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Sign, strength and shape of stream fish-based metric responses to geoclimatic and human pressure gradients



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ABSTRACT

Efficient community indices and indicators are crucial for the adequate management and design of measures ensuring the ecosystem integrity. In this study we analyse the shape, sign and strength of the response of some biotic integrity indices and indicators of structure and function of fish communities along geo-climatic and human pressures gradients at catchment scale. To that purpose, > 300 sites all over the Iberian Peninsula were characterized at the catchment scale by means of two anthropogenic drivers (agricultural and urban land proportion) and seven natural environmental descriptors covering geographical and climatic aspects. Regarding to fish-based metrics, a set of the most frequently used in stream health assessment studies have been selected, including taxonomic classic indicators, size related indicators and also recent multimetric indices created in the European context (WFD). We applied boosted regression trees that allow estimating the sign and strength of the response as well as considering non-linearity and impact thresholds. Our results show that the jointly contribution of anthropic drivers was lower than geo-climatic drivers. For most of indices and indicators, one single land cover contributed more markedly to the total deviance explained than the other, and they responded rather consistently to land-use variables, i.e., most of them responded negatively to the increase of anthropic use in the catchment. Size diversity, Fish Region Index (FRI) and maximum weight were those more sensible to agriculture land, while EFI+, mean weight, distinctness and FRI were those more sensitive to urban land. Regarding the shape of the response, urban land proportion affects normally at extremely low values, while agriculture land proportion induces smoother changes on a wider range. Our results may have practical implications, such as the selection of an efficient array of fish-based metrics to be included in ecological assessment and monitoring programs.

1. Introduction

An effective environmental assessment of aquatic ecosystems is crucial for the adequate management and design of measures that ensure the ecosystem integrity (Carballo et al., 2009). Current national and international environmental regulations such as the U.S. Clean Water Act and the E.U. Water Framework Directive (WFD) (2000/60/ EC 23 October 2000), have boosted the development of indices of biotic integrity (IBI sensu Karr, 1981) [henceforth indices] and bioindicators, i.e., variables informing about something different from what they actually measure (Daan, 2005) [henceforth indicators], based on biological communities (*bioassessment*) (Hering et al., 2006).

Efficient community indices and indicators should ideally offer reliable information from an integrated assessment of relevant structural and functional key variables of the ecosystem (Bonada et al., 2006). Then, these metrics should not only mirror large-scale diversity patterns originally shaped by natural geo-climatic drivers (Mittelbach et al., 2007; Field et al., 2009; Oberdorff et al., 2011) as it has been evidenced by previous authors (Mittelbach et al., 2007; Field et al., 2009; Oberdorff et al., 2011; Marzin et al., 2013; Feld et al., 2016), but specially they should be sensitive to common anthropogenic disturbances. For example, land use conversion to more anthropic uses (agriculture or urban settlements) has been found to affect ecosystem functions and diversity in different directions and strengths (Allan, 2004; Feld et al., 2013).

Within the wide variety of indices and indicators referring to different biological communities (Hering et al., 2006; Vidal-Abarca et al., 2016), specially fish are a suitable group to assess ecological status in rivers (Schiemer, 2000; Birk et al., 2012; Blevins et al., 2013; Izzo et al., 2016) since they have found to be sensitive to anthropogenic disturbances (Pont et al., 2007; Casatti et al., 2009; Aparicio et al., 2011). However, a detailed understanding of the responses of fish communities

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to human disturbance at the catchment scale is still lacking, with frequent evidences of opposing responses.

In fact, Allan (2004) highlighted the covariation of natural and anthropogenic gradients use as one of the causes that complicate our understanding of the relationships between land cover and the ecological integrity of streams. Another reason for the lack of success in identifying pathways of influence with observed effects of anthropic land use on stream biota is the presence of nonlinear responses (Allan, 2004). Nonlinear, and even non-monotonic, responses may be caused by interactions among factors and the existence of impact thresholds. The detail of such effects has been improved by the application of machine learning techniques to the adjustment of models that reflect nonlinear responses (Clapcott et al., 2012). The shape of the response could be characterized by the identification of impact initiation and cessation thresholds, and the presence of abrupt changes in variables behaviour (Wagenhoff et al., 2017). Then, knowing the thresholds of the non-linear response could profitably increase our detailed knowledge about the response of the indices and indicators to the artificial land cover intensity and that should help to identify appropriate management actions.

In summary, there is still an active debate about whether indices and indicators really offer a reliable and generalizable indication of overall ecosystem status and the consequences of appraisal results (Hering et al., 2010; Feld et al., 2016) and studies analysing simultaneously the response of different indices and indicators to geo-climatic and anthropogenic gradients are scarce.

In this study we analyse the shape, sign and strength of the response of some biotic integrity indices and indicators of structure and function of fish communities along geo-climatic and anthropic gradients at catchment scale. A set of the most frequently used metrics in stream health assessment studies have been selected, including taxonomic classic indicators, size related indicators and also recent multimetric indices created in the European context (WFD). We applied a statistical analysis method that allows estimating the sign and strength of the response as well as considering potential non-linearities and impact thresholds. In accordance with previous studies of community indicators of freshwater fish (Dahm et al., 2013; Kail and Wolter, 2013; Benejam et al., 2016; Feld et al., 2016) our hypothesis is that the higher the degraded land proportion in the catchments, i.e., larger relative proportions of different anthropogenic covers, the poorer the ecological status according to biotic community metrics, being this relationship not necessarily linear neither monotonic. To test this hypothesis, 10 community metrics have been calculated in > 300 sites in rivers from Iberian Peninsula.

2. Methods

2.1. Study area

The study was conducted in the Spanish part of the Iberian Peninsula. Located in southern Europe (latitudinal range of 36.0° – 43.7° N), it is characterized by a high variability of physiographical conditions. This region is divided in two macrobioclimatic areas (the Temperate and the Mediterranean zone, the latter mainly characterized by strong summer water stress, Rivas-Martínez et al. 2004). The altitudinal range goes from the sea level to > 3,400 m. The diversity of geo-hydrological conditions makes flow regimes of rivers highly variable, from temporary (seasonal flow) to perennial (continuous flow). All of these characteristics provide a high heterogeneity of habitats (from arid steppes to moist fir-beech forest, see Blondel and Vigne, 1993) and together with other Mediterranean basins, they constitute some of the most important hotspot in the world (Myers et al., 2000).

In addition to the diverse physiographical conditions, the Iberian Peninsula presents a wide variety of anthropogenic pressures, from the long history of deforestation related to political and cultural changes and pressures exerted by agriculture and cattle breeding (López-



Fig. 1. Location of sample sites in the Iberian Peninsula selected for this study and watershed areas to selected sample sites.

Bermúdez and García-Ruiz, 2008; Cerdá, 2008), to the heterogeneous distribution of human population, from extremely low densities of 9 inhabitants·km⁻², to high densities of 810 inhabitants·km⁻², (source: Instituto Nacional de Estadística, http://www.ine.es/).

2.2. Fish data

Sample sites were selected from the Spanish section of the database originally collected within the EU EFI+ project (http://efi-plus.boku. ac.at/) with 1741 fishing occasions in 1507 sampling sites. Fish data were sampled by means of electrofishing surveys and determined at species level. Only captures in the first pass were considered since not all fishing occasions had multiple passes. Fish samplings took place between 1991 and 2007 (see Table S1 for a complete list of sampling sites). Length data of every individual were available in the original database (resolution 1 mm). A total of 326 sample sites were finally selected from the original database to attain similar sample densities all over the study area (Fig. 1) since some regions as North and Northwest had been more densely represented in the original sampling network than the rest.

2.3. Geo-climatic and land use descriptors

All sites were characterized at the catchment scale by means of two anthropogenic descriptors, in addition to seven natural environmental descriptors covering geographical and climatic aspects. CORINE land cover data (European Environmental Agency; www.eea.europa.eu) was used to calculate the proportion of agricultural and urban land within the catchment. Both descriptors were selected because they are known to affect aquatic communities (Allan, 2004; Feld et al., 2013) although the influence degree is still uncertain (Benejam et al., 2016; Feld et al., 2016). The land cover classes that aggregate the type 1 of CORINE level 1 "Artificial surfaces" were considered as "urban land". As "agricultural land" it was considered the type 2 of CORINE level 1, "Agricultural areas", except classes 2.4.3 (Land principally occupied by agriculture, with significant areas of natural vegetation) and 2.4.4. (Agro-forestry areas) due to their higher level of naturalness. Since fish sampling took place between 1991 and 2007 and different CORINE land cover data are available (1990, 2000 and 2006), three sample periods were considered

Table 1

CORINE land cover versions and climatic period within Spain02 database considered according to sample year.

Fish sample period	Land use data source	Climatic period used
1991–1993	CORINE 1990	1960–1990
1994–2002	CORINE 2000	1970–2000
2003–2007	CORINE 2006	1976–2006

to attribute the closest land cover version in each case (see Table S1): CORINE 1990 for samplings prior 1992, CORINE 2000 for samplings between 1993 and 2002, CORINE 2006 for samplings between 2003 and 2007 (Table 1, Table S1).

Of the seven geo-climatic descriptors, latitude, longitude and altitude were collected from the original database EFI+ . The upstream catchment area was derived from digital maps using ArcGIS 10.3°. For temperature related variables, the climatic database Spain02 (see details in Herrera et al. 2012) was used, which comprises monthly temperature data for the period 1950–2008 in a 0.2° regular grid. A 30-year period, prior to the sampling date, was selected to calculate climatic variables for each site. In a similar way to land cover assignation procedure three different periods were used (Table 1, Table S1). From the monthly data base, annual mean temperature was calculated as the average of mean annual temperature along the period. With the aim of considering the frequent high variability along the year and between years in the Iberian Peninsula, coefficients of variation of the temperature along the year (T_{intra}CV) and along the period (T_{inter}CV) were calculated.

2.4. Fish community indices and indicators

In total, ten fish community metrics were calculated for each sampling site for the entire fish community. It has not been the objective of this study to consider all types of indices or indicators described previously in literature, and thus the indices and indicators considered in this study should be viewed as a sample of some of the most commonly used and we have tried to group them to facilitate the interpretation of results. Among taxonomic indicators, species richness, Shannon index, rareness and taxonomic distinctness were calculated. Species richness describes the total number of different taxa encountered at a site, while Shannon index accounts also for the relative abundance of each species within a site (Shannon, 1948). Taxon rareness provides a measure of the summed relative frequencies of rare taxa within a community, based on the overall frequency of the taxa in the entire dataset (Crisp et al., 2001; Linder, 2001). Taxonomic distinctness describes the phylogenetic connections of the taxa within a community (Clarke and Warwick, 1998). These classical taxonomic indicators have been widely used among ecological assessments, although they have been found to present variable responses to environmental and human disturbance gradients (Benejam et al., 2016; Feld et al., 2016).

Regarding the size-related indicators, from the original database based on lengths (mm) of individuals, individual body weights (g) were estimated by means of length-weight relationships reported in FishBase (www.fishbase.org, Froese and Pauly 2016). Mean weight, total weight range, maximum weight and weight diversity were calculated. Weight diversity was calculated following the non-parametric approach of Quintana et al. (2008). This metric is based on Shannon diversity and integrates the amplitude of the weight range and the evenness as Shannon integrates the number of species and their relative abundance. Size diversity metrics have been found to mirror environmental and human disturbance gradients (Brucet et al., 2006; Emmrich et al., 2011, Benejam et al., 2016).

Finally, two bioassessment fish-based indices were calculated: European Fish Index (EFI+ hereafter) and Fish Region Index (FRI hereafter). The EFI+ is a multimetric index designed to assess the human-induced impact on the biotic condition in rivers by measuring the deviation of the actual fish fauna from a predicted fish assemblage for each specific river type using 13 abiotic variables (Pont et al., 2007; EFI+ Consortium, 2009), and thus a priori controlling the effects of geoclimatic variables. On a European scale the EFI+ represents the first fish-based assessment method applicable on a large geographical scale (Logez and Pont, 2011; Segurado et al., 2014). For its calculation free software (available at http://fame.boku.ac.at) was used. The result, ranging from zero (very impaired) to one (reference conditions), provides information about the impaired degree of the site. The FRI (Wolter et al., 2013) is based on previous indices developed and harmonized for Austria (Schmutz et al., 2000) and Germany (Dußling et al., 2004). Based on the natural probabilities of occurrence of every single species in a given sample in the river regions relevant for fish (Epirhithral, Metarhithral, Hyporhitral, Epipotamal, Metapotamal, and Hypopotamal), the FRI is calculated as average of the present species averages with unequal variances and random samples. This kind of fish specific indexes have been less explored and compared with other indexes or indicators in scientific literature, being scarce their application in Mediterranean areas (Segurado et al., 2014).

2.5. Data analysis

Spearman correlation matrix was calculated for all geo-climatic and land cover variables with the aim to exclude highly correlated variables, considered as those with a Spearman correlation > [0.7]. Spearman correlation was also evaluated between community metrics to assess its relationship. All values were ln(x + 1) transformed to reduce the influence of extreme values. To allow comparing the observed effects of the factors on different fish community metrics, all values were standardized: $x_{st,I} = (x_i - \mu)/\sigma(x)$, being xi each value of the ln (x + 1) transformed data base, μ the average value and $\sigma(x)$ the standard deviation of the ln(x + 1) transformed data base.

The relations between every fish community metric and the selected (uncorrelated) geo-climatic and land cover variables were quantified by means of boosted regression tree analyses (BRT hereafter, Elith et al., 2008), a method that allows to detect potential non-linear responses. A BRT model was fitted for every fish community index or indicator and the selected geo-climatic and land cover variables. Model settings were chosen according to Elith et al. (2008) criteria, setting a learning rate lr slow enough to increase the number of trees required to achieve the lowest predictive deviance up to 1000. Since the number of observations varies around 250, and accordingly to Elith et al. (2008) recommendations, tree complexity was set at 3 to allow us to test 3-way interactions, bag fraction was set at 0.5, using 5-fold cross validation. For the model analysis and visual interpretation of the shape of response (partial dependence plots), we used an approach similar to Wagenhoff et al. (2017) calculating total deviance explained (TDE) as (%TDE = [mean total deviance - mean residual deviance]/mean total deviance). Moreover, we identified thresholds of interest along geoclimatic gradients, such as abrupt changes in the metric response to gradual changes in a geo-climatic variable, and along stressor gradients, such as impact cessation point (i.e., last change of a positive or a negative response rate to no stressor influence) or abrupt changes (i.e., special case when impact initiation matches impact cessation). The relative position of this thresholds in the stressor range of variation would indicate the sensitiveness of the metric to that specific stressor (i.e. the lower the stressor value to initiate the metric response, the higher the sensitiveness of the metric to that stressor).

To allow comparing the strength of the response to variables across models, the absolute contribution of each variable to the total deviance has been calculated as *relative contribution* \times %*TDE*. By this way, it is possible to compare contributions even if the models result in very different explicative power.

All the analyses were conducted using R v. software (R Development Core Team 2017). The function *cor.test* of package "*stats*" and the

Table 2

Range of variation of geo-climatic and anthropogenic descriptors, coefficient of variation (%) and descriptors used to proceed with the standardization $[x_{st,l}=(x_i-\mu)/\sigma(x)]$ of Ln(x + 1) transformed data base: μ is the average value and $\sigma(x)$ is the standard deviation of the Ln(x + 1) transformed data base.

Descriptor	Code	Range (min; max)	CV (%)	μ; σ(x)	
Geo-climatic					
Latitude (°)	Lat	36.21; 43.65	4.57	3.75; 0.05	
Longitude (°)	Lon	-0.89; -9.12	45.30	1.70; 0.41	
Altitude (m.a.s.l.)	Alt	1; 1650	67.89	5.80; 1.29	
Catchment area (ha)	Cat	342.5;	385.19	9.60; 1.82	
		4,139,000			
Mean annual air temperature (°C)	Temp	7.45; 17.87	18.38	2.57; 0.17	
Interannual CV temperature	TinterCV	3.0; 14.0	28.92	0.06; 0.02	
Intra-annual CV temperature	TintraCV	22.0; 84.0	25.02	0.39; 0.08	
Anthropogenic Agriculture land (%) Urban land (%)	Ag Ur	0; 95.3 0; 21.3	112.67 306.56	0.15; 0.15 0.01;0.02	
				-	

**Note*: μ is the average value and $\sigma(x)$ is the standard deviation of the ln(x + 1) transformed data base.

function *gbm.step* of package "*dismo*" (Hijmans et al., 2017) were used to run the Spearman correlation test and the BRT analysis respectively.

3. Results

3.1. Characterization and correlation of geo-climatic variables and indices/ indicators

Differences in range and coefficient of variation (CV) were remarkable for geo-climatic and anthropogenic descriptors that characterize selected sites (Table 2). The highest variability occurred in catchment area, agriculture land and urban land (> 100% in all cases). Intermediate variabilities were found for altitude and interannual CV of temperature; altitude of sampled sites ranged from almost the sea level (1 m) to 1650 m and interannual CV of temperature ranged from 3 to 14%. Thus, studied sites present a wide variety of conditions. Except for the geographic coordinates, the lowest variability was found for mean annual air temperature and intra-annual CV temperature. Two of the geo-climatic variables (altitude and Intra-annual CV temperature) were significantly correlated (Spearman test, p < 0.05, $|\mathbf{r}| > 0.7$) with temperature (Table 3), thus in the subsequent statistical analysis these variables (both altitude and Intra-annual CV temperature) were not included and temperature variable was preserved as climatic variable.

Several community indices and indicators were also correlated (Spearman test, p < 0.05, Table 4). With the exception of Distinctness, taxonomic indicators were highly positively correlated between them (r > 0.75, p < 0.001), weakly negatively correlated with EFI+

Table 3

Correlation matrix of the geo-climatic and anthropogenic descriptors: longitude (Lon), latitude (Lat), altitude (Alt), temperature (Temp), interannual CV temperature (TinterCV), intra-annual CV temperature (TintraCV), Agricultural land (Ag), Urban land (Ur). Significant correlations > |0.7| are displayed in bold type.

	Lon	Alt	Cat	Temp	TinterCV	TintraCV	Ag	Ur
Lat Lon Alt Cat Temp TinterCV TintraCV Ag	-0.2	-0.2 0.4	-0.3 0.0 -0.1	-0.5 -0.1 - 0.7 0.3	-0.2 -0.0 0.5 -0.1 -0.4	-0.1 0.4 0.9 -0.1 -0.7 0.5	-0.2 -0.0 -0.1 0.3 0.3 -0.2 -0.1	-0.0 0.1 -0.1 0.2 -0.2 -0.1 0.1

(r < |0.5|, p < 0.001) and positively correlated with FRI (r > 0.6, p < 0.001)p < 0.001). This later relation is rather counterintuitive, since FRI normally increases while RI, SW and RA decrease with impairment. As a matter of fact, FRI increases with impairment within a given fish region in the gradient epirhithral-metapotamal; but also increases among fish regions in that gradient. Size related indicators also exhibited a high positive correlation within them (r > 0.69, p < 0.001), but scarcely correlated with other types of metrics (although significant, p < 0.01, $r \le 0.22$ in the case of taxonomic indicators, and r < |0.3| in the case of multimetric indices). Finally, multimetric indices were weakly correlated between them (r = -0.48, p < 0.001) and, as expected, in a negative way as more impaired sites present higher values of FRI and lower values of EFI+. Therefore, metrics within a given type (taxonomic, size related and multimetric) provide redundant information (all indices or indicators within the same group are well correlated, r > 0.69, p < 0.001) with the exception of Distinctness and multimetric indices given that they are weakly correlated.

The range and the coefficient of variation (CV) were also substantial for indices and indicators (Table 5). In general, size related indicators (with the exception of Size Diversity) present the highest variability together with rareness (CV > 170%), while multimetric indices exhibited low variability together with distinctness (CV < 30%). Therefore, the wide variety of geo-climatic and anthropogenic descriptors offered a remarkable context to deal with the hypothesis.

3.2. BRT models: strength, sign and shape of the response

All BRT models explained > 25% of the total deviance (Table 6) and five of the ten BRT models explained \geq 50% of the total deviance. The model fitted to FRI exhibited the highest goodness-of-fit (78.4% TDE). Taxonomic indicators models, such as those resulting from richness, Shannon and distinctness explained ~60% TDE, and performed better than those referred to size-related indicators which explained roughly half that amount (~30% to 40% TDE). EFI+ yielded intermediate values of %TDE (49.5% TDE). The predictive performance was the lowest for rareness and size diversity model explaining 26.5 and 27.6% TDE respectively.

In relative terms, the proportion of variance jointly attributable to geo-climatic descriptors is higher than 65% TDE in all cases, arising values of 84.1%; 86.2%; 88.1% and 88.7% in the case of distinctness, FRI, Shannon and richness respectively (Table 6). Latitude and/or longitude together with catchment area explained a considerable proportion of variance in many community descriptors models. For example, in seven out of ten BRT models, catchment area was the strongest geo-climatic variable with a relative contribution to the model higher than 22%, arising a contribution of 28.8% in the case of richness. Latitude presented a relative contribution higher than 20% in five out of ten models, and longitude was the strongest geo-climatic variable in the case of rareness (23.9%) and distinctness (23.5%). Temperature explained around 25% of TDE in the case of rareness and FRI, being relevant in three taxonomic indicators while less relevant for size related indicators (relative contribution lower than 11%). In absolute terms, geo-climatic descriptors have a stronger effect in the case of FRI (67.5%), Shannon (54.6%) and richness and distinctness (~52%), while these contributions decrease below 30% in the case of size related indicators (Table 6).

The jointly contribution of anthropic drivers was lower than geoclimatic drivers, although variedly (Table 6): strength of anthropic drivers was up to eight times less than geo-climatic drivers when explaining richness and Shannon indicators, between four and six times less when explaining rareness, total range, distinctness and FRI; and a half or a third less when explaining EFI+ and size related indicators (except total range).

In seven out of the ten models, one single land cover contributes more markedly to the total deviance explained than the other (Table 6). Agriculture land contributes more markedly in the case of richness,

Table 4

Correlation matrix of the ten community descriptors variables: RI (Richness), SH (Shannon diversity), RA (Rareness), DI (Distinctness), TR (Total Range), MW (Mean weight), MXW (Maximum weight), SD (Size Diversity), FRI (Fish Region Index) and EFI+ (European Fish Index). See methods section for the description of community descriptors. Significant correlations > 0.6 are displayed in bold type.

	SH	RA	DI	TR	MW	MXW	SD	FRI	EFI +
RI SH RA DI TR MW MXW SD FRI	0.92***	0.83*** 0.77***	- 0.39*** - 0.367*** 0.50***	0.21** 0.134* 0.10 ns 0.06 ns	-0.13 ns -0.13* -0.18** 0.22** 0.69***	0.19** 0.12* 0.09 ns 0.07 ns 0.72*** 0.99***	0.11* 0.08 ns 0.04 ns 0.07 ns 0.73*** 0.90*** 0.91***	0.63*** 0.62*** 0.83*** -0.49*** -0.16** 0.05 ns 0.05 ns 0.01 ns	- 0.41*** - 0.41*** - 0.41*** 0.09 ns - 0.31*** - 0.15 ns - 0.15* - 0.12 ns - 0.48***

Note: Significance after Spearman test (P < 0.05): *** P < 0.001, ** P < 0.01, * P < 0.05, n.s. non-significant.

Table 5

Range of variation of community metrics and indicators, coefficient of variation (%) and descriptors used to proceed with the standardization $[x_{st,I} = (x_i - \mu)/\sigma(x)]$ of Ln(x + 1) transformed data base: μ is the average value and $\sigma(x)$ is the standard deviation of the Ln(x + 1) transformed data base.

Community metric	Code	Range (min; max)	CV (%)	μ; σ(x)
Taxonomic indicators				
Richness	RI	1; 8	58.99	1.20; 0.42
Shanon diversity	SH	0; 2.45	89.76	0.48; 0.40
Rareness	RA	0; 1.03	230.30	0.05; 0.10
Taxonomic distinctness	DI	1; 4	22.5	1.43; 0.19
Size-related indicators				
Total range (mm)	TR	0; 5081.1	171.34	4.85; 1.36
Mean weight (g)	MW	0.31; 1516.1	253.28	3.19; 1.07
Max weight (g)	MXW	0.31; 5082.10	170.12	4.92; 1.26
Size diversity	SD	0; 7.05	41.64	1.42; 0.52
Multimetric indexes				
EFI index	EFI	1; 0	29.93	1.77; 0.19
Fish Region Index FRI		3.75;7.99	22.90	0.53; 0.15

Shannon, rareness and size diversity indicators, being the latter the most noticeable case with agriculture land explaining 2.7 times as much deviance as the urban land; while the influence of urban land is higher than agriculture in the case of distinctness, mean weight and EFI+, being the latter the most differentiated explaining 2.8 times as much as deviance as agriculture land. Both land uses present similar contribution to the total deviance explained of models in the rest of cases (i.e., for total range, maximum weight and FRI). Taking into account the absolute explained variance by each variable, calculated as relative contribution \times %TDE (see methods section); it is possible to compare variables contribution between models. Size diversity, FRI and maximum weight are the most sensible to agriculture land, being the absolute contribution of this variable 6.7; 5.4 and 5.0% to the total deviance. In the case of urban land, the most sensible were EFI+, mean weight, distinctness and FRI being the urban land contribution to the models 10.5, 8.8; 5.7 and 5.5%. FRI is the only index whose sensitiveness to both agricultural and urban land-use is higher than 5% in absolute terms, which could indicate that FRI is a good candidate index to measure both land-use effects on stream fish communities, although EFI+, mean weight and size diversity could be better when analysing an specific single land type.

Indicators and indices respond rather consistently to land-use

Table 6

BRT model outputs: total % deviance explained (% TDE) (a measure of model fit) and mean CV correlation coefficient of observed vs predicted values derived from 5 folds (model predictive performance), and the relative contribution (%) of each geo-climatic and land use factor on the models. The contributions of the 3 highest-ranked predictors in each model are presented in bold. The absolute contribution of each variable to the models calculated as *relative contribution* \times *%TDE* appears in italics in a second line each time. RI (Richness), SH (Shannon diversity), RA (Rareness), DI (Distinctness), TR (Total Range), MW (Mean weight), MXW (Maximum weight), SD (Size Diversity), FRI (Fish Region Index) and EFI + (European Fish Index).

			Relative contributions of descriptors							
Index	TDE(%)	CV correlation	Lat	Lon	Catch	Т	TinterCV	Ag	Ur	
RI	59.2	0.55	20.0	14.6	28.8	15.6	9.1	7.4	4.6	
			11.8	8.6	17.1	9.2	5.4	4.4	2.7	
SH	61.5	0.61	22.1	13.2	26.3	19.0	8.1	7.4	3.8	
			13.6	8.1	16.2	11.7	5.0	4.6	2.4	
RA	26.5	0.35	5.9	23.9	15.9	24.7	9.1	13.4	7.2	
			1.6	6.3	4.2	6.5	2.4	3.5	1.9	
DI	62.0	0.55	21.5	23.5	19.3	9.9	9.9	6.8	9.2	
			13.3	14.6	12.0	6.2	6.1	4.2	5.7	
TR	34.2	0.32	21.9	15.7	26.0	7.1	8.3	11.1	9.9	
			7.5	5.4	8.9	2.4	2.8	3.8	3.4	
MW	41.1	0.31	11.9	11.8	24.4	10.9	8.9	10.7	21.3	
			4.9	4.9	10.0	4.5	3.7	4.4	8.8	
MXW	39.4	0.37	17.2	16.9	24.8	7.4	8.3	12.7	12.8	
			6.8	6.6	9.8	2.9	3.3	5.0	5.0	
SD	27.6	0.24	20.5	12.1	19.2	7.0	7.9	24.3	9.1	
			5.7	3.3	5.3	1.9	2.2	6.7	2.5	
FRI	78.4	0.78	14.7	15.5	23.4	24.5	8.1	6.9	7.0	
			11.5	12.1	18.4	19.2	6.3	5.4	5.5	
EFI+	49.5	0.35	15.0	16.4	22.0	7.2	10.6	7.6	21.2	
			7.41	8.10	10.91	3.55	5.25	3.76	10.51	



Fig. 2. Partial dependence plots of boosted regression tree (BRT) models showing the fitted functions (smoothed curves drawn in red dashed lines) and the relative contribution in % (number within each plot) of fish community indices/indicators to geo-climatic and land use variables. Impact cessation points, i.e., last change of a positive or a negative response rate to no stressor influence, are indicated by a shaded grey area when they are patent.

variables (Fig. 2). Indices that penalize the more impaired conditions used to show a negative response to urban and agricultural area in the catchment, whereas FRI, which increases with impact intensity, responds positively. Most of indices and indicators respond to extremely low values of urban land proportion, and the impact cessation point, i.e., the stressor intensity where community metric stabilizes, is reached around 2.7% (1 standardized unit in Fig. 2, hereafter st. unit), even less in the case of FRI and EFI+ being around 1.2% (0.25 st. unit). This evidences that they become saturated by very low values of urban area in the catchment. However until reaching that cessation point, the response pattern of indices and indicators is variable. Richness, Shannon and EFI+ exhibited a decreasing trend, i.e., the higher the pressure, the lower the values of indices and indicators until reach that early saturation point. Meanwhile, the rest of indicators and indices, seem to experience an inflection point meaning that very low values, around 1.7% (0.5 st. unit) of urban land proportion has a positive effect in the indices and indicators values.

On the contrary, agriculture land proportion induces a similar response in most of the metrics presenting relatively high values when agricultural land proportion is very low, and subsequently decreasing more or less sharply arising a stable value when pressure increase (i.e., impact cessation point) (Fig. 2). Some metrics present an abrupt change at variable values depending on the metric, e.g., around 50.8% (1.75 st. unit) in the case of richness, Shannon, total range, mean weight, maximum weight and size diversity, and around 15.7% (0.5 st. unit) for rareness. Exceptions to this pattern are distinctness and FRI which present a gradual decrease and a gradual increase respectively until reach the saturation point around 45.2% (1.5 st. unit) and 50.8% (1.75 st. unit) respectively; and EFI+ that present an abrupt increase with lower values of agricultural land until reach 2.5% (-0.75 st. unit) and then slightly decrease.

Independently of land-use, geo-climatic factors induce the strongest responses on fish community indices and indicators (Table 6). Except in the case of distinctness and EFI+, most of indices exhibited a gradual positive relation with catchment area throughout a wide range of values (between 24 km^2 (-1 st. unit) and 5605 km^2 (2 st. unit), and in the case of size diversity an abrupt positive change around 900 km² (1 st. unit) is found. Latitude induces similar responses for most of the indices and indicators with higher values at higher latitudes, except for FRI that present a negative response and for rareness and mean weight where no clear response is found. Longitude effect is more variable across fish community metrics (Fig. 2). The strongest effect is found for rareness where a negative relation is observed presenting an abrupt change around -1.9° E (-1.5 st. unit). Negative responses are also found for richness, Shannon, total range, maximum weight, FRI and EFI+ although exhibiting varied shapes. In the case of temperature, a clear relation is found for those community metrics where the relative contribution of temperature is higher than 15%, positive for richness, Shannon, rareness and FRI, and negative for EFI+, while the shape is fuzzier where a poorer relation exist, i.e., for size-related variables and distinctness.

4. Discussion

As hypothesized, we found that fish community metrics respond rather consistently to anthropogenic land cover proportion, i.e., community metrics reflect a poorer status when land cover proportion in the catchment increase. Moreover, geo-climatic variables induce the most noticeable responses on fish community as found by previous authors (Brucet et al., 2013; Marzin et al., 2013; Feld et al., 2016), who have highlighted the relevance of environmental conditions in the response of ecological indicators.

4.1. Strength, sign and shape of the response to land use gradients

agricultural land proportion used to be similar or higher than urban land proportion, except in the case of distinctness, mean weight and EFI + where urban land proportion contributed more to explained variance. Both agricultural and urban land uses produced a response on the tested metrics that was consistent with their consideration as anthropogenic stressors. The sign of the responses to urban and agricultural land uses are also consistent with previous comparable studies. Fish community richness and diversity have been frequently reported to negatively respond to agricultural and urban land use (Burcher et al., 2007; Clapcott et al., 2012; Benejam et al., 2016). However, this is controversial since some other studies concluded that the response might be lacking (Marzin et al., 2012). Even a positive response of richness and taxonomic rareness to agricultural land use has been reported for fish (Feld et al., 2016). In our study, richness and Shannon diversity slightly increase with increasing agricultural land use proportion at low values of this stressor. This might be due to the inclusion of alien species in the calculation of these indexes. Some of these species will benefit from impacts derived from agricultural land use in the catchment (Cooper et al., 2013). For instance, crucian carp (Carassius carassius) and common carp (Cyprinus carpio) would tolerate eutrophy related deoxygenation (Jeppesen et al., 2010), and have in reservoirs and regulated reaches a focus of invasions and habitat refuge.

In the case of body size based metrics, we have found a stronger effect of agricultural land proportion on all size related metrics than urban land, with larger values of size related metrics in less degraded catchments. This results are consistent with previous authors (Emmrich et al., 2011; Maceda-Veiga et al., 2018), but the opposite relation for size diversity and total range has been also detected (Murphy et al., 2013; Benejam et al., 2016). Larger body size is frequently related to higher trophic levels, which is the basis for hypothesis linking larger sizes to large food chains and, consequently, less impaired conditions. But some clade-specific adaptations to herbivory (e.g., Cyprinids, see Burress et al., 2016), along with the existence of large bodied tolerant species (e.g., certain catfish species, see Benejam et al., 2016), may add regional variations to such generalization; thus producing regional deviations from what could be expected. Regarding to multimetric indices, it had been found that overall fish biotic metric (FAME) at basin scales have a weaker response to perturbation in Mediterranean regions (Ferreira et al., 2007). However, EFI+ (an extension of the original FAME) was improved using a greater Spanish fish data. FRI was developed in Austria and not harmonized for Spain, although its scientific basis were applied to Spanish fish communities in the past (García de Jalón and González del Tánago, 1983). In our case, both indices responded accordingly to their purpose; although FRI responded similarly to both types of land use and EFI+ responded more markedly to urban land use. EFI+ has been tested as a sensitive metric to detect both global and specific stressors (Marzin et al., 2012; Almeida et al., 2017).

In general, fish community responses to land use, as an anthropogenic stressor, have been frequently reported as weak (Brucet et al., 2013), or at least weaker than other biological quality elements (Clapcott et al., 2012; Dahm et al., 2013; Kail and Wolter, 2013). This weaker response may be caused by the higher mobility of fish relative to other taxa. The existence of accessible favourable habitats would buffer the effect of perturbations, and the responses of the metrics would remain undetected (Marzin et al., 2012). In that sense, a scaledependent analysis considering also hydromorphodynamic drivers of biological communities acting at lower spatial-scale (e.g., reach or segment scale), such as channel features, riparian vegetation structure, upstream river network characteristics considering the presence of barriers could greatly contribute to a better comprehension of fish community responses. More extensive data sets could also include a higher resolution of land use types to identify the key specific artificial land covers that affect fish communities more intensely.

Among the anthropic drivers, the strength of the response of

4.2. Non-linear responses along the stressor gradients

The analysis of the shape of the response curve has allowed us to detect how anthropic and geo-climatic variables affect each fish community metric, identifying thresholds of interest along the variable gradient. The technique used for this purpose (BRT) has been tested in previous similar studies and has been proven to give substantial predictive advantage over methods such as GLM and GAM (Elith et al., 2008), and even over specific techniques like piecewise linear regression models (Wagenhoff et al., 2017). At this respect, the proportion of agricultural land induces a similar response in most of the community metrics presenting higher values at less degraded catchment and exhibiting abrupt changes more or less sharply over a wider range of proportions, while urban land effects appear at very low values of its proportion, which becomes rapidly saturated. In some cases, as in rareness, distinctness, total range, mean weight, maximum weight and size diversity, very low values (around 1.7%) of urban land proportion has a positive effect in the indices and indicators values. This kind of positive effect at low pressure values has been previously described, e.g., initial increase of nutrients or sediments had a positive effect on ecological attributes (Odum et al., 1979), such as an increase in macroinvertebrate production and diversity (Wagenhoff et al., 2011, 2012). This early response at so low values makes the effect of the urban area very difficult to quantify, beyond determining whether it affects (totally) or not (at all). Therefore, the effect of an increase in the agricultural area is easier to quantify, and makes agricultural land a pressure whose impacts on the fish community are easier to modulate by planners than impacts caused by urban land use. FRI is the metric that is globally more sensitive to the two types of land uses considered. Both cause an increase in the value of FRI, which is consistent with the response of this index to the impoverishment of the ecological conditions (Kail and Wolter, 2013; Wolter et al., 2013).

Many evidences of a non-linear effect with very low thresholds of initiation (10–20%) are collected in Allan (2004), and are considered as one of the characteristics of the effect of the impervious area, as a consequence of the increment of urban land use on stream biota. We have seen that the most sensitive metrics to this pressure (which are EFI +, mean weight, FRI, and maximum weight) show an equally non-linear and abrupt response, starting at low values and producing a saturation of the response (impact cessation threshold) at values around 3%. In the case of agriculture land the response is more gradual obtaining a saturation of the response at values close to 50%. This observed range is highly consistent with those compiled (30%-50%) by Allan (2004) in New Zealand and United States watersheds.

4.3. Strength, sign and shape of the response to geo-climatic gradients

As expected from literature review, geo-climatic variables were noticeably more influential than land use variables and different shapes of response were found across the different community metric here considered. Taxonomic based indicators, except distinctness, show a direct positive response to catchment area. This somewhat expectable since the size of the basin increases along the river continuum (Vannote et al., 1980); and species diversity increases from the heterotrophic upper tributaries to the autotrophic middle sections, where diversity usually reaches a maximum. In the lower reaches the river becomes heterotrophic again, and the number of species becomes reduced. However, all the sampling sites in this study were located in wadeable sections, far upstream from those lower reaches. Regarding body size related indicators, they are quite sensitive to catchment area and latitude according to our results, showing a positive response in most of cases. This is to some extent expectable since, at similar hydrological conditions, micro and mesohabitat characteristics vary with the catchment area, producing a more suitable habitat to larger fish (i.e., reduced water velocity and increased channel depth). Both multimetric indices, FRI and EFI+, are also highly dependent on geo-climatic

variables. EFI+ could be expected to be almost independent of geoclimatic variables, since it was designed to be applicable in all Europe (Logez and Pont, 2011). During the process of data input in the software to calculate this index, longitude, latitude, upstream drainage area, and mean air temperature of the sampled site are required. However, latitude, longitude, catchment area and interannual variability of temperature account a no negligible (> 10%) proportion of TDE in the EFI + model. This index increases abruptly with latitude and decreases gradually with catchment area, which could be related with the normally lesser extension and better ecological status of rivers in the North relative to the South of the Spanish surface water bodies (WISE WFD Database https://www.eea.europa.eu/data-and-maps/data/wise-wfd). In the case of FRI, it is an index that increases along the gradient rhithral-pothamal conditions, and for a given river region, increases with increasing impact (Wolter et al., 2013). Latitude and longitude induce gradual changes in FRI over a wide range of values. At higher latitudes the value of the FRI decreases, which is understandable, since at higher latitudes in the Iberian Peninsula rivers source closer to their mouth, without generating lower reaches with potamal conditions. There is also an associated boreo-alpine gradient by which latitude compensates the rhithral-potamal gradient caused by altitude. Longitude induces a similar response: the farther west the lower the value of FRI. Longitude apparently should not produce an effect on the rhithral-potamal conditions of streams, unless sites are not equally located along that gradient in the dataset. Including sites in Portugal (Fig. 1) would correct the over-representation of the northwest of the Iberian Peninsula (with short streams and rhithral conditions) relative to the southwest (long rivers with abundant potamal reaches).

The present study contributes to the understanding of the effects of stressors acting at large scales (i.e., catchment scale) on fish communities by analysing a set of the most frequently used metrics in stream health assessment studies. The combination of taxonomic classic indicators, with size related indicators, which are novel in lotic ecosystems, and also recent fish specific multimetric indices created in the European context (WFD) could be effective tools to evaluate the impact of land use changes as they show different sensitiveness to different values of geo-climatic conditions and stressor levels. Therefore, we would recommend combining all these types of metrics to build an efficient array of indicators to be used in the same way physicians employ a diagnosis toolbox (Elosegi et al., 2017). This 'river doctor diagnosis toolbox' should not include redundant indicators such as those highly correlated to each other (e.g., all the taxonomic indicators); selecting the ones that are most sensitive to stressors and independent of geo-climatic variables. Further advances on this approach should come from testing other currently used indicators. Moreover, the combination with cause-effect approaches to elucidate mechanistic ecosystem functioning, could be of paramount importance contributing to conservation and restoration goals and anticipating management initiatives, given the incessantly alteration at the landscape scale worldwide with relevant implications for biodiversity monitoring.

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Appendix A. Supplementary data

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