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Fine-scale determinants of conservation value of river reaches in a hotspot of native and non-native species diversity

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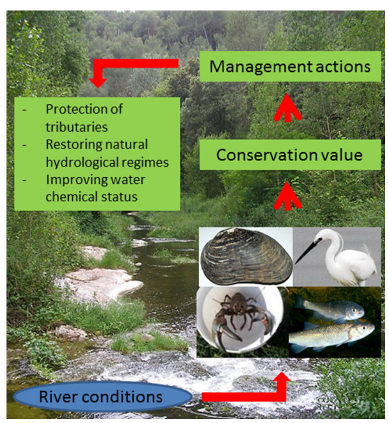
HIGHLIGHTS

- A moderate relationship was observed among indicators of conservation value.
- Protected areas offered limited coverage to imperilled freshwater fauna.
- River tributaries were identified as native fish refugees.
- Restoring water quality and the natural hydrological regime are priority tasks.
- Multiple components of diversity should be examined in resource management.

GRAPHICAL ABSTRACT



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ABSTRACT

Global freshwater biodiversity is declining at unprecedented rates while non-native species are expanding. Examining diversity patterns across variable river conditions can help develop better management strategies. However, many indicators can be used to determine the conservartion value of aquatic communities, and little is known of how well they correlate to each other in making diagnostics, including when testing for the efficacy of protected areas. Using an extensive data set (99,700 km^2, $n = 530$ sites) across protected and unprotected river reaches in 15 catchments of NE Spain, we examine correlations among 20 indicators of conservation value of fish communities, including the benefits they provide to birds and threatened mammals and mussels. Our results showed that total native fish abundance or richness correlated reasonably well with many native indicators. However, the lack of a strong congruence led modelling techniques to identify different river attributes for each indicator of conservation value. Overall, tributaries were identified as native fish refugees, and nutrient pollution, salinization, low water velocity and poor habitat structure as major threats to the native biota. We also

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Threatened taxa
Natura 2000
Environmental degradation

found that protected areas offered limited coverage to major components of biodiversity, including rarity, threat and host-parasite relationships, even though values of non-native indicators were notably reduced. In conclusion, restoring natural hydrological regimes and water chemical status is a priority to stem freshwater biodiversity loss in this region. A complementary action can be the protection of tributaries, but more studies examining multiple components of diversity are necessary to fully test their potential as fluvial reserves in Mediterranean climate areas.

1. Introduction

Biodiversity loss is occurring at unprecedented rates driven by global change (Foley et al., 2005; Halpern et al., 2008; Tittensor et al., 2014). Although global change effects are visible across a wide range of habitats, freshwater ecosystems are particularly affected (Strayer and Dudgeon, 2010). A good example are Mediterranean rivers, where many endemic species live and some of them are at the brink of extinction (Smith and Darwall, 2006; Marr et al., 2010). Human pressure in the Mediterranean area date back to ancient times when humans settled along main rivers and began to exploit water and biological resources, including on the riverbanks (Hooke, 2006). This pressure intensified with modern civilisations that also diversified the type of impacts, including emergent pollutants (Petrovic et al., 2011; Kuzmanović et al., 2015) and the release of non-native species (Leprieur et al., 2008a; Cobo et al., 2010). The situation is expected to worsen due to climate change and human population growth (Vörösmarty et al., 2010); therefore, conservation of freshwater diversity and the goods and services they provide to society requires urgent management actions.

Protected areas are considered as a mainstay of biodiversity conservation as well as contributing to human well-being (Gaston et al., 2008). In rivers, the most effective conservation strategy is proposed to be framed at the basin scale (Allan et al., 1997; Saunders et al., 2002; Linke et al., 2012). This framework considers that basins are biogeographic units (Doadrio, 1988; Reyjol et al., 2007), and that rivers are linear systems through which major threats to freshwater diversity such as pollution can easily propagate (Allan et al., 1997; Nel et al., 2007). Environmental quality standards have been proposed at the basin scale driven by international legislation, such as EU's River Basin Management Plans (Directive 2000/60/EC). At this scale, however, a strict protection is unrealistic. It generates many socio-economic conflicts and is logistically unfeasible for large basins (Saunders et al., 2002); therefore, river reaches need to be prioritised according to their conservation value (Margules and Usher, 1981; Filipe et al., 2004; Hermoso et al., 2015). Nevertheless, this raises the question of which are the best indicators to assess the conservation value of a community.

Traditionally, conservation priorities have been based on indicators such as species richness, rarity, and threatened status (Margules and Usher, 1981). The threatened status is often based on the International Union for the Conservation of Nature (IUCN) Red List (http://www.iucnredlist.org/). However, the conservation status of a species can be unknown or vary across regions due to discrepancies in classifications; for example, the river blenny *Salaria fluviatilis* is listed as least concern in the IUCN Red list and as endangered in the Spanish Red Data Book (Doadrio et al., 2011). Therefore, the focus on international criteria can bias setting conservation priorities at the national level; the target of most conservation actions since they are more politically than biogeographically driven (O'Riordan and Stoll-Kleeman, 2002; Battisti and Fanelli, 2015). Likewise, prioritising rarity to reduce extinction risk may leave unprotected species with a less restricted distribution, including species of major importance for other threatened taxa as food source (e.g. Ruiz-Olmo et al., 2001; Lopes-Lima et al., in press) or for the functioning of the fluvial ecosystem (Winfield and Townsend, 1991; Flecker et al., 2010). Thus, the ideal conservation action would be one that secures threatened species while maximising the protection of species diversity at the basin scale.

Since a major ecological rule is that biodiversity increases with surface area (Lomolino, 2000; but see Allouche et al., 2012), and river size increases downstream (Strahler, 1964), protecting downstream areas could maximise the number of species protected at the basin scale. However, these reaches are usually neighboured by large urban areas and hence the most disturbed, including the presence of non-native species (Marchetti et al., 2004; Closs et al., 2015). As biological invasions pose a significant threat to biodiversity and ecosystem services (Vilà et al., 2009; Simberloff et al., 2013), the presence of non-native species may jeopardise conservation goals in rivers. Studies examining diversity patterns help identify hotspots of high conservation value, but also the mechanisms behind these patterns (Baselga, 2010; Gutiérrez-Cánovas et al., 2013). For instance, if turnover dominates diversity patterns, it suggests that stress generates new communities in which tolerant species may replace those sensitive (Baselga, 2010). In contrast, if species poor sites are a subset of species of those enriched (high degree of nestedness), it suggests that stress causes a progressive loss of sensitive species and that conservation efforts may focus on species rich sites (Baselga, 2010). However, hotspots of native species richness may not be congruent with rarity or threat (Orme et al., 2005; Collen et al., 2014), further increasing the complexity of setting conservation targets.

In this study, we examine indicators that can be used to determine the conservation value of fauna across 15 catchments (99,700 km^2) in the Western Mediterranean area, a world hotspot of biodiversity (Myers et al., 2000) but also highly prone to biological invasions (Leprieur et al., 2008a). The selected basins typify common threats to other Mediterranean-type rivers, including pollution, overharvesting, hydrological alterations, and riparian removal (Moyle et al., 2011). We mainly focus on fish because the distribution of many native species has markedly declined worldwide (Closs et al., 2015), including in the study area (Maceda-Veiga et al., 2010). Firstly, we used pair-wise correlations to test whether one indicator of conservation value could act as surrogate of the others to plan management actions, including measures of fish species diversity, rarity, and nativeness plus indicators of conservation value of fish for other fauna, such as host for freshwater mussels or prey for mammals and birds. Secondly, we tested whether current protected areas meet conservation indicators of the aquatic fauna because they were designed primarily to protect terrestrial taxa (Filipe et al., 2004; Lawrence et al., 2011; Hermoso et al., 2015). Finally, we examined relationships between these indicators of conservation value, and geographical, water and habitat variables to identify the river attributes in which management policies can act to enhance the conservation value of fish communities. These river attributes were further confirmed via a fish community analysis, which also identified the mechanisms behind community variation across rivers and their conditions.

2. Materials and methods

2.1. Study area

We assembled environmental and fish data from our own surveys performed in NE Spain from 2002 to 2009 (Maceda-Veiga et al., 2010; Maceda-Veiga and De Sostoa, 2011; Figuerola et al., 2012, and unpublished data). Briefly, this data set comprised 530 sampling sites that involved all Catalonian catchments from the Muga to Riudecanyes basins, plus the complete River Ebro and part of the Garonne basin (Fig. 1). Our

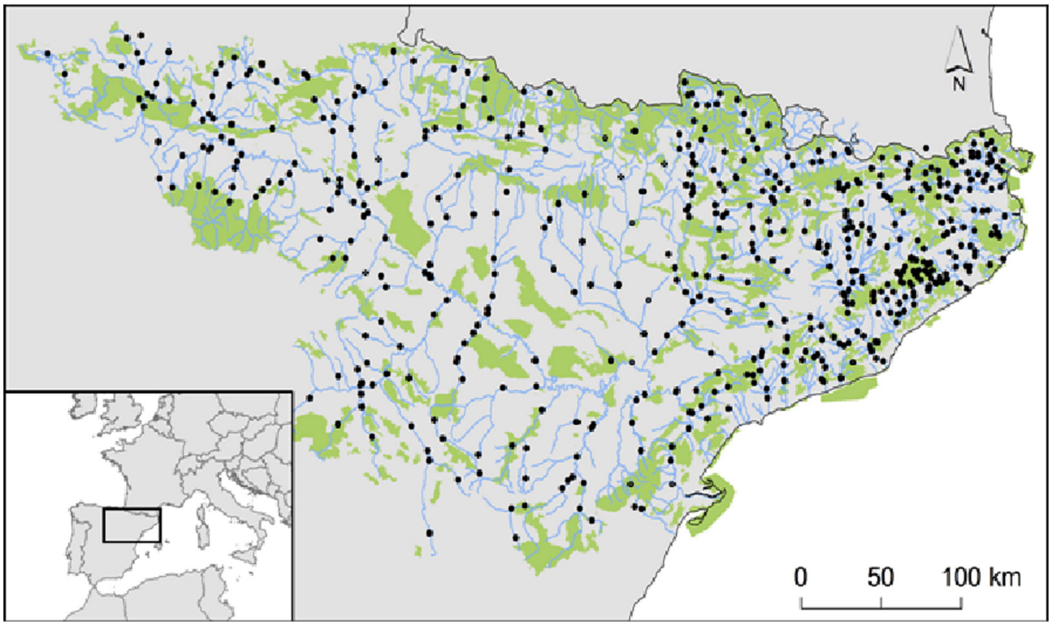

Fig. 1. Location of the 530 sampling sites surveyed for the current study in NE Spain with protected areas highlighted in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

data set accounted for all river typologies present in this region in terms of hydrological alterations, riparian characteristics, geology, water quality and flow. Most of these rivers are small and follow a typical Mediterranean hydrological regime, with severe droughts in summer and torrential floods in autumn. Large rivers, however, peak in flow in spring because of snowmelt. We surveyed in low flow conditions because this is when fish populations are more stable and can be properly sampled using electrofishing (see below). Low flow conditions also represent more intense stressful conditions in Mediterranean rivers (Gasith and Resh, 1999) and consequently, we could better identify the environmental drivers of fish fauna.

Sampled fish included species exclusively found in Iberian rivers (endemisms), such as the Ebro barbel (*Luciobarbus graellsii*) and the Iberian red-fin barbel (*Barbus haasi*), in addition to some of the world's worst invasive species, such as the largemouth bass (*Micropterus salmoides*) and the common carp (*Cyprinus carpio*). Only strictly freshwater fish were included in our analyses with the exception of the critically endangered European eel (*Anguilla anguilla*). We did not include brackish species (e.g. mugilids) that mostly occur in coastal lagoons or river mouths, which are more influenced by marine than freshwater conditions.

Other fauna included threatened freshwater mussels that depend on fish as host to complete their life cycle (Lopes-Lima et al., in press) and piscivorous animals, such as the European otter (*Lutra lutra*) and many waterbirds, including the common kingfisher (*Alcedo atthis*), the Grey heron (*Ardea cinerea*) and cormorants (*Phalacrocorax carbo*). We did not survey them but used their potential distribution in our study area (e.g. Palomo et al., 2007; Lopes-Lima et al., in press; SEO Birdlife, 2012a,b) to estimate the conservation value of fish for freshwater mussels, mammals and birds.

2.2. Fish survey

We followed an international standardised fish sampling method (CEN standards EN 14962 and EN 14011), as driven by the European Water Framework Directive. Fish were sampled by a single-pass electrofishing using a portable unit which generated up to 200 V and 3 A pulsed D.C in an upstream direction, covering the whole wetted width of the 100-m long reaches surveyed at each location (see also Maceda-Veiga et al., 2010; Benejam et al., 2012). We selected the location of each sampling site based on accessibility and representativeness, including a variety of habitat types (pools, rifles and runs). The same equipment was used across sites to avoid bias in fish captures (Benejam et al., 2012), and the crew had a standardised time devoted to the electrofishing passes according to their own experience and the reach features. Fish captures were standardised to captures per unit of effort (CPUE - fish abundance divided by fishing time in minutes and the area surveyed in square meters). Although sites were only surveyed once due to the vast geographical area covered, the methodological consistency across sites should accurately reveal relative changes in fish abundance or richness depending on river conditions. Our estimates of species richness and abundance from 4-pass electrofishing were reasonably high with 80–100% of the species detected and 50–90% of the individuals captured (A. Sostoa, unpublished data).

Fish were identified to species level, counted, and released in each site. Species nomenclature was updated from previous studies (Maceda-Veiga et al., 2010; Maceda-Veiga and de Sostoa, 2011) after an exhaustive examination of recent literature (Doadrio et al., 2011; Aparicio et al., 2013) and fish collections at the Natural History Museum of Madrid, Spain. Fish species were defined as non-native if they did not historically occur in a basin and in Spain, and as translocated if their presence is the result of an introduction from another basin within Spain where they are native (Table 1). Non-native and translocated species were grouped as introduced.

2.3. Indicators of conservation value

We calculated 20 indicators of conservation value to describe the fish community at each sampling site along the 15 basins surveyed in NE Spain. We defined an indicator of conservation value as any trait of the fish community composition that can be used to determine its conservation interest and guide management strategies (e.g. presence of threatened and non-native species, overall native richness). We first calculated the total abundance (captures per unit of effort) and richness for native, non-native, translocated and introduced fish species separately. We then calculated the proportion of native, non-native and translocated species in relation to the total fish abundance and richness in each site as a measure of the degree of nativeness and invasiveness of the fish community. We also calculated the number of species listed as the world's worst invaders (http://www.issg.org/). To better determine

Table 1
Occurrence (%) of freshwater fish species in NE Spain ($n = 530$ sites) with indication of their distribution (endemic, native, and non-native) and threatened status (catalogued as endangered in the IUCN red list, Habitats Directive, Spanish legislation or the Red data book of fish), the presence of spawning migratory behaviour in native fish, and the value of all fish species for waterbirds, threatened mussels and mammals (see methods for further details).

Scientific name	Occurrence	Threatened status	Distribution	Migratory	Mussel host	Piscivorous
Achondrostoma arcasii	**4.72**	Endangered	Endemism	No	No	No
Anguilla anguilla	11.51	Endangered	Native	Yes	Yes	No
Barbatula quignardi	10.5	Not endangered	Native	No	No	Yes
Barbus haasi	26.06	Endangered	Endemism	No	Yes	Yes
Barbus meridionalis	15.66	Endangered	Native	No	Yes	Yes
Cobitis calderoni	**2.45**	Endangered	Endemism	No	Yes	No
Cobitis palludica	**0.19**	Endangered	Endemism	No	Yes	No
Cottus hispaniolensis	**0.57**	Endangered	Native	No	No	No
Gobio lozanoi	19.06	Not endangered	Native	No	Yes	Yes
Luciobarbus graellsii	28.11	Not endangered	Endemism[t]	Yes	Yes	Yes
Parachondrostoma miegii	21.89	Endangered	Endemism[t]	Yes	Yes	Yes
Phoxinus bigerri	24.34	Not endangered	Native	No	Yes	Yes
Salaria fluviatilis	**4.53**	Endangered	Native	No	Yes	No
Salmo trutta	34.34	Not endangered	Native[t]	Yes	Yes	Yes
Squalius laietanus	16.23	Not endangered	Native	Yes	Yes	No
Gasterosteus aculeatus	**0.94**	Endangered	Native	No	Yes	No
Alburnus alburnus	9.06	–	Non-native	No	Yes	Yes
Ameirus melas	0.75	–	Non-native	No	Yes	Yes
Barbatula barbatula	1.5	–	Non-native	No	No	Yes
Carassius auratus	1.51	–	Non-native	No	No	No
Cyprinus carpio	14.91	–	Non-native[w]	No	Yes	Yes
Esox lucius	0.38	–	Non-native	No	Yes	No
Gambusia holbrooki	2.45	–	Non-native[w]	No	Yes	No
Gobio occitaniae	2.45	–	Non-native	No	Yes	No
Gobio spp.	5.40	–	Non-native[*]	No	Yes	No
Lepomis gibbosus	4.15	–	Non-native	No	Yes	No
Micropterus salmoides	1.32	–	Non-native[w]	No	Yes	No
Oncorhynchus mykiss	1.89	–	Non-native	No	No	No
Phoxinus spp.	6.60	–	Non-native[*]	No	Yes	No
Pseudorasbora parva	0.19	–	Non-native	No	No	No
Rutilus rutilus	0.38	–	Non-native	No	Yes	No
Sander lucioperca	0.38	–	Non-native	No	Yes	No
Scardinius erythrophthalmus	3.4	–	Non-native	No	Yes	No
Silurus glanis	1.89	–	Non-native	No	No	No

[t] translocated native species in some catchments of NE Spain.
[w] Listed as world's worst invasive species (http://www.issg.org/).
[*] Introduced taxa pending of genetic studies to confirm species identify in some catchments of Catalonia.

the contribution of native and non-native species richness in fish diversity hotspots, we also calculated the ratio between the current native species present in each site and the total number of native species historically occurring in each basin following Doadrio et al. (2011).

To assess the conservation value of fish communities based on their endangerment degree, we calculated the number of species in each site using three classifications: the IUCN red list of globally endangered species (http://www.iucnredlist.org/), the red data book of the fish of Spain (Doadrio et al., 2011), and the list of fish species protected by Spanish legislation (Real Decreto 139/2011). A species was considered as threatened if it was catalogued as 'critically endangered', 'endangered' or 'vulnerable'. We also considered the fish species listed in Annexes of the EU's Habitats Directive, which denotes threatened species at the European level. As a regional fish conservation index, we used the scores of species provided by Maceda-Veiga et al. (2010) that were summed to describe the conservation value of each location. Finally, we calculated the number of species whose range occupied <5% of our sites as a measure of rarity, and the number of native species exclusively found in Spain as a measure of endemicity (Table 1).

To further determine the importance of the fish fauna in each site, we calculated total richness of migratory species (Doadrio et al., 2011, Table 1). This indicator informs about river connectivity, as migratory species play a major role in energy transfer along rivers (Flecker et al., 2010). We also calculated the proportional abundance of fish suitable as hosts for freshwater mussels (see Lopes-Lima et al., in press in relation to the total fish abundance in each site (Table 1). Freshwater mussels that use fish as hosts for their larvae are worldwide-threatened taxa (Strayer et al., 2004; Lopes-Lima et al., in press). For this analysis, we only considered basins where we had historical evidence of occurrence of freshwater mussel species (see Lopes-Lima et al., in press). As fish are a key food item for the European otter and many waterbird species, we also calculated the total abundance of potential preferred prey based on diet studies of these consumers in our study area (e.g. Lekuona and Campos, 1997; Ruiz-Olmo et al., 2001; Vilches et al., 2012).

As a by-product of electrofishing, we also captured the red-swamp crayfish (*Procambarus clarkii*) and the signal crayfish (*Pacifastacus leniusculus*). Although both non-native species are a potential valuable food resource for mammals and waterbirds (Tablado et al., 2010), both cause several ecological impacts due to their trophic and non-trophic activities (Gherardi, 2006; Carvalho et al., 2016). Thus, the abundance (expressed as CPUE) of the two crayfish species was also included as a neutral indicator of conservation value in our analyses.

2.4. Geographical and environmental descriptors

We characterised each sampling site with 27 variables related to geography, habitat quality and water properties.

As geographical features, we recorded the basin name and elevation (m.a.s.l.) in each sampling site using Google Earth®. Elevation was used as a surrogate for the position of the sampling site in the river, and summarise the role of natural spatial gradients in fish indicators, as previously validated in this region (Maceda-Veiga et al., 2013; Murphy et al., 2013). We also calculated the Strahler stream order number on a map (1:50,000) as a measure of river size. It ranks rivers from a small, first order tributary all the way to the largest main river based on a hierarchy of tributaries. Strahler stream order number is directly proportional to relative watershed dimensions, channel size and stream discharge at that place in the system (Strahler, 1964). Because stream order number is dimensionless, two drainage basins differing greatly in linear scale can be easily compared with respect to corresponding points into their geometry.

Prior to each fish survey we analysed 7 water quality variables in situ. A digital multiparametric YSI® sonde was used for temperature (°C), conductivity (μS/cm) and pH, and the colourimetric test kit VISOCOLOR® for ammonium (NH_4^+, mg/l; detection limit (dl) = 0.2 mg/l), nitrite (NO_2^-, mg/l; dl = 0.02 mg/l), nitrate (NO_3^-, mg/l; dl = 1 mg/l) and phosphate (PO_4^{3-}-P, mg/l; dl = 0.2 mg/l) concentrations. To characterise habitat quality, we incorporated 17 variables from two widely used habitat quality indices in this region: the riparian vegetation quality index QBR (Munné et al., 2003), and a version of the U.S. Rapid Bioassessment (RBA) protocol (Barbour et al., 1999) for Mediterranean rivers. Briefly, RBA ranked 10 features of the local habitat (habitat structure, habitat diversity, river channelization, channel morphology, water flow, degree of silting, erosion of river margins, macrophyte coverage, and the coverage and width of riparian canopy) on an ordinal scale of 1–10 for RBA and 0–25 for QBR (score increases with quality). RBA includes more variables related to physical habitat for aquatic fauna than the QBR (total vegetation cover and structure, vegetation cover quality, and river channel alterations) but both consider the status of riparian vegetation.

To assess whether the sampling site was located in a protected area, we combined the layers of regional protected areas and the Natura 2000 network from the Autonomous Government of Catalonia, the Ebro Water Authority and the Spanish Government with the layer containing all our sampling points using the Geographical Information System (GIS) software ArcGis®. Subsequently, we obtained a matrix with our sampling points and the value of the landscape attribute, resulting in 27% of the sampling sites within protected areas ($n = 139$).

2.5. Statistical analyses

All statistical analyses were performed in R v.2.15.3 (R Core Team, 2013) using the libraries 'MASS' (Venables and Ripley, 2002), 'vegan' (Oksanen et al., 2015), 'lme4' (Bates and Maechler, 2009), 'car' (Fox and Weisberg, 2011), and 'betapart' (Baselga and Orme, 2012) and the functions outlined below. Spearman rank correlation (ρ) was used to examine congruence among indicators of conservation value in fish communities. Correlation coefficients around 0.1 were considered to be weak, 0.3 as moderate, 0.5 as moderately strong, and 0.7 and above as strong (modified from Lamoreux et al., 2006 and Tisseuil et al., 2013).

To assess if protected areas fulfil conservation values, we compared values of the 15 least correlated indicators of conservation value ($\rho < 0.7$) between protected and unprotected areas using a series of generalised linear mixed models (GLMMs) with the function 'lmer'. Basin was included as random factor in GLMMs to account for spatial autocorrelation of sites within each basin. Sampling year was also included as random factor to control possible inter-annual variation in fish captures, but it was removed from the final models because it did not influence the significance of predictors, as reported in a sub-set of the current data-set (Murphy et al., 2013).

To test whether indicators of conservation value identify the same river attributes, we determined relationships between the least correlated indicators of conservation value and the predictors related to geography, water and habitat quality (Appendix S1) using a series of GLMMs with the function and random term stated above. Elevation was included as fixed factor in models to account for the longitudinal position of the reach in the stream, and the role of spatial gradients in the fish community structure. Main and interactive effects of Strahler stream order number with elevation were also included to account for the differences in the conservation value of tributaries and main rivers at different elevations. For each indicator of conservation value we built a saturated model (containing all predictors) and followed a manual step-wise deletion of non-significant terms (Crawley, 2007; Zuur et al., 2009). Significance of predictors in GLMMs was tested using a likelihood-ratio test within the function 'Anova'. The comparison of nested models (models with and without a predictor) via a likelihood-ratio test led to the same minimum adequate model.

Final models were validated via diagnostic plots of model residuals against fitted values and against each predictor, Q-Q plots of model residuals and the Cox statistic to verify the assumptions of normality, homoscedasticity and detect unduly observations following Zuur et al. (2009) and Thomas et al. (2015). Relationships between each indicator and the selected or excluded predictors were also visually inspected to further determine their relevance. Log-transformation was applied to continuous predictors and arcsine squared root transformation to proportions to increase model fitting and meet statistical assumptions. Although we are aware of a vibrant debate on "the best" model selection procedure, all have pros and cons (e.g. Aho et al., 2014; Cade, 2015; Leek and Peng, 2015) and we considered a backward stepwise procedure is appropriate in our case given the clear effects of the selected predictors on the response variables and their ecological relevance.

To further determine the role of geography, habitat quality and water properties in the fish community, we examined relationships among the composition of the fish community (presence/absence) and the 15 least correlated predictors used in the GLMMs. For this analyses, predictors were grouped in three sets: (i) geographical features (basin, elevation and the Strahler river order number), (ii) water properties (conductivity, pH, concentrations of ammonia, nitrites, nitrate, and phosphate), and (iii) habitat quality (water velocity, aerial coverage, riparian coverage, habitat diversity, and macrophyte coverage, percentage of dead wood, and channel morphology. The variation in community composition attributed to each of these three sets of predictors was computed using variation-partitioning analyses (VP). Whilst causality cannot be determined in observational studies, VP decomposes the variation of dependent variables in independent and joined effects of a set of predictors (Borcard et al., 1992).

To determine the mechanisms behind community variation across sites, we used Baselga's method (2010) that decomposes total dissimilarity (i.e. beta diversity) in the community into its turnover (species replacement) and nestedness-resultant components (species loss). The relationship between community variation (either turnover or nestedness-resultant dissimilarity) and the predictors was assessed using distance-based Redundancy Analyses (db-RDA, function 'capscale' in R). We ranked the predictors within each group according to their unique explained variance (from greater to the least), introduced them in db-RDA models in this order, and tested for significance using the function 'anova.cca'. Only significant predictors were retained to avoid overfitting due to the inclusion of non-significant terms. Finally, we ran series of db-RDA models containing all combinations of the selected predictors per set to estimate the unique and shared fractions of explained variation. Significance was reached at $P \leq 0.05$ in all statistical procedures.

3. Results

We found 16 native and 18 non-native fish species in rivers of NE Spain, including two introduced uncertain taxa (*Phoxinus* spp. and *Gobio* spp.), and translocated native species from the Ebro basin (Table 1). Threatened species at national and international levels represented 56% of the fish fauna, including all endemic fish species (Table 1). However, five out of six are not listed as threatened at the European level (EU's Habitats Directive), even though they occurred in ≤5% of the sampling sites (Table 1).

3.1. Relationships among the indicators of conservation value and their coverage by protected areas

Most pair-wise correlations among native-fish indicators of conservation value were moderately strong ($\rho \sim 0.5$) to moderate ($\rho \sim 0.3$). The strongest positive relationships ($\rho \geq 0.7$) occurred between richness

Table 2
Spearman rank correlation coefficients (ρ) among 20 indicators of conservation value used to describe the fish community of rivers in NE Spain (n = 530). Note that most indicators of conservation value were not strongly correlated to each other ($r \geq 0.70$ in bold). See methods for further details.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. Total native fish abundance																			
2. Total native fish richness	0.67																		
3. Endemic fish richness	0.49	0.61																	
4. IUCN threatened fish richness	0.36	0.32	0.47																
5. Local conservation index	0.63	**0.93**	**0.71**	0.35															
6. Legally threatened fish in Spain	0.28	0.35	0.29	0.28	0.31														
7. Spanish Red Book fish	0.40	**0.72**	0.10	0.08	0.64	0.20													
8. EU's threatened fish species	0.20	0.24	−0.21	0.13	0.16	0.29	0.45												
9. Rare fish richness	0.23	0.35	0.22	0.16	0.36	0.62	0.35	0.06											
10. Migratory fish richness	0.41	**0.74**	0.34	0.15	0.68	0.12	**0.80**	0.15	0.18										
11. Historical versus actual richness	0.40	0.19	0.02	0.32	0.15	−0.09	0.22	0.35	−0.03	0.12									
12. Non-native fish abundance	0.01	0.19	0.12	0.04	0.25	0.12	0.27	0.05	0.21	0.14	−0.29								
13. Non-native fish richness	0.01	0.22	0.15	0.06	0.28	0.14	0.30	0.04	0.23	0.18	−0.29	**0.96**							
14. Traslocated fish abundance	−0.13	−0.07	−0.06	0.00	0.09	−0.06	0.21	0.28	−0.06	0.14	0.08	0.14	0.13						
15. Traslocated fish richness	−0.08	−0.09	0.01	0.06	0.14	−0.04	0.15	0.21	−0.03	0.10	0.00	0.17	0.17	**0.90**					
16. Introduced fish abundance	−0.05	0.12	0.05	0.00	0.25	0.07	0.34	0.18	0.15	0.18	−0.19	**0.81**	**0.80**	0.60	0.54				
17. Introduced fish richness	−0.01	0.15	0.13	0.07	0.30	0.10	0.33	0.11	0.19	0.19	−0.26	**0.86**	**0.88**	0.45	0.53	**0.91**			
18. Worst invasive fish richness	0.18	0.42	0.37	0.04	0.48	0.20	0.42	0.00	0.20	0.25	−0.25	0.65	0.68	0.02	0.08	0.49	0.59		
19. Preferred fish prey	**0.94**	0.61	0.48	0.34	0.62	0.24	0.41	0.22	0.19	0.41	0.32	0.15	0.14	0.07	0.09	0.15	0.15	0.25	
20. Mussel hosts	0.48	0.43	0.32	0.36	0.47	0.04	0.36	0.45	0.03	0.21	0.61	0.07	0.07	0.22	0.17	0.16	0.13	0.08	0.47

and abundance-based indicators, and between total native richness and that of migratory and threatened species in the Spanish Red Book (Table 2). A highly positive relationship was also found between total native richness and the local conservation index score, which also correlated well with endemic species richness (Table 2). However, total native richness was moderately related to that of threatened species according to the IUCN, European and Spanish legislation (Table 2). The latter only correlated well with the richness of rare species. A moderately strong relationship also occurred between total native richness and the number of potentially preferred prey for birds and mammals and world worst invasive species (Table 2). Hotspots of introduced and non-native fish species correlated well to each other. However, both were weakly related to mussel hosts, as opposed to native fish indicators, including the current hotspots of richness in relation to the historical fish occurring in each basin (Table 2).

The lack of strong congruence ($\rho \geq 0.7$) among the majority of indicators suggests that factors affecting them differ markedly, including the effect of protected area (Table 3). The abundance and total richness of native fish species, together with the proportion of native fish species, increased in protected compared to unprotected areas (Table 3). Protected areas also had a higher abundance of potential preferred fish prey for birds and mammals, which was strongly correlated with total native fish richness (Table 2). Protected status was negatively associated with all indicators related to introduced fish species (Table 3) but neutral for the two introduced crayfish species. This was also the case of the indicator related to threatened mussels and most indicators of the native fish fauna, including number of threatened and rare species (Table 3).

3.2. Influence of geography, habitat quality and water properties on the indicators of conservation value

The relative influence of geography, habitat and water quality variables varied with the indicators of conservation value (Table 4). Overall, both native and introduced fish indicators were negatively related to elevation. A negative relationship was also found between native fish indicators and nutrient pollution (ammonium, nitrites, nitrate and phosphate), whereas introduced-fish indicators increased in reaches

Table 3
Estimates and their associated standard errors and tests for the effect of protected area on indicators of conservation value of river reaches in NE Spain (n = 530). The direction of the effect on each indicator is shown based on the sign of estimate and significance (at $P \leq 0.05$) in generalised linear mixed models.

	Estimate	SE	χ^2	P value	Effect
Native fish					
Total native fish abundance (Nat abun)	0.20	0.05	15.46	**<0.001**	**Positive**
Total native fish richness (Nat rich)	0.12	0.05	4.90	**0.02**	**Positive**
Endemic fish richness (End rich)	0.04	0.09	0.18	0.66	Neutral
IUCN threatened fish richness (IUCN rich)	0.09	0.12	0.65	0.41	Neutral
Legally threatened fish in Spain (Leg Spain)	−0.15	0.25	0.33	0.56	Neutral
EU's threatened fish species (Leg Habitats)	0.04	0.18	0.04	0.85	Neutral
Rare fish richness (Rare rich)	0.29	0.27	1.19	0.28	Neutral
Historical versus actual richness (Hist rich)	0.33	0.04	62.86	**<0.001**	**Positive**
Ratio native:total fish richness (Ratio nat rich)	0.05	0.03	4.26	**0.03**	**Positive**
Introduced fish					
Non-native fish richness (Non-native rich)	−0.86	0.24	12.57	**<0.001**	**Negative**
Traslocated fish richness (Tras rich)	−1.19	0.23	27.06	**<0.001**	**Negative**
Worst invasive fish richness (Worst inv. rich)	−0.42	0.18	5.43	**0.02**	**Negative**
Ratio non-native:total fish richness (Ratio nnative rich)	−1.03	0.27	14.58	**<0.001**	**Negative**
Ratio introduced:total fish richness (Ratio intr rich)	−1.08	0.21	25.17	**<0.001**	**Negative**
Other categories					
Procambarus clarkii abundance (Proc abun)	−0.10	0.19	0.27	0.60	Neutral
Pacifastacus leniusculus abundance (Pacif abun)	0.45	0.29	2.33	0.13	Neutral
Mussel hosts	0.19	0.27	4.01	0.06	Neutral

Table 4
Predictors related to geography, habitat quality and water properties retained as having a significant effect (at $P \leq 0.05$) on the indicators of conservation value of fish communities in NE Spain according to generalised linear mixed models with basin as random factor (see methods for further details). The direction of effect is based on regression coefficients of predictors for each indicator of conservation value (+, positive; −, negative; +/−, inconsistent). See Table 3 for Acronyms and Appendix S2 for descriptive statistics of the predictors.

Indicators of conservation value	Elevation	Stream order	Aerial coverage	Channel morphology	Dead wood	Habitat diversity	Macrophytes	Riparian coverage	Water velocity	Ammonia-Nitrites	Conductivity	Nitrate	pH	Phosphate
Native fish fauna														
Nat abun	ns	ns	ns	ns	ns	−	+	ns	+	−	ns	ns	ns	−
Nat rich	−	ns	ns	ns	ns	+	+	ns	ns	−	ns	ns	+	−
End rich	−	+/−	ns	ns	ns	ns	+	ns	ns	−	+	ns	+	ns
IUCN rich	−	ns	−	ns	ns	ns	ns	ns	ns	ns	−	ns	ns	−
Leg Spain	ns	+/−	−	ns	ns	+/−	ns	ns	+	ns	+	ns	ns	−
Leg Habitats	ns	ns	−	ns	ns	ns	ns	ns	ns	−	+	ns	ns	ns
Rare rich	−	ns	ns	ns	ns	ns	ns	ns	+	−	+	ns	ns	−
Migr rich	ns	+	ns	ns	ns	+/−	ns	ns	ns	−	−	ns	+	ns
Hist rich	ns	−	ns	ns	ns	+/−	+	ns	ns	ns	ns	−	ns	ns
Ratio nat rich	ns	−	ns	+/−	ns	ns	+/−	ns	ns	ns	−	ns	−	ns
Introduced fish fauna														
Exo rich	−	+	ns	+/−	ns	ns	ns	ns	−	ns	+	ns	+	ns
Tras rich	ns	+	ns	ns	ns	+	ns	ns	ns	ns	ns	ns	ns	ns
Worst rich	−	+	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	+	ns
Ratio exo rich	−	+	ns	−	ns	ns	ns	ns	ns	ns	+	ns	+	ns
Ratio intr rich	ns	+	ns	−	ns	ns	−	ns	ns	ns	+	ns	+	ns
Other categories														
Proc abun	−	+	ns	ns	ns	−	ns	ns	ns	ns	ns	+	ns	ns
Pacif abun	ns	ns	+	+/−	+	+	ns	ns	ns	ns	−	+	+	ns
Mussels' hosts	ns	ns	ns	ns	ns	+	+	ns	ns	ns	+	ns	ns	ns

with low water velocity, low macrophyte coverage, and high pH and conductivity, as proxy of salinity (Fig. 2). Interestingly, the nativeness of fish communities declined with conductivity and river size, as defined by Strahler stream order (Table 4). Small streams typically had the most well-preserved native fish fauna (Appendix S3), including the largest proportion of native species historically present in each basin (Fig. 3). As for introduced fish, the abundance of the crayfish *P. clarkii* increased with river size and nitrate pollution (Table 4). The latter was also related to the presence of the crayfish *Pacifastacus leniusculus*, which was also associated with reaches with a higher habitat complexity and lower salinity (Table 4).

The total variation in the composition of fish communities (R^2 = 24%) was mostly driven by geographical features (15%) followed by habitat quality (3%) and water properties (2%, Fig. 4). The total explained variance by the dissimilarity analysis (beta diversity) was higher, either in its turnover (69%) or nestedness-resultant component (79%). Geographical features made the largest contribution to variation in the two components of beta diversity, representing 35% for turnover and 39% for the nestedness-resultant dissimilarity (Fig. 4). However, the latter was more related to water (14%) and habitat quality (8%) than was turnover (7 and 4%, respectively), suggesting that disturbed river reaches had a subset of species of those enriched.

4. Discussion

Our intensive survey used 20 indicators of conservation value to assess fish communities and the associated benefits for other riverine taxa. We found that hotspots of native and introduced fish richness were weakly correlated, but that the former encapsulated at least reasonably well variation in one indicator of rarity and threatened status and the benefits of fish for mussels, birds and mammals. However, protected areas had a neutral effect on most native indicators, as opposed to the introduced ones, highlighting the need to increase their value for the former. We identified tributaries as native fish refugees, and nutrient pollution, salinization, low water velocity and poor habitat structure as major threats to the native biota at the basin scale.

4.1. Low congruence among most indicators of conservation value and the limited coverage of imperilled fauna by protected areas

Our results are consistent with previous data showing that regional and Natura 2000 protected areas did not markedly favour European and IUCN threatened aquatic taxa (Abellán and Sánchez-Fernández, 2015; Guareschi et al., 2015). Our study expands fish research in the Natura 2000 network by Hermoso et al. (2015) including regional protected areas and introduced species, and showing the congruence among 20 indicators of conservation value. Since many native indicators did not strongly correlate to each other, we suggest that the design of new protected areas should use indicators that balance richness and rarity, such as the conservation index by Filipe et al. (2004). In our study though, this index generated a similar pattern of total native fish richness, highlighting the difficulties of setting conservation priorities and nourishing the debate of what to conserve (Wilson et al., 2006; Polasky et al., 2008). This debate also applies to the use of richness or abundance data in ecological research (Brotons et al., 2004; Howard et al., 2014). Despite their strong correlation in our study, it is advisable that the former provides unique information, such as population viability (Morris et al., 2002).

In our study, total native fish richness and abundance also correlated reasonably well with the number of potential preferred fish prey for birds and mammals compared to mussel hosts. This can be attributed to the host specificity of mussel's larvae, which also varies across species (Lopes-Lima et al., in press). However, it may be related to the fact that this aspect of mussels' biology is still poorly studied (Lopes-Lima et al., in press). Differences in congruence among these indicators also resulted in a different coverage by protected areas, having a neutral effect on the mussel hosts and a positive effect on the prey for birds and mammals. These results support the notion that current protected areas were designed primarily to protect terrestrial taxa (Lawrence et al., 2011; Hermoso et al., 2015). Likewise, they illustrate that the protection of species interactions is largely neglected in conservation (Valiente-Banuet et al., 2015), even though freshwater mussels are one of the most imperilled faunal groups (Lopes-Lima et al., in press). For instance,

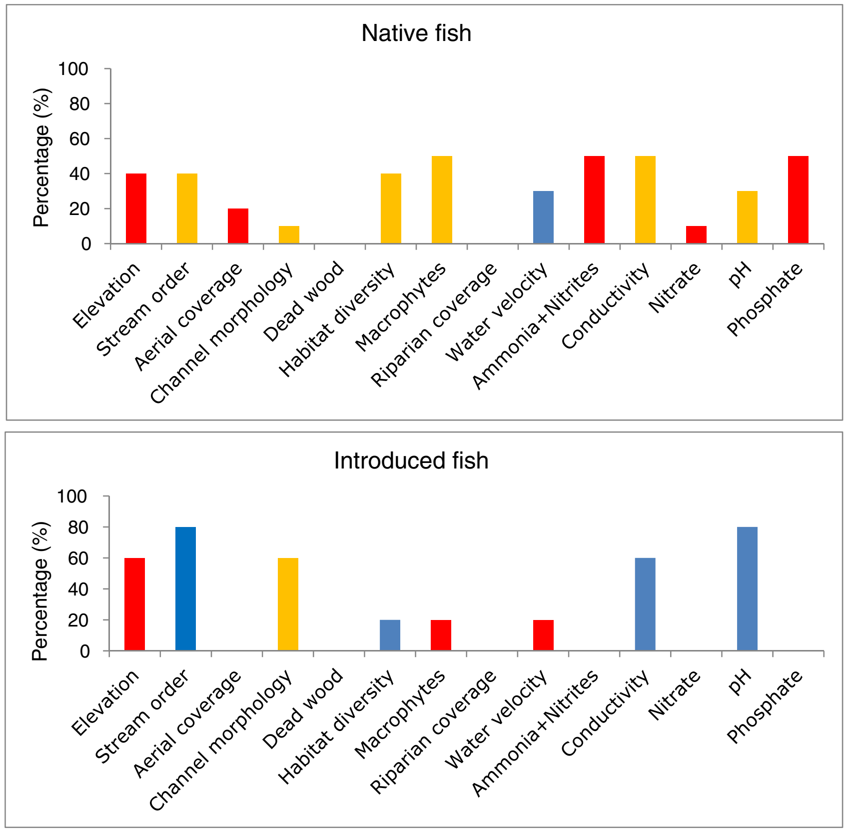

Fig. 2. Percentage of generalised linear mixed models (from Table 4) in which each predictor was retained as having a significant effect (positive: blue, negative: red, and both: orange) on indicators of conservation value related to native and introduced (non-native + translocated) fish species in river reaches of NE Spain. See Appendix S2 for predictor value ranges. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the river blenny (*Salaria fluviatilis*) is not listed as threatened in the IUCN but if left unprotected in Spain, the conservation of the highly threatened mussel *Margaritifera auricularia* can be seriously compromised. This blenny is the unique living host for the larvae of *M. auricularia*, a species that is only found in the Ebro basin (Araujo et al., 2001; Lopes-Lima et al., in press).

Although protected areas did not favour all indicators of threatened status in our study area, they had higher native and less introduced richness and abundance than unprotected areas. We are not aware of eradication or control campaigns for introduced fish in protected areas of NE Spain, therefore our results are likely to be attributed to the fact that protected areas restrict activities such as angling, which is a major pathway of fish introductions (Marr et al., 2010; Maceda-Veiga, 2013). Low human disturbance may also explain why protected areas had more native fish species. However, focal reach protection often does not guarantee good conditions for aquatic taxa, as upstream and downstream insults can jeopardise conservation goals (Nel et al., 2007; Linke et al., 2008). As in other Mediterranean-climate areas (Hermoso et al., 2013; Moyle, 2014), tributaries acted in our study as major refugia for native fish. We thus urge their protection to arrest the decline in native fish species observed in NE Spain (Maceda-Veiga et al., 2010) and especially for 38% of native fish species with a focal distribution. These protected areas could also act as green corridors for many terrestrial species (Baschak and Brown, 1995).

4.2. Management actions should also focus on improving water quality and restoring the natural hydrological regime

Besides the creation of fluvial reserves, we argue that the protection of aquatic fauna requires improving water chemical status, as reported in other Mediterranean regions (Petrovic et al., 2011; Moyle, 2014). Our results show that salinization (Cañedo-Argüelles et al., 2013) and nutrient pollution (e.g. nitrite, nitrate, phosphate) (Camargo and Alonso, 2006; Johnson et al., 2010) are two of the major threats to native fish. However, their interaction has to be considered in management practices. Salinity can alleviate nitrite toxicity to freshwater taxa (Alonso and Camargo, 2008; Noga, 2011) and be anti-parasitic, as reported in aquaculture (Noga, 2011; Maceda-Veiga and Cable, 2014). Likewise, nitrate can protect fish against some monogenean infections (Smallbone et al., 2016). Thus, it may happen that a partial removal of pollutants can worsen the status of a species, and highlights the urgent need of studies examining the context-dependence of effects of pollutant interactions on wild fish populations (Hamilton et al., 2015; Colin et al., 2016). This is particularly important given the complex mixtures of pollutants occurring in rivers (Petrovic et al., 2011; Kuzmanović et al., 2015; Hukari et al., 2016), including water quality hazards such as phosphates for which there is limited insight into their direct toxicity to aquatic taxa.

Poor water and habitat quality were also related in our study to the proliferation of introduced fish species. In particular, they were mostly found in reaches with low water velocity and altered channel morphology, including embankments and weirs, supporting the notion that the natural hydrology of Mediterranean rivers protects native fish (Marchetti et al., 2004; Kiernan et al., 2012). Restoring river connectivity is of major importance to allow migratory species (e.g. *A. anguilla*, *Luciobarbus graellsii*) access to fluvial reserves, although they may transport toxicants and diseases from downstream areas (see Flecker et al., 2010). However, restoring the natural hydrological regime may not control the spread of introduced species, as translocated native species, which represent 26% of fish introductions in our study, have evolved under the Mediterranean climate. Further, non-native species can occur in natural Mediterranean streams (Moyle, 2014; Closs et al., 2015), even though most species, including the worst invaders *Micropterus salmoides, Cyprinus carpio* and *Gambusia holbrooki*, perform

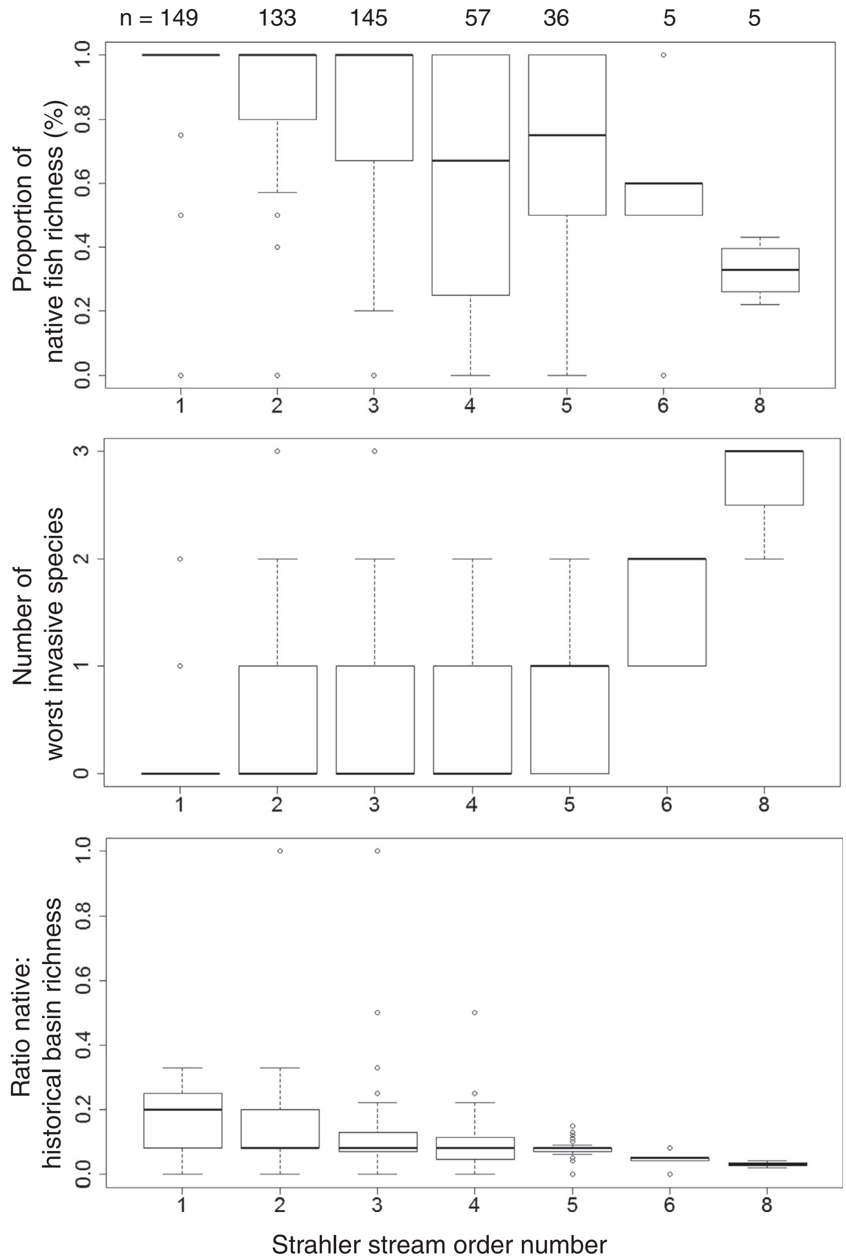

Fig. 3. Relationships between river size as defined by the Strahler river order number and the degree of nativeness of the fish community, including the number of world worst invasive fish species in river reaches of NE Spain (see methods for further details). The number of sampling sites per stream order is shown on the top, and details on fish species composition according to stream order are provided in Appendix S3.

better in low water velocities (Marr et al., 2010; Doadrio et al., 2011). Nonetheless, it is worth noting that their eradication may not be desirable, as long-term introduced species can be playing a key role in recipient communities (Schlaepfer et al., 2011).

Since *C. carpio* and *P. clarkii* were common non-native species in our study area, and profoundly alter aquatic ecosystems (e.g. bioturbation, macrophyte removal) (Gherardi, 2006; Shin-ichiro et al., 2009), it is likely that some of the associations found between introduced species and river conditions are partly explained by their activity. Whether induced by non-native species or not, according to our partitioning analysis of dissimilarity, environmental degradation seem to cause the loss of sensitive species in fish communities, as reported in aquatic invertebrates (Gutiérrez-Cánovas et al., 2013). In our study, the relationship between the nestedness-resultant dissimilarity and water and habitat degradation was poorer (8–14%) than that (31–51%) reported by Gutiérrez-Cánovas et al. (2013). However, our results support previous data on aquatic organisms showing a poor relationship between beta diversity (or related measures) and environmental conditions (Beisner et al., 2006; Heino et al., 2015). Although the reasons are not fully understood, the low explanatory power could be related to the presence of rare species (i.e. numerous absences in the site-by-site species matrix) (see Heino et al., 2015). However, it may also be attributed to the fact that aquatic ecosystems are highly dynamic and a single snapshot sampling of biota and abiotic conditions fails to reveal strong community-environmental relationships (Beisner et al., 2006; Erős et al., 2012; Heino et al., 2015).

The loss of sensitive species does not necessarily mean that the fish community was dominated by non-native species, as taxa sensitive to poor water and habitat quality exist among native and non-native species (Kennard et al., 2005; Maceda-Veiga and de Sostoa, 2011; Segurado et al., 2011). In-depth knowledge of the physiological response of fish to multiple stressors (Maceda-Veiga et al., 2015; Colin et al., 2016) can

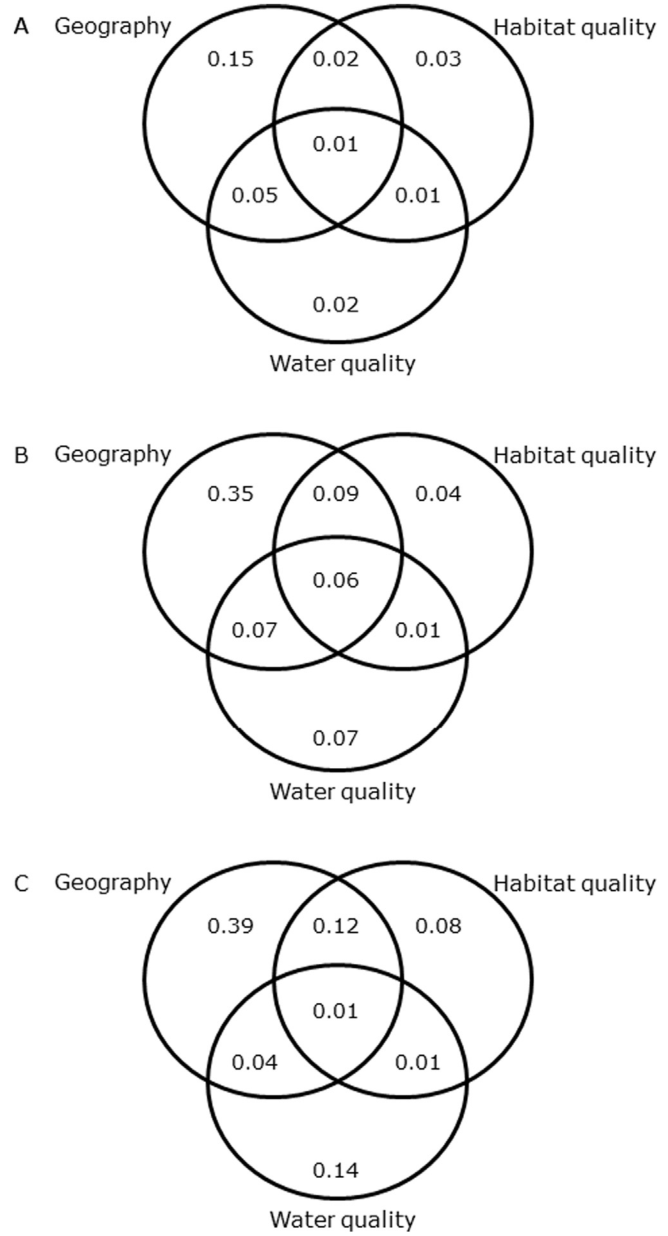

Fig. 4. Venn diagrams showing the unique and shared fractions of variation in fish communities of NE Spain (adjusted R^2) explained by geography, habitat and water quality, for (A) total variation in species composition, and for the two components of total dissimilarity: (B) spatial turnover and (C) nestedness-resultant dissimilarity.

then recommend special protection to particular species, coupled to tributaries as general native fish refugees. In our study, however, the largest fraction of variation in species composition was related to geography, supporting that each basin is a biogeographic unit and has its own history of biological invasions (Doadrio, 1988; Leprieur et al., 2008b). Thus, fine-scale studies in each basin are needed to fully test the potential of tributaries as fluvial reserves, including indicators of taxonomic, functional and phylogenetic diversity that consider interspecific relationships (Strecker et al., 2011; Guareschi et al., 2015; Valiente-Banuet et al., 2015).

5. Conclusions

Our study shows that fish fauna in Mediterranean rivers is at risk by multiple stressors. Different indicators of conservation value are related to different sets of stressors, but restoring water quality and natural flow regimes were identified as management priorities. It will help conserve riverine aquatic diversity and ensure, at a lower cost, the quality of freshwater resources upon which human populations depend on (Vörösmarty et al., 2010; Green et al., 2015). As a complementary action, we propose careful monitoring and focal removal of introduced species in tributaries as current native fish diversity refugees (see also Hermoso et al., 2013). The efficacy of common fish catching methods (electrofishing) is also higher in small than in large rivers (Bohlin et al., 1989). However, the design of fluvial reserves is complex and requires the selection of multiple protected zones with different management regimes (Linke et al., 2008, 2012; Hermoso et al., 2016). Nonetheless, we believe that the design of new protected areas should not change the focus on the management of hydrological regimes and sewage discharges at the basin scale, as this is the most effective way to conserve fluvial diversity. In this regard, our study suggests the need of establishing safe thresholds of pollutant mixtures for the native fauna, especially under forecast climate conditions, and the use of genetic tools to reveal taxonomic gaps.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.09.097.

References

Abellán, P., Sánchez-Fernández, D., 2015. A gap analysis comparing the effectiveness of Natura 2000 and national protected area networks in representing European amphibians and reptiles. Biodivers. Conserv. 24 (6), 1377–1390.
Aho, K., Derryberry, D., Peterson, T., 2014. Model selection for ecologists: the worldviews of AIC and BIC. Ecology 95, 631–636.
Allan, D., Erickson, D., Fay, J., 1997. The influence of catchment land use on stream integrity across multiple spatial scales. Freshw. Biol. 37 (1), 149–161.
Allouche, O., Kalyuzhny, M., Moreno-Rueda, G., Pizarro, M., Kadmon, R., 2012. Area–heterogeneity tradeoff and the diversity of ecological communities. Proc. Natl. Acad. Sci. 109 (43), 17495–17500.
Alonso, A., Camargo, J.A., 2008. Ameliorating effect of chloride on nitrite toxicity to freshwater invertebrates with different physiology: a comparative study between amphipods and planarians. Arch. Environ. Contam. Toxicol. 54 (2), 259–265.
Aparicio, E., Carmona-Catot, G., Kottelat, M., Perea, S., Doadrio, I., 2013. Identification of Gobio populations in the northeastern Iberian Peninsula: first record of the non-native Languedoc gudgeon *Gobio occitaniae* (Teleostei, Cyprinidae). BioInvasions Records 2 (2), 163–166.
Araujo, R., Bragado, D., Ramos, M.A., 2001. Identification of the river blenny, *Salaria fluviatilis*, as a host to the glochidia of *Margaritifera auricularia*. J. Molluscan Stud. 67, 128–129.
Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish. 2ª edició. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C..
Baschak, L.A., Brown, R.D., 1995. An ecological framework for the planning, design and management of urban river greenways. Landsc. Urban Plan. 33, 211–225.
Baselga, A., Orme, C.D.L., 2012. Betapart: an R package for the study of beta diversity. Methods Ecol. Evol. 3 (5), 808–812.
Baselga, A., 2010. Partitioning the turnover and nestedness components of beta diversity. Glob. Ecol. Biogeogr. 19 (1), 134–143.

Bates, D., Maechler, M., 2009. lme4: Linear Mixed-Effects Models Using S4 classes. R Package Version 0.999375–31. Available in http://CRAN.R-project.org/package=lme4.
Battisti, C., Fanelli, G., 2015. Don't think local! Scale in conservation, parochialism, dogmatic bureaucracy and the implementing of the European directives. J. Nat. Conserv. 24, 24–30.
Beisner, B.E., Peres-Neto, P.R., Lindström, E., Barnett, A., Longhi, M.L., 2006. The role of environmental and spatial processes in structuring lake communities from bacteria to fish. Ecology 87, 2985–2991.
Benejam, L., Alcaraz, C., Benito, J., Caiola, N., Casals, F., et al., 2012. Fish catchability and comparison of four electrofishing crews in Mediterranean streams. Fish. Res. 123, 9–15.
Bohlin, T., Hamrin, S., Heggberget, T.G., Rasmussen, G., Saltveit, S.J., 1989. Electrofishing—theory and practice with special emphasis on salmonids. Hydrobiologia 173 (1), 9–43.
Borcard, D., Legendre, P., Drapeau, P., 1992. Partialling out the spatial component of ecological variation. Ecology 73 (3), 1045–1055.
Brotons, L., Thuiller, W., Araújo, M.B., Hirzel, A.H., 2004. Presence-absence versus presence-only modelling methods for predicting bird habitat suitability. Ecography 27 (4), 437–448.
Cade, B.S., 2015. Model averaging and muddled multimodel inferences. Ecology 96, 2370–2382.
Camargo, J.A., Alonso, Á., 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ. Int. 32 (6), 831–849.
Cañedo-Argüelles, M., Kefford, B.J., Piscart, C., Prat, N., Schäfer, R.B., Schulz, C.J., 2013. Salinisation of rivers: an urgent ecological issue. Environ. Pollut. 173, 157–167.
Carvalho, F., Pascoal, C., Cássio, F., Sousa, R., 2016. Direct and indirect effects of an invasive omnivore crayfish on leaf litter decomposition. Sci. Total Environ. 541, 714–720.
Closs, G.P., Krkosek, M., Olden, J.D., 2015. Conservation of Freshwater Fishes (Vol. 20). Cambridge University Press.
Cobo, F., Vieira-Lanero, R., Rego, E., Servia, M., 2010. Temporal trends in non-indigenous freshwater species records during the 20th century: a case study in the Iberian Peninsula. Biodivers. Conserv. 12 (19), 3471–3487.
Colin, N., Porte, C., Fernandes, D., Barata, C., Padrós, F., et al., 2016. Ecological relevance of biomarkers in monitoring studies of macro-invertebrates and fish in Mediterranean rivers. Sci. Total Environ. 540, 307–323.
Collen, B., Whitton, F., Dyer, E.E., Baillie, J.E., Cumberlidge, N., et al., 2014. Global patterns of freshwater species diversity, threat and endemism. Glob. Ecol. Biogeogr. 23 (1), 40–51.
Crawley MJ. The R book. Wiley, England; 2007.
Doadrio, I., Perea, S., Garzón-Heydt, P., JL, G., 2011. Ictiofauna continental española. Bases para su seguimiento. DG Medio Natural y Política Forestal, MARM, Madrid, Spain.
Doadrio, I., 1988. Delimitation of areas in the Iberian Peninsula on the basis of freshwater fishes. Bonner Zoologische Beiträge 39 (2–3), 113–128.
Erős, T., Saly, P., Takacs, P., Specziar, A., Biro, P., 2012. Temporal variability in the spatial and environmental determinants of functional metacommunity organization – stream fish in a human-modified landscape. Freshw. Biol. 57, 1914–1928.
Figuerola, B., Maceda-Veiga, A., de Sostoa, A., 2012. Assessing the effects of sewage effluents in a Mediterranean creek: fish population features and biotic indices. Hydrobiologia 694, 75–86.
Filipe, A.F., Marques, T.A., Tiago, P., Ribeiro, F., Da Costa, L.M., Cowx, I.G., Collares-Pereira, M.J., 2004. Selection of priority areas for fish conservation in Guadiana River Basin, Iberian Peninsula. Conserv. Biol. 18 (1), 189–200.
Flecker, A.S., McIntyre, P.B., Moore, J.W., Anderson, J.T., Taylor, B.W., Hall, J.R.O., 2010. Migratory Fishes as Material and Process Subsidies in Riverine Ecosystems. In Community Ecology of Stream Fishes: Concepts, Approaches, and Techniques. American Fisheries Society, Symposium. Vol. 73 pp. 559–592.
Foley, J.A., DeFries, R., Asner, G.P., Barford, C., et al., 2005. Global consequences of land use. Science 309 (5734), 570–574.
Fox, J., Weisberg, S., 2011. An {R} Companion to Applied Regression, Second Edition.Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.
Gasith, A., Resh, V.H., 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annu. Rev. Ecol. Syst. 51-81.
Gaston, K.J., Jackson, S.F., Cantú-Salazar, L., Cruz-Piñón, G., 2008. The ecological performance of protected areas. Annu. Rev. Ecol. Evol. Syst. 93-113.
Gherardi, F., 2006. Crayfish invading Europe: the case study of *Procambarus clarkii*. Mar. Freshw. Behav. Physiol. 39, 175–191.
Green, P.A., Vörösmarty, C.J., Harrison, I., Farrell, T., Sáenz, L., et al., 2015. Freshwater ecosystem services supporting humans: pivoting from water crisis to water solutions. Glob. Environ. Chang. 34, 108–118.
Guareschi, S., Bilton, D.T., Velasco, J., Millán, A., Abellán, P., 2015. How well do protected area networks support taxonomic and functional diversity in non-target taxa? The case of Iberian freshwaters. Biol. Conserv. 187, 134–144.
Gutiérrez-Cánovas, C., Millán, A., Velasco, J., Vaughan, I.P., Ormerod, S.J., 2013. Contrasting effects of natural and anthropogenic stressors on beta diversity in river organisms. Glob. Ecol. Biogeogr. 22 (7), 796–805.
Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., et al., 2008. A global map of human impact on marine ecosystems. Science 319 (5865), 948–952.
Hamilton, P.B., Cowx, I.G., MF, O., Griffiths, A.M., Grahn, M., et al., 2015. Population-level consequences for wild fish exposed to sublethal concentrations of chemicals–a critical review. Fish Fish. Early view.
Heino, J., Melo, A.S., Bini, L.M., Altermatt, F., Al-Shami, S.A., Angeler, D.G., et al., 2015. A comparative analysis reveals weak relationships between ecological factors and beta diversity of stream insect metacommunities at two spatial levels. Ecology and Evolution 5, 1235–1248.
Hermoso, V., Ward, D.P., Kennard, M.J., 2013. Prioritizing refugia for freshwater biodiversity conservation in highly seasonal ecosystems. Divers. Distrib. 19 (8), 1031–1042.
Hermoso, V., Filipe, A.F., Segurado, P., Beja, P., 2015. Effectiveness of a large reserve network in protecting freshwater biodiversity: a test for the Iberian Peninsula. Freshw. Biol. 60 (4), 698–710.
Hermoso, V., Filipe, A.F., Segurado, P., Beja, P., 2016. Catchment zoning to unlock freshwater conservation opportunities in the Iberian Peninsula. Divers. Distrib. (Early view).
Hooke, J.M., 2006. Human impacts on fluvial systems in the Mediterranean region. Geomorphology 79 (3), 311–335.
Howard, C., Stephens, P.A., Pearce-Higgins, J.W., Gregory, R.D., Willis, S.G., 2014. Improving species distribution models: the value of data on abundance. Methods Ecol. Evol. 5 (6), 506–513.
Hukari, S., Hermann, L., Nättorp, A., 2016. From wastewater to fertilisers—technical overview and critical review of European legislation governing phosphorus recycling. Sci. Total Environ. 542, 1127–1135.
Johnson, P.T., Townsend, A.R., Cleveland, C.C., Glibert, P.M., Howarth, R.W., et al., 2010. Linking environmental nutrient enrichment and disease emergence in humans and wildlife. Ecol. Appl. 20 (1), 16–29.
Kennard, M.J., Arthington, A.H., Pusey, B.J., Harch, B.D., 2005. Are alien fish a reliable indicator of river health? Freshw. Biol. 50 (1), 174–193.
Kiernan, J.D., Moyle, P.B., Crain, P.K., 2012. Restoring native fish assemblages to a regulated California stream using the natural flow regime concept. Ecol. Appl. 22 (5), 1472–1482.
Kuzmanović, M., Ginebreda, A., Petrović, M., Barceló, D., 2015. Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers. Sci. Total Environ. 503, 289–299.
Lamoreux, J.F., Morrison, J.C., Ricketts, T.H., Olson, D.M., Dinerstein, E., et al., 2006. Global tests of biodiversity concordance and the importance of endemism. Nature 440 (7081), 212–214.
Lawrence, D.J., Larson, E.R., Liermann, C.A.R., Mims, M.C., Pool, T.K., et al., 2011. National parks as protected areas for US freshwater fish diversity. Conserv. Lett. 4 (5), 364–371.
Leek, J.T., Peng, R.D., 2015. Statistics: *P* values are just the tip of the iceberg. Nature 520, 612.
Lekuona, J.M., Campos, F., 1997. Foraging ecology of Cormorants (*Phalacrocorax carbo*) wintering in northern Spain. Folia Zool. 46 (3), 243–252.
Leprieur, F., Beauchard, O., Blanchet, S., Oberdorff, T., Brosse, S., 2008a. Fish invasions in the world's river systems: when natural processes are blurred by human activities. PLoS Biol. 6 (2), e28.
Leprieur, F., Beauchard, O., Hugueny, B., Grenouillet, G., Brosse, S., 2008b. Null model of biotic homogenization: a test with the European freshwater fish fauna. Divers. Distrib. 14 (2), 291–300.
Linke, S., Norris, R.H., Pressey, R.L., 2008. Irreplaceability of river networks: towards catchment-based conservation planning. J. Appl. Ecol. 45 (5), 1486–1495.
Linke, S., Kennard, M.J., Hermoso, V., Olden, J.D., Stein, J., Pusey, B.J., 2012. Merging connectivity rules and large-scale condition assessment improves conservation adequacy in river systems. J. Appl. Ecol. 49, 1036–1045.
Lomolino, M.V., 2000. Ecology's most general, yet protean pattern: the species-area relationship. J. Biogeogr. 27 (1), 17–26.
Lopes-Lima, M., Sousa, R., Geist, J., Aldridge, D.C., Araujo, R., et al., 2016. Conservation status of freshwater mussels in Europe: state of the art and future challenges. Biol. Rev. (in press).
Maceda-Veiga, A., Cable, J., 2014. Efficacy of sea salt, metronidazole and formalin-malachite green baths in treating *Ichthyophthirius multifiliis* infections of mollies (*Poecilia sphenops*). Bull. Eur. Assoc. Fish Pathol. 34 (5), 183.
Maceda-Veiga, A., De Sostoa, A., 2011. Observational evidence of the sensitivity of some fish species to environmental stressors in Mediterranean rivers. Ecol. Indic. 11 (2), 311–317.
Maceda-Veiga, A., Monleon-Getino, A., Caiola, N., Casals, F., De Sostoa, A., 2010. Changes in fish assemblages in catchments in north-eastern Spain: biodiversity, conservation status and introduced species. Freshw. Biol. 55, 1734–1746.
Maceda-Veiga, A., De Sostoa, A., Sánchez-Espada, S., 2013. Factors affecting the establishment of the invasive crayfish *Procambarus clarkii* (Crustacea, Decapoda) in the Mediterranean rivers of the northeastern Iberian Peninsula. Hydrobiologia 703 (1), 33–45.
Maceda-Veiga, A., Figuerola, J., Martínez-Silvestre, A., Viscor, G., Ferrari, N., Pacheco, M., 2015. Inside the Redbox: applications of haematology in wildlife monitoring and ecosystem health assessment. Sci. Total Environ. 514, 322–332.
Maceda-Veiga, A., 2013. Towards the conservation of freshwater fish: Iberian Rivers as an example of threats and management practices. Rev. Fish Biol. Fish. 23 (1), 1–22.
Marchetti, M.P., Light, T., Moyle, P.B., Viers, J.H., 2004. Fish invasions in California watersheds: testing hypotheses using landscape patterns. Ecol. Appl. 14 (5), 1507–1525.
Margules, C.R., Usher, M.B., 1981. Criteria used in assessing wildlife conservation potential: a review. Biol. Conserv. 21, 79–109.
Marr, S.M., Marchetti, M.P., Olden, J.D., García-Berthou, E., Morgan, D.L., et al., 2010. Freshwater fish introductions in mediterranean-climate regions: are there commonalities in the conservation problem? Divers. Distrib. 16 (4), 606–619.
Morris, W.F., Bloch, P.L., Hudgens, B.R., Moyle, L.C., Stinchcombe, J.R., 2002. Population viability analysis in endangered species recovery plans: past use and future improvements. Ecol. Appl. 12 (3), 708–712.
Moyle, P.B., 2014. Novel aquatic ecosystems: the new reality for streams in California and other Mediterranean climate regions. River Res. Appl. 30 (10), 1335–1344.
Moyle, P.B., Katz, J.V.E., Quinones, R.M., 2011. Rapid decline of California's native inland fishes: a status assessment. Biol. Conserv. 144, 2414–2423.
Munné, A., Prat, N., Solà, C., Bonada, N., Rieradevall, M., 2003. A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. Aquat. Conserv. Mar. Freshwat. Ecosyst. 13, 147–163.

Murphy, C.A., Casals, F., Solà, C., Caiola, N., de Sostoa, A., García-Berthou, E., 2013. Efficacy of population size structure as a bioassessment tool in freshwaters. Ecol. Indic. 34, 571–579.
Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403 (6772), 853–858.
Nel, J.L., Roux, D.J., Maree, G., Kleynhans, C.J., Moolman, J., et al., 2007. Rivers in peril inside and outside protected areas: a systematic approach to conservation assessment of river ecosystems. Divers. Distrib. 13, 341–352.
Noga, E.J., 2011. Fish Disease: Diagnosis and Treatment. John Wiley & Sons.
Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Peter, R., et al., 2015. Vegan: Community Ecology Package. R package version 2.3–0. http://CRAN.R-project.org/package=vegan.
O'Riordan, T., Stoll-Kleeman, S., 2002. In: O'Riordan, T., Stoll-Kleeman, S. (Eds.), Biodiversity, Sustainability and Human Communities: Protecting Beyond the Protected. Cambridge Univ. Press, Cambridge, pp. 87–112.
Orme, C.D.L., Davies, R.G., Burgess, M., Eigenbrod, F., Pickup, N., et al., 2005. Global hotspots of species richness are not congruent with endemism or threat. Nature 436 (7053), 1016–1019.
Palomo, L.J., Gisbert, J., Blanco, J.C., 2007. Atlas y Libro Rojo de los Mamíferos Terrestres de España. Dirección General para la Biodiversidad-SECEM-SECEMU (Madrid, 588 pp).
Petrovic, M., Ginebreda, A., Acuña, V., Batalla, R.J., Elosegi, A., et al., 2011. Combined scenarios of chemical and ecological quality under water scarcity in Mediterranean rivers. Trends Anal. Chem. 30 (8), 1269–1278.
Polasky, S., Nelson, E., Camm, J., Csuti, B., Fackler, P., et al., 2008. Where to put things? Spatial land management to sustain biodiversity and economic returns. Biol. Conserv. 141 (6), 1505–1524.
R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria 3-900051-07-0 http://www.R-project.org/.
Reyjol, Y., Hugueny, B., Pont, D., Bianco, P.G., Beier, U., et al., 2007. Patterns in species richness and endemism of European freshwater fish. Glob. Ecol. Biogeogr. 16 (1), 65–75.
Ruiz-Olmo, J., López-Martín, J.M., Palazón, S., 2001. The influence of fish abundance on the otter (Lutra lutra) populations in Iberian Mediterranean habitats. J. Zool. 254 (03), 325–336.
Saunders, D.L., Meeuwig, J.J., Vincent, A.C.J., 2002. Freshwater protected areas: strategies for conservation. Conserv. Biol. 16 (1), 30–41.
Schlaepfer, M.A., Sax, D.F., Olden, J.D., 2011. The potential conservation value of non-native species. Conserv. Biol. 25 (3), 428–437.
Segurado, P., Santos, J.M., Pont, D., Melcher, A.H., Jalon, D.G., Hughes, R.M., Ferreira, M.T., 2011. Estimating species tolerance to human perturbation: expert judgment versus empirical approaches. Ecol. Indic. 11 (6), 1623–1635.
SEO/BirdLife, 2012a. Atlas de las aves en invierno en España 2007-2010. Ministerio de Agricultura, Alimentación y Medio Ambiente-SEO/ BirdLife (Madrid).
SEO/BirdLife, 2012b. Atlas de las reproductoras de España 2007-2010. Ministerio de Agricultura, Alimentación y Medio Ambiente-SEO/ BirdLife (Madrid).
Shin-ichiro, S.M., Usio, N., Takamura, N., Washitani, I., 2009. Contrasting impacts of invasive engineers on freshwater ecosystems: an experiment and meta-analysis. Oecologia 158 (4), 673–686.
Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D., et al., 2013. Impacts of biological invasions: what's what and the way forward. Trends Ecol. Evol. 28, 58–66.
Smallbone, W., Cable, J., Maceda-Veiga, A., 2016. Chronic nitrate enrichment decreases severity and induces protection against an infectious disease. Environ. Int. 91, 265–270.
Smith, K.G., Darwall, W.R.T., 2006. The Status and Distribution of Freshwater Fish Endemic to the Mediterranean Basin. IUCN, Gland, Switzerland and Cambridge, UK (v + 34 pp).
Strahler, A.N., 1964. Quantitative geomorphology of drainage basin and channel networks. Handb. Appl. Hydrol.
Strayer, D.L., Dudgeon, D., 2010. Freshwater biodiversity conservation: recent progress and future challenges. J. N. Am. Benthol. Soc. 29 (1), 344–358.
Strayer, D.L., Downing, J.A., Haag, W.R., King, T.L., Layzer, T.B., Newton, T.J., Nichols, S.J., 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. Bioscience 54, 429–439.
Strecker, A.L., Olden, J.D., Whittier, J.B., Paukert, C.P., 2011. Defining conservation priorities for freshwater fishes according to taxonomic, functional, and phylogenetic diversity. Ecol. Appl. 21 (8), 3002–3013.
Tablado, Z., Tella, J.L., Sánchez-Zapata, J.A., Hiraldo, F., 2010. The paradox of the long-term positive effects of a north American crayfish on a European Community of predators. Conserv. Biol. 24 (5), 1230–1238.
Thomas, R.J., Lello, J., Medieros, R., Pollard, A., Seward, A., Smith, J., et al., 2015. Data analysis with R statistical software: a guidebook for scientists. Eco-Explore.
Tisseuil, C., Cornu, J.F., Beauchard, O., Brosse, S., Darwall, W., et al., 2013. Global diversity patterns and cross-taxa convergence in freshwater systems. J. Anim. Ecol. 82 (2), 365–376.
Tittensor, D.P., Walpole, M., Hill, S.L.L., Boyce, D.G., Britten, G.L., et al., 2014. A mid-term analysis of progress towards international biodiversity targets. Science 346, 241–244.
Valiente-Banuet, A., Aizen, M.A., Alcántara, J.M., Arroyo, J., Cocucci, A., et al., 2015. Beyond species loss: the extinction of ecological interactions in a changing world. Funct. Ecol. 29 (3), 299–307.
Venables WN, Ripley BD. Modern Applied Statistics with S·Fourth Edition. Springer, New York. 2002; ISBN 0-387-95457-0
Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., et al., 2009. How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. Front. Ecol. Environ. 8 (3), 135–144.
Vilches, A., Miranda, R., Arizaga, J., 2012. Fish prey selection by the Common Kingfisher Alcedo atthis in Northern Iberia. Acta Ornithol. 47 (2), 167–175.
Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., et al., 2010. Global threats to human water security and river biodiversity. Nature 467 (7315), 555–561.
Wilson, K.A., McBride, M.F., Bode, M., Possingham, H.P., 2006. Prioritizing global conservation efforts. Nature 440 (7082), 337–340.
Winfield, I.J., Townsend, C.R., 1991. The role of cyprinids in ecosystems. In: Winfield, I.J., Nelson, J.S. (Eds.), Cyprinid Fishes. Systematics, biology and exploitation. Fish and Fisheries Series, 3, Great Britain, pp. 552–567 Chapman & Hall.
Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Gail M, Krickeberg K, Samet JM, Tsiatis a, Wong W, Editors. Spring Science and Business Media, New York, NY.