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Research article

Response of a pan-European fish index (EFI+) to multiple pressures in rivers across Spain

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ABSTRACT

Rivers are ecosystems highly threatened by human activities and fish are an invaluable tool to measure and communicate environmental degradation and restoration. Fish bioassessment is crucial but notoriously difficult in Mediterranean-climate streams for a number of reasons, including low local species richness, faunas with high spatial turnover and generalist species, and scarcity of reference sites. In this study, we conducted the most comprehensive test of the pan-European fish index (EFI+) in the Iberian Peninsula, analysing its response to multiple anthropogenic pressures. We compiled a database, which we provide online, with 2970 electrofishing samples across Spain, involving 100,732 fish of 69 species. Principal component analyses of many quantitative variables were used to create new synthetic anthropogenic pressure indices. Correlation and multiple linear regression analyses were used to test the relationship between these pressures and the fish index (EFI+) and its four individual metrics scores (i.e., density of species intolerant to oxygen depletion, density of fish ≤150 mm of species intolerant to habitat degradation, richness of species of rheophilic reproduction habitat, and density of species of lithophilic reproduction habitat). We also obtained the same models but including the river basin district to test for spatial or methodological differences. Our results indicate that both the EFI+ index and its individual metrics respond to various anthropogenic pressures. These pressures explained about 36% of the variance of EFI+ values. Notably, downstream and mainstream reaches with higher agricultural or urban land uses, increased hydrologic alteration, and water and habitat quality impairment exhibited lower EFI+ values. Although less variance was explained for the individual metrics than for the fish index, they responded as expected to the different pressures. For instance, the richness of rheophilic species and the number of lithophilic fish decreased with hydrologic alteration, while the number of fish intolerant to oxygen depletion decreased with water quality impairment. Similar correlations were observed when river basin district was included in the model, but with higher explained variation and greater significance of the pressures. While it is possible to develop regional indices with more metrics and a stronger correlation with anthropogenic pressures, EFI+ is the only fish index that has been validated throughout the Spanish peninsular territory. Our results support the use of EFI+ in intercalibration exercises across Spain until better regional indices are developed.

1. Introduction

Human activities have altered the quality and quantity of fresh waters, causing detrimental effects on freshwater species and their assemblages worldwide (Best, 2019; Dias et al., 2017). Despite the essential role of freshwater ecosystems in sustaining human livelihoods and providing essential ecosystem services, they are under constant threat from persistent and emerging stressors. These include habitat degradation, hydrologic alteration, climate change, land use change, water pollution and emerging contaminants, over-exploitation, invasive

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alien species, and the joint effects of these and other stressors (Dudgeon et al., 2006; Reid et al., 2019). As a consequence, freshwater ecosystems are facing losses of ecological integrity and unprecedented rates of biodiversity decline that are greater than in the most terrestrial ecosystems (Sala et al., 2000). Thus, urgent and coordinated action is needed to mitigate threats to freshwater ecosystems and protect the invaluable ecosystem services that they provide.

Mediterranean rivers, particularly those in the Iberian Peninsula, are extremely threatened ecosystems. They boast a high number of endemisms, but also various invasive species, and intense anthropogenic perturbations (e.g., Aparicio et al., 2000; Maceda-Veiga, 2013). Human impacts including habitat degradation, river regulation, water pollution, land use alterations, and climate change are considered major threats to the integrity of Iberian riverine ecosystems and their fishes (Almeida et al., 2017; Jarić et al., 2019; Maceda-Veiga, 2013; Radinger et al., 2019a). For instance, Radinger et al. (2019a) observed that alien species thrived in the lower reaches and the mainstem of the Ebro River, characterized by warmer temperatures, intensive land use, and strong hydrologic alteration through damming; conversely, native species richness was higher in the tributaries, which are more preserved. Similarly, Colin et al. (2018) argued that functional diversity indices declined due to the effects of physical habitat degradation and the ratio of alien fish biomass.

The need to monitor freshwater ecosystems and to assess their ecological integrity is well-recognized and there are many bioassessment tools that have provided valuable information about the status and change of freshwater systems (Vadas et al., 2022). In particular, freshwater fish are widely used since they have several advantages as ecological indicators (Radinger et al., 2019b). For instance, the taxonomy, ecological requirements and life history of fish are generally better known than for many other taxa (Oberdorff et al., 2001; Simon, 1999). Moreover, fish have comparably long lifespans and large home ranges and thus respond to disturbances at larger spatial and temporal scales (Simon, 1999). Therefore, fish can be used to evaluate the impact of hydrological and morphological deterioration (Birk et al., 2012) and loss of ecological connectivity (Schiemer, 2000).

Fish-based indices involve assessing the deviation of the assemblage structure from what is expected under minimum human disturbance (Bailey et al., 2004; Stoddard et al., 2006). Fish indices serve as valuable tools for assessing the effects of human activities on aquatic ecosystems and for determining their ecological condition, in an effort to improve water and habitat quality (Karr, 1981; Roset et al., 2007). A pan-European initiative, driven by the demands of the European Water Framework Directive (WFD) (European Commission, 2000), developed the European Fish Index (EFI+) to provide a standardized assessment method for the ecological status of European rivers (EFI+ Consortium, 2009; Bady et al., 2009; Logez and Pont, 2011). The EFI+ index follows a multimetric approach that quantifies the deviation between the observed and a reference fish community with a specific focus on functional aspects and distinguishing between salmonid and cyprinid river types (Noble et al., 2007; Pont et al., 2006, 2007). In salmonid river types, the metrics used are related to water and habitat quality, while in cyprinid rivers they are linked to reproductive traits and flow requirements (EFI+ Consortium, 2009). The EFI+ index offers several advantages when applied in ecological assessments (Segurado et al., 2014). Firstly, it demonstrates a high degree of generalizability, as it is not contingent upon regional variation in taxonomic composition. This characteristic enables its application to large regions and diverse ecological sites. Nevertheless, it is noteworthy that EFI+ relies on a low number of metrics, which may result in reduced sensitivity when used in regions with particular faunas or environmental conditions. In particular, a number of limitations were identified by the developers of the EFI+ index, including poor performance in sites with natural lakes upstream or with winter dry periods and no reference sites for calibration in lowland reaches of very large rivers (EFI+ Consortium, 2009).

Fish bioassessment in Mediterranean river ecosystems is crucial, but it presents challenges due to a number of reasons, including low local species richness, fauna with high spatial turnover and generalist species, and scarcity of reference sites (Benejam et al., 2008; Magalhães et al., 2008; Segurado et al., 2011, 2014). A number of fish-based assessment indices have been developed, often with a regional focus and related to environmental characteristics of local basins (Segurado et al., 2014) or with varying criteria regarding the selection of contributing metrics (Logez and Pont, 2011). For example, in Spain, regional fish-based indices have been developed for Catalan rivers (García-Berthou et al., 2015; Sostoa et al., 2010), the Guadiana River basin (Hermoso et al., 2010), the Júcar River basin (Aparicio et al., 2011), rivers of the northern Iberian Peninsula (Gartzia de Bikuña et al., 2017), or the Guadalquivir River basin (Ramos-Merchante and Prenda, 2018). Although these indices have provided valuable assessments of the status of specific river basins, they were calibrated/tested in limited regional extents and do not work well in other Iberian regions. For instance, the IBIMED, which was the Spanish fish index intercalibrated for the WFD does not respond to pressures prevalent in most of Spain (García-Berthou and Bae, 2014). EFI+, on the other hand, responds well in the Ebro River basin (Almeida et al., 2017; García-Berthou and Bae, 2014). Although fish data from the Iberian Peninsula (and throughout Europe) were used to develop the EFI+, calibration samples (i.e. unimpacted and slightly disturbed sites) mostly originated from the northernmost rivers and the Tagus River district (see e.g., Fig. 2 in EFI+ Consortium, 2009). To the best of our knowledge, no publication has shown the statistical response of EFI+, or other fish indices, to multiple pressures throughout Spain. In this context, the European Commission reported that there are important gaps in the assessment methods for the WFD in Spain that "have resulted in an important number of water bodies with unreliable or unknown status", and that there is no classification system for fish in rivers (European Commission, 2015). Therefore, we consider that validating the use of EFI+ throughout Spain and developing new fish indices is an urgent need that can help management and conservation, particularly in a country with unsustainable water over-exploitation (e.g., European Environment Agency, 2021).

Considering all the above, the main objective of this study was to analyse the response of EFI+ to multiple anthropogenic pressures across the Iberian Peninsula, including land use changes, hydrologic alteration, and habitat and water quality deterioration. Therefore, we used principal component analyses to develop synthetic pressure gradients and tested their relationship to the fish index and metric scores with correlation and multiple regression analyses. Our hypothesis was that EFI+ would exhibit a weak (i.e., low percentage of explained variation) but significant response to anthropogenic pressures in Iberian rivers, since the index consists of only two metrics per river type and few calibration sites from Spain (except from the northernmost part) were used to develop it (EFI+ Consortium, 2009). We also hypothesized that the different metrics of EFI+ demonstrate distinct responses to different pressure types such as hydrologic alteration or water quality impairment (Logez and Pont, 2011; Zajicek et al., 2018).

2. Material and methods

2.1. Study area

The Iberian Peninsula is a transcontinental region in southwestern Europe characterized by a remarkable geographic and climatic diversity. Annual mean air temperature ranges from above 17 °C in the south to below 2.5 °C in the highest mountains (>3000 m) of the Pyrenees (AEMET, 2011). Hot- and warm-summer Mediterranean climates (Csa and Csb in Köppen classification) are the most prevalent. Temperate oceanic climate (Cfb) with less seasonality in precipitation is more frequent in the north. Humid continental climate dominates in the uppermost reaches of the Pyrenees, and arid climates (group B) are present in the southeastern part (AEMET, 2011). Consequently, Iberian rivers show marked spatial heterogeneity in flow variability (Baeza et al., 2005; Gasith and Resh, 1999). The river basins in the northern and northwestern (humid) parts are small, with short permanent streams that flow into the Cantabrian Sea. The rest of the Iberian basins show typical irregular periods of torrential rainfall in autumn and spring and droughts in summer. Except for the Ebro, basins draining into the Mediterranean Sea are rather small (<22,000 km^2). Larger basins such as Douro, Tagus, Guadiana and Guadalquivir (all >55,000 km^2) drain into the Atlantic Ocean (Fig. S1). The flow regime of many rivers in the Iberian Peninsula has been profoundly altered by over a thousand large reservoirs and water abstraction through many weirs and wells (e.g., Benejam et al., 2010; Radinger et al., 2018).

2.2. Fish data compilation

We obtained electrofishing fish data of Spanish rivers and streams from the Spanish Ministry of Environment, sampled by environmental and governmental agencies between 1985 and 2015. The sampling followed the methodology recommended by the European Standard EN 14011 (CEN, 2003). Suitable samples for computing EFI+ scores were preselected following the WFD and the EFI+ protocols (EFI+ Consortium, 2009). These included samples from streams or rivers, obtained only from single pass electrofishing, and with reported transect length, river width, and individual fish lengths, the sampled transect length had to be at least 10–20 times the river width with a minimum of 100 m. These criteria resulted in electrofishing samplings of 2970 sites from 15 river basin districts across Spain (Table S1 and Fig. S1). Individual fish wereidentified to species level and nomenclature followed Doadrio et al. (2011); some former synonyms were used to calculate the fish index in EFI+ software (Table S2). All samples comprised a total of 100,732 fish belonging to 48 native and 21 non-native species (Table S2). Most streams were dominated by endemic Iberian cyprinids of the genus *Achondrostoma*, *Parachondrostoma*, *Pseudochondrostoma*, *Barbus*, or *Luciobarbus*, except for headwaters where the native *Salmo trutta* prevailed. Species non-native to the Iberian Peninsula, such as *Cyprinus carpio*, *Gambusia holbrooki*, or *Alburnus alburnus* were found at 839 sites (Table S2 and Fig. S2).

2.3. European fish index

EFI+ is a site-specific pan-European fish index that uses a complex predictive modelling approach to compare the observed fish community with that expected from models for reference conditions in absence of human disturbances (EFI+ Consortium, 2009; Logez and Pont, 2011). EFI+ calculation requires multiple data obtained in situ or from geographic information systems (GIS) (EFI+ Consortium, 2009), such as: i) sampling method (wading, boat or mixed), fished area, wetted width, and sediment size (organic, silt, sand, gravel or boulder), which were obtained in field; ii) elevation and river slope, which were estimated from a national raster digital elevation model with a spatial resolution of 50 × 50 m; iii) air temperature (annual, January and July averages), which were obtained from raster maps of the Global Climate Monitor (www.globalclimatemonitor.org); and iv) upstream drainage area, distance from source, and other geomorphological and hydrological categorical data, which were obtained with ArcGIS 10.4.1 software, using layers available at the geoportal of the Spanish Ministry of the Environment (https://sig.mapama.gob.es/geoportal/). However, the six environmental variables finally used to compute the metric values (and thus EFI+) are: actual river slope, July temperature, thermal amplitude, naturally dominant sediment (categorical), and two geomorphological variables (synthetic gradients) obtained through multivariate analysis of the drainage area, presence of flood plain and distance from the source (geomorphological variable 1) and geomorphological and water source types (variable 2) (EFI+ Consortium, 2009).

Although EFI+ is a site-specific index, each sampling site is first classified by the software into salmonid or cyprinid river type, according to seven physiographic characteristics (longitude, latitude, altitude, distance from source, mean temperature, wetted width and actual river slope) (EFI+ Consortium, 2009). The river types thus obtained in our study are given in Fig. S2 and the Supplementary raw data. The two metrics that form the EFI+ in the salmonid river type are: (1) number of fish intolerant to oxygen depletion (Ni.O2.Intol) and (2) number of fish ≤150 mm intolerant to habitat degradation (Ni.Hab.Intol.150). For the cyprinid river type, the two metrics are: (1) richness of rheophilic species, i.e. species requiring fast-flowing habitats (Ric.RH.Par) and (2) number of lithophilic fish, i.e. species requiring gravel habitats for reproduction (Ni.Litho). See Table S2 for the fish typologies according to EFI+. For each sampling site, EFI+ is the average of the two metric scores, depending on the fish river type. The metric scores are obtained as differences between observed and expected metric values for the site, standardized and rescaled to the 0–1 range (EFI+ Consortium, 2009; Logez and Pont, 2011). We used the official software (EFI+ Consortium, 2009) to calculate EFI+ and its four metric scores. EFI+ produces a score that indicates the ecological status of the river on a range from zero to one, where a value of less than 0.252 for salmonid river types indicates a bad status, and a value of more than 0.911 indicates a high status. For cyprinid river types, a value of less than 0.218 or 0.187 (for wading or boat electrofishing, respectively) indicates a bad status, while a value of more than 0.939 or 0.917 (for wading or boat, respectively) indicates a high status. There is a range of values in between for the categories good, moderate, and poor in both river types (see Table S5).

2.4. Anthropogenic pressures

To analyse the response of EFI+ and its metric scores, we considered thirteen variables that capture various anthropogenic pressures, including land use, hydrologic alteration, and water and habitat quality (Radinger et al., 2018; Segurado et al., 2011). We also used physiographical variables to account for spatial variation (see Table S3 for details). Specifically, we used GIS to calculate the reservoir area and the number of weirs and reservoirs upstream of the sampling sites as indicators of hydrologic alteration. We used the Corine land cover map (25 m resolution raster) to calculate the percentages of agriculture, forest, and urban land use in the basin upstream of sampling site. Additionally, we obtained data on ammonia, nitrite, nitrate, dissolved oxygen concentrations, conductivity, pH, and in situ temperature from the national database NABIA (RD, 2015) to measure physicochemical water quality. To assess hydromorphological quality, we used the index of fluvial habitat (IHF) and the Riparian Forest Quality index (QBR), both obtained from national databases. The IHF evaluates the heterogeneity and diversity of physical structures of river habitats (Pardo et al., 2002), while the QBR measures riparian habitat quality through vegetation cover, structure, and channel alterations (Munné et al., 2003). Detailed information on data sources, units, and transformations used for statistical analyses of these variables can be found in Table S3. Summary statistics are provided in Table S4.

2.5. Statistical analyses

We developed anthropogenic pressure indices through three separate principal component analyses (PCAs) encompassing: i) land use, including percentages of agricultural, forested, and urban land; ii) hydrologic alteration, including the number of weirs, reservoirs, and the accumulated area of reservoirs; and iii) water quality parameters, including ammonium, nitrate, nitrite, oxygen concentration, conductivity, pH, and water temperature (Tables S3 and S4). We also used a PCA of physiographical descriptors (e.g., elevation or upstream drainage area) to describe altitudinal and longitudinal gradients and to analyse their relationship with EFI+ and anthropogenic pressures. For each PCA, we generally retained two axes, which are independent and not correlated by definition, as synthetic variables because of their high percentage of explained variation and the structure of the factor loadings; however, only one axis was used for the PCAs of hydrologic alteration and of water quality because they explained most of the available variables (i.e., the factor loadings were mostly related to the first axis) (see Figs. S8–S19). The signs of some PCA scores were reversed to indicate that positive values reflect higher pressures (degradation). As a descriptor of habitat quality and hydromorphological pressure, we finally used only IHF because it is widely used by the Spanish agencies and was more related to EFI+ than to QBR (Fig. S20). QBR mainly reflects the composition and structure of the vegetation rather that instream habitat quality.

To test the relationship of EFI+ and its metric scores with the five anthropogenic pressures (the four PCA axes abovementioned and IHF), we first used scatterplots with linear correlation analyses using the R package PerformanceAnalytics (Peterson and Carl, 2020). We then conducted four regression analyses to analyse the independent effects of different pressures. Note that the correlation between these five pressures was always low ($|r| < 0.34$) (Fig. S21), suggesting that they indicate indeed different anthropogenic disturbances. We developed two different regression models (with or without the index of fluvial habitat, IHF) because habitat quality is known to be important for fish, but IHF was not available for some river districts (and so sample size was increased without using IHF). Finally, we also added the river basin district as a further categorical predictor and repeated the two models to test for spatial or methodological differences. All statistical analyses were performed using base packages in R version 3.5 (R Core Team, 2019).

3. Results

We observed relationships between EFI+ and various spatial descriptors and anthropogenic pressures using univariate correlation analyses (Figs. S8, S11, S14, S17, and S20). The PCA of the physiographical descriptors showed that the first axis, which was mostly related to upstream drainage area and distance to source, accounted for 40% of the variation, while the second axis, related to elevation and distance from mouth, accounted for 34% (Fig. S9). EFI+ showed significant associations with several physiographical descriptors, as indicated by Pearson correlation coefficients (r) ranging from −0.32 to 0.19 (Fig. S8) and the two PCA axes (Fig. S10, $r = -0.25$ for PCA1 and 0.13 for PCA2). These findings illustrate that EFI+ values decrease in the downstream direction ($r = -0.20$) and at low elevation ($r = 0.19$). Indeed, our findings revealed that the highest EFI+ values were observed in mountainous, headwater reaches, particularly in the Pyrenees and Cantabrian mountain ranges, while the lowest values were found in downstream reaches, such as in the mainstem of the Guadiana, Ebro, and Douro Rivers, as well as in parts of Catalonia (Fig. 1).

Forest and agricultural land cover upstream of the sampling site were, negatively correlated ($r = -0.99$), whereas urban land use was less predominant and less dependent on those (Figs. S11–S12). Forest cover was more predominant in mountain areas, while agricultural land use was more common in downstream reaches, particularly in the Douro and Guadiana basins (Fig. 2a). EFI+ correlated positively to forest land cover ($r = 0.30$) and negatively to agricultural ($r = -0.29$) and urban land use ($r = -0.19$) (Figs. S11 and S13).

The three hydrologic alteration indicators (number of weirs, number of reservoirs, and accumulated area of reservoirs upstream of the sampling point) were strongly correlated with each other (Fig. S14, $r =$ 0.63–0.86) and the first PCA axis thus explained most of the variation (81%, Fig. S15). EFI+ was correlated with these three variables and the PCA axis indicated that it took lower values with increased hydrologic alteration (Fig. S16). Hydrologic alteration was most prevalent in the mainstems of large rivers such as Ebro, Douro, Guadiana, Guadalquivir, and Júcar (Fig. 2b). Similarly, most water quality variables were correlated to each other and EFI+ decreased with water quality degradation (Figs. S17–S19). Water quality was most affected in areas with high levels of industrial and population density, such as northeastern Spain (Catalonia), the Basque Country, Madrid, Valencia, and the lower Guadalquivir (Fig. 3a). Finally, EFI+ increased with habitat quality (IHF) (Fig. S20, $r = 0.18$), which was better in headwater, mountain

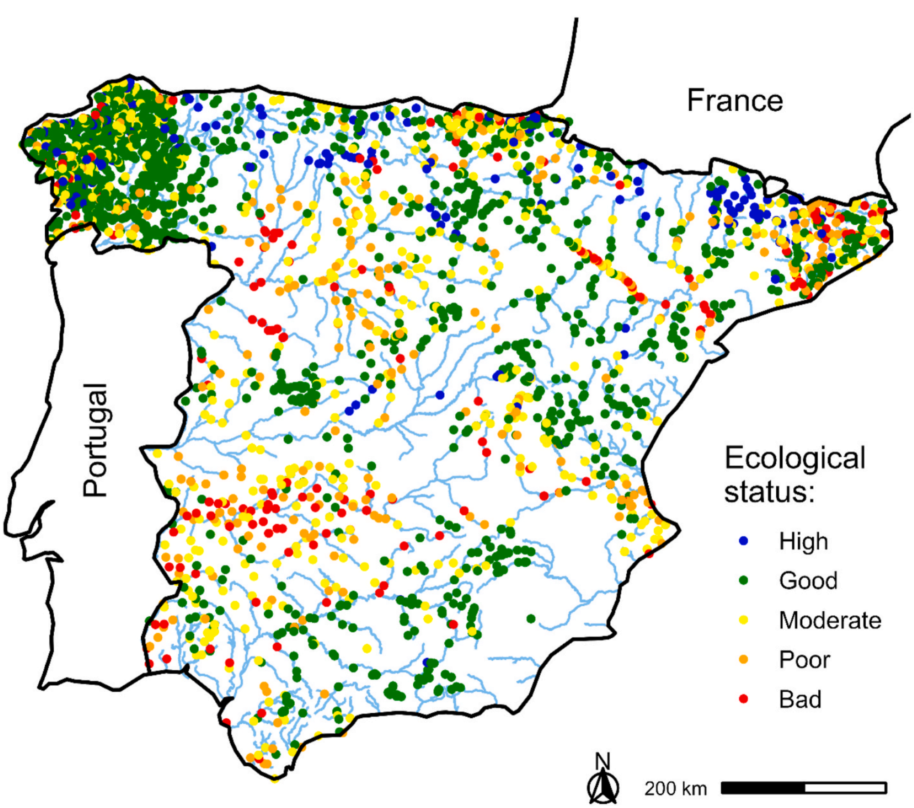

Fig. 1. Ecological status of the fish assemblages of Spanish streams and rivers according to the EFI+ index. See Fig. S3 for the numerical results of the EFI+ index.

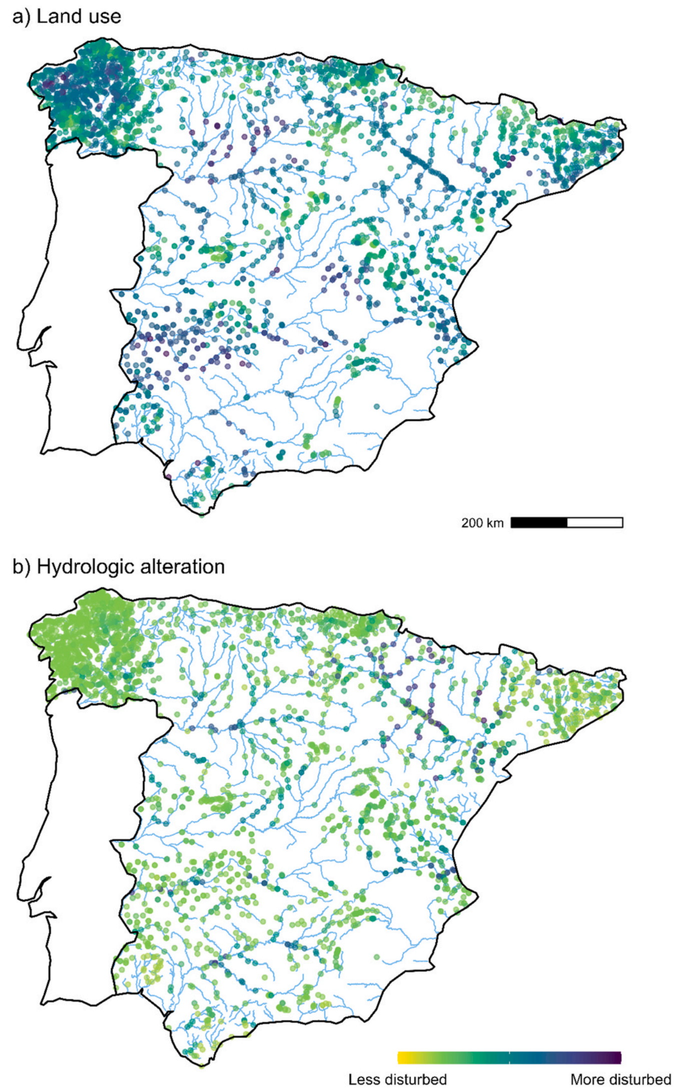

Fig. 2. Spatial variation of a) land use and b) hydrologic alteration in Spanish streams and rivers. The two pressures correspond to the first axis of two separate principal component analyses (see Figs. S11–S16) and vary with color (i.e. more blue color indicates more disturbance). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

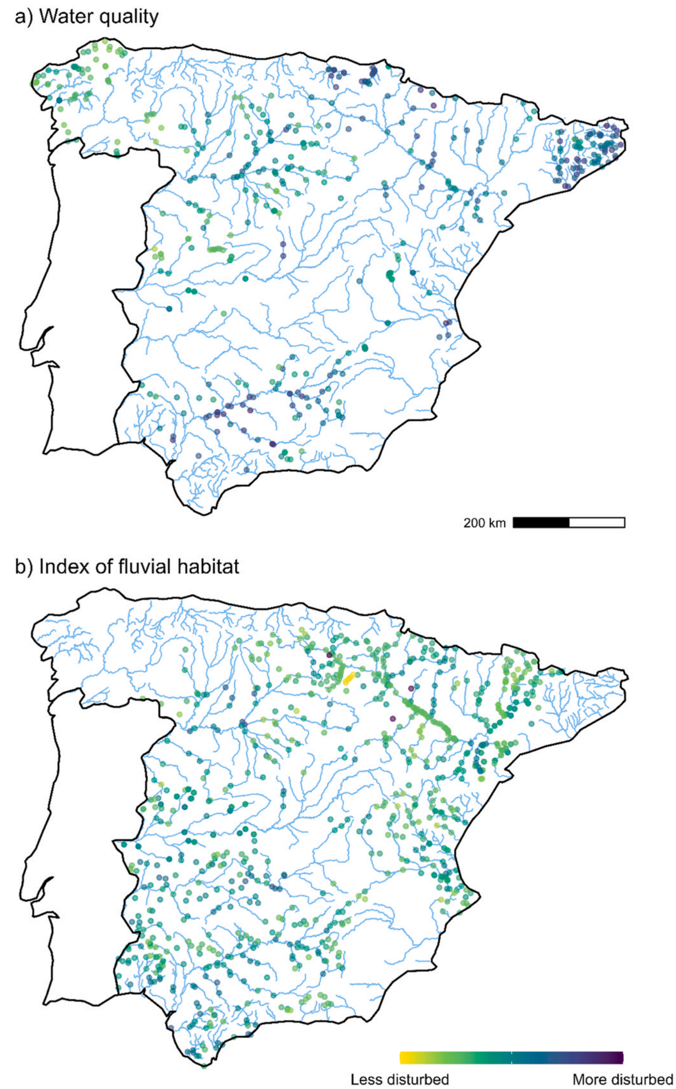

Fig. 3. Spatial variation of a) water quality and b) habitat quality (IHF) in Spanish streams and rivers. The water quality pressure corresponds to the first axis of a principal component analysis (see Figs. S17–S19). More blue color indicates more disturbance (poorer water quality or habitat quality). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

reaches that in lowland reaches (Fig. 3b).

The multiple linear regression model of EFI+ with the five pressures explained 36% of the variance, but only the contributions of land use (PCA1) and water quality (PCA1) were statistically significant (Table 1). Excluding the habitat quality index (IHF) as a predictor led to an increase in sample size and significance of the four pressures (i.e., agricultural land use (PCA1), urban land use (PCA2), hydrologic alteration and water quality), although the overall explained variation was lower (Table 2). Additionally, separate models for the individual metric scores (Ric.RH.Par, Ni.Litho, Ni. Hab.Intol.150 and Ni.O2.Into) that compose the EFI+ explained less variance than the index itself (Tables 1 and 2), indicating their complementarity. Specific metrics showed differential responses to the pressures, in agreement with expectations. For instance, the richness of rheophilic species (Ric.RH.Par metric score) varied with hydrologic alteration, and the number of lithophilic fish (Ni.Litho) responded to both hydrologic alteration or change in land use (Tables 1 and 2). Similarly, the number of fish intolerant to oxygen depletion (Ni. O2.Into) responded mainly to urban land use, hydrologic alteration, and water quality when the habitat quality index was removed (Table 2).

When river basin was added as a categorical factor to the same models (Tables 3 and 4), the findings remained consistent. However, a higher proportion of the explained variation (R^2) and increased significance of the pressure variables were observed. These outcomes indicate that the river basin district plays a substantial role in explaining EFI+, in addition to the pressures considered. According to the EFI+ scores, the ecological status of the rivers in the northwestern part of Spain (Galicia), which has an Atlantic climate with higher precipitation, was generally good (Fig. 1). Conversely, the ecological status of the rivers in the Guadiana basin was generally poor (Fig. 1), with highest agricultural land use (50% of the sites had more than 61% of agricultural land use) compared all other river districts (with less than 35%) (Fig. 4c). Consequently, the observed values of the number of rheophilic species and density of lithophilic fish metrics were lowest in the Guadiana basin (GDN in Fig. 4a and b, Figs. S4–S5). Although Guadiana is one of the Iberian river basins with the highest number of endemic species, which are typically rheophilic and lithophilic (Table S2), 25% of its available samples (first quartile) had no rheophilic species and 75% of the samples only one or fewer. In contrast, most sites (second quartile) in all the

Table 1
Multiple linear regression analyses of EFI+ and the two metric scores of the cyprinid river type sites with pressures of land use (PCA1 and PCA2), hydrologic alteration (PCA1), water quality (PCA1), and habitat quality (IHF).

Response variables (R^2_{adj})	Predictors	*SS*	*df*	*P*
EFI+ (0.359)	Land Use (PCA1)	2.214	1	**< 0.001**
	Land Use (PCA2)	0.064	1	0.180
	Hydrologic alteration (PCA1)	0.001	1	0.865
	Water quality (PCA1)	0.353	1	**0.002**
	Habitat quality (IHF)	0.055	1	0.214
	Residual	4.299	122	
Ric.RH.Par (0.171)	Land Use (PCA1)	0.102	1	0.103
	Land Use (PCA2)	0.009	1	0.633
	Hydrologic alteration (PCA1)	0.519	1	**< 0.001**
	Water quality (PCA1)	0.398	1	**0.001**
	Habitat quality (IHF)	0.140	1	0.056
	Residual	4.567	122	
Ni.Litho (0.246)	Land Use (PCA1)	1.876	1	**< 0.001**
	Land Use (PCA2)	0.025	1	0.578
	Hydrologic alteration (PCA1)	1.761	1	**< 0.001**
	Water quality (PCA1)	0.001	1	0.890
	IHF	0.042	1	0.471
	Residual	9.748	122	

Sum of squares (*SS*), degrees of freedom (*df*), *P* values and adjusted coefficients of determination (R^2_{adj}) are given. The two metrics of EFI+ are: Ric.RH.Par (richness of species requiring a rheophilic reproduction habitat), and Ni.Litho (number of fish requiring a lithophilic reproduction habitat). Significant results ($P \leq 0.05$) are bolded.

other basins had one or two rheophilic species (Fig. 4a).

4. Discussion

4.1. Response of EFI+ to multiple pressures

Iberian rivers, particularly those in the southern half of the peninsula, are among the most affected in Europe in terms of nitrogen and phosphorous concentrations and alteration of natural low flow regimes (Grizzetti et al., 2017). Despite this region also being among the richest in Europe in fish endemisms (Doadrio et al., 2011), Spain has no national fish index that responds well to anthropogenic pressures throughout the country, as mandated by the European WFD (European Commission, 2015, 2019). Our results confirm the initial hypothesis that the EFI+ and its metrics respond to anthropogenic pressures in rivers across Spain. Specifically, we found that headwater streams, characterized by natural forests, low agricultural impact, and minimal hydrologic alterations, generally have a high ecological status. In contrast, there is a progressive ecological degradation in Iberian rivers, consistent with observations from other European rivers. This degradation occurs along the upstream-downstream gradient due to multiple pressures on freshwater ecosystems, such as pollution and hydrological and hydromorphological alterations (Grizzetti et al., 2017).

Our validation of the EFI+ index was conducted using multiple anthropogenic pressures and the most comprehensive electrofishing dataset compiled so far in Spain. Environmental pressures overall explained up to 36% of the variability observed in EFI+ in Spanish rivers, a percentage comparable to other fish bioassessment studies conducted in Mediterranean regions (Aparicio et al., 2011; García-Berthou and Bae, 2014; Magalhães et al., 2008). Interestingly, we observed independent effects of agricultural land use, water quality impairment and hydrologic alteration on the fish index through multiple linear regression. These pressures tend to covary because the conversion of forests to agricultural land brings increased number of dams and weirs to meet water demands, physical habitat degradation, and pollution through the use of fertilizers and pesticides (e.g., Grizzetti et al., 2017).

Table 2
Multiple linear regression analyses of EFI+ and its four metric scores of salmonid and cyprinid river types with pressures of land use (PCA1 and PCA2), hydrologic alteration (PCA1) and water quality (PCA1).

Response variables (R^2_{adj})	Predictors	*SS*	*df*	*P*
EFI+ (0.172)	Land Use (PCA1)	3.077	1	**< 0.001**
	Land Use (PCA2)	0.387	1	**0.007**
	Hydrologic alteration (PCA1)	0.271	1	**0.024**
	Water quality (PCA1)	0.328	1	**0.013**
	Residual	18.312	348	
Ric.RH.Par (0.091)	Land Use (PCA1)	0.264	1	**0.008**
	Land Use (PCA2)	0.090	1	0.121
	Hydrologic alteration (PCA1)	0.940	1	**< 0.001**
	Water quality (PCA1)	0.172	1	**0.032**
	Residual	12.926	346	
Ni.Litho (0.206)	Land Use (PCA1)	3.047	1	**< 0.001**
	Land Use (PCA2)	0.060	1	0.357
	Hydrologic alteration (PCA1)	3.144	1	**< 0.001**
	Water quality (PCA1)	0.428	1	**0.014**
	Residual	24.356	346	
Ni.Hab.Intol.150 (0.116)	Land Use (PCA1)	0.181	1	0.152
	Land Use (PCA2)	0.412	1	**0.032**
	Hydrologic alteration (PCA1)	0.916	1	**0.001**
	Water quality (PCA1)	0.159	1	0.179
	Residual	9.737	112	
Ni.O2.Intol (0.411)	Land Use (PCA1)	0.128	1	0.248
	Land Use (PCA2)	2.285	1	**< 0.001**
	Hydrologic alteration (PCA1)	1.198	1	**< 0.001**
	Water quality (PCA1)	4.487	1	**< 0.001**
	Residual	10.665	112	

Sum of squares (*SS*), degrees of freedom (*df*), *P* values and adjusted coefficients of determination (R^2_{adj}) are given. The four metrics of EFI+ are: Ric.RH.Par (richness of species requiring a rheophilic reproduction habitat), Ni.Litho (number of fish requiring a lithophilic reproduction habitat), Ni.Hab.Intol.150 (number of fish ≤150 mm intolerant to habitat degradation), and Ni.O2.Intol (number of fish intolerant to oxygen depletion). Significant results ($P \leq 0.05$) are bolded.

However, our results show that for the same agricultural land use (upstream of a certain site), increased hydrologic alteration or water quality deterioration decrease the values of EFI+.

As hypothesized, individual metrics within EFI+ also exhibited significant responses to different pressures, confirming the complementary nature of these metrics within each river type. For example, in the cyprinid river type, rheophilic species (Ric.RH.Par) and lithophilic species (Ni.Litho) were mainly associated with hydrologic alteration, while in the salmonid river type, species intolerant to oxygen depletion (Ni.O2.Intol) predominantly responded to water quality (see Tables 1–4). The differential response of individual metrics to different pressures provides a mechanistic basis for the patterns observed, emphasizing the robustness and applicability of the index across Mediterranean streams. Hydrologic alteration is known to regulate and decrease water flows, thus increasing siltation and hindering rheophilic species with lithophilic reproduction habitat (Almeida et al., 2017; García-Berthou and Bae, 2014). By contrast, the Ni.O2.Intol metric score responded to the water quality pressure, because the latter seems to be more limiting for the species present in salmonid river types, which often display intolerance to low oxygen concentration (Logez and Pont, 2011).

However, it is likely that a regional fish index developed specifically for Spain, similarly than EFI+ but with more metrics, would respond better to the unique fish assemblages and anthropogenic pressures of the country (Logez et al., 2010; Segurado et al., 2014). In fact, the purpose of EFI+ was to develop an index at the European scale and although it is "reasonably accurate at the European scale, its applicability varied

Table 3
Multiple linear regression models of EFI+ and the two metric scores in the cyprinid river type sites with pressures of land use (PCA1 and PCA2), hydrologic alteration (PCA1), water quality (PCA1), habitat quality (IHF) and river basin as a categorical factor.

Response variables (R^2_{adj})	Predictors	*SS*	*df*	*P*
EFI+ (0.386)	Land Use (PCA1)	2.214	1	**< 0.001**
	Land Use (PCA2)	0.064	1	0.171
	Hydrologic alteration (PCA1)	0.001	1	0.862
	Water quality (PCA1)	0.353	1	**0.002**
	Habitat quality (IHF)	0.055	1	0.204
	River basin	0.348	5	0.075
	Residual	3.950	117	
Ric.RH.Par (0.315)	Land Use (PCA1)	0.101	1	0.074
	Land Use (PCA2)	0.009	1	0.600
	Hydrologic alteration (PCA1)	0.519	1	**< 0.001**
	Water quality (PCA1)	0.398	1	**< 0.001**
	Habitat quality (IHF)	0.140	1	**0.036**
	River basin	0.947	5	**< 0.001**
	Residual	3.620	117	
Ni.Litho (0.344)	Land Use (PCA1)	1.876	1	**< 0.001**
	Land Use (PCA2)	0.025	1	0.551
	Hydrologic alteration (PCA1)	1.761	1	**< 0.001**
	Water quality (PCA1)	0.001	1	0.883
	Habitat quality (IHF)	0.042	1	0.440
	River basin	1.624	5	**< 0.001**
	Residual	8.125	117	

Sum of squares (*SS*), degrees of freedom (*df*), *P* values and adjusted coefficients of determination (R^2_{adj}) are given. The two metrics of EFI+ are: Ric.RH.Par (richness of species requiring a rheophilic reproduction habitat), and Ni.Litho (number of fish requiring a lithophilic reproduction habitat). Significant results ($P \leq 0.05$) are bolded.

Table 4
Multiple linear regression models of EFI+ and its four metric scores of salmonid and cyprinid river types with pressures of land use (PCA1 and PCA2), hydrologic alteration (PCA1), water quality (PCA1) and river basin as a categorical factor.

Response variables (R^2_{adj})	Predictors	*SS*	*df*	*P*
EFI+ (0.329)	Land Use (PCA1)	3.077	1	**< 0.001**
	Land Use (PCA2)	0.387	1	**0.003**
	Hydrologic alteration (PCA1)	0.271	1	**0.012**
	Water quality (PCA1)	0.328	1	**0.006**
	River basin	3.933	11	**< 0.001**
	Residual	14.379	337	
Ric.RH.Par (0.353)	Land Use (PCA1)	0.264	1	**0.002**
	Land Use (PCA2)	0.090	1	0.066
	Hydrologic alteration (PCA1)	0.940	1	**< 0.001**
	Water quality (PCA1)	0.172	1	**0.011**
	River basin	4.016	11	**< 0.001**
	Residual	8.910	335	
Ni.Litho (0.317)	Land Use (PCA1)	3.047	1	**< 0.001**
	Land Use (PCA2)	0.060	1	0.304
	Hydrologic alteration (PCA1)	3.144	1	**< 0.001**
	Water quality (PCA1)	0.428	1	**0.006**
	River basin	5.479	11	**< 0.001**
	Residual	18.878	335	
Ni.Hab.Intol.150 (0.325)	Land Use (PCA1)	0.181	1	0.102
	Land Use (PCA2)	0.412	1	**0.014**
	Hydrologic alteration (PCA1)	0.916	1	**< 0.001**
	Water quality (PCA1)	0.159	1	0.125
	River basin	2.701	6	**< 0.001**
	Residual	7.036	106	
Ni.O2.Intol (0.480)	Land Use (PCA1)	0.128	1	0.220
	Land Use (PCA2)	2.285	1	**< 0.001**
	Hydrologic alteration (PCA1)	1.198	1	**< 0.001**
	Water quality (PCA1)	4.487	1	**< 0.001**
	River basin	1.749	6	**0.004**
	Residual	8.916	106	

Sum of squares (*SS*), degrees of freedom (*df*), P values and adjusted coefficients of determination (R^2adj) are given. The four metrics of EFI+ are: Ric.RH.Par (richness of species requiring a rheophilic reproduction habitat), Ni.Litho (number of fish requiring a lithophilic reproduction habitat), Ni.Hab.Intol.150 (number of fish ≤150 mm intolerant to habitat degradation), and Ni.O2.Intol (number of fish intolerant to oxygen depletion). Significant results ($P \leq 0.05$) are bolded.

among different biogeographical regions and countries" (Segurado et al., 2014). This applicability is probably better for coolwater Mediterranean streams, which share a fauna similar than the rest of Europe, than for warmwater streams and rivers (Logez and Pont, 2011). We encourage the responsible administrations of Mediterranean countries to develop such national indices with the state-of-the-art methodology established by EFI+ (EFI+ Consortium, 2009; Logez and Pont, 2011), i. e., a multimetric guild-based site-specific index based on predictive modelling. Increasing the number of metrics in such indices to more than two per type as used in EFI+ seems essential to improve its response to environmental degradation and to provide a really multimetric index. However, there has been a proliferation of fish indices and many other aspects need to be considered for proper bioassessment developments (see a recent review in Vadas et al., 2022) and EFI+ is an excellent benchmark that has many virtues (predictive modelling, large sample sizes, excellent statistical techniques, accounting for environmental heterogeneity, etc.).

Although the relationship between the fish index and anthropogenic pressures could be expected to be stronger, limitations in the data used in our study may have influenced the results. For instance, many different crews sampled the fish along the years and in different river districts and this is known to introduce variability particularly in habitat assessments but also fish abundance estimates (Benejam et al., 2012; García-Berthou et al., 2015). In some large river reaches the length of the fishing transect was not so long as requested by the CEN standard (CEN, 2003). EFI+ has been shown to depend on this length (Almeida et al., 2017), thus limiting the representativeness of the sample. Moreover, wading electrofishing was used in many large rivers, where boat electrofishing would be much more efficient. Unfortunately, the habitat quality index (IHF) was not available for some river districts, limiting the sample size, the statistical power, and the analyses. Excluding IHF as a predictor in the models led to an increase in sample size and significance of the four pressures, although the overall explained variation was lower. This makes sense because fish are known to depend markedly on habitat quality (hence the use of litophilic and rheophilic guild metrics in EFI+) (EFI+ Consortium, 2009; Pont et al., 2006, 2007) and suggests that models with IHF but larger sample sizes would have larger explained variation and more significance of other predictors. The hydrologic alteration pressure (number of dams and weirs and accumulated reservoir area upstream of the sampling sites) is frequently used (e.g., Radinger et al., 2019a) but coarse. More direct measures of hydrologic alteration (e.g., flow indicators) would be preferable. For instance, many river reaches run dry artificially in the Iberian Peninsula due to small weirs and water abstraction and this affects fish abundance when the flow resumes (e.g., Benejam et al., 2010). However, this has not been comprehensively analysed and it is difficult to prove if the dryness is natural or not. It is important to note that EFI+ excludes sites with no fish captures (which were thus not included in this study). These sites, which are increasingly found in the Iberian Peninsula and elsewhere, are sometimes the result of water abstraction and other pressures. Finally, mostly nutrient concentrations were considered for the water quality pressure and as usually many other pollutants were not considered because they were not widely available.

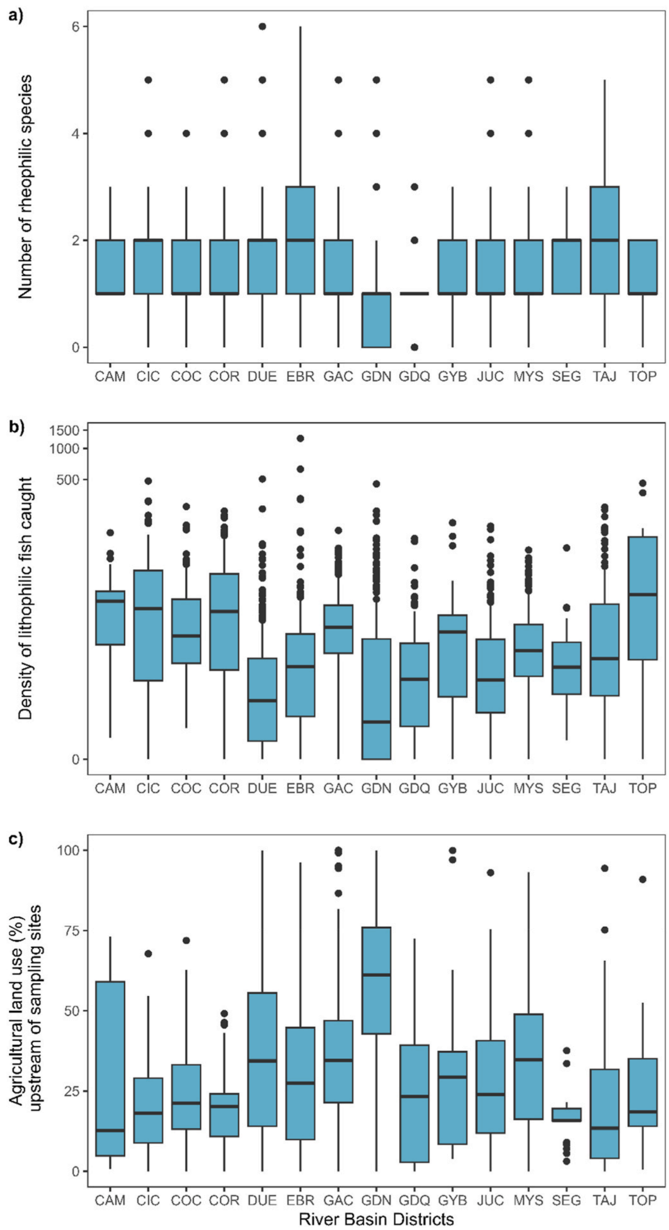

Fig. 4. a) Observed number of rheophilic fish species (per sampling site), b) observed number of fish specimens belonging to the lithophilic guild (per site), and c) percentage of agricultural land use upstream of sampling sites (per site) by river basin districts. See abbreviations of the river basin districts in Table S1 (Supplementary Information).

4.2. Differences among river basins

We observed an increase in explained variation when incorporating the river basin district in the models (Tables 3 and 4). These results are similar than those observed by Logez et al. (2010), who observed differences in fish functional structure between Iberian and French rivers (i.e., Mediterranean and temperate Europe). However, the spatial differences found in our study have a number of possible explanations, including real differences in ecological status that are not explained by the pressures (e.g., due to pressures not considered such as the impacts of invasive alien species), insufficient performance of the predictive models of EFI+ in some regions, and heterogeneity in the available data (e.g., due to methodological variations in fish sampling as mentioned above).

For instance, the Guadiana River in general had a poor or bad ecological status (Fig. 1) due to the lowest observed values of the Ric.RH.Par and Ni.Litho metrics across Spain (Fig. 4a and b), in contrast to neighbouring basins. Guadiana basin has numerous native rheophilic and lithophilic species, particularly four species of native barbel (*Luciobarbus comizo*, *L. guiraonis*, *L. microcephalus*, *L. sclateri*). However, these species are currently less frequent and abundant in the Guadiana basin compared to neighbouring basins with similar latitudes, climates, and ichthyological faunas. Conversely, a total of twelve non-native species were found in the sampled fish community of Guadiana basin, and except for rainbow trout, none of these species exhibit rheophilic or lithophilic characteristics. This indicates a progressive decline in the presence of native rheophilic and lithophilic species that contribute positively to the EFI+ metrics, coupled with an increase in non-native species that generally do not contribute to the index. The rivers in the Guadiana basin are among the most degraded in the Iberian Peninsula due to extensive damming, and intensive agricultural land use (e.g., Collares-Pereira et al., 2000; Corbacho and Sánchez, 2001; Hermoso et al., 2010). However, the abundance of invasive alien species has been suggested as a main driver of decline of native species in this river basin irrespective of habitat degradation (Hermoso et al., 2011).

The EFI+ index focuses on the functional traits of fish species, regardless of whether they are native or alien. This is frequent in bioassessments for the WFD, since this regulation does not mention alien species although they constitute a major pressure in many aquatic ecosystems (Boon et al., 2020; Vandekerkhove et al., 2013). However, it has been shown that the EFI+ implicitly considers native and alien species characteristics (Almeida et al., 2017; García-Berthou and Bae, 2014). Native fish species, which in the Iberian Peninsula mostly belong to the rheophilic and lithophilic reproduction guilds, dominate in abundance at upstream sites. By contrast, invasive alien fish species, which in the Iberian Peninsula and possibly elsewhere mostly belong to the limnophilic and phytophilic reproduction guilds, proliferate at downstream sites due to impaired water and habitat quality and hydrologic alteration (Almeida et al., 2017; García-Berthou and Bae, 2014). Therefore, a possible explanation for the spatial differences in the EFI+ index is that this index takes low values when alien species are very abundant and native species are rare. Although the index does not explicitly consider the native status of fish species, it indirectly accounts for the abundance of alien species as a pressure on native species and the overall ecosystem.

In summary, our research provides evidence that EFI+ and its individual metrics exhibit significant responses to various anthropogenic pressures, particularly changes in land use, hydrologic alteration, and water quality throughout Spanish rivers and streams. Therefore, we deem that EFI+ is an adequate tool to estimate the ecological status and invaluable for intercalibration exercises, which are the processes used within the framework of the European WFD to ensure consistency and comparability of ecological assessment methods among different countries and regions (see e.g., Segurado et al., 2014). However, our results also reveal spatial variability between basins that could not be fully explained. These findings underscore the need to develop and evaluate a national index specifically adapted for Iberian rivers, which would produce more precise responses to human pressures in Mediterranean regions, building on the successful, state-of-the-art experience of this pan-European fish index.

CRediT authorship contribution statement

Juan Diego Alcaraz-Hernández: Data curation, Formal analysis, Methodology, Software. **Johannes Radinger:** Writing - original draft. **Yaiza Luque:** Data curation. **Emili García-Berthou:** Conceptualization, Funding acquisition, Methodology, Project administration, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

https://doi.org/10.6084/m9.figshare.23500512

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.120043.

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