire et al., 2015; Mouillot et al., 2013; Petchey and Gaston, 2002). It is widely recognised that FD measures are a superior alternative to taxonomic-based approaches to detect the consequences of human impacts on animal assemblages (e.g. Gagic et al., 2015; Hooper et al., 2005; Villéger et al., 2010). However, their advantages over IBQs have not been specifically investigated. IBQs often use community traits in making diagnoses, but resultant scores do not explicitly account for functional diversity.

Here, we assess how taxonomic richness and the functional structure of freshwater fish assemblages respond to fish invasions and environmental degradation in an extensive area of north-eastern Spain. This region has a long-history of anthropogenic disturbances (e.g. water pollution, physical habitat degradation, and non-native invasions; Figuerola et al., 2012; Maceda-Veiga et al., 2017a; Mas-Marti et al., 2010) and allow us to assess the relative contribution of these three factors to variation in the structure of fish assemblages in a wide range of orographic conditions (Sabater et al., 2009). If suitable for river biomonitoring programs, fish diversity measures should respond to three major threats to riverine ecosystems, namely water pollution, physical habitat degradation and non-native fish invasions and, to a minor degree, to natural factors, including altitude. However, the low fish species richness in Mediterranean rivers (often <4 species, e.g. Maceda-Veiga et al., 2017a) contrasts with the higher richness in other European rivers and may limit the performance of FD indices to detect effect of stressors (see Maire et al., 2015). Nonetheless, this region is highly prone to non-native fish invasions, with tributaries containing mostly native species and lowland mainstems mostly non-native species (up to six invasive species in Maceda-Veiga et al., 2017a).

The life-histories of native fish populations, including endemic (*Barbus haasi*

Mertens 1925, *Luciobarbus graellsii* Steindachner, 1866) and widely distributed species (*Salmo trutta* Linnaeus, 1758, *Anguilla anguilla* Linnaeus, 1758), are adapted to the hydrological dynamism of Mediterranean rivers (e.g. Vinyoles et al., 2010; Doadrio, 2011). However, fish species introduced in this area, including globally distributed invaders (e.g. *Alburnus alburnus* Linnaeus, 1758, *Cyprinus carpio* Linnaeus, 1758), appear to perform better in hydrological regimes generated by damming and water abstractions than in natural rivers (Maceda-Veiga et al., 2017a).

The objectives of our study are: i) to test whether three FD indices (functional specialization, originality and entropy) identify the impacts of human activities better than does taxonomic richness in species-poor fish assemblages, and ii) to compare the diagnostic value of traditional IBQs and FD indices in detecting two major threats to rivers, namely habitat degradation and the release of non-native species, which may not necessarily co-occur (see Benejam et al., 2009). We expected that FD indices would provide better inferences of how fish invasions, water pollution and physical habitat degradation affect fish assemblages than would do species richness alone, because community-habitat relationships should be mediated via functional traits (e.g. Suding et al., 2008). If FD indices have potential to become new monitoring tools in species-poor systems, we expected them to perform better than a regional fish index and other IBQs widely used by water agencies in compliance with the EU's Water Framework Directive.

2. Materials and methods

2.1. Study area

The study area is located in north-eastern Spain and comprises 15 catchments, including the complete Ebro River and part of the Garonne basin (Fig 1). Except the Garonne, all rivers flow east from the Cantabrian, Pyrenean or low mountains to the sea. Overall, the selected river basins drain an extensive area of up to 99,700 km^2 and the variety of human impacts over large-scale natural gradients provide an excellent study system (see also Sabater et al., 2009). Approximately 40% of all sampling sites (N = 389) have non-native species. The range of values for widely used indicators of pollution (e.g. conductivity and nutrients) is wide in invaded (conductivity = 79-4108 µS cm-1; nitrate = 0-25 mg l-1) and non-invaded sites (conductivity = 20-4108 µS cm-1; nitrate = 0-30 mg l-1). Similarly, the altitudinal range of sites with non-native (3-984 m.a.s.l.) and native species was wide (3-1814 m.a.s.l.) (see Maceda-Veiga et al., 2017b for further details).

Most of these rivers are small and follow a typical Mediterranean hydrological regime, with severe droughts in summer and torrential floods in autumn. In large rivers, however, streamflow peaks in spring because of snowmelt. We surveyed in low flow conditions because this is when fish populations can be most efficiently sampled using electrofishing (see below). These conditions are also likely to intensify the effects of anthropogenic stressors on aquatic organisms (Petrovic et al., 2011).

2.2. Fish surveys

We assembled fish data from 430 surveys performed in north-eastern Spain from 2002 to 2008 (e.g. Sostoa et al., 2003; Maceda-Veiga and de Sostoa, 2011). Our surveys followed an international standardized fish sampling method (CEN standards EN 14962 and EN 14011), in compliance with the EU's Water Framework Directive.

We used a single-pass electrofishing approach using a portable unit which generated up to 200 V and 3 A pulsed D.C. in an upstream direction. We covered the whole wetted width of the 100-m long reaches surveyed in each site, which included a variety of habitat types (pools, rifles and runs) (see Maceda-Veiga et al., 2017a for further details). Fish were kept in buckets provided with air pumps until the end of the survey when they were released into the river. There was no mortality. Fish were anaesthetized with a buffered MS222® solution (0.02% Tricaine methane-sulfonate, Sigma) to reduce stress. Fish were identified to species level, counted and a representative set of individuals of each species (40 individuals when possible) weighed to the nearest g. Fish biomass was expressed as total fish weight divided by the area surveyed and sampling time in minutes (kg/ha x min).

2.2. Functional characterization of fish

To describe the functional identity of each fish species, we used 9 traits that are related to key biological functions such as food acquisition, locomotion and reproduction weighted by the biomass of each species in each sampling site (Table 1) (Buisson et al., 2013; Olden et al., 2006, Villéger et al., 2013). These traits have major implications for ecosystem functions. For example, migratory species, including the European eel (*A. anguilla*) are important for the transfer of energy along rivers (Flecker et al. 2010), and prey consumption is related to predator's body size (e.g. Jardine et al., 2017).

Traits were coded as continuous or ordinal variables. We used regional fish descriptions (Doadrio, 2011; Kottelat and Freyhof, 2007; Sostoa et al., 1990), electronic databases (http://www.fishbase.org), the scientific literature, and our own expertise to provide a functional description of all fish species (Table 2). Ordinal traits were assigned a single state based on a majority rules approach according to adult preferences following Olden et al. (2006). The lack of an in-depth ecological knowledge of some fish species precluded the use of more traits. We acknowledge that species traits can differ among populations (see Ackerly and Cornwell, 2007), but we lack this specific information for the present study. Species mostly found in river mouths such as mullets (*Liza* spp., *Chelon labrosus* Risso, 1827 and *Mugil cephalus* Linnaeus, 1758) were excluded from the analysis, as they play a minor ecological role at the basin scale.

2.3. Measuring functional diversity

Functional diversity (FD) indices of fish assemblages were computed using different measures of dissimilarity among the traits of a given species in relation to the trait composition of the overall data-set (N = 430 sites) (Maire et al., 2015; Mouillot et al., 2013; Villéger et al., 2008). We calculated overall differences in traits among species using the Gower distance (Gower, 1966). We then used a principal coordinates analysis to identify the number of axes that best represent the differences in trait composition (i.e. the multidimensional Euclidean space in Villéger et al., 2008). The first four axes (mSD: 0.012) provided the best result based on the criterion of Maire et al. (2015) for species-poor systems, where the species more separated had the most extreme traits. However, the relative importance of the traits of a given species in relation to the complete data-set can be weighted using different algorithms. This is why we used the three indices of FD that can be calculated even with one species in each sampling site, namely functional entropy (FEnt), functional specialization (FSpe), and functional originality (FOri; Maire et al., 2015).

FEnt was calculated as 1/(1-Q), where Q is Rao's quadratic entropy computed as the biomass-weighted sum of pairwise functional distance among species within the community (Ricotta and Szeidl, 2009). FEnt increases when species with the greatest biomass are functionally distinct (Mouillot et al., 2013). FSpe was calculated as the biomass-weighted mean distance in the functional space to the average value of all the species present at the regional scale (Bellwood et al., 2006). It reaches high values when the species with the greatest biomass has the most extreme traits from the regional pool. Finally, we calculated FOri as the biomass-weighted mean distance to the nearest species within functional space (Mouillot et al., 2013). It increases when species with unique trait combinations have the greatest biomass in the community.

2.4. Environmental variables and indices of biotic quality

In each sampling site, we quantified seven water quality variables (pH, conductivity, ammonium, nitrite, nitrate and phosphate-P concentrations) prior to fish sampling (e.g. Maceda-Veiga et al., 2017a). These variables provide an overview of major stressors to aquatic ecosystems, including nutrient pollution and changes in overall ionic composition (e.g. Nielsen et al., 2003; Smallbone et al., 2016; Maceda-Veiga et al., 2017a). To describe physical habitat, we used 17 variables from two widely used habitat quality indices in this region, namely the QBR (Munné et al., 2003) and RBA indices (a modified version of the U.S. Rapid Bioassessment by Barbour et al., 1999). As geographical features, we recorded the basin name and altitude (m.a.s.l.) in each site using Google Earth®. Altitude was used as a surrogate for the position of the sampling site in the river, and summarises the role of natural spatial gradients in fish indicators, as previously validated in this region (Murphy et al., 2013).

As indices of biotic quality, we downloaded scores of three indices based on diatoms, fish and invertebrates from the Catalan Water Agency (http://aca-web.gencat.cat/aca/appmanager/aca/aca/) for 50 sites that match with our fish surveys. We used the Specific Polluosensitivity Index for diatoms (IPS, Coste, 1982), the Index of Biotic Integrity for Catalan rivers for fish (IBICAT, Sostoa et al., 2003), and the Index of the Iberian Biomonitoring Working Party for invertebrates (IBMWP, Alba-Tercedor et al., 2002). Last, we calculated the median tolerance of all fish species to water and habitat degradation in each sampling site using the tolerance indicator values (TIV) developed by Maceda-Veiga and de Sostoa (2011).

2.5. Statistical analyses

All analyses were computed in R (R Development Core Team, 2013) using the packages *stats*, *MASS*, *lme4* (Bates et al., 2016), *ade4* (Dray & Dufour, 2007), *psych* (Revelle and Revelle, 2016), and *hier.part* (Walsh et al. 2013) and the functions outlined below. Continuous variables were log-transformed and % were arc-sine square-root transformed to aid in model fitting. The original set of 24 environmental variables was reduced by excluding highly correlated variables (Spearman's rho > 0.7), as reported in Maceda-Veiga and de Sostoa (2011). A principal component analysis (the function *principal*) was then applied to summarize variation in the remnant 11 water and habitat variables in form of principal component axes, which we re-named as 'gradients of anthropogenic impact'. The 'varimax' rotation facilitated the interpretation of axes, and the number of axes was selected based on explanatory power.

2.5.1. Modelling taxonomic and functional fish diversity as function of natural factors, environmental degradation and non-native fish biomass

Generalized linear mixed models (GLMM, the function *glmer*) were used to examine relationships among fish diversity measures (taxonomic richness, FEnt, FSpe, and FOri), altitude, and the gradients of anthropogenic impact. As other anthropogenic stressor, we included in the models the proportion of non-native fish species in relation to the total fish biomass to explore their contribution to the variation in taxonomic richness and FD indices. Basin was included as random effect to control for potential systematic differences among basins. Sampling year was also included as random factor but was excluded from final models because the explained variance was close to 0. Proportional data (FSpe, FOri, and FEnt) was analysed using binomial errors/logit link, and patterns in species richness were examined using Poisson errors/log link. Models were validated by visually inspecting diagnostic plots of residuals. The statistical threshold was established at $P < 0.05$.

To further test the robustness of our results, we used a hierarchical partitioning (HP) analysis (the function *hier.part*) using the error distributions validated in the GLMM approach. HP models deal with collinearity among predictors (e.g. between altitude and habitat degradation, see Murphy et al., 2013), which even in small amounts can bias regression parameters (Freckleton, 2011). Whilst causality cannot be determined in observational studies, HP decomposes the variation of dependent variables in unique and joined fractions of a set of predictors (Mac Nally and Walsh, 2004). We assessed the significance of HP models using a randomization test for hierarchical partitioning analysis (the function *rand.hp*). As many regressors can generate rounding errors in HP models, we validated their outputs by changing the order of predictors, as recommended by Mac Nally and Walsh (2004).

2.5.2. Effects of environmental degradation and non-native fish biomass on fish traits

To determine the relative contribution of each functional trait in the fish community-environment relationships, we used fourth-corner and RLQ analyses following the guidelines of Dray et al. (2014). Both methods are based on the analysis of the fourth-corner matrix, which crosses traits and environmental variables weighted by species biomass. RLQ is a multivariate technique that provides ordination scores to summarize the joint structure among species distributions across sampling sites, environmental variables and species traits. In contrast, the fourth-corner method mainly tests for individual trait-environment relationships (one trait and one environmental variable at a time). We included the non-native status of fish as additional trait in this analysis to avoid circularity in our reasoning (as non-native species contributed to the trait matrix). We used model type 6 to avoid inflated rates of type I error (Dray and Legendre, 2008). The significance of trait-environment relationships was assessed through Monte Carlo permutation (999 iterations using the approach of ter Braak et al., 2012).

2.5.3. Comparing the diagnostic value of fish diversity measures and traditional indices of biotic quality in environmentally degraded or invaded river reaches

We used Spearman rank correlation coefficients (at $P < 0.05$) to test to which extent the fish diversity measures used (taxonomic richness, FEnt, FSpe and FOri) were associated with other measurements of river health status, namely indicators of nutrient pollution, conductivity and the number of non-native species) using an independent data-set from the same study area ($N = 50$ sampling sites). Moreover, we compared these correlation coefficients with those obtained from correlations with indices of biotic quality to assess the potential superiority of FD indices in determining river health status.

3. Results

3.1. Description of the taxonomic and functional diversity of fish assemblages

We captured 26 fish taxa from 11 families, of which 12 species were Cyprinidae (Table 2). Fourteen taxa were native and 12 non-native, and the maximum total species richness in each site was 11. The most frequently captured native species (>100 sampling sites) were brown trout (*S. trutta*), Iberian redfin barbel (*B. haasi*) and common barbel (*L. graellsii*). The most common non-native species (captured in > 40 sampling sites) were carp (*C. carpio*) and bleak (*A. alburnus*). Functional specialization (FSpe) and functional originality (FOri) reached their maximum value (1), whereas the functional entropy index (FEnt) ranged from 1 to 1.8. Species richness was significantly correlated to the three functional diversity (FD) indices, with correlation coefficients slightly higher for FEnt (r = 0.54; $P < 0.01$) than for FSpe (r = 0.24; $P < 0.01$) and FOri (r = 0.26; $P < 0.01$).

3.2. Defining gradients of water and physical habitat degradation

Only the first two axes of PCA were considered gradients of anthropogenic impact and explained altogether 42.88 % of variance (Table 3). PC1 accounted for 29.6% of the variance and was mainly driven by water pollution (e.g. nitrite, nitrate, and phosphates-P). PC2 explained 13.8% of variance and was mainly related to physical habitat degradation (riparian cover, habitat structure, and channel morphology).

3.3. Relative contribution of natural factors, environmental degradation and non-native fish biomass to variation in taxonomic and functional diversity of fish assemblages

Since human activities often concentrate in the lowlands, it was necessary to disentangle the relative effects of natural factors, water pollution, physical habitat degradation, and the proportion of non-native fish species expressed as biomass on the three FD measures. Hierarchical partitioning (HP) models revealed that altitude made the largest individual contribution to the variation in all four fish diversity measures (Fig 2). Patterns in taxon richness were mainly attributed to natural factors, namely altitude and basin (Fig 3). All three functional diversity (FD) indices followed the altitudinal trend observed for species richness (Figs 3 and 4). Physical habitat degradation also explained a significant unique fraction of variation in FD indices (Fig 2).

HP results were mostly concordant with those from generalized linear mixed models (Table 4). However, the two modelling techniques ranked the contribution of altitude, physical habitat degradation and non-native fish biomass to variation in FD measures in a different order (Fig 2, Table 4). While FSpe and FOri indices were influenced by altitude and the biomass of non-native fish species in the same way, only FSpe was significantly related to physical habitat degradation (Table 4). Water pollution was not retained as having a significant effect in any of the four fish diversity measures (Table 4). The random effect of basin was of major importance to explain variation in species richness but not in FD (Table 5).

3.4 Associations of fish traits with environmental degradation and non-native biomass

The first of three axes in the RLQ analysis explained the vast majority (68%) of variation in the trait-environment relationship, which was largely driven by responses of non-native species, velocity preference, shape factor and vertical position (Figure 5). Overall, there was a significant link between traits and the environment (RLQ: $p = 0.04$). However, only one individual environment-trait link was significant, namely a positive relationship between water pollution (PC1) and non-native fish status (fourth-corner: $r = 5.06$, $p_{adj} = 0.04$; Table S1). There was a further notable, yet non-significant, negative relationship between altitude and velocity preference (fourth-corner: $r = -2.66$, $p_{adj} = 0.11$; Table S1).

3.5. Comparing the diagnostic value of fish diversity measures and traditional indices of biotic quality in environmentally degraded or invaded river reaches

The strongest correlations were found among all three FD indices (FSpe, FOri, and FEnt) and the proportion of non-native fish in the fish assemblage expressed as richness or biomass (Table 6). A weak but negative relationship was observed for the regional fish index IBICAT and the two non-native fish metrics (Table 6). In contrast, a strong negative relationship was found among non-native fish the diatom (IPS) and benthic macroinvertebrate (IBMWP) indices, and the tolerance indicator values of fish to water pollution (TIV_WATER) and physical habitat degradation (TIV_RBA) following the methods outlined of Maceda-Veiga and de Sostoa (2011) (Table 6).

At least one indicator of environmental degradation was significantly correlated, either with a fish diversity measure or an index of biotic quality (Table 6). IPS and IBMWP showed a highly negative correlation with conductivity and total nutrient concentrations. Conversely, nutrients were positively related to FSpe, FOri and the TIV for water quality (Table 6). A positive association was also found for the habitat index RBA and the index IPS and the TIV for physical habitat quality (Table 6). In contrast, the physical habitat index RBA was negatively associated with FSpe and FOri (Table 6). All water and habitat indicators were poorly related to total fish richness, the fish index IBICAT, and the FD index FEnt (Table 6).

4. Discussion

Our study is a first in showing the potential of FD measures to identify the mechanisms behind changes in the structure of species-poor fish assemblages. We showed the superior performance of three functional diversity (FD) indices (specialization, FSpe; originality, FOri; and entropy; FEnt) over taxonomic richness and a regional fish index in response to habitat degradation and non-native fish invasions, two major drivers of the freshwater biodiversity crisis around the world (Marr et al., 2010; Strayer and Dudgeon, 2010; Vörösmarty et al., 2010).

4.1. Relative contribution of natural factors, environmental degradation and non-native fish biomass to variation in taxonomic and functional diversity of fish assemblages

Our results support the notion that geographical features, including altitude, are major shaping forces of the composition of fish assemblages (Maceda-Veiga et al., 2017a;

Richards et al., 1996; Williams et al., 2003). In upstream sites above 1000 m, FD indices and richness values were very low. As altitude declined below 500 m FD indices increased rapidly, whereas species richness exhibited a more gradual change (Fig 3). The most plausible explanation for these results is that rivers increase in size downstream as do resources available (Angermeier and Schlosser, 1989; Lomolino, 2000). Such a trend in the three indices of FD indicates that more species with extreme, unique trait values were found downstream, as reported by Karadimou et al. (2016).

The relative effect of non-native fish biomass and physical habitat quality on FD was index- and model-specific. Incongruence between generalized linear mixed models (GLMMs) and hierarchical partitioning analyses of variance (HP) can be due to collinearity. Even when highly correlated variables are excluded before running any model, predictors are always correlated (see details in Mac Nally, 2002). Non-native fish often occur in hydrologically-impacted rivers (e.g. dams, Maceda-Veiga et al., 2017a; Marchetti et al., 2004). This may have obscured our relationships between non-native fish species and physical habitat degradation and FD indices, but HP models can shed light into these associations (see also Buisson et al., 2008; Murphy et al., 2013). GLMMs and HPs ranked biotic and abiotic predictors in a different order, which has management implications because different actions are required to extirpate species and to restore habitat. Nonetheless, hydromorphological restoration might solve both issues, as rivers with natural flow regimes are often the least invaded (Maceda-Veiga et al., 2017a, Bernardo et al., 2003; Marchetti and Moyle, 2001; Poff et al., 1997).

In our study, the indices FSpe and FOri were strongly associated with non-native fish biomass and physical habitat degradation as opposed to species richness and FEnt, both mostly affected by natural factors (Fig 3, Fig 4). These results proved the utility of FD indices to detect human impacts, as has been demonstrated in many taxa including birds (Huijbers et al., 2016), plants (Laliberté et al., 2013), aquatic invertebrates (Gutiérrez-Cánovas et al., 2015) and fish (Villéger et al., 2010), but it had not yet been tested in species-poor fish assemblages. A high number of endemic species but low richness is a common trait in freshwater fish assemblages in Mediterranean basins, which are home to among the most threatened faunas in the world (Smith and Darwall, 2006). Studies examining species composition provide useful insights into how human perturbations affect these assemblages (e.g. Maceda-Veiga et al., 2017a). However, functional trait-based ecology transforms taxonomic information into a matrix of ecological traits and allows researchers to make cross-taxa and cross-system comparisons more easily (Mouillot et al., 2013; Villéger et al., 2010).

4.2. Identifying the traits that best represent differences in the functional composition of fish assemblages

FSpe and FOri indices differed in their response to human impacts, supporting the idea that multiple indices are required to fully describe functional diversity (Mouillot et al., 2013; Villeger et al., 2008). FOri reduced if non-native fish dominated in biomass because successful invaders shared traits (e.g. high fecundity, Marr et al., 2010). These traits are very different from those of native species, and non-native fish traits have a strong biomass weighting because of the large size of the fish (e.g. the predator *Silurus glanis* Linnaeus, 1758, >100 Kg; Doadrio, 2011). The native fish fauna in our study-system naturally lacks many predators, with most species being medium-size omnivorous-invertivorous cyprinids (Doadrio, 2011), which may also explain an increase in FSpe with the proportion of non-native fish species. A remarkable exception is the native fish *A. anguilla* which has unique trait sets among native fish (e.g. predator, catadromous) (de Sostoa et al., 1990). However, the traits of *A. anguilla* probably had a minor weight in computing the FD indices because this species is currently rare in our study area (Maceda-Veiga et al., 2010). Interestingly, traits such as fecundity seemed to be related to water quality even when this factor did not have an overall effect on FD indices. These results can be explained because nutrient pollution was one of our water quality variables and energetic reserves increase body condition and hence fecundity (Peig and Green, 2009), but may have not affected other functional traits. This result indicates that it may be important to use a trait which has the strongest link with a function of interest, instead of combining many traits in scores of multi-trait indices (see also Colin et al., 2016b).

Although the indices FSpe and FOri better identified anthropogenic impact than did species richness, this does not mean that modern ecology can be disconnected from taxonomy. A good taxonomical and ecological knowledge of species is crucial to assign traits properly (e.g. Rodríguez-Lozano et al., 2016; Sánchez-Hernández et al., 2011). Even though the marine fish *Sarpa salpa* Linneo, 1758 and *Siganus luridus* Rüppell 1829 are both herbivores, the former feeds exclusively on vascular plants whereas the latter feeds on macroalgae, affecting the ecosystem in a different manner (Vergés et al., 2014). Similarly, detailed studies of two omnivorous species in our study (e.g. *B. meridionalis*, *Squalius laietanus*) have shown differences in prey consumed in clean and polluted sites among seasons (Colin, In preparation). These changes provide evidence further that a good knowledge of natural history is essential to avoid a coarse assignment of traits, but pragmatism is also needed as function of time invested in data acquisition and model outcomes. In our study, there were also issues with species identity (genera *Phoxinus*, *Gobio*, *Carassius*, *Barbatula*) which requires identification using genetic data (Maceda-Veiga et al., 2017a).

4.3. Congruence among fish diversity measures and other indicators of river health

Riverine taxa integrate the effects of multiple stressors over variable spatio-temporal scales based on their different life-histories (Barbour et al., 1999). Thus, biotic indices may not correlate strongly with fluctuating variables such as water variables but inform about diagnostic ability. However, strong correlations can indicate that aquatic taxa are exposed to chronic pollution (e.g. Colin et al., 2016a), which is suggested in our study by a strong relationship between diatom (IPS) and invertebrate-based indices (IBMWP) and nutrient concentrations. These results support the idea that biotic indices were originally developed to detect organic pollution, even though they are now used to determine the ecological impact of many pollution sources (e.g. Colin et al., 2016a; Juttner et al., 2012; Munné and Prat, 2009). A strong negative relationship was found in our study between non-native fish and the indices IPS and IBMWP. As stated above, this association can be attributed to the fact that non-native fish species tend to occur in degraded sites, although these species are often ecosystem engineers which directly cause habitat degradation (Shin-Ichiro et al., 2009). Nonetheless, there is still limited insight into how non-native fish invasions affect native fish responses to water pollution and physical habitat degradation (but see Maceda-Veiga et al., 2017b).

The confounding effect of non-native fish in ecosystem health diagnostic was particularly problematic for fish-based measures. For example, the regional fish index IBICAT did not significantly respond to water quality and physical habitat, and was markedly affected by non-native fish occurrence, as has been highlighted by Benejam et al., (2009). Fish richness was also poorly related to environmental degradation and mostly driven by the presence of non-native fish. Conversely, functional indices (FSpe and FOri) responded to both, even though the direction of the effects often differed from our large-scale data-set, which indicates that the outputs of functional analyses depend on the pool of traits of the assemblage under study (Mouillot et al., 2013; Villeger et al., 2008).

Last, our study showed that invaded sites were dominated by fish species relatively tolerant to poor water and habitat quality according to tolerance indicator values developed by Maceda-Veiga and de Sostoa (2011). These results support the prevailing assumption in the literature that non-native fish species are highly tolerant (Hermoso et al., 2011; Ribeiro et al., 2008; but see Kennard et al., 2005; Maceda-Veiga and de Sostoa, 2011). However, it may also indicate that native species living there have wider tolerance ranges, if say, non-native fish species that typically occur in upstream reaches (e.g. *Oncorhynchus mykiss*, *Phoxinus* spp.) are likely to be more tolerant than native fish species from downstream reaches (e.g. *Luciobarbus graellsii*) (Maceda-Veiga and de Sostoa, 2011). However, these inferences need testing with specific experiments because the response of a species to stress is affected by many factors at play, including acclimation, adaptation, and phenotypic plasticity (Biagianti-Risbourg et al., 2013).

5. Conclusions

Official biomonitoring schemes are firmly rooted in more than 200 indices of biotic quality which appraise the ecological status of water-bodies in Europe alone (Birk et al., 2012). The principal disadvantages are that they are not generalisable beyond the region they were designed for and they do not reflect ecological processes. Without underestimating their value, our study provides evidence of the potential of the indices FSpe and FOri based on fish traits as new diagnostic tools in species-poor systems. The FD indices were sensitive to both abiotic and biotic degradation, and showed more sensitivity to anthropogenic impacts than did species richness and a regional fish index. By identifying which traits make species more vulnerable to human actions in extensive data-sets collected through official monitoring schemes, it is possible to identify the taxa at most-risk in the highly threatened Mediterranean rivers (Smith and Darwall, 2006). Therefore, FD indices are a promising tool to better understand the causes of decline in freshwater fish and develop more effective conservation strategies.

Acknowledgments

We thank all people who assisted in the field and Dr Lluís Cardona for useful insights into the use of functional guilds in ecological studies. NC was funded by the National Commission of Scientific and Technological Research (CONICYT PhD fellowship, Ref: 72100771) and AMV by the Severo Ochoa Program for Centres of Excellence in R+D+I (Ref: SEV-2012-0262).

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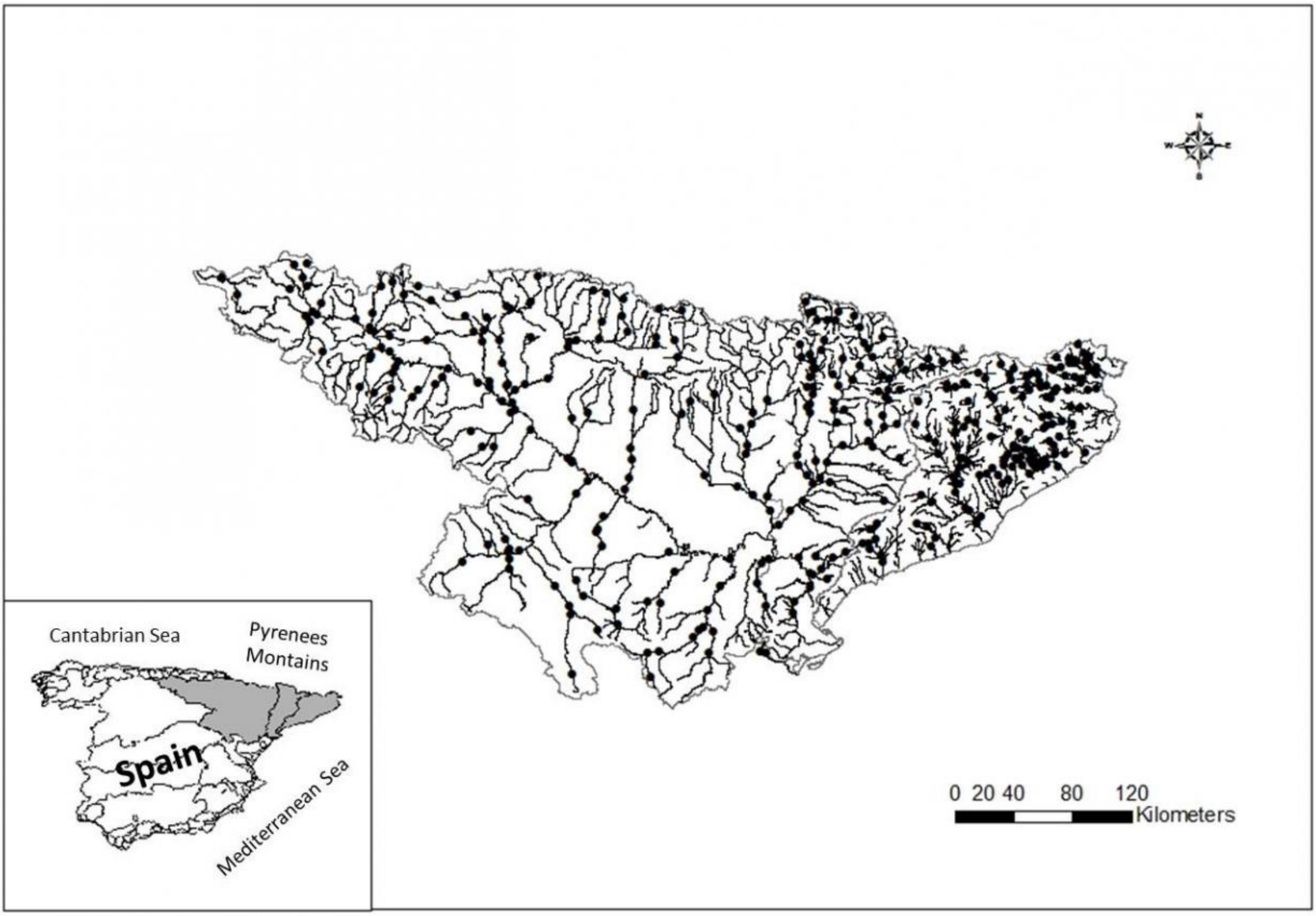
Figure 1. Location of study area in northeastern Spain. The 389 sampling sites are shown with black points.

Figure 2. Independent contribution (%) of the five predictors to explain variation of 3 functional diversity measures (FSpe, FOri, FEnt) and taxonomic richness. All predictors were significant at the 95% confidence interval based on a randomized permutation test.

Figure 3. Relationship (mean fitted values from GLMM) between taxon richness and altitude (with basin as random effect) at three levels of physical habitat quality (PC2): PC2 = maximum (a); PC2 = median (b); and PC2 = minimum (c).

Figure 4. Relationships between FD indices (FSpe, FOri and FEnt) and significant drivers according to GLMMs accounting for altitude, physical habitat (PC2) and ratio of non-native to native biomass (‘ratio’); a-c) FSpe vs altitude for 3 levels of non-native fish biomass (100%, 50% and 0%, colors) and three types of physical habitat (maximum, median , minimum value of PC2, respectively); d) FOri vs altitude for 3 levels of non-native fish biomass ; e) FEnt vs altitude for 3 levels physical habitat habitat (maximum, median , minimum value of PC2, respectively).

Figure 5. Results of the first two axes of the RLQ analysis: (a) species scores (see Table 1 for species codes); (b) coefficients for environmental variables (PC1=water quality, PC2=physical habitat); (c) coefficients for traits (see Table 1 for descriptions); and (d) eigenvalues with the first two axes in grey. Note: water quality is negatively correlated with PC1.


Cantabrian Sea
Pyrenees Montains
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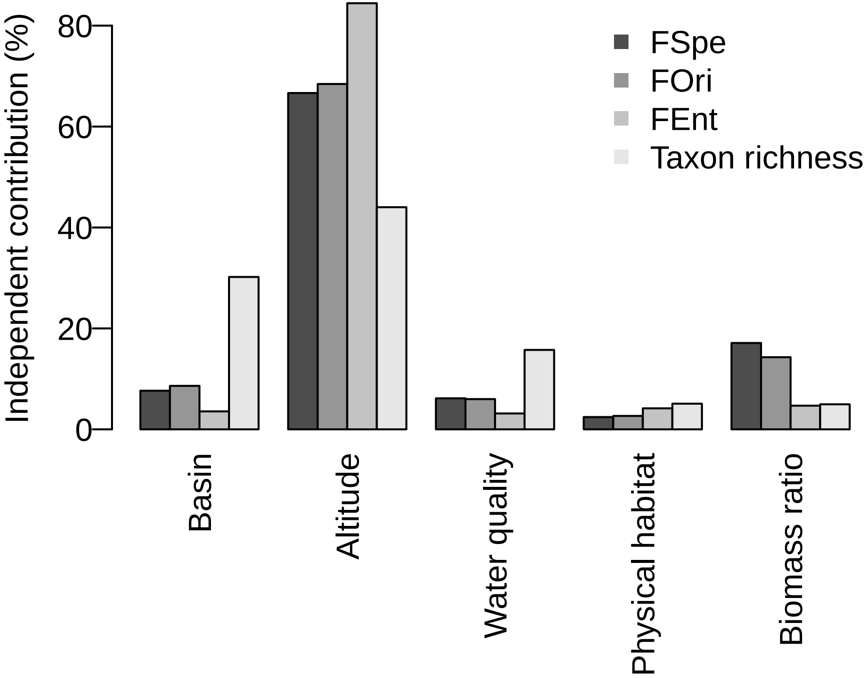
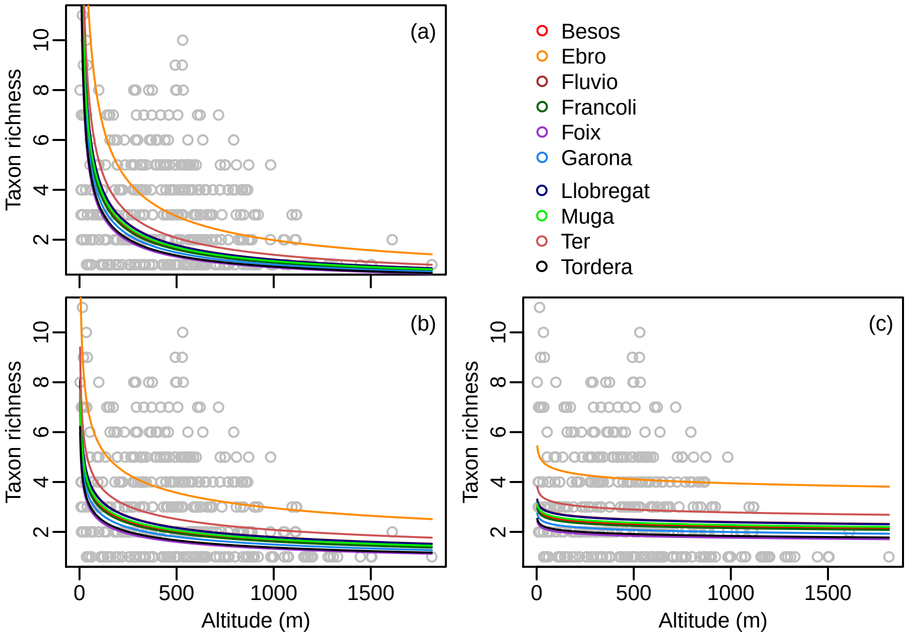
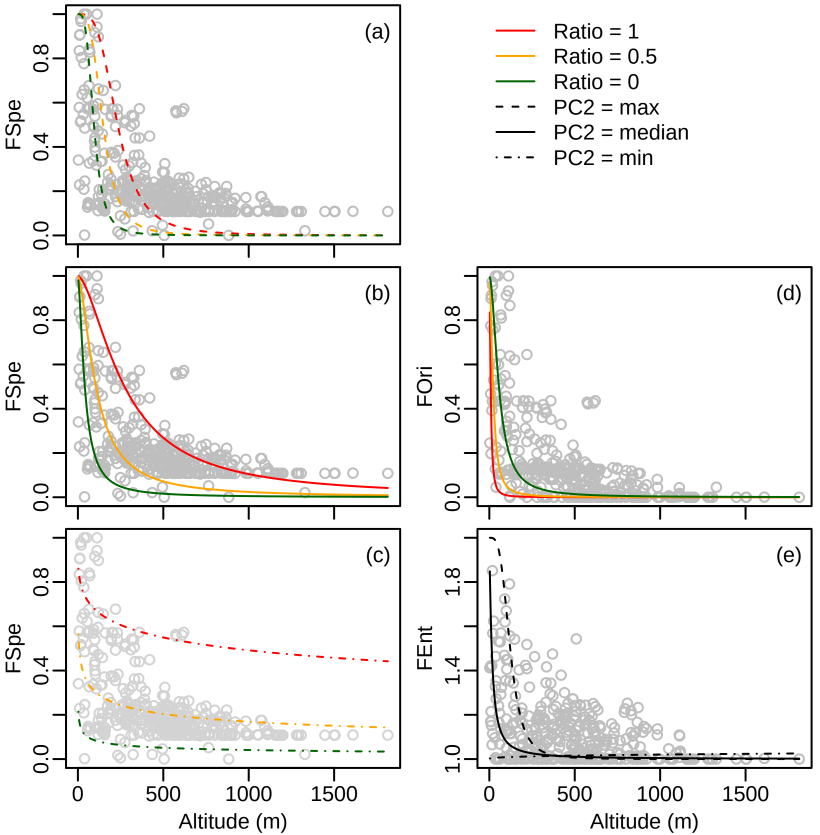
0 20 40 80 120
Kilometers



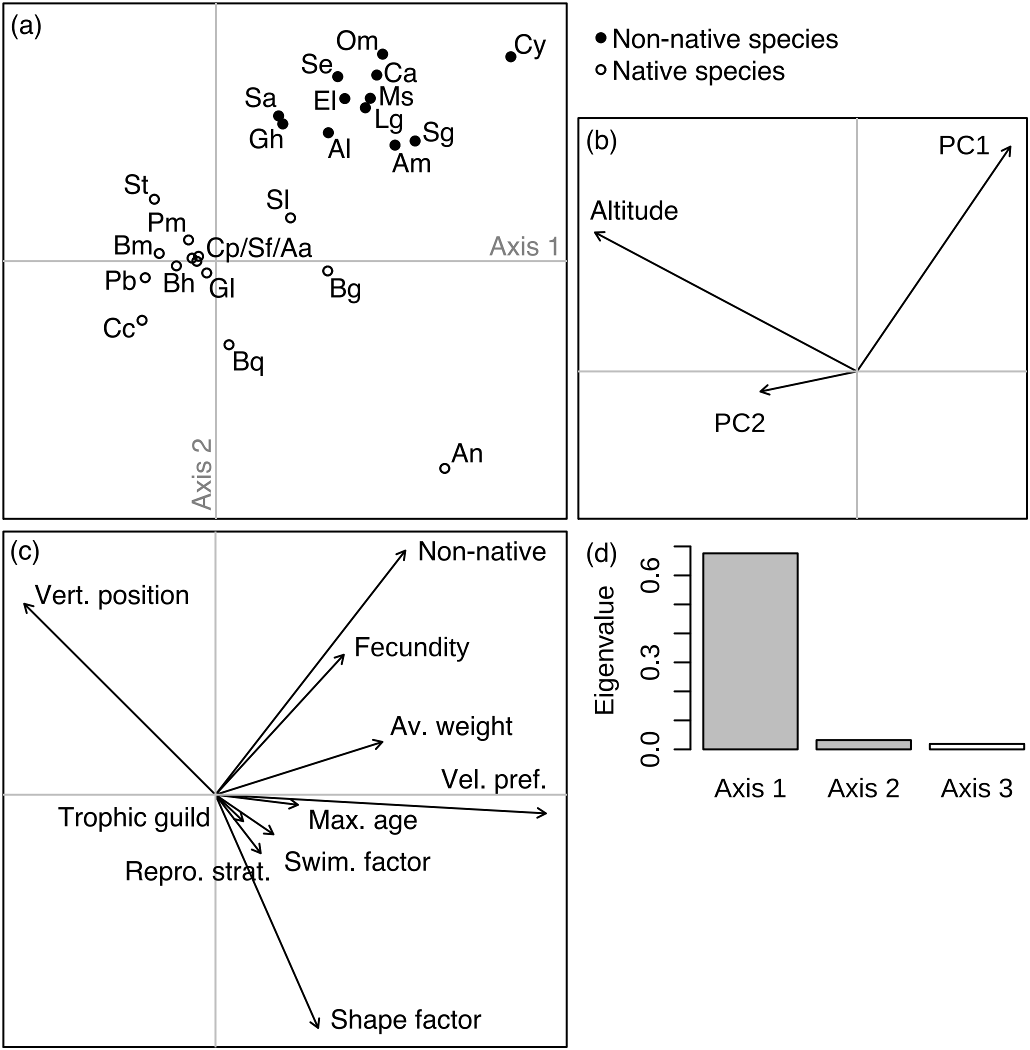
Independent contribution (%)
0
20
40
60
80
FSpe
FOri
FEnt
Taxon richness
Basin
Altitude
Water quality
Physical habitat
Biomass ratio



(a)
(b)
(c)
Taxon richness
Altitude (m)
Besos
Ebro
Fluvio
Francoli
Foix
Garona
Llobregat
Muga
Ter
Tordera



(a)
(b)
(c)
(d)
(e)
FSpe
FOri
FEnt
0.0
0.4
0.8
1.0
1.4
1.8
0
500
1000
1500
Altitude (m)
Ratio = 1
Ratio = 0.5
Ratio = 0
PC2 = max
PC2 = median
PC2 = min



(a)
Non-native species
Native species
Om
Cy
Se
Ca
Sa
El
Ms
Lg
Gh
Al
Sg
Am
St
Sl
Pm
Bm
Cp/Sf/Aa
Axis 1
Pb
Bh
Gl
Bg
Cc
Bq
An
Axis 2
(b)
PC1
Altitude
PC2
(c)
Non-native
Vert. position
Fecundity
Av. weight
Vel. pref.
Trophic guild
Max. age
Swim. factor
Repro. strat.
Shape factor
(d)
Eigenvalue
0.0
0.3
0.6
Axis 1
Axis 2
Axis 3

Table 1. Functional traits used to characterize fishes in the present study.

Function	**Trait**	Trait type	**Categories**	**Categorical value**
Reproduction	Reproductive strategy	Ordinal	Fractional Spawner	1
			Up the river	2
			Migratory	3
			Parental care	4
			Ovoviviparous	5
			Spawns several substrate	6
	Fecundity (egg number)		<2000	1
			2000-10000	2
			>10000	3
Trophic interaction	Trophic guilds		Herbivore-detritivore	1
			Omnivore	2
			General invertivore	3
			Surface/water column invertivore	4
			Benthic invertivore	5
			Piscivore	6
Habitat use	Vertical position		Benthic	1
			Benthopelagic	2
			Pelagic	3
	Velocity preference		Fast	1
			Moderate	2
			Slow-None	3
			General	4
Locomotion	Shape factor	Continuous	(Ratio)	
	Swimming factor		(Ratio)	
Survival	Average weight		(Continuous)	
	Maximal age		(Integer)	

Table 2. List of fish species captured with indication of their status (N, native or A, non-native) in rivers from north-eastern Spain

Family	Species	Code	Common name	Origin
Anguillidae	*Anguilla anguilla*	An	European eel	N
Blenniidae	*Salaria fluviatilis*	Sf	Freshwater blenny	N
Centrarchidae	*Lepomis gibbosus*	Lg	Pumpkinseed	A
	Micropterus salmoides	Ms	Largemouth bass	A
Cobitidae	*Barbatula quignardi*	Bq	Pyreneann stone loach	N
	Cobitis calderoni	Cc	Northern iberian spined-loach	N
	Cobitis paludica	Cp	Southern iberian spined-loach	N
Cyprinidae	*Achondrostoma arcasii*	Aa	Bermejuela	N
	Alburnus alburnus	Al	Bleak	A
	Barbus haasi	Bh	Iberian redfin barbel	N
	Barbus meridionalis	Bm	Western Mediterranean barbel	N
	Carassius auratus	Ca	Goldfish	A
	Cyprinus carpio	Cy	Carp	A
	Gobio lozanoi	Gl	Pyrennean gudgeon	N
	Luciobarbus graellsii	Bg	Ebro barbel	N
	Parachondrostoma miegii	Pm	European nase	N
	Phoxinus spp.	Pb	Pyrennean minnow	N
	Squalius laeitanus	Sl	Ebro chub	N
	Scardinius erythrophthalmus	Se	Rudd	A
Ictaluridae	*Ameiurus melas*	Am	Bullhead	A
Esocidae	*Esox lucius*	El	Pike	A
Percidae	*Sander luciperca*	Sa	Pike-perch	A
Poeciliidae	*Gambusia holbrooki*	Gh	Mosquitofish	A
Salmonidae	*Oncorhynchus mykiss*	Om	Rainbow trout	A
	Salmo trutta	St	Brown trout	N
Siluridae	*Silurus glanis*	Sg	Welscatfish	A

Table 3. Loadings for axes 1 and 2 according to PCA built using water physico-chemical variables and habitat quality features measured in rivers from north-eastern Spain. Values ≥ 0.4 are in bold font.

Environmental Variables	PC1	PC2
Habitat structure	-0.24	**0.67**
Riparian coverage	-0.09	**0.79**
Channel conservation	-0.05	**0.80**
pH	0.03	0.02
Temperature	0.27	-0.28
Ammonium	**0.80**	-0.08
Nitrite	**0.79**	-0.13
Nitrate	**0.76**	-0.09
Phosphates	**0.49**	-0.23
Conductivity	**0.43**	-0.31
Macrophytes	-0.06	0.04

Table 4. Statistics from generalized linear mixed models for fish diversity measures (taxon richness and FSpe, FOri and FEnt functional diversity indices) as a function of non-native fish biomass and indicators of water and physical habitat quality. Bold values indicate significance at *P* <0.05.

	Estimate	**SE**	***z***	***p***
Taxon richness				
(Intercept)	2.540	0.234	10.876	**<0.001**
log(altitude + 1)	-0.294	0.038	-7.829	**<0.001**
Physical habitat	0.733	0.218	3.361	**<0.001**
Water quality	-0.184	0.131	-1.407	0.1594
Non-native biomass ratio	0.148	0.102	1.446	0.1481
log(altitude + 1)*Physical habitat	-0.128	0.036	-3.565	**<0.001**
log(altitude + 1)*Water quality	0.022	0.027	0.817	0.4141
FSpe				
(Intercept)	6.640	1.432	4.636	**<0.001**
log(altitude + 1)	-1.742	0.282	-6.177	**<0.001**
Physical habitat	3.691	1.443	2.559	**0.0105**
Water quality	-0.692	0.719	-0.962	0.3362
Non-native biomass ratio	3.072	0.546	5.625	**<0.001**
log(altitude + 1)*Physical habitat	-0.700	0.278	-2.518	**0.0118**
log(altitude + 1)* Water quality	0.164	0.160	1.026	0.3048
FOri				
(Intercept)	9.147	1.840	4.971	**<0.001**
log(altitude + 1)	-2.189	0.364	-6.017	**<0.001**
Physical habitat	1.970	1.681	1.172	0.2413
Water quality	-0.720	0.723	-0.996	0.3190
Non-native biomass ratio	-3.423	1.279	-2.677	**0.0074**
log(altitude + 1)*Physical habitat	-0.400	0.341	-1.175	0.2399
log(altitude + 1)* Water quality	0.145	0.176	0.826	0.4088
FEnt				
(Intercept)	4.529	1.472	3.077	**0.002**
log(altitude + 1)	-1.478	0.298	-4.958	**<0.001**
Physical habitat	5.808	2.111	2.752	**0.006**
Water quality	-0.991	0.987	-1.005	0.315
Non-native biomass ratio	0.343	0.973	0.353	0.724
log(altitude + 1)*Physical habitat	-0.981	0.406	-2.416	**0.016**
log(altitude + 1)* Water quality	0.186	0.227	0.820	0.412

Table 5. Spearman rank correlation coefficients between water biochemistry**,** habitat quality indices (RBA), relative biomass and taxon richness of non-native species, and several biodiversity indicators: taxon richness, 3 functional diversity measures (FEnt, FSpe and FOri), 5 bioindicators based on biotic integrity of fish (IBICAT), diatoms (IPS) and macroinvertebrates (IBMPW) as and tolerance indicator values of fish communities to water (TIV_WATER) and habitat deterioration (TIV_RBA).

	Functional diversity measures and IBIs								
Environmental stressors	Taxon Richness	FSpe	FOri	FEnt	IBICAT	IBMWP	IPS	TIV_RBA	TIV_WATER
Conductivity	-0.059	0.197	0.124	-0.060	-0.104	-0.481***	-0.396**	-0.498**	0.306
Total Nitrogen	-0.001	0.436**	0.427**	0.160	0.112	-0.576***	-0.684***	-0.398*	0.439*
RBA	-0.072	-0.469***	-0.454**	-0.060	-0.017	0.357	0.466***	0.401*	-0.531**
Non-native relative biomass	0.332*	0.609***	0.666***	0.445**	-0.282*	-0.440**	-0.592***	-0.804***	0.900**
Non-native relative richness	0.305*	0.525***	0.575***	0.325*	-0.297*	-0.584***	-0.688***	-0.762***	0.793***

Note: Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'